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Présentée par EMILIE DELETRE

DE LA REPULSION CHEZ LES INSECTES
- Cas d'étude du moustique *Anopheles gambiae* et de la mouche blanche *Bemisia tabaci* -

« My perfume? Actually, it's called *insect repellent* but at the moment it doesn't seem to be working? »

Soutenue le 24 novembre 2014 devant le jury composé de

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Merci…

‘Le cœur peut s’émouvoir souvent à la rencontre d’un autre être, car chacun exerce sur chacun des attractions et des répulsions’ *Fort comme la mort* (1889), Guy De Maupassant

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Sommaire

1. Prospects for repellency in pest control – current developments and future challenges (Submitted) **E Deletre**, B Schatz, LWilliams, D Bourguet, A Ratnadass, F Chandre, T Martin, *Biological review*
2. Repellent, Irritant and Toxic Effects of 20 Plant Extracts on Adults of the Malaria Vector *Anopheles gambiae* Mosquito. (2013) **E Deletre**, T Martin, P Campagne, D Bourguet, A Cadin, C Menut, R Bonafos, F Chandre, *PLoS ONE*, 8(12):e82103.
3. Electrophysiological and behavioral characterization of bioactive compounds of four essential oils on *Anopheles gambiae* and prospects for use in treated bed nets (Submitted), **E Deletre**, F Chandre, L Williams, C Duménil, C Menut, T Martin, *J. Chemical ecology*
4. Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Submitted) **E Deletre**, M Mallent, , C Menut, F Chandre, T Martin, J. of Economic Entomology
5. Naturally Occurring Bioactive Compounds from four repellent essential oils against the whitefly *Bemisia tabaci* (Submitted) **E Deletre**, F Chandre, B Barkman, C Menut, T Martin, *J. Chemical ecology*
6. Repellent effect of alphacypermethrin-treated netting against *Bemisia tabaci* (Hemiptera: Aleyrodidae). (2014) T Martin, A Kamal, E Gogo, M Saidi, **E Delétré**, R Bonafos, S Simon, M Ngouajio *J. Econ. Entom*. 107(2):684-90.
7. Repellent net as a new technology to protect cabbage crops. (2013) T Martin, R Palix, A Kamal, **E Delétré**, R Bonafos, S Simon, M Ngouajio, *J. Econ. Entom*. 106(4):1699-706.
8. Evaluation of acaricide-treated string curtains for control of two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) on greenhouse roses and impact of the string curtain on the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (2014) **E. Deletre**, R. Bonafos, T. Martin, *Crop Protection*, 60:34–43
9. The repellency of lemongrass oil against stable flies, tested using video tracking. (2013) F Baldacchino, C Tramut, A Salem, E Liénard**, E Delétré**, M Franc, T Martin, G Duvallet, P Jay-Robert, *Parasite*, 20:21.

Table des tableaux :

Table des figures :

Introduction générale: Attraction vs Répulsion

‘Notre langage ne vaut rien pour décrire le monde des odeurs’
Le parfum (1985), Patrick Süskind.

D’après la FAO, il faudra nourrir 9 milliards de personnes en 2050 ce qui équivaudrait à augmenter de 70% la production actuelle. Une manière simple d’augmenter la production serait de limiter les pertes de rendement et de récolte en luttant contre les insectes. Il faudra donc produire plus mais il faut produire mieux. Les insecticides ont été et sont largement utilisés mais avec l’apparition de résistances, la pollution de l’environnement et l’impact sur la santé nous amène à chercher de nouvelles alternatives de lutte. La FAO a également montré que l’Afrique avait le meilleur potentiel de changement. En effet, le développement du secteur agricole permettrait de lutter contre la faim et la pauvreté. Les insectes ne sont pas qu’un problème pour l’agriculture, ils transmettent également des maladies humaines. La lutte contre les insectes peut donc aussi améliorer la santé. Cette thèse a pour objectif de trouver une solution qui réduit l’utilisation des pesticides qui soit respectueuse de l’environnement et de la santé humaine par l’identification de nouvelles substances actives dans la lutte contre les insectes nuisibles pour la santé humaine et l’agriculture. La solution étudiée ici est une barrière physico-chimique c’est à dire la combinaison entre un filet anti-insecte et un répulsif.

Deux insectes modèles importants pour l’afrique ont été choisis pour cette étude : *Anopheles gambiae,* moustique vecteur du paludisme et *Bemisia tabaci,* ravageur polyphage vecteur de virus. Ces deux insectes sont donc des suçeurs-piqueurs, vecteurs d’agents pathogènes dont le seuil de tolérance est très faible puisqu’un seul insecte suffit à rendre l’hôte malade. Le filet « répulsif » constituera une double barrière physico-répulsive qui va empêcher le moustique de piquer à travers la moustiquaire et empêcher la mouche blanche de passer à travers le filet. Dans cette introduction, dans un premier temps, l’interaction entre un insecte et son hôte sera présentée. Puis nous présenterons plus en détails l’interaction entre nos insectes modèles et leurs hôtes et leur principal moyen de lutte avant de décrire les différents phénomènes de répulsion. Enfin nous décrirons les produits répulsifs utilisés pendant la thèse.

Le règne végétal produit plusieurs centaines de milliers de substances chimiques qui affectent le comportement des insectes (Weenström *et al.*, 2010). Dans un premier temps, les composés secondaires ont été considérés comme des déchets métaboliques puis ils se sont révélés comme les éléments essentiels de la recherche, de la reconnaissance, de la sélection et de l'acceptation de la plante hôte par les insectes (Bruce *et al*., 2005a). Ils sont le moteur majeur de l’évolution de la biodiversité, résultant de la coévolution entre plantes et insectes (Thiéry *et al.*, 2013). Les interactions plantes-insectes peuvent être positives comme dans le cas de la pollinisation ou négative comme dans le cas de l’herbivorie (De boer et Dicke, 2005 ; Vet et Dicke, 1992). En effet, les insectes recherchent un hôte pour la nutrition, un site de refuge, d’accouplement ou de ponte (Schoonoven *et al.*, 2005). Ils sont sensibles à une grande variété de stimuli émis par l’hôte qui peuvent être olfactifs, visuels, tactiles et gustatifs (Thorsteinson, 1960). La sélection de l'hôte se ferait en plusieurs étapes distinctes. La première étape de «choix» est la recherche et la reconnaissance de l'hôte à distance en utilisant les signaux olfactifs et visuels, et la deuxième étape de «sélection» est l'acceptabilité de l'hôte à l'aide des indices tactiles, olfactifs et gustatifs au contact de celui-ci (Visser, 1988).

Tout comme les espèces phytophages, les insectes hématophages se nourrissant sur des hôtes : humain ou animal utilisent des stimuli olfactifs, visuels, tactiles et gustatifs pour trouver leurs hôtes. Ainsi, l'interaction animal-insecte partage un grand nombre de

ressemblance à l'interaction plante-insecte pour ce qui est de la recherche, la reconnaissance et l'acceptation de l'hôte.

1. Recherche et reconnaissance de l'hôte à distance

Deux grands types de stimuli sont en jeu pour rechercher l'hôte à distance : les stimuli olfactifs et les stimuli visuels. Par exemple, chez la mouche des pommes, *Rhagoletis pomonella,* la femelle détecte l'hôte grâce aux composés volatils émis par les pommes et une fois arrivée à l'arbre, les fruits sont localisés par leurs caractéristiques visuelles (Aluja et Propoky, 1993).

Les signaux chimiques perceptibles à distance émis par les plantes sont généralement des composés organiques volatils perçus par les organes sensoriels des insectes. Deux espèces végétales, mêmes proches, ne présentent jamais quantitativement ou qualitativement un bouquet odorant identique même si certains composés sont identiques. Ce bouquet odorant change en fonction des facteurs abiotiques (cycles journaliers, éclairement, sol, etc) ou biotique (pollinisation, maturation, etc) (Bernays et Chapman, 1994 ; Dudareva et Pichersky, 2006). Mais l'ensemble du bouquet odorant est rarement perçu par l'insecte et seulement quelques composés perçus sont réellement impliqués dans le choix de l'hôte.

Dans la recherche de l'hôte, il y a 2 phases distinctes : la recherche du stimulus et l'orientation (Bernays et Chapman, 1994). Pour s'orienter les insectes utilisent les informations de leur environnement et les informations internes inscrites dans leurs patrimoines génétiques (Bell, 1990). Deux stratégies existent chez les insectes afin d'optimiser la recherche d'informations olfactives ou visuelles : 1) le *ranging* où l'insecte se déplace au hasard ou de manière rectiligne et 2) le *perching* où l'insecte se positionne afin de rencontrer des signaux (Bell, 1990 ; Cardé, 1996 ; Cardé et Bell, 1995). Dans le premier cas, pour optimiser la recherche de son hôte, dans un vent régulier l'insecte volera perpendiculairement au vent, mais dans un vent instable il volera parallèlement et contre le vent (Zanen *et al.,* 1994 ; Dusenberry, 1990). Les antennes et les mécanorécepteurs des insectes leur permettent de déterminer la direction du vent. Une fois que les stimuli olfactifs de l'hôte sont perçus, l'insecte vole contre le vent (anémotaxie) jusqu'à l'hôte. En effet, un insecte en vol perçoit des poches ou bouffées d'odeurs, l'intervalle de temps qui les sépare augmente avec l'éloignement de la source. Dans le second cas, certains insectes choisissent un autre type d'approche : ils s'orientent dans le vent et lorsqu'ils perçoivent un stimulus, ils décollent et atterrissent un peu plus loin en direction de la source. Ils effectuent ainsi des petits vols en direction de la source. Un insecte dont les plantes hôtes sont éloignées utilisera la première stratégie, au contraire d'un insecte où son environnement est riche en hôtes. Mais dans les deux stratégies, l'approche se fait en zigzag afin d'augmenter les chances de l'insecte de trouver son hôte (Kennedy, 1983 ; Willis et Baker, 1984).

Les insectes reconnaissent leurs hôtes par leurs odeurs, caractérisées par le mélange de leur composés volatils, leur ratio et leur diffusion dans l'espace et le temps (Sachse et Galizia, 2003; Bruce et al, 2005a). En effet, le contraste entre le signal et le paysage chimique semble jouer un rôle clé dans la reconnaissance (Bernays, 2001). Par contre, les mécanismes de diffusion spatio-temporelle des signaux olfactifs sont très peu connus (Bruce *et al*., 2005a). Les herbivores peuvent être attirés sur des distances relativement longues, ce qui prouve la sensibilité de leur système olfactif, à savoir des récepteurs spécialisés pour identifier les volatils. Par exemple, le charançon de la graine du chou *Ceutorhynchus assimilis* est attiré à

plus de 20m par les isothiocyanates de sa plante hôte (Evans & Allen-Williams, 1993), et 30% de ses neurones olfactifs répondent aux isothyocianates (Blight et al., 1989). Les insectes ont deux paires d'organes olfactifs, les antennes et les palpes maxillaires, couverts par différents types de sensilles: sensilles basiconiques, sensilles trichoïdes, et sensilles coelonics. Pourquoi deux « nez »? Ces deux organes peuvent avoir des fonctions distinctes, comme les palpes maxillaires du moustique *An. gambiae* qui servent à détecter le CO_2 alors que les antennes servent à détecter les composés volatils de la sueur de l'homme. Mais les palpes et les antennes peuvent également montrer des spectres de réponse qui se superposent comme la détection du CO_2 qui se fait par les antennes et les palpes chez *D. megalonaster* (Hansson et Stensmyr, 2011).

Le système olfactif des insectes est composé : 1) de sensilles chémoréceptrices possédant des pores, 2) du lobe antennaire, formé de glomérules qui sont composés des axones des neurones olfactifs, 3) des corps pédonculés et 4) du lobe latéral qui analyse le code olfactif et entraine une réponse comportementale associée (Figure 1). Chaque sensille abrite 1 à 4 neurones olfactifs, chaque neurone exprime une combinaison unique de récepteurs olfactifs et de corécepteurs et 'projette' son axone dans un seul glomérule olfactif du lobe antennaire (Kim, 2013). Dans le lobe antennaire, les glomérules sont reliés par des interneurones et les neurones de projection relient le lobe antennaire aux centres de traitement des commandes supérieures: les corps pédonculés et le lobe latéral (Chritensen & White, 2000).

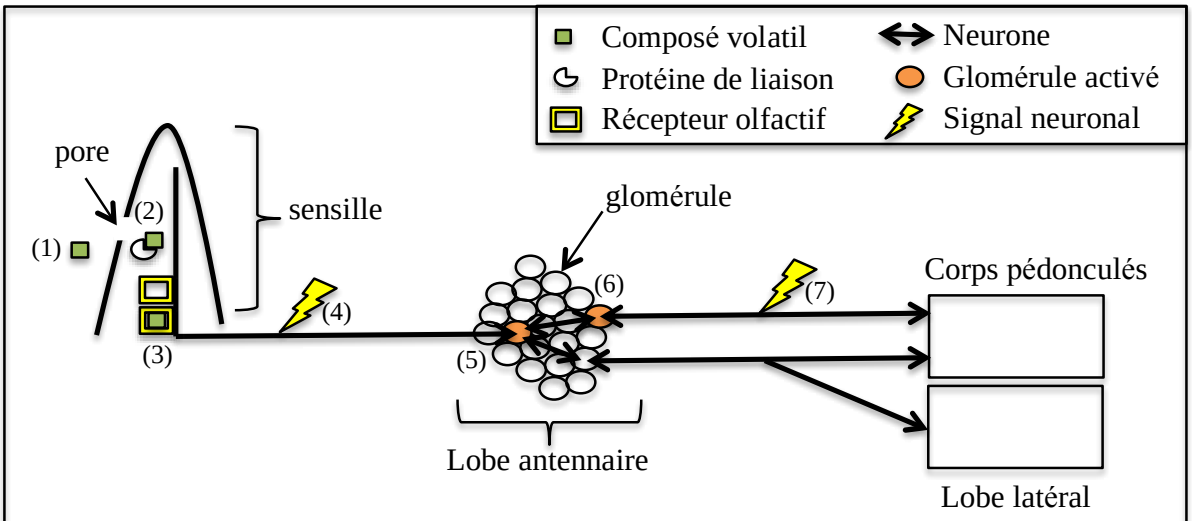
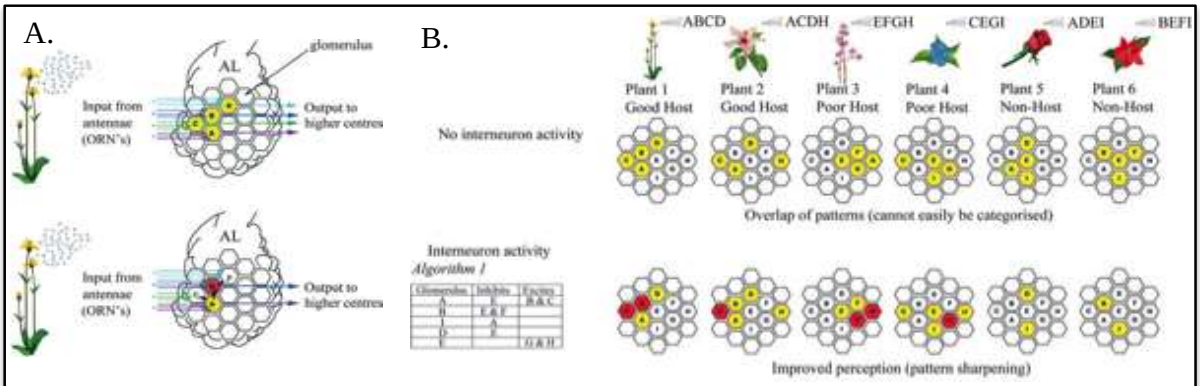


Figure 1: Schéma de transduction d'un signal olfactif

Les molécules odorantes traversent les pores des sensilles[1], se diffusent dans l'endolymphe grâce à des protéines de liaison[2] qui les transportent jusqu'aux récepteurs olfactifs[3] (Leal, 2013). Le complexe substance odorante – protéine de liaison conduit à l'ouverture de canaux ioniques des récepteurs olfactifs puis d'une dépolarisation dans les neurones olfactifs[4] (Kaupp, 2010). Dans le lobe antennaire les glomérules activés par le signal neuronal[5] activent à leur tour les interneurones qui modulent l'activité des neurones de projection par l'activation ou l'inhibition d'autres glomérules[6] (Ache & Young, 2005). Les glomérules activés et les interneurones entrainent la dépolarisation des neurones de projections, qui transmettent l'information jusque dans les corps pédonculés et le lobe latéral[7].

Ce schéma a été adapté de la revue '*Prospects for repellency in pest control – current developments and future challenges*' (Deletre *et al.*, soumis à Biological Review).

Le système olfactif utilise un système de codage pour identifier les différentes odeurs grâce aux récepteurs olfactifs activés. L'information est codée de manière qualitative, quantitative, temporelle et spatiale et ce code est transmis au cerveau qui induit une réponse comportementale (De Bruyne *et al*., 1999). Un récepteur olfactif peut être activé par différentes molécules odorantes et une molécule odorante peut être reconnue par plusieurs récepteurs olfactifs. De plus, la même molécule odorante peut activer certains récepteurs olfactifs et en inhiber d'autres (De Bruyne *et al*., 1999). La sensibilité aux odeurs est proportionnelle au nombre de récepteurs olfactifs disponibles, leur spécificité et leur affinité pour la molécule odorante (Gomez-Martin *et al*., 2010). Les molécules odorantes peuvent activer des récepteurs plus ou moins longtemps, le codage temporel améliore ainsi la capacité des insectes à reconnaître et surtout différencier les odeurs (Kaupp, 2010).
Mais tous les neurones olfactifs expriment le même récepteur et convergent vers le même glomérule (Gomez-Martin *et al*., 2010). Dans un premier temps, l'odeur est donc codée par la distribution des glomérules activés correspondant aux récepteurs olfactifs activés par la molécule odorante (Séjourné *et al*., 2011). Ce code est ensuite modulé par les interneurones, au sein du lobe antennaire, par l'inhibition ou l'activation de glomérules ce qui modifient l'activité des neurones de projection (Cunningham, 2012 ; Figure 2). Ce traitement de l'information permet de faire ressortir le signal des autres bruits olfactifs, les glomérules amplifient l'information. Ces informations sont transmises aux centres nerveux centrales qui analysent le code olfactif (Galizia *et al.*, 1999). Les corps pédonculés sont probablement un site pour la mémoire olfactive et l'apprentissage olfactif modulant le comportement en fonction de l'expérience de l'individu alors que le lobe latéral semble être un site pour les comportements innés (Heinseberg, 2003 ; Menzel, 2001 ; Gomez-Martin et al., 2010).



A. How plant volatiles form spatial patterns within the antennal lobe (AL). (a) Elemental patterns. Out of the complex blend of volatile compounds that make up a plant's unique odour, a subset of volatiles (in this example, the four coloured volatiles) are detected by receptors on sensory neurons [olfactory receptor neurons (ORNs)] in the insect antennae. Different ORN classes bear different receptor types (and are thus triggered by different volatiles), and each class of ORN relays information to specific regions (called glomeruli) in the AL. For example, in this schematic, the volatile represented by the green dots activates specific (green) ORNs, which relay information to glomerulus C. As a result, blends of volatiles are translated into patterns of excitation in the AL (yellow hexagons). The activated glomeruli evoke synchronized firing in output neurons (broader arrows), which send information to higher centres of the insect brain. Further processing then leads to behavioural responses such as upwind flight towards the odour source. (b) Pattern sharpening (blend-specific patterns). When a glomerulus is activated, interneuron activity (black arrows) can influence the level of excitation in neighbouring glomeruli by increasing (+) or decreasing (-) output activity. The global response of interneurons can be represented as a processing algorithm (the example here is A excites B, A inhibits C, B inhibits D), which sharpens output firing patterns. Red shading denotes an increased level of glomerular activity (increased output strength) evoked by excitation from both ORNs and interneurons.

B. How an interneuron network could influence plant odour perception in the antennal lobe (AL). The schematic represents spatial patterns in the AL evoked by odours from six plant species. Each plant odour is comprised of four volatiles from a possible 9 (A–I), and each volatile increases excitation in (activates) the corresponding lettered glomeruli. Plants 1–4 are all host species (Plants 1 and 2 are good hosts and Plants 3 and 4 are poor hosts), whereas Plants 5 and 6 are non-host species. Level of excitation: clear = activity below a threshold for recognition by higher centres of the insect brain, yellow = moderate (behaviourally relevant) excitation, red = strengthened excitation. (a) In the absence of interneuron effects (elemental representation of volatiles), host plants have overlapping patterns, which cannot be simply categorized into good, poor and non-hosts. (b) Interneuron effects detailed in Algorithm 1 give rise to sharper, more distinct patterns, which can be more easily categorized (e.g. good host = red C, poor host = red G, non-host £ 3 active glomeruli).

Figure 2: Codage de l'information olfactive

Le schéma ci-dessus tiré de '*Can mechanism help explain insect host choice*' (Cunningham, 2012) illustre les mécanismes d'intégration de l'information olfactive reçue par l'insecte.

Mais encore aujourd'hui le système olfactif des insectes réserve des mystères. L'activation des récepteurs olfactifs pourrait être due à la substance odorante seule ou nécessiterait le complexe : protéine de liaison - molécule odorante (Leal, 2013). Il est maintenant admis que les récepteurs olfactifs jouent un rôle important dans la sélectivité des odeurs mais les autres protéines du système olfactif telles que les protéines de liaisons contribueraient à la spécificité et à la sensibilité globales du système olfactif des insectes (Leal, 2013). Les mécanismes de médiation de la transduction de l'information olfactive chez les insectes sont encore controversés, les récepteurs seraient soit des récepteurs ionitropics soit des protéines à sept domaines transmembranaires couplés à des protéines G mais tous deux associés à un corécepteur très conservé chez les insectes (Touhara & Vosshall, 2009; Leal, 2013). Mais la vitesse de transduction du signal par un récepteur à protéine-G, observé dans le système olfactif des vertébrés, est relativement lente par rapport à celle observée chez les insectes. De plus, les récepteurs ionotropiques évitent une consommation d'énergie importante dans la seconde cascade de messagerie par rapport à l'utilisation d'ATP ou de GTP qui sont couteux (Ha & Smith, 2009; Touhara & Vosshall, 2009). Le corécepteur n'est pas directement impliqué dans la reconnaissance de l'odeur, mais il participerait à la transduction du signal (Hansson et Stensmyr, 2011) et contribuerait à la sélectivité et à l'activation des récepteurs (Kaupp, 2010). Par exemple, De Genarro et al. (2013) ont montré que des moustiques *Anopheles gambiae* avec des corécepteurs mutants étaient moins attirés par le miel et ne répondaient pas à l'odeur humaine en l'absence de CO_2 : les réponses aux odeurs et l'activité spontanée des neurones olfactifs des mutants ont été réduits.

Dans des cas spécifiques les repères visuels peuvent être encore plus importants que les odeurs. Certains insectes spécialistes choisissent de rester sur une forme de plante ou de feuille particulière, surtout si leur plante hôte a une forme de feuille qui est caractéristique (Bernays, 2001), et d'autres cherchent un site de ponte ou de nutrition grâce aux signaux visuels, une fois arrivés dans l'habitat de l'hôte. Par exemple, la femelle *R. pomonella* visite autant les vrais fruits que les leurres visuels suspendus dans le même arbre (Prokopy et Roitberg, 1984). Chez les pucerons, la recherche de l'hôte peut être basée sur la composition du mélange des composés volatils des hôtes et/ou sur le rapport des volatils et/ou sur la couleur et la forme de l'hôte en fonction des espèces et du morphe (Webster, 2012). Après que le puceron se soit posé sur un hôte potentiel, les signaux chemotactiles et gustatifs de l'hôte jouent un rôle clé dans son acceptation (Powell *et al*., 2006).

2. Sélection et acceptation de l'hôte au contact

Même si les odeurs continuent à jouer un rôle sur le comportement de l'insecte après contact avec l'hôte, certains indices gustatifs spécifiques jouent également un rôle prépondérant (Bernays, 2001 ; De Boer, 2006). Le système olfactif permet aux insectes de distinguer leur hôte (source de nourriture) des non-hôtes et le système gustatif faciliterait ce processus (Glendinning *et al*., 1998) et permettrait d'évaluer la qualité des aliments (Vosshall & Stocker, 2007). Par exemple, le chemoréception de contact joue un rôle important dans la sélection de l'hôte pour la mouche des racines du chou, *Delia radicum*, très sensible à un produit chimique appelé «facteur d'identification du chou" présente dans la surface de la feuille de *Brassica oleacera* (Roessingh *et al*., 1997).

La sélection d'une plante s'explique également par ses caractéristiques physiques : texture, relief, épaisseur de la feuille, densité de trichomes, de stomates,… Mais les informations chimiques fournies restent prépondérantes sur les indices physiques. Le contact de l'insecte avec la plante lui permet de la refuser ou de l'accepter en fonction des signaux chimiques qu'il rencontre. La plante a un intérêt évolutif à produire des signaux de défense à sa surface. Ainsi l'insecte s'adapte et interprète ces signaux afin d'éviter d'ingérer des molécules toxiques et de dépenser de l'énergie inutilement à sonder une plante non hôte. Les stimuli qui guident la prise de nourriture sont donc modelés par l'évolution mais aussi par l'expérience immédiate.

Chez les insectes piqueurs-suceurs, la sélection de la plante hôte se fait par une perception gustative grâce à des sensilles (Backus, 1988 ; Walker et Gordh, 1989 ; Calatayud et Le Ru, 2006). Ils 'goûtent' la surface de la plante hôte puis le stylet pénètre dans les tissus de la plante pour 'goûter' les différents contenus cellulaires. Sur une plante non hôte, la durée de piqûre est réduite, le temps d'accès à la nourriture est rallongé et la plante est alors rejetée.

La sélection de l'hôte par des propriétés gustatives est régie par l'équilibre des stimuli phagostimulants et dissuasifs décryptés au niveau des centres nerveux centraux (Chapman, 2003). Les drosophiles et les insectes de manière générale sont attirés par les sucres et une faible concentration de sel et sont repoussés par des composés toxiques et/ou amers (Yarmolinsky et *al.*, 2009). Les organes du système gustatif sont répartis sur plusieurs parties du corps: les pièces buccales (palpes maxillaires, cavité buccale, pharynx), les pattes et les ailes (De Boer, 2006; Vosshall & Stocker, 2007). Les sensilles sur ces organes permettent à l'insecte de détecter des sources potentielles de nourriture sans les consommer (Sturcow 1959; Montell, 2009). Ces mêmes sensilles sont présentes sur les tarses et sont impliquées dans d'autres comportements tel que l'accouplement (Ozaki *et al*., 2011). Par exemple, les

récepteurs gustatifs (e.g. Gr68a), principalement localisés sur les pattes des mâles, sont impliqués dans la détection d'une phéromone femelle non-volatil (Isono & Morita, 2010).

Les sensilles externes du système gustatif sont unipores contrairement aux sensilles olfactives qui sont multipores (Chapman, 2003 ; Figure 3). Les sensilles gustatives contiendraient deux types de neurones gustatifs répondant aux molécules de goût attrayant ou de goût anti-appétant ou quatre types de neurones gustatifs répondant au sucre (cellule S), à l'eau (cellule W), aux faibles concentrations de sel (cellule L1), et aux fortes concentrations en sel et à l'amertume (cellule L2). Elles comprennent également un neurone chemosensoriel et plusieurs types de cellules accessoires (Vosshall & Stocker, 2007; Montell, 2009; Yarmolinsky et al, 2009.). Par exemple, dans le ganglion sous oesophagien de la drosophile, un des neurones moteurs, qui contrôle l'extension du proboscis, est stimulé par l'activité des neurones Gr5a (activés par des phagostimulants) et inhibé par l'activité des neurones Gr66a (activés par des anti-appétants) (Yarmolinsky et al., 2009). Le ganglion sous oesophagien ne contient aucune subdivisions structurelles tels que les glomérules dans le lobe antennaire (Vosshall & Stocker, 2007). Mais les axones des neurones gustatifs se terminent dans le ganglion sous oesophagien dans des domaines spatialement distincts (Yarmolinsky *et al*., 2009;. Isono & Morita, 2010). Les axones des neurones gustatifs provenant du pharynx, du labelle, et des tarses se terminent dans les zones distinctes du ganglion oesophagien et certains de ces neurones se terminant dans des zones distinctes expriment pourtant les mêmes récepteurs, ce qui suggère qu'une molécule gustative donnée peut déclencher des comportements différents en fonction du site de stimulation (Vosshall & Stocker, 2007).

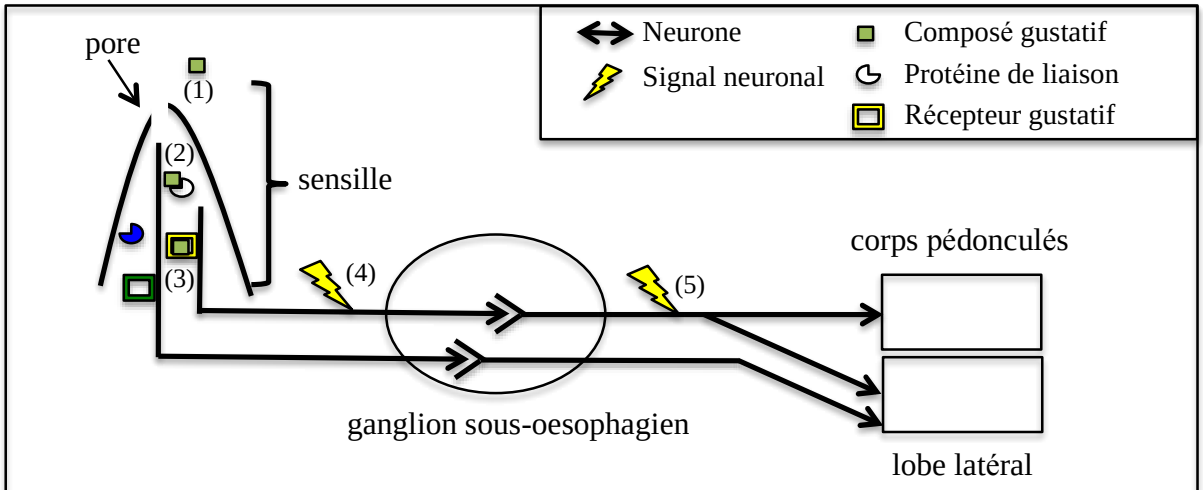


Figure 3: Schéma de transduction d'un signal olfactif

Les composés gustatifs se dissolvent dans le bouchon visqueux exsudé au niveau du pore[1] et se diffusent dans l'endolymphe grâce à des protéines de liaison[2] qui les transportent jusqu'aux récepteurs gustatifs[3] ((Amrein & Thorne, 2005; Isono & Morita, 2010). L'activation des récepteurs gustatifs conduit à l'ouverture de canaux ioniques des récepteurs gustatifs puis d'une dépolarisation dans les neurones gustatifs dont les axones arrivent dans le ganglion sous-oesophagien[4] (Schoonhoven et Van Loon, 2002). Au niveau du ganglion sous oesophagien, des neurones sont alors activés et transmettent l'information jusque dans les corps pédonculés et le lobe latéral[5] (Vosshall & Stocker, 2007).

Ce schéma a été adapté de la revue '*Prospects for repellency in pest control – current developments and future challenges*' (Deletre *et al*., soumis à Biological Review).

Le système gustatif est bien moins connu que le système olfactif. Comme le système olfactif, le système gustatif est codé quantitativement, qualitativement, spatialement et temporellement (Schoonhoven et Van Loon, 2002; Koul, 2008). Par exemple, la cellule S sensible aux sucres de *Manduca sexta* répond différemment au saccharose ou au glucose. Ces différences de sensibilités peuvent être attribuées à la morphologie des différents sites de liaisons des récepteurs gustatifs (Lam et Frazier, 1991). Mais contrairement au système olfactif : un récepteur - un neurone, différent récepteurs gustatifs peuvent être exprimés sur un même neurone (Montell, 2009). Cela permet d'augmenter le spectre de molécules actives mais cela diminue les capacités de discrimination des différentes molécules (Isono & Morita, 2010). Les récepteurs gustatifs étaient supposés fonctionner comme des récepteurs couplés à des protéines G, mais récemment, une nouvelle hypothèse a été émise : les récepteurs seraient de types canaux ioniques s'ouvrant avec des ligands spécifiques comme les récepteurs olfactifs (Yarmolinsky et al., 2009). Les récepteurs gustatifs seraient des structures hétérodimères avec un co-récepteur commun tels que le Gr64f (Isono & Morita, 2010).

3. *Anopheles gambiae* et son hôte

Anopheles gambiae est le vecteur majeur responsable de la transmission de *Plasmodium* spp., en particulier *Plasmodium falciparum*, qui est le parasite responsable du paludisme chez l'homme (WHO, 1993). En 2010, 3,3 milliard de personnes ont été exposées au paludisme et 655000 en sont morts (WHO, 2011). Le développement de tests de dépistage rapides, de traitements efficaces, l'utilisation de moustiquaires imprégnées et les traitements intra-domiciliaires avec des pyréthrinoides ont permis de faire diminuer de 17% de 2010 à 2011 les cas de paludisme (WHO, 2008).

La moustiquaire imprégnée de pyréthrinoïdes est à la fois une barrière physique qui empêche le moustique d'atterir sur l'homme et chimique. En effet, les pyréthrinoïdes ont un effet excito-répulsif ou irritant, 'knock-down' et toxique (Zaim *et al.*, 2000). L'effet 'knock-down' ou paralysant est une tétanisation passagère de l'insecte (White, 2007). Les pyréthrinoïdes agissent sur l'ouverture des canaux sodium voltage-dépendant : les pyréthrinoïdes de type 1 empêchent les canaux de se fermer créant ainsi une succession de potentiels d'action (Saldago *et al.*, 2000). Leur effet irritant empêche le moustique de rester longtemps sur la moustiquaire et de passer à travers. Leur effet paralysant et toxique permet de diminuer la pression des populations Et leur effet répulsif permettrait de protéger les personnes ne dormant pas sous filet puisqu'un nombre plus faible de moustiques entrerait dans la maison mais cet effet est encore discuté.

Mais des populations résistantes aux pyréthrinoides ont été découvertes dans 27 pays (WHO, 2011 ; Ranson *et al.*, 2011). Une forme de résistance d'*Anopheles gambiae* aux pyréthrinoïdes est la résistance à l'effet 'knock-down' (*Kdr*) qui résulte de la mutation du canal sodium voltage dépendant (L1014F) (Marinez-Torres, 1998). Cette mutation réduit l'affinité des pyréthrinoïdes au canal sodium voltage dépendant (Pauron *et al.*, 1998). Ils peuvent également être résistants grâce à la surproduction ou hyperactivités des enzymes dégradant les pyréthrinoïdes. Ces populations sont donc moins sensibles aux pyréthrinoïdes ce qui a pour effet de diminuer l'effet toxique des pyréthrinoïdes, il faut donc augmenter les doses. L'effet irritant est également moins fort, le moustique peut ainsi rester plus longtemps sur la moustiquaire et piquer à travers. L'effet répulsif des pyréthrinoïdes étant encore discuté, les conséquences des résistances sur cet effet ne sont pas connues.

D'autres insecticides sont utilisés contre les moustiques comme les organophosphates et les carbamates qui agissent sur l'acétylcholinestérase. Cette enzyme dégrade le neurotransmetteur acétylcholine au niveau des synapses. Lorsque la synapse est inhibée le neurotransmetteur s'accumule et les récepteurs restent actifs jusqu'à entrainer la paralysie et la mort (Aldridge, 1950; Fournier and Mutero, 1994; Djogbenou *et al.*, 2007). Mais il existe aussi des populations résistantes comme la population résistante AcerKis qui porte la mutation *Ace.1*. En protection personnelle, les répulsifs comme le DEET sont également utilisés. Le mode d'action du DEET n'est pas encore bien défini, nous discuterons des hypothèses dans la suite du manuscrit.

Chez *Anopheles gambiae*, les mâles se nourrissent de jus sucré et de nectar de fleurs alors que les femelles se nourrissent comme les mâles mais sont également hématophages, ainsi les organes olfactifs et gustatifs des mâles et des femelles sont différents (Carnevale et Robert, 2014). Les antennes présentent un fort dimorphisme sexuel : les antennes mâles ont des soies longues et plumeuses avec des récepteurs olfactifs impliqués dans la perception des phéromones et des mécanorécepteurs impliqués dans la perception de certaines vibrations alors que les antennes des femelles ont des soies verticillées et courtes avec des récepteurs olfactifs qui servent au repérage et à la localisation de l'hôte (Figure 4). Les pièces buccales sont également dimorphiques : les mâles ont un appareil de type suceur avec des palpes maxillaires, des mandibules et des maxilles réduites et un hypopharynx soudé à la lèvre inférieure alors que les femelles ont un appareil de type vulnérant avec des palpes maxillaires, des maxilles et des mandibules développés, un labium se terminant par deux labelles, un labium souple et le labre par lequel passe le sang aspiré.

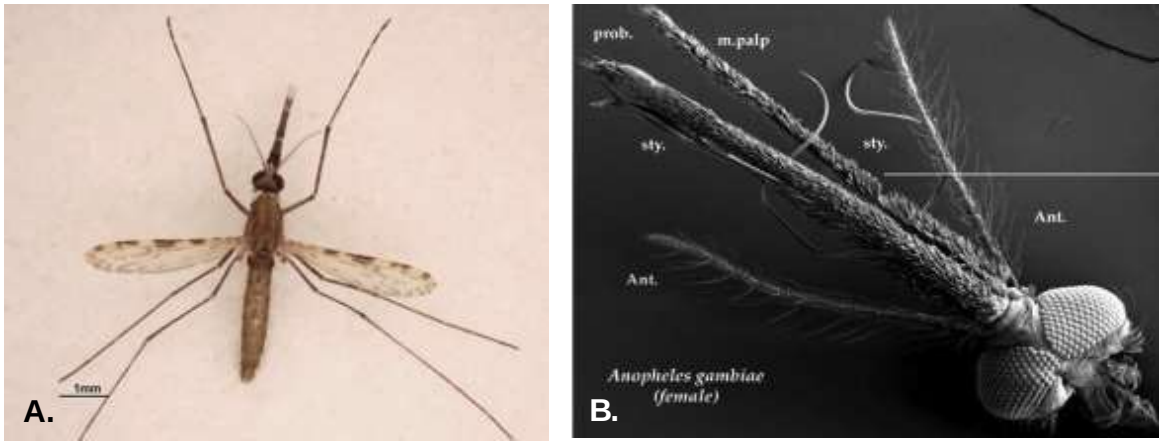


Figure 4: *Anopheles gambiae*
A. Photo d'une femelle *Anopheles gambiae*
B. Photo par microscope electronique d'une tête d'*Anopheles gambiae* femelle: Ant.: antenne, Sty.: stylet, Prob.: proboscis, M.Palp: palpe maxillaire

Le comportement de recherche de l'hôte chez la femelle moustique se décompose en trois phases continues : pour trouver son hôte, le moustique effectuerait un vol en zigzag, au hasard à la recherche de stimuli attractifs puis un vol orienté vers l'hôte après la perception du stimulus en remontant le vent dont l'intensité augmente en se rapprochant de l'hôte et un vol attiré lié à l'attractivité de l'hôte lorsque le moustique est proche de son hôte et décide de se poser (Lehane, 1991). Pour les moustiques nocturnes comme l'espèce Anophèles, l'olfaction est le facteur le plus important. En effet, la localisation de l'hôte par le moustique se fait à longue distance grâce aux stimuli olfactifs perçus par les chémorécepteurs des antennes et à

courte distance par des stimuli visuels (Meijerink & Van Loon, 1999 ; Van Den Broek & Den Otter, 2000). Plusieurs stimuli attractifs ont été identifiés comme le CO_2 présent dans la respiration de l'hôte, des stimuli sécrétés par la peau comme l'acide lactique ou des composés de la sueur humaine comme l'acide (E/Z)-3-méthyl-2-hexénoique et l'acide 7-octonéique (Costantini *et al.*, 2001). En ce qui concerne les stimuli visuels, les moustiques sont attirés par le bleu, le rouge et le noir et repoussés par le blanc et le jaune mais il sont surtout sensibles aux UV, aux variations de formes, aux contrastes et à l'intensité lumineuse (Lehane, 1991 ; Gouagna et Robert, 1993). L'endroit où le moustique se pose sur l'hôte et choisit de piquer a fait l'objet de nombreuses études : *Anopheles gambiae* choisirait les zones fraiches et bien irriguées comme les chevilles (De Jong et Knols, 1995 ; De Jong et Knols, 1996). La prise du repas de sang se divise en quatre phase : l'exploration, phase où la femelle se pose et où les stylets commencent à entrer dans la peau, le sondage : pénétration des stylets dans la peau et apparition du sang dans les stylets, ingestion : apparition du sang et arrêt du gorgement et retrait : raidissement des pattes antérieures et reprise de la mobilité des palpes et retrait des stylets (Clements, 1992).

Les essais sur *Anopheles gambiae* effectués au cours de cette thèse ont été faits avec la souche sensible de référence 'Kisumu' et deux souches résistance de référence 'KdrKis' et 'AcerKis' qui ont le même fond génétique que la souche Kisumu à une mutation près. Les 3 souches ont été élevées dans des salles différentes à 27 ± 2°C, 80 ± 10% RH, 12L:12D. Les larves ont été nourries avec de la nourriture pour poisson et les adultes avec du jus sucré à 10% ou du sang de lapin pour la reproduction.

4. *Bemisia tabaci* et son hôte

Bemisia tabaci est un polyphage et donc un ravageur majeur de nombreuses cultures aussi bien en plein champ qu'en serre dans les régions tempérées et tropicales (Jing *et al.*, 2003). Ce ravageur cause trois types de dommages : des dommages directs en consommant le phloème, des dommages indirects via le développement de *Cladosporium* spp. et *Alternaria* spp. qui se développent sur les fèces excrétées sur les feuilles et qui diminue la photosynthèse et via la transmission de virus, comme par exemple le *tomato yellow leaf curl virus* (Berlinger, 1986). Aujourd'hui faire une culture de tomates de plein champ est un réel challenge à cause de la transmission de ce virus par *B. tabaci* (Berlinger *et al.*, 1986). De plus, les mouches blanches sont très difficiles à contrôler avec des insecticides parce qu'elles vivent sous les feuilles (Muniz *et al.*, 2002 ; Zhang *et al.*, 2004). Les insecticides systémiques comme les néonicotinoïdes ou les insecticides qui affectent le développement des insectes sont couramment utilisés en serre ou au champ mais des résistances à ces insecticides ont été trouvés dans des populations de mouches blanches en Europe et en Afrique (Elbert et Naulen, 2000; Houndete *et al.*, 2010). En effet, des résistances peuvent apparaître rapidement à cause du cycle de développement rapide de *Bemisia tabaci* et de sa forte fécondité (Perring, 2001). Le développement de serres, de tunnels et de filets anti-insectes a permis de faire une production rentable de tomates et autres légumes dans les régions méditerranéennes (Berlinger *et al.*, 2002). Martin *et al.* (2014) ont montré qu'un filet traité à l'alphacypermthrin était plus efficace pour lutter contre la mouche blanche, en retardant l'infestation, qu'un non traitée. Mais à cause des populations résistantes et du risque de pollution il faut d'ores et déjà trouver une alternative. Notre nouvelle stratégie de barrière physique et répulsive semble donc intéressante dans le cas de la lutte contre cet insecte en Afrique. De plus, la lutte biologique comme l'utilisation de *Encarsia formosa* et l'utilisation de variétés tolérantes aux virus

permettent de contrôler la population dans les serres et diminuer son impact. Mais *Bemisia tabaci* reste un gros problème en Afrique où son contrôle se fait quasi exclusivement par des traitements chimiques qui se montrent peu efficaces à cause des problèmes de résistance (Huat *et al.,* 2013). L'utilisation de variétés tolérantes y est impossible parce qu'elles sont trop sensibles aux autres maladies.

La stratégie de *Bemisia tabaci* pour trouver son hôte est différente du moustique, il effectue des vols de courte distance jusqu'à trouver un hôte permanent. Mais le vol jusqu'à un nouvel hôte permanent dépend également du vent qui les transporte. En effet, il n'est pas rare de voir des aleurodes sur des hôtes temporaires pendant les périodes chaudes de la journée : leurs petites tailles leur permettent peu de réserves d'énergie et d'eau et les obligent à rester sur ces hôtes et de se nourrir à partir de leur phloème pour synthétiser du sorbitol. Cette stratégie permet de se rapprocher par des vols successifs vers un hôte permanent en augmentant la probabilité de chance de rencontre et sans trop de perte d'énergie. Il est important de noter également que les aleurodes ne choisissent pas uniquement leur hôte pour se nourrir, comme *Anopheles gambiae*, mais aussi pour déposer leurs œufs ce qui induit que l'hôte doit être de bonne qualité pour la descendance.

Lors de la recherche de l'hôte, le vol orienté et l'atterrissage, les signaux visuels et olfactifs jouent des rôles importants (Visser, 1988). Quand l'insecte veut se disperser il est attiré par les UV ce qui leur permet de voler en direction du ciel (Mound, 1962 ; Blackmer et Byrne, 1993a ; Blackmer et Byrne, 1993b). Mais quand l'insecte est à la recherche d'un hôte il est attiré par le jaune-vert ce qui lui permet de descendre vers les plantes (Van Lenteren et Noldus, 1990). Lorsque les stimuli olfactifs sont associés à une plante, ils initient le vol dirigé alors que les stimuli visuels permettent une meilleure précision pour l'atterrissage. Mais le rôle de l'olfaction dans le choix de l'hôte n'a pas encore été très bien étudié chez *Bemisia tabaci.* Les antennes de *Bemisia tabaci* ne présentent que 7 sensilles, ce qui pourrait suggérer que l'olfaction n'est pas très développée chez cet insecte. Après contact avec l'hôte, la mouche blanche évalue la qualité de l'hôte par l'analyse de la surface de la feuille par les pièces buccales tel que le labium puis par l'analyse interne de la feuille grâce aux pièces buccales en particulier le stylet (Ghanim *et al.,* 1998 ; Rosell *et al.,* 1999). Lors de la piqure, des virus peuvent être transmis par la salive des aleurodes et leurs pièces buccales, il faut donc empêcher les insectes de piquer. La phase la plus importante est le 'probing' et des produits anti-appétants comme l'imidacloprid interviennent dans cette phase lors de l'ingestion de ce produit systémique (Isaacs *et al.,* 1999).

Récemment il a été montré que certains volatils de tomates attiraient *Bemisia tabaci* mais que d'autres diminuaient l'attractivité des tomates comme le p-cymène, le β-myrcène, l'α-terpinène ou l'α-phellandrène (Bleeker *et al.,* 2009). Des composés comme le 7-épizingibérène et le R-curcumène seraient également des composés répulsifs de la tomate parce qu'ils seraient toxiques pour les aleurodes et leur descendance (Bleeker *et al.,*2011).

Figure 5: *Bemisia tabaci*
A. Photo de *Bemisia tabaci*
B. Diagramme de la surface ventrale de l'antenne femelle de *Bemisia tabaci*, les points noirs correspondent aux différentes sensilles (Mellor *et al*., 1994).

Les essais sur *Bemisia tabaci* effectués au cours de cette thèse ont été faits sur le biotype Q2. Les aleurodes ont été élevés sur des plants de tomate, *Solanum lycopersicum*, à 27 ± 1°C, 50 ± 10% RH, 12L:12D. Par contre au Kenya en 2014, les essais ont été effectués sur *Trialeurodes vaporarium.* En effet, du à une année avec des températures fraiches, les mouches blanches présentent sur le terrain et élever au laboratoire était des *Trialeurodes vaporarium* et non des *Bemisia tabaci.*

5. Les différents phénomènes de répulsion

Le mot 'répulsif' est utilisé pour décrire différents types de comportement. Il dérive du latin *repellere* qui signifie rejeter. Au sens strict une substance répulsive devrait être uniquement des substances qui induisent un mouvement de fuite de l'insecte. Mais cette définition est clairement trop restrictive. Une définition plus large doit être retenue pour définir une substance répulsive: substance qui empêche un insecte de chercher, suivre, localiser, sélectionner, reconnaître ou accepter son hôte. Toute substance qui modifie l'attraction d'un insecte pour son hôte est considérée comme répulsive (Ramirez *et al.*, 2012). A l'heure actuelle, un répulsif est défini par la réponse comportementale de l'insecte face à un stimuli (Miller *et al.,* 2009). Mais les différences entre les réponses comportementales peuvent être subjectives. Le problème le plus important pour les scientifiques travaillant sur la répulsion est de trouver le 'bon' bioessai. En effet en fonction du stimulus et de la manière dont il est utilisé, il est possible de discriminer les différents répulsifs. Mais dans un premier temps, il faut identifier et définir les différents répulsifs. Cinq phénomènes de répulsion ont été identifiés : la répulsion-expulsion, la répulsion-masquante, la répulsion-irritation, la répulsion-anti-appétante et la répulsion visuelle (Figure 6).

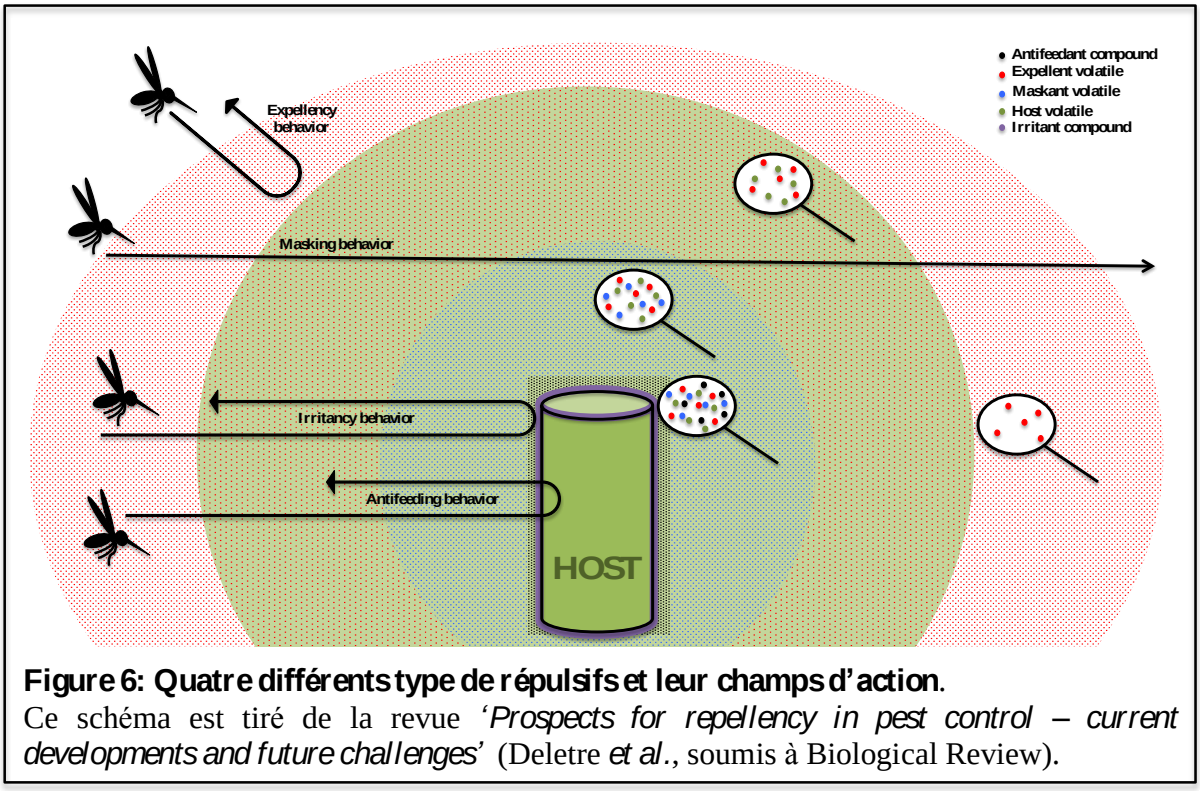


Figure 6: Quatre différents type de répulsifs et leur champs d'action.
Ce schéma est tiré de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

La répulsion-expulsion. Un répulsif 'expulsif' est une substance qui agit à distance engendrant un mouvement de fuite de l'insecte face au stimulus (Mathews and Mathews, 1978; Bernier *et al.*, 2007; Nerio *et al.*, 2010). Par exemple, la phéromone d'alarme du puceron, (E)-β-farnesène, est un répulsive 'expulsif', les pucerons fuient leur plante hôte en présence de ce composé (Cook *et al.*, 2006). Ce type de répulsif crée donc une barrière olfactive protectrice autour de l'hôte. Ils sont généralement très volatils et peuvent se montrer toxique à haute dose. L'évitement est un comportement vis à vis d'une substance qui agit à distance et fait fuir l'insecte, cette substance est répulsive-expulsive (White, 2007). Mais dans ce cas, l'insecte a appris à éviter le composé c'est un comportement de protection, ces composés sont volatils et souvent toxiques par fumigation. En effet dans le verbe éviter, il y a une connotation de choix qui ferait partie du mécanisme de la répulsion. Ce terme peut donc être utilisé quand la réponse comportementale d'un individu naïf est différente d'un individu expérimenté vis à vis d'un même composé. Par exemple, *Bemisia tabaci* évite les plantes protégées par les prédateurs mais uniquement quand il a déjà rencontré le prédateur (Nomikou *et al.*, 2003).

La répulsion-masquante. Un répulsif dit 'masquant' est un composé qui diminue l'attractivité de l'hôte en interférant avec sa localisation. De tels composés ne sont pas répulsifs par eux mêmes, ils modifient le profil chimique de l'hôte soit en ajoutant soit en supprimant des composés du profil odorant empêchant l'identification de l'hôte. Nolen *et al.* (2002) le définit comme un inhibiteur volatil qui inhibe la recherche et la localisation de l'hôte. Par exemple, l'émission de Rutgers 612 (2-ethyl-1,3-hexanediol) inhibe l'attraction des moustiques vis à vis des gradients croissants de CO_2 (Simpson & Wright, 1971). Ce composé inhibe l'attraction de l'hôte vis à vis de l'insecte et s'appelle d'ailleurs également inhibiteur d'attraction. Le but de ce type de composé est de cacher l'hôte à l'insecte.

La répulsion-irritation. Un répulsif irritant engendre un mouvement de fuite vis à vis du stimulus après un contact physique avec celui-ci. En entomologie médicale, ce type de

répulsif est également appelé ‘landing inhibitor’ (WHO, 2013). Les pyrétrinoïdes sont des répulsifs de ce type appelés également excito-répulsifs puisqu’ils augmentent l’activité locomotrice des moustiques. Le but de ce type de répulsifs est d’empêcher le contact physique entre l’insecte et son hôte pour l’empêcher de reconnaitre et accepter son hôte et donc l’empêcher de s’en nourrir.

La répulsion-antiappétante. Les répulsifs dits anti-appétants agissent sur le comportement alimentaire en l’empêchant, l’interrompant ou le gênant (Koul, 2008). Ils agissent juste après le contact avec la substance ou juste après le début de l’ingestion (Cook *et al.*, 2006). Contrairement à un produit irritant, il ne cause pas de mouvement de fuite instantané. Un suppresseur inhibe l’initiation de l’alimentation, un ‘deterrent’ empêche la prolongation du procédé et un anorexigenique provoque une perte d’appétit (Glendinning *et al.*, 1998). Le but de ces substances est de réduire ou de stopper l’activité alimentaire. Un célèbre anti-appétant est l’extrait de neem utilisé contre un grand nombre d’insectes (Cook *et al.*, 2006). En entomologie médical, ces composés sont également appelés ‘feeding inhibitor’ (WHO, 2013).

La répulsion-visuelle. Un composé ou un objet qui modifie la forme ou la couleur d’un hôte diminue l’attractivité de l’insecte pour son hôte et est dit répulsif-visuel. Le but est de cacher l’hôte à l’insecte et de l’empêcher de le reconnaître. Par exemple, certains plastiques absorbent les UV ce qui modifie le comportement des insectes et surtout leur dispersion (Raviv & Antignus, 2004). Ces plastiques perturbent le vol de ces insectes qui utilisent les UV pour se repérer ainsi les insectes ont à la fois des difficultés pour trouver leur hôte et/ou un partenaire sexuel.

Ces différents phénomènes de répulsion sont donc caractérisés en fonction de leurs réponses comportementales face à un stimulus (Figure 7).

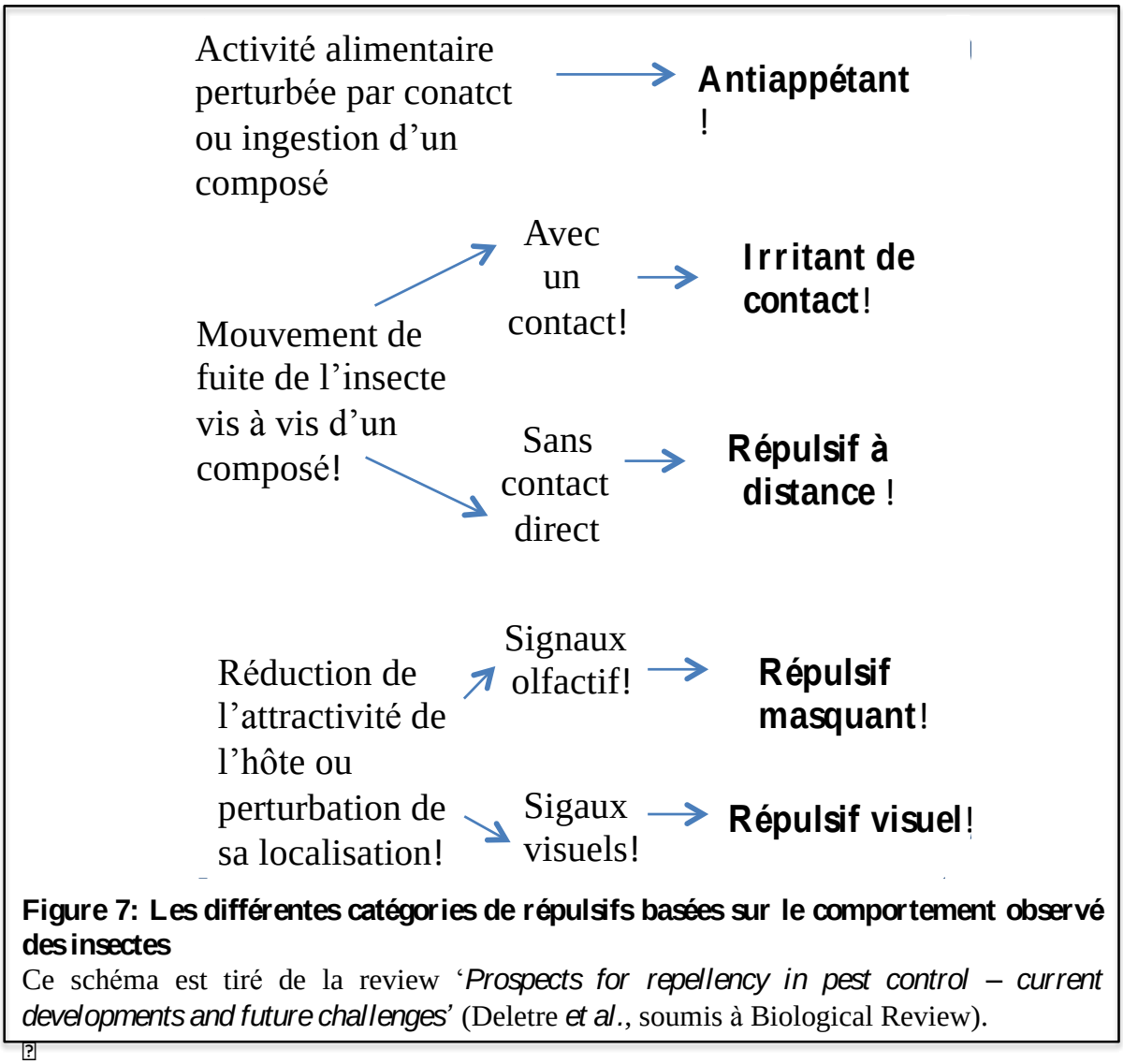


Figure 7: Les différentes catégories de répulsifs basées sur le comportement observé des insectes

Ce schéma est tiré de la review '*Prospects for repellency in pest control – current developments and future challenges*' (Deletre *et al.*, soumis à Biological Review).

6. Quels produits utiliser avec les filets anti-insectes?

Les pyréthrinoïdes, actuellement utilisés avec les moustiquaires, sont des produits répulsifs-expulsifs et répulsifs-irritants. Le premier critère est donc que les produits devaient à la fois être expulsifs et irritants. Les pyréthrinoïdes sont des dérivés du pyrètre qui provient de la plante *Tanacetum cinerariifolium*. Comme expliqué précédemment les métabolites secondaires synthétisés par les plantes, comme les terpènes, jouent un rôle majeur dans leurs fonctions biologiques comme l'attraction des pollinisateurs ou la répulsion des insectes. Pour cette thèse, des composés d'origine végétale semblaient donc appropriés. Ils devaient être facilement accessibles, peu couteux, biodégradables, avoir un impact limité sur la santé humaine et l'environnement et avoir des propiétés biologiques. Les huiles essentielles et végétales regroupaient tous ces critères.

Ainsi pour étudier les différents phénomènes de répulsion, 20 extraits de plantes ont été testés sur *Anopheles gambiae* et *Bemisia tabaci* (Table 1). Ces plantes ont été choisies après une recherche bibliographique et ont été sélectionnées parce qu'elles avaient des composés majoritaires relativement différents et qu'elles avaient déjà montré une certaine activité biologique sur d'autres insectes. Pour les témoins positifs, la perméthrine et aussi le DEET ou diethyltoluamide, qui est le répulsif de référence sur les moustiques, ont été utilisés.

Tableau 1. Extrait de plantes sélectionnées (Regnault-Roger *et al*, 2002; Hilje & Mora, 2006; Amer & Melhorn, 2006; Boer *et al*, 2010; Nerio et al, 2010; Maia & Moore, 2011; Zoubiri & Baalioumer, 2011; Regnault-Roger *et al*, 2012). Ces résultats sont présentés dans l'article: Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013).

Nom commun	**Nom latin**	**Type d'extrait, organe extrait**	**Composés majeurs (%)**[1]	**Fournis par**
Aframomum	*Aframomum pruinosum*	Huile essentielle, feuille	*(E)*-(R)-nerolidol (95%)	IBMM*, France
Aneth	*Anethum graveolens*	Huile essentielle, graine	(+)-carvone(60%) – limonene (30%)	IBMM, France
Cannellle	*Cinnamomum zeylanicum*	Huile essentielle, écorce	Cinnamaldehyde (80%)	Nactis, France
Citron	*Citrus limon*	Huile essentielle ,fruit	(D)-limonene (95%)	Capua, Italy (Lot 20500)
Citronelle	*Cymbopogon winterianus*	Huile essentielle, feuille	citronellal (34%) – geraniol (22%) –citronellol (12%)	Nactis, France (Lot 4001850)
Coleus	*Plectranthus tenuicaulis*	Huile essentielle, feuille	Epoxyocimene (74.4%)	IBMM, France
Coriandre	*Coriandrum sativum*	Huile essentielle, graine	(+)-linalool (72%)	Fabster, France
Cumin	*Cuminum cyminum*	Huile essentielle, graine	Cuminaldehyde (30%)	Ipra, France (Lot 902560)
Eucalyptus	*Eucalyptus globulus*	Huile essentielle, graine	1,8-cineole (81%)	Huiles & Sens, France (Lot B38037)
Geranium	*Pelargonium graveolens*	Huile essentielle, graine	citronellol (41%) – geraniol (18%)	IBMM, France
Gingembre	*Zingiber officinalis*	Huile essentielle, racine	Zingiberene (30%)	Ipra, France (Lot 902724)
Lemongrass	*Cymbopogon citratus*	Huile essentielle, feuille	Geranial (45%), neral (30%)	IBMM, France
Litsea	*Litsea cubeba*	Huile essentielle, feuille	Geranial (45%), neral (32%)	IBMM, France
Pennyroyal	*Mentha pulegium*	Huile essentielle, feuille	(+)-pulegone (87%)	IBMM, France
Neem	*Melia azadirachta*	Huile végétale, feuille	azadirachtin (<1%)	Huiles & Sens, France (Lot 00028/11)
Poivre	*Piper nigrum*	Huile essentielle, graine	β-caryophyllene (30%), limonene (14%), pinenes (14%)	IBMM, France
Romarin	*Rosmarinus officinalis*	Hydrolat biologique, feuille	eau (98%), 1,8-cineole (<1%), camphene (<1%), camphor (<1%)	Huiles & Sens, France (Lot EB815N002)
Sariette	*Satureja montana*	Huile essentielle, feuille	Carvacrol (47%), γ-terpinene (18%), p-cymene (13%)	Huiles & Sens, France (Lot B854002)
Solidage	*Solidago canadensis*	Huile essentielle, feuille	Germacrene-D (32%) - Limonene (13%)	Huiles & Sens, France (Lot A2)
Thym	*Thymus vulgaris L.*	Huile essentielle, feuille	Thymol (35%), p-cymene (23%), carvacrol (15%)	Huiles & Sens, France (Lot A2)

[1] La composition en pourcentage de l'extrait de plante a été calculée à partir de l'analyse GC/FID selon la méthode de normalisation, les facteurs de réponse de tous les composés ont été considérés comme unique.

Les huiles essentielles sont des mélanges complexes de plusieurs molécules. La littérature est bien documentée sur l'effet des huiles essentielles sur le comportement des insectes, mais très peu sur l'effet de leurs composés majeurs pris un à un ou en mélange, illustrant le manque de connaissances sur le ou les mécanismes d'action des huiles essentielles. Certains extraits qui se sont montrés efficaces ont été analysés plus détails afin de comprendre le mécanisme d'action des huiles essentielles et de leurs composés (Table 2). Deux hypothèses ont été formulées pour leur mécanisme d'action : 1) l'activité biologique des huiles essentielles est due au seul composé majoritaire ou 2) à un effet additif/synergique de plusieurs composés. Afin de tester ces hypothèses, les composés majoritaires (> 3%) et le mélange, dans les proportions naturelles, des composés majoritaires ont été testés sur *Anopheles gambiae* et *Bemisia tabaci.* Les composés ont été mélangés en suivant le ratio trouvé dans l'huile essentielle c'est à dire dans les proportions naturelles.

Pour finir, 5 composés intéressants : carvacrol, géraniol, linalool, cinnamaldéhyde, cuminaldéhyde ont été testés sur des populations de moustiques résistants aux insecticides: KdrKis et AcerKis afin d'obtenir plus d'informations sur les modes d'action et les cibles potentiels et d'identifier le risque de résistances croisées aux composés bioactifs découverts. De plus, ces populations sont présentes sur le terrain, il est donc intéressant de connaître les réponses comportementales des insectes résistants avant de faire des tests d'efficacité sur le terrain.

Tableau 2: Composition[1] des huiles essentielles de citronnelle, de cannelle, de cumin, de thym et de lemongrass. Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Citronelle *Cymbopogon winterianus*	Cannelle *Cinnamomum zeylanicum*	Cumin *Cuminum cyminum*	Thym *Thymus vulgaris*	Lemongrass *Cymbopogon citratus*
34.7 citronellal	78.5 trans-	30.1 cuminaldehyde	30.5 thymol	74.1 citral
22.5 geraniol	9.6 2-metoxy-	12.2 β-pinène	23.7 *p*-cymene	4.5 geraniol
12.0 citronellol	3.1 cinnamyl-acetate	11.6 γ-terpinene	13.6 carvacrol	3.9 geranyl acétate
3.5 geranyl-acétate	**91.2 sous-total**	9.7 *p*-cymene	8.4 α-terpinene	**82.5 Sous-total**
3.3 limonene	1.1 benzaldéhyde	**63.6 sous-total**	4.0 linalool	1.9 limonene
76.0 Sous-total	0.9 coumarine	16.6 p-mentha-1,3-dien-7-al	3.5 β-caryophyllene	1.8 β-caryophyllene
3.2 elemol	0.7 phenyl ethyl alcool	8.8 p-mentha-1,4-dien-7-al	**83.7 sous-total**	0.7 linalool
2.9 citronellyl	0.4 cis-cinnamaldehyde	0.6 α-pinène	1.7 myrcene	1.5 borneol
2.5 β-elemene	**94,3 total**	0.4 myrcene	1.1 borneol	0.6 nerol
2.2 δ-cadinene		0.4 limonene	1.1 α-pinène	**89.0 total**
0.9 linalol		**90.4 total**	1.4 γ-terpinene	
0.8 eugenol			1.2 terpinen-4-ol	
89.5 total			0.9 limonene	
			0.8 α-thujene	
			91,9 total	

[1] La composition en pourcentage de l'extrait de plante a été calculée à partir de l'analyse GC/FID selon la méthode de normalisation, les facteurs de réponse de tous les composés ont été considérés comme unique.

Dans ce mémoire, nous traiterons dans un premier temps de l'effet répulsif à distance appelé répulsion-expulsion puis de l'effet répulsif de contact appelé répulsion-irritation. Ensuite nous présenterons les résultats d'efficacité de la barrièrre physico-chimique. Enfin nous étudierons et discuterons d'autres pistes d'amélioration de cette stratégie de lutte. Outre s'intéresser à des alternatives aux pyréthrinoïdes et des nouvelles stratégies de lutte contre les insectes vecteurs d'agents pathogènes, nous nous intéresserons aux modes d'action des répulsifs et des effets répulsifs de la perméthrine et du DEET.

Au lieu de simplement présenter chaque article par une courte introduction, les résultats obtenus pour un même effet sont regroupés au sein d'une partie. Cette organisation particulière permettra une lecture fluide et surtout de comparer les résultats obtenus sur les 2 insectes. Une synthèse des méthodes et des résultats est donnée dans chaque partie et ils sont détaillés dans les articles placés à la suite du texte principal.

Recherche d’un répulsif-expulsif

‘*Le beau parfum est celui qui nous procure un choc*’,
Edmont Roudnista

1. Introduction

Par définition, la répulsion-expulsion est donc un comportement de fuite de l'insecte vis à vis d'une odeur répulsive. Combiné avec un filet, un produit expulsif repoussera l'insecte de son hôte et créera un espace sain autour de celui-ci. Notre hypothèse est que les répulsifs-expulsifs agissent sur les récepteurs olfactifs. Avant de tester la combinaison de la barrière physique et chimique, il faut trouver un produit expulsif.

Pour tester cet effet, il faut donc permettre à l'insecte de sentir le composé testé mais l'empêcher d'entrer en contact afin d'être sûre que le comportement de fuite est du à l'odeur. L'hôte n'a ici aucun rôle à jouer, au contraire, il perturberait l'essai et ne nous permettrait pas de savoir si notre composé est répulsif-expulsif ou répulsif-masquant. Une manière simple de tester un répulsif-expulsif est d'utiliser un olfactomètre tubulaire. Lorsque le produit est répulsif l'insecte va dans le sens contraire du stimulus.

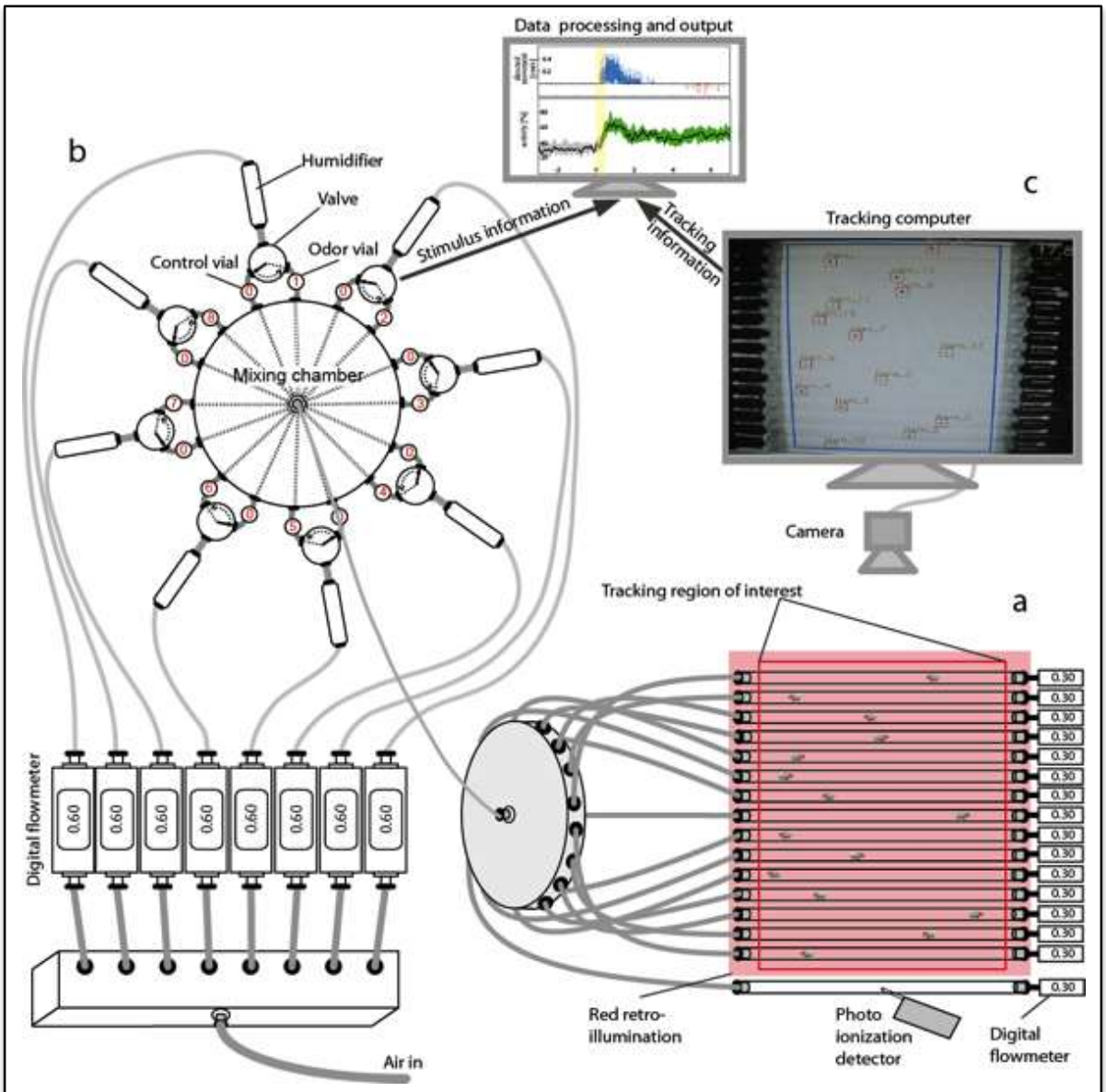


Figure 8 : Olfactomètre tubulaire complexe dont l'odeur délivrée est contrôlée par ordinateur et le comportement de l'insecte suivi par video-tracking
Cet dispositif est tiré de '*a high-throughput behavioral paradigm for Drosophila olfaction – The flywalk*' (Steck *et al.*, 2012)

Steck *et al.* (2012) ont récemment inventé un olfactomètre tubulaire très perfectionné (Figure 8). L'odeur délivrée est contrôlée par ordinateur afin de mettre la concentration et le ratio souhaités et le comportement des insectes est suivi par vidéo tracking. Les olfactomètres à quatre ou six branches peuvent également être utilisés pour tester l'effet répulsif mais restent plus difficiles à utiliser que les olfactomètres tubulaires (Bruce *et al.*, 2005a; Webster *et al.*, 2010; Togni *et al.*, 2010 Ukeh *et al.*, 2012). Cependant l'olfactomètre en Y ne peut être utilisé dans le cas présent : l'insecte testé n'avancera pas jusqu'à la zone de choix si le produit est répulsif.

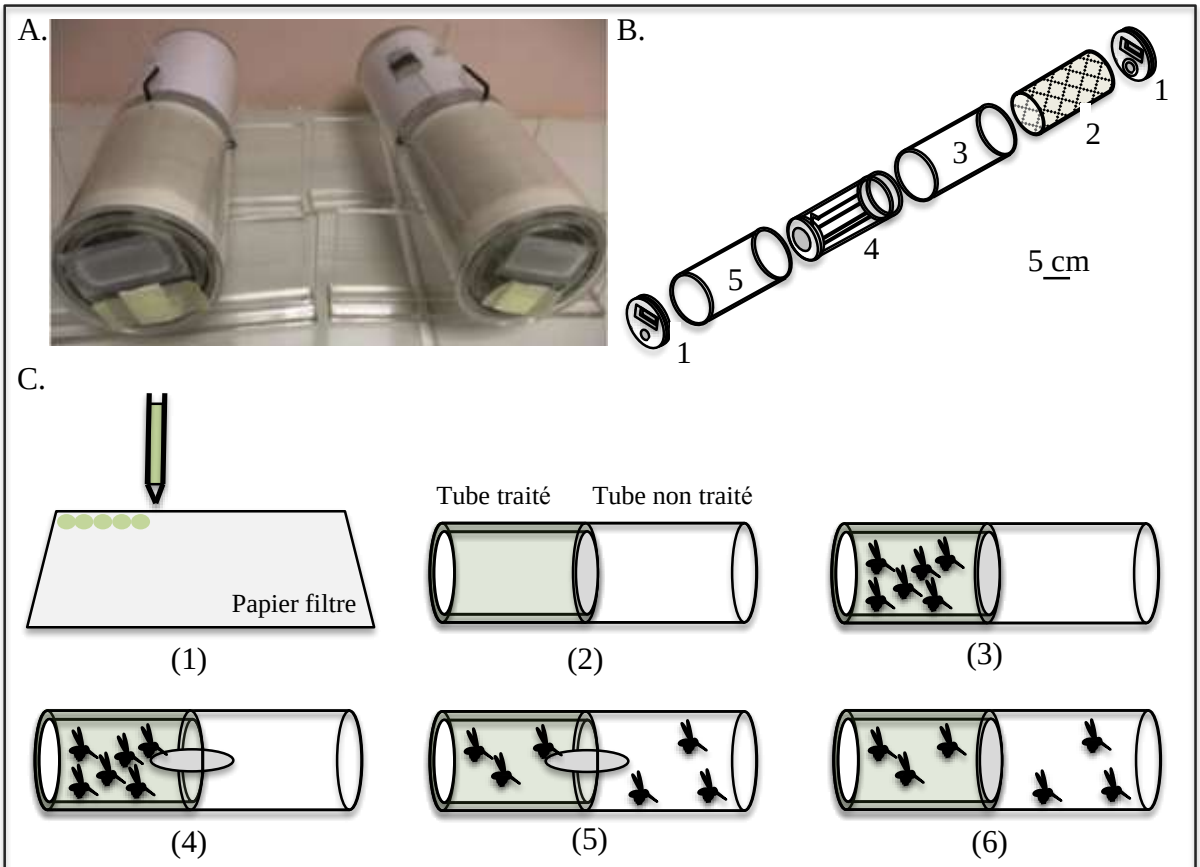


Figure 9: Test pour la répulsion-expulsion pour *Anopheles gambiae*

A. Photo du dispositif
B. Schéma du dispositif, 1:bouchon, 2:filet métallique, 3:tube traité, 4: section de jointure, 5:tube non traité.
C. Séquence de test, 1: un papier filtre (13*30cm) est traité avec 3,3mL de solution, 2: le papier est introduit dans le tube traité à l'extérieur de la section de jointure, 3: les moustiques sont introduits dans le tube traité, 4: la porte est ouverte après 30s d'acclimatation, 5: les moustiques sont laissés libres pendant 10 min, 6: après fermeture de la porte, le nombre de moustiques échappés sont calculés.

Pour ce test, 3 concentrations ont été testé: 0,01%, 0,1% et 1%. Pour chaque concentration et le témoin (éthanol pur), 3 lots de 20 moustiques ont été testé.

Les proportions du nombre de moustiques échappés ont été comparé par paire avec le témoin avec le test de Fisher et corrigées avec la méthode de Bonferroni-Holm. Un modèle linéaire généralisé (distribution binomiale) a été étudié pour connaître l'effet du produit, de la concentration et de l'interaction concentration-produit (dose-dépendance) sur l'effet répulsif sur le moustique. Enfin une classification ascendante hiérarchique (Ward) a été effectué pour classer les extraits de plantes.

Pour plus de détails, cf. Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013).

2. Test de répulsion chez le moustique

Pour tester l'effet répulsion-expulsion, le dispositif de Grieco *et al.* (2005) créé pour *Aedes aegypti* a été adapté pour *Anopheles gambiae* (Figure 9). Les moustiques sont introduits dans un tube où ils peuvent détecter le produit testé uniquement par l'olfaction mais ne peuvent être en contact avec lui. En effet, un filet métallique empêche le contact entre le moustique et le papier traité. Si le produit est répulsif les moustiques peuvent s'échapper dans

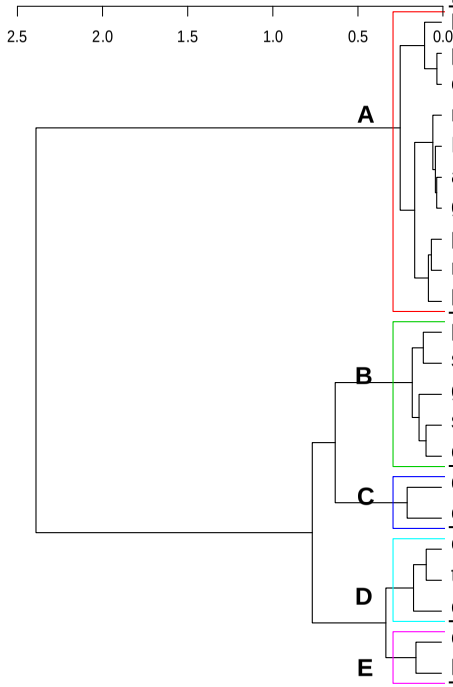
un autre tube, donc plus le produit est répulsif plus le nombre de moustiques qui s'échappe est important.

L'effet répulsif-expulsif a été différent en fonction du produit testé et s'est montré dose-dépendant (Figure 10). De manière générale, plus la dose était forte plus l'effet répulsif était important (GLM, $P < 0.001$, coefficient: 0.82). Huit extraits de plantes n'ont montré aucun effet : le citron, l'eucalyptus, le neem, l'aframomum, le géranium, le pennyroyal, le romarin, et le litsea. Les douze autres se sont montrés répulsifs à au moins une concentration, par ordre croissant : le poivre, la sarriette, le gingembre, le solidage, le cumin, l'aneth, le coléus, le coriandre, le thym, la citronnelle, la cannelle et le lemongrass. Il faut noter que le DEET et la perméthrine n'ont pas montré d'effet répulsif-expulsif.

Afin de mieux comprendre les mécanismes d'action des huiles essentielles, les quatre huiles essentielles les plus irritantes ont été analysées par GC et GC-MS afin d'identifier, de quantifier et de tester l'effet irritant de leurs composés. Ainsi les composés majoritaires des huiles essentielle de cannelle, de cumin, de thym et de citronnelle présentés dans le tableau 2 ainsi que les mélanges dans les proportions naturelles des composés majoritaires de chaque huile ont été testés afin d'identifier quels composés étaient actifs dans le mélange.

Les mélanges des composés majoritaires ont également montré un effet répulsif-expulsif à haute dose mais pas à faible dose et ils ont été aussi répulsifs que leur huile essentielle correspondante (Figure 11). Les composés minoritaires n'auraient donc aucun rôle dans la répulsion-expulsion. Les produits suivants ont montré le même degré de répulsion que leurs huiles essentielles correspondantes: carvacrol, citronellal, geraniol, citronellol, cuminaldehyde, γ-terpinene. Il faut noter que le cinnamaldéhyde a été répulsif mais pas autant que l'huile essentielle de cannelle.

L'effet répulsif-expulsif de l'huile essentielle de thym est donc du à l'effet du carvacrol, celui de la citronnelle au citronellal et/ou geraniol, celui du cumin au cuminaldehyde et celui de la cannelle à l'effet additif/synergique du cinnamaldehyde et de l'acétate de cinnamyle.



product	0.01 %	0.1 %	1%	p-value[2]
lemon	-5.2 (-13.4 – 3.0)[1]	13.6 (1.8 - 25.4)	14.2 (2.00 - 26.4)	0.003**
permethrin	5.1 (-2.7 - 13.0)	10.3 (1.0 - 19.5)	7.7 (-0.7 - 16.1)	–
eucalyptus	2.2 (-1.8 - 6.3)	9.2 (2.3 - 16.1)	4.4 (-1.1 – 9.9)	0.300
neem	4.1 (-2.3 - 10.4)	2.7 (-3.8 - 9.3)	10.3 (2.0 - 18.6)	–
DEET	0.9 (-5.1 - 6.8)	2.0 (-4.3 - 8.2)	8.1 (0.3 - 15.9)	–
aframomum	2.0 (-6.1 - 10.0)	-3.2 (-10.0 - 3.6)	10.6 (0.5 - 20.7)	1.000
geranium	-6.9 (-13.2 - -0.6)	-1.3 (-9.2 - 6.7)	11.1 (0.6 - 21.5)	0.929
pennyroyal	12.5 (1.2 - 23.7)	-10.6 (-21.8 - 0.6)	7.5 (-4.7 - 19.6)	<0.001***
rosemary	13.2 (1.9 - 24.6)	-3.1 (-10.9 - 4.8)	13.0 (2.2 - 23.7)	0.042*
litsea	7.0 (0.4 - 13.7)	1.0 (-2.7 - 4.7)	16.4 (6.7 - 26.0)	–
pepper	17.2 (6.5 - 27.8)	5.7 (-2.6 - 13.9)	**30.0 (17.1 - 42.9)**	0.004**
savory	12.5 (2.6 - 22.3)	2.1 (-4.8 - 8.9)	**40.6 (27.9 - 53.3)**	<0.001***
ginger	10.7 (-4.6 – 18.0)	16.0 (27.9 - 50.6)	**35.9 (-4.6 – 18.0)**	<0.001***
solidage	2.2 (-6.9 - 11.3)	-4.7 (-11.7 - 2.3)	**36.9 (24.8 - 48.9)**	<0.001***
cumin	2.5 (-6.9 - 11.8)	9.2 (-1.9 - 20.2)	**42.4 (30.0 - 54.8)**	<0.001***
dill	9.3 (-1.3 – 20.0)	**35.9 (23.9 - 47.8)**	**37.3 (26.0 - 48.7)**	<0.001***
coleus	**32.1 (20.0 - 44.1)**	**43.9 (31.4 - 56.3)**	**26.5 (14.0 – 39.0)**	0.050.
coriander	8.8 (0.8 - 16.8)	9.8 (1.7 - 17.9)	**53.1 (42.6 - 63.7)**	<0.001***
thyme	6.7 (-2.4 - 15.7)	**39.2 (26.7 - 51.8)**	**63.4 (52.0 - 74.8)**	<0.001***
citronnella	-3.7 (-9.3 - 1.9)	17.4 (5.9 - 28.9)	**69.5 (60.0 - 79.0)**	<0.001***
cinnamomum	**26.3 (14.9 - 37.7)**	14.8 (5.6 – 24.0)	**73.5 (64.9 - 82.1)**	<0.001***
lemongrass	**26.0 (14.6 - 37.5)**	**23.5 (11.8 - 35.1)**	**60.2 (49.1 - 71.3)**	<0.001***

Figure 10: Réponse comportementale de femelles *Anopheles gambiae*[0] à l'effet répulsif-expulsif du DEET, de la Perméthrine et de 20 extraits de plantes à 3 différentes concentrations (0,01; 0,1 et 1% de produit dans la solution de traitement des papiers).

A. Dendrogramme établi par classification ascendante hiérarchique

B. Proportion corrigée par la formule d'Abbot de moustiques échappés (intervalle de confiance calculé selon la méthode de Wald) par concentration

[0] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs en gras se sont montrées significativement différentes du témoin

[2] P-value du modèle linéaire généralisé de l'interaction concentration-produit (dose-dépendance) sur l'effet répulsif sur le moustique. Le coefficient a été comparé à 0 et seul les p-values des coefficients positifs ont été données.

Ces résultats sont présentés dans l'article: Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013).

A.

Product	C1	C2
permethrin	-0.2 (-5.3 - 4.9)[1]	6.3 (-1.5 – 14.0)
DEET	-3.3 (-8.0 - 1.4)	15.0 (5.2 - 24.7)
thyme oil	-1.3 (-5.1 - 2.5)	**51.4 (39.3 - 63.4)**
thyme blend	8.6 (-0.1 - 17.2)	**83.0 (75.2 - 90.9)**
thymol	16.6 (6.2 - 27)	-2.4 (-5.7 – 1.0) *
p-cymene	-0.1 (-4.0 - 3.7)	-1.5 (-4.3 - 1.4) *
carvacrol	9.4 (1.7 – 17.0)	**36.6 (25.0 - 48.2)**
α-terpinene	25.7 (14.4 – 37.0) *	4.3 (-2.4 - 11.1) *
linalool	12.7 (4.5 - 20.9)	17.6 (8.6 - 26.7) *
β-caryophyllene	-8.7 (-14.3 - -3.1)	10.4 (-0.2 - 21.1) *
citronella oil	-1.3 (-8.1 - 5.4)	**54.3 (42.7 - 65.8)**
citronella blend	**32.9 (21 - 44.8)** *	**76.2 (67.3 - 85.2)**
citronellal	8.9 (0.3 - 17.4)	**40.1 (28.1 - 52.2)**
geraniol	5.1 (-2.7 – 13.0)	**50.5 (38.0 - 63.1)**
citronellol	10.6 (1.1 – 20.0)	25.2 (13.8 - 36.6)
limonene	-0.1 (-4.4 - 4.2)	-3.3 (-3.3 - -3.3) *
geranyl-acetate	13.1 (4.6 - 21.6)	15.5 (7.1 - 23.9) *
cumin oil	4.7 (-0.5 - 9.9)	**37.3 (25.7 - 48.9)**
cumin blend	0.0 (0.0 - 0.0)	**30.6 (19.2 - 42.1)**
cuminaldehyde	1.6 (-1.5 - 4.7)	**25.4 (14.3 - 36.5)**
β-pinene	3.9 (-1.2 – 9.0)	2.8 (-1.9 - 7.5) *
γ-terpinene	4.5 (-1.1 - 10.1)	21.0 (11.0 – 31.0)
p-cymene	0.2 (-6.1 - 6.5)	0.2 (-6.1 - 6.5) *
cinnamon oil	8.5 (0.9 - 16.1)	**84.0 (75.7 - 92.3)**
cinnamon blend	12.4 (3.3 - 21.5)	**66.2 (54.4 – 78.0)**
cinnamaldehyde	16.5 (7.8 - 25.3)	**43.0 (32.1 – 54.0)** *
2-methoxy-cinnamaldehyde	-6.8 (-12.2 - -1.5)	-3.0 (-10.3 - 4.2) *
cinnamyl-acetate	7.6 (-0.8 - 16)	15.8 (6.0 - 25.7) *

B.

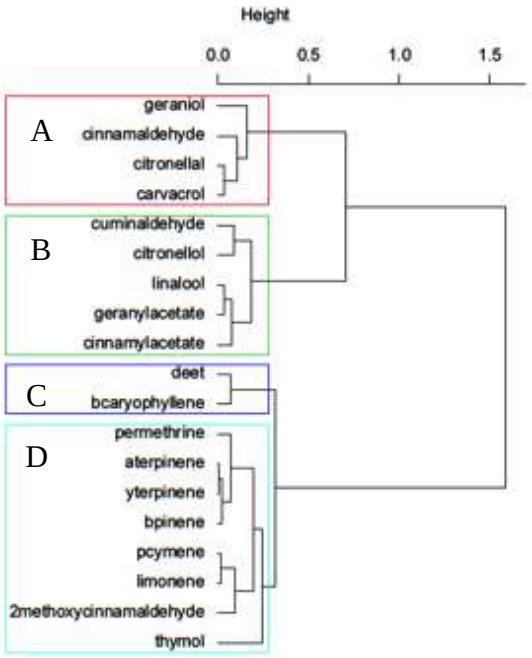


Figure 11: Réponse comportementale de femelles *Anopheles gambiae*[0] à l'effet répulsif-expulsif du DEET, de la Perméthrine, des composés majoritaires des huiles essentielles de cannelle, de citronnelle et de cumin et du mélange des composés majoritaires de chaque à 2 différentes concentrations.

A. Proportion corrigée par la formule de Sun-Sheppard de moustiques échappés (intervalle de confiance calculé selon la méthode de Wald) par concentration

B. Dendrogramme établi par classification ascendante hiérarchique

Citronella blend: 34,74% citronellal, 22,50% geraniol, 12,03% citronellol, 3,51% geranyl acetate, 3,34% limonene. Cinnamon blend: 78,51% cinnamaldehyde, 9,65% 2-metoxycinnamaldehyde, 3,15% cinnamylacetate. Cumin blend: 30,1% cuminaldehyde, 12,2% β-pinene, 11,6% γ-terpinene, 9,7% *p*-cymene. Thyme blend: 30,5% thymol, 23,7% p-cymene, 13,6% carvacrol, 8,4% α-terpinene, 4,0% linalool, 3,5% b-caryophyllene

[0] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs en gras se sont montrées significativement différentes du témoin

Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Afin de mieux comprendre le mode d'action et les cibles des composés expulsifs les produits ont été testés sur deux souches résistantes de moustiques. De plus pour une utilisation sur le terrain, il était intéressant de savoir si les réponses comportementales seraient les mêmes pour des moustiques résistants ou sensibles. Parmi les composés les plus efficaces, le cinnamaldéhyde, le cuminaldéhyde, le carvacrol et le géraniol ont été choisis pour être testés sur les deux souches résistantes KdrKis et AcerKis, présentées en introduction, afin de comparer les effets répulsifs sur des populations sensibles et résistantes.

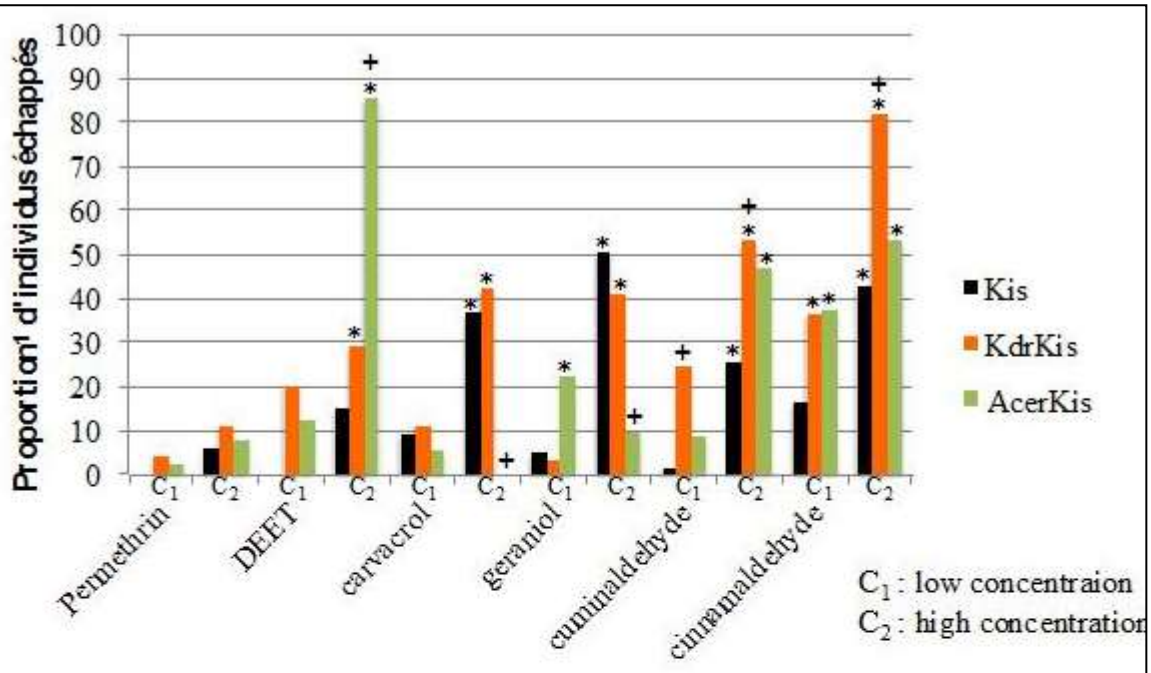


Figure 12: Réponse comportementale de femelles *Anopheles gambiae*[2] à l'effet répulsif-expulsif du DEET, de la Perméthrine, du carvacrol, du géraniol, du cuminaldéhyde et du cinnamaldéhyde à 2 différentes concentrations sur souche sensible Kis et sur 2 souches résistantes KdrKis et AcerKis.

[1] Proportion corrigée par la formule de Sun-Sheppard de moustiques échappés par concentration.

[2] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre.

* Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une * se sont montrées significativement différentes du témoin.

\+ Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une + se sont montrées significativement différentes des valeurs de la souche sensible.

Ces résultats sont présentés dans l'article: Behavior modulation inducing by kdr and ace1 genes on the repellent effect of DEET, Permethrin and natural compounds on the malaria vector Anopheles gambiae (Deletre *et al.*, en préparation).

La permethrine n'a été répulsive-expulsive pour aucune population (Figure 12). Le DEET n'a pas été répulsif-expulsif pour la population sensible mais l'a été pour les deux populations résistantes. A forte concentration, le carvacrol et le géraniol ont été répulsifs pour les populations Kis et KdrKis mais pas pour la population AcerKis alors que le cinnamaldéhyde et le cuminaldéhyde ont été répulsifs pour toutes les populations. A faible concentration, seul le cinnamaldéhyde a montré un effet répulsif et seulement sur les deux populations résistantes.

3. Test de répulsion chez la mouche blanche

Pour tester l'effet répulsion-expulsion sur *Bemisia tabaci*, un olfactomètre vertical sans courant d'air a été utilisé (Zhang et al, 2004) (Figure 13).

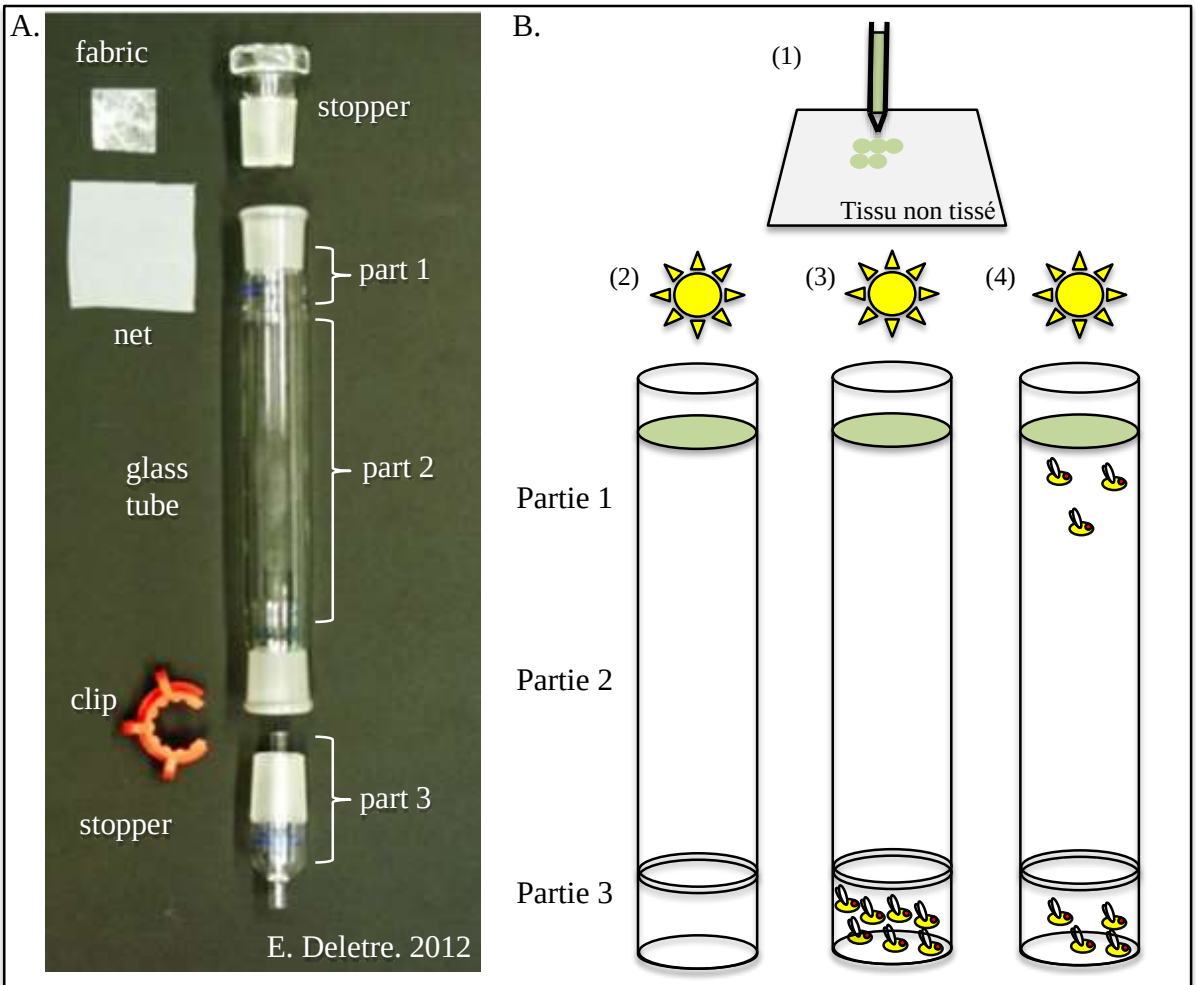


Figure 13: Test pour la répulsion-expulsion pour *Bemisia tabaci*

A. Schéma du dispositif

B. Séquence de test, 1: le tissu est traité avec le produit, 2: le tissu est placé en haut de l'olfactomètre tubulaire, 3: les aleurodes sont introduits dans la partie 3 de l'olfactomètre, 4: après une heure, la distribution des aleurodes dans les différentes parties est enregistrée.

2 à 4 concentrations (10 à 0,01%) ont été testé avec une application de 10 µl/cm^2 de produit. 4 répétitions de 10 à 20 aleurodes ont été effectués pour chaque concentration et témoin. Pour comparer les distributions d'aleurodes entre le témoin et le traitement un test de Fisher a été effectué. Pour plus de détails, cf. Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Deletre *et al.*, soumis à Journal of Economic Entomology).

Les aleurodes sont introduits dans un tube où ils peuvent détecter le produit testé uniquement par l'olfaction mais un filet à mailles fines empêche le contact entre le produit et les tarses des insectes et leurs pièces buccales ne peuvent être en contact avec lui. Le cylindre a été orienté verticalement sous une source de lumière et à cause de la tendance innée des aleurodes à aller vers la lumière, ils vont rapidement se retrouver en haut de l'olfactomètre (phototaxie positive). Après 1h, le nombre d'aleurodes dans chaque partie a été enregistré. Lorsque le produit testé était répulsif, les aleurodes restaient dans la partie basse du cylindre au lieu d'aller dans la partie haute du cylindre.

L'effet répulsif-expulsif a été différent en fonction du produit testé et de sa concentration. Deux extraits de plantes ont été significativement répulsifs aux trois concentrations testées: l'aframomum et la citronnelle (Tableau 3). Douze extraits de plantes ont été significativement répulsifs à deux concentrations: cannelle, cumin, aneth, géranium,

gingembre, lemongrass, litsea, menthe pouliot, sarriette, solidage et thym. Cinq extraits de plantes ont été significativement répulsifs à une seule concentration: coriandre, eucalyptus, citron, poivre et romarin. Le neem n'a pas été répulsif quelle que soit la concentration utilisée. Le DEET a été sensiblement répulsif à 0,1% et 1 % alors que la perméthrine n'a pas été répulsive quelle que soit la concentration. Les extraits de plantes et les témoins positifs, le DEET et perméthrine, ont provoqué un taux de mortalité de moins de 2%.

Tableau 3: Réponse comportementale de *Bemisia tabaci* à l'effet répulsif-expulsif du DEET, de la perméthrine et des 20 extraits de plantes à 3 différentes concentrations (0,01; 0,1 et 1% de produit dans la solution de traitement des tissus). Ces résultats sont présentés dans l'article : Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Deletre *et al.*, soumis à Journal of Economic Entomology).

Nom commun	**Nom latin**	**Effet répulsif-expulsif**[1]
DEET	composé synthétique	+ +
Permethrine	composé synthétique	**0**
Aframomum	*Aframomum pruinosum*	+ + +
Aneth	*Anethum graveolens*	+ +
Cannelle	*Cinnamomum zeylanicum*	+ +
Citron	*Citrus limon*	+ +
Citronelle	*Cymbopogon winterianus*	+++
Coleus	*Plectranthus tenuicaulis*	+ +
Coriandre	*Coriandrum sativum*	+ +
Cumin	*Cuminum cyminum*	+
Eucalyptus	*Eucalyptus globulus*	+ +
Geranium	*Pelargonium graveolens*	+ +
Gingembre	*Zingiber officinalis*	+
Lemongrass	*Cymbopogon citratus*	+ +
Litsea	*Litsea cubeba*	+ +
Pennyroyal	*Mentha pulegium*	**0**
Neem	*Melia azadirachta*	+ +
Poivre	*Piper nigrum*	+
Romarin	*Rosmarinus officinalis*	+
Sariette	*Satureja montana*	+ +
Solidage	*Solidago canadensis*	+ +
Thym	*Thymus vulgaris L.*	+ +

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. + : une concentration a eu un effet répulsif, 0 : aucune concentration n'a eu d'effet répulsif.

Afin de mieux comprendre le mode d'action des huiles essentielles, les composés majoritaires des huiles essentielles de cannelle, de cumin, de lemongrass et de citronnelle

présentés dans le tableau 2 ainsi que les mélanges dans les proportions naturelles des composés majoritaires de chaque huile ont été testés afin d'identifier quels composés étaient actifs dans le mélange.

Les 4 mélanges de composés majoritaires se sont montrés aussi répulsifs que leurs huiles essentielles associées. Le mélange de composés majoritaires de la citronnelle s'est montré répulsif à 0,1 % et à 1 % avec une toxicité de vapeur élevée. Parmi les composés seuls, le géraniol, le citronellol, l'acétate de géranyle se sont montrés répulsifs. A la plus forte concentration testée, le citronellal a montré une forte toxicité de vapeur à 0,34 $mg.L^{-1}$ (Tableau 4).

Tableau 4: Réponse comportementale de *Bemisia tabaci* à l'effet répulsif-expulsif du mélange des composés majoritaires des huiles essentielles de citronnelle, de cumin, de cannelle, et de lemongrass et de chaque composé majoritaire à 2 différentes concentration. Ces résultats sont présentés dans l'article: Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Deletre *et al.*, soumis à Journal of Economic Entomology).

Produit	Effet répulsif-expulsif
citronellal	+*
geraniol	+++
citronellol	+
geranyl acétate	+
limonene	++
Mélange 'Citronelle'[1]	+
cuminaldehyde	+
β-pinène	0
γ-terpinene	0
p-cymene	0
Mélange 'cumin'[2]	++
(E)-cinnamaldehyde	+++
2-methoxy-cinnamaldehyde	0
cinnamylacetate	0
Mélange 'cannelle'[3]	++
citral	+
Mélange 'lemongrass'[4]	++

[1] Mélange 'citronnelle: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acétate, 3.34% limonene. Mélange 'cannelle': 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate. Mélange 'cumin' : 30.1% cuminaldehyde, 12.2% β-pinène, 11.6% γ-terpinene, 9.7% *p*-cymene. Mélange 'lemongrass' : 74,1% citral, 4,5% geraniol, 3,9% geranyl acétate.

* Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm.

+ : une concentration a eu un effet répulsif, 0 : aucune concentration n'a eu d'effet répulsif.

Le mélange des composés majoritaires du cumin s'est montré répulsif aux 2 concentrations testées et parmi ses composés majoritaires, seul le cuminaldéhyde a montré un effet répulsif à forte concentration. Le mélange des composés majoritaires de la cannelle a été répulsif à 0,1 % avec une forte toxicité de vapeur à 1 %. Parmi les composés du mélange, le

cinnamaldehyde s'est montré répulsif quelle que soit la dose mais avec une mortalité de 30%. Le mélange des composés majoritaires du lemongrass a été répulsif à 0,1 % avec une forte toxicité de vapeur à 1 %. Parmi les composés majoritaires testés seuls, le géraniol et l'acétate de géranyle ont été répulsifs à forte concentration et le citral a provoqué une forte mortalité de vapeur à haute concentration. En conclusion, l'effet répulsif de l'huile essentielle de cannelle est sûrement du au cinnamaldéhyde, celui du cumin au cuminaldéhyde, celui de la citronnelle à l'effet synergique et/ou additif du géraniol, du citronellol et de l'acétate de géranyle et l'effet répulsif du lemongrass serait du au citral, au geraniol, et à l'acétate de géranyle.

4. Discussion

Le DEET et la perméthrine ne se sont pas montrés répulsif-expulsif sur *Anopheles gambiae*. Comme l'ont montré également Achee *et al.* (2009) sur *Aedes aegypti* la perméthrine n'est pas un expulsif même si, lorsqu'une moustiquaire en est imprégnée, un nombre moins important de moustiques semble entrer dans la maison (Adeogun *et al.*, 2012). La tension de vapeur de ces composés est faible 0,27 Pa et $7*10^6$ Pa à 25°C ce qui pourrait expliquer leur faible effet répulsif par rapport aux terpènes. Pourtant le DEET est connu pour être un bon répulsif d'où une tension de vapeur très différente tout de même par rapport à la perméthrine. Comme il est généralement appliqué sur la peau qui est à une température plus élevée que 25°C son efficacité pourrait en être augmentée. Cependant, comme nous le verrons dans la suite du manuscrit, le DEET ne serait peut être pas un expulsif mais un produit irritant, masquant ou anti-appétant. Par contre, le DEET s'est montré répulsif sur *Bemisia tabaci.* Kain *et al.* (2014) ont montré que le DEET active les neurones I40a+ chez *Drosophila melanogaster* et lorsque ces neurones sont désactivés les mouches perdent leur répulsion-expulsion vis-à-vis du DEET (Kain *et al.*, 2014). Ces neurones semblent être très conservés chez les insectes, cela pourrait donc expliquer l'effet expulsif du DEET sur *Bemisia tabaci.*

Parmi les 20 extraits de plantes testés nous remarquons que le neem (huile végétale) ne s'est pas montré répulsif ce qui s'explique par le fait que ses composants sont très peu volatils. De plus, le romarin (hydrolat biologique) n'aurait pas été répulsif parce que ses composés bioactifs étaient en quantité trop faible. En effet, la répulsion augmente avec la concentration quelque soit l'extrait testé.

Les huiles essentielles les plus répulsives contre *Anopheles gambiae* ont été le lemongrass et la cannelle ainsi que la citronnelle, le thym et la coriandre ; contre *B. tabaci* l'aframomum et la citronnelle ont été les plus répulsives, bien que la cannelle, le géranium et la sarriette ont également été répulsifs à des doses plus élevées. Pour les 4 huiles essentielles retenues pour chaque insecte, les mélanges des composés majoritaires se sont également montrés aussi répulsifs que leurs huiles essentielles associées ce qui laisse supposer que les composés minoritaires n'interviennent pas dans la répulsion expulsion.

La citronnelle et la cannelle se sont donc montrées répulsives pour les deux insectes. Le mélange des composés majoritaires de l'huile essentielle de cannelle s'est montré également répulsif pour les deux insectes et le composé bioactif trouvé a été le cinnamaldéhyde. Le cinnamaldéhyde agirait donc sur un récepteur bien conservé chez les insectes et cela aurait tendance à corroborer notre hypothèse sur le mécanisme d'action d'un répulsif-expulsif. Le cinnamaldehyde active le récepteur TRPA1 impliqué dans la détection des hautes températures. Or il a été démontré chez Drosophila que ce récepteur était nécessaire pour obtenir l'effet répulsif du citronellal et chez *Anopheles gambiae* le TRPA1 est directement activé par le citronellal (Kwon *et al.*, 2010). Pour la citronnelle, le mélange des composés

majoritaires s'est montré également répulsif pour les deux insectes. Mais le citronellal n'a pas été le seul composé répulsif, le citronellol et particulièrement le géraniol se sont également montrés répulsifs. Au niveau neurophysiologique, il a été montré que le géraniol avait un effet sur les impulsions nerveuses spontanées (Chen et Viljoen, 2010).

Nous avions émis l'hypothèse que les répulsifs-expulsifs étaient reconnus par les récepteurs olfactifs, nous avons donc effectué des électroantennogrammes (EAG) sur des moustiques (Figure 14). L'EAG permet de mesurer la réponse électrique d'une antenne à une odeur et de savoir ainsi si l'insecte est capacle de détecter cette odeur grâce à ses récepteurs olfactifs.

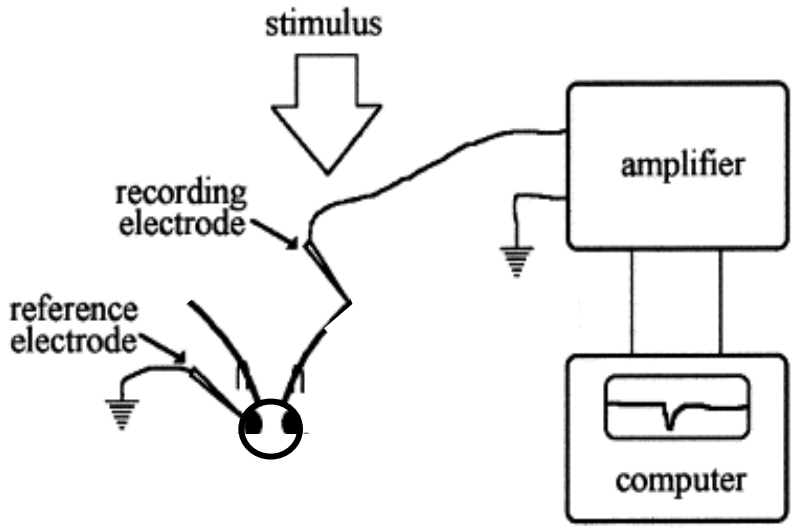


Figure 14: Test EAG sur *Anopheles gambiae* de 17 composés majeurs provenant des huiles essentielles de cannelle, citronelle, thym et cumin.

Chaque composé a été testé à 1% (25µl sur un papier filtre) dans l'éthanol sur 28 moustiques femelles dont la tête et le bout de l'antenne ont été excisés. Les composés ont été testé après 6 min par un pulse de 0,5s au hasard toutes les 30s. La séquence témoin (air, ethanol, octanal, air) a été testée au début, au milieu et à la fin des 17 composés.

L'amplitude des réponses (moyenne ± ES) a été corrigée par rapport au témoin : $0{,}5(\sqrt{x}+\sqrt{(x+1)})$ et présenté comme une réponse relative au standard, 100 µM octanal. Elles ont été analysées par une analyse de variance suivie d'un test de comparaison par paire de Tukey.

Pour plus de détails, cf. Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Les réponses d'EAG des femelles d'*Anopheles gambiae* montrent clairement que les insectes détectent les composés testés (F=23,5, DF=18, P-value>0,001) (Figure 15). Les réponses les plus fortes ont été élicitées par deux benzaldéhyde aromatique : le cinnamaldéhyde et le cuminaldéhyde, un alcool monoterpène acyclique, le linalool, et un aldéhyde monoterpène acyclique, le citronellal. Les moustiques semblent peu sensibles à deux phénols monoterpènes cycliques : le carvacrol et le thymol. Le cinnamaldéhyde et le citronellal en élicitant une forte réponse sur l'EAG corroborent notre hypothèse sur un mécanisme d'action par le système olfactif. Dekker *et al.* (2011) a également montré que plusieurs composés répulsifs étaient détectés par les antennes d'*Aedes aegypti* mais tous les

composés actifs sur l'EAG ne se sont pas obligatoirement montrés répulsifs. Cependant les résultats de comportement ne sont pas toujours en accord avec les résultats d'électrophysiologie. En effet, le carvacrol s'est montré répulsif mais ne semble pas être détecté par les antennes du moustique. Une des hypothèses est que le carvacrol soit détecté par les appendices bucaux du moustique tels que les palpes. Le cinnamaldéhyde, le linalol, le cuminaldehyde, le citronellal, le β-caryophyllène, l'acétate de cinnamyle et le géraniol ont donné des réponses EAG significatives par rapport à l'éthanol et certains composés comme le linalol n'ont pas été répulsifs. Ces composés actifs pourraient donc agir en tant que répulsif ou attractif comme suggéré par Knaden *et al.* (2012).

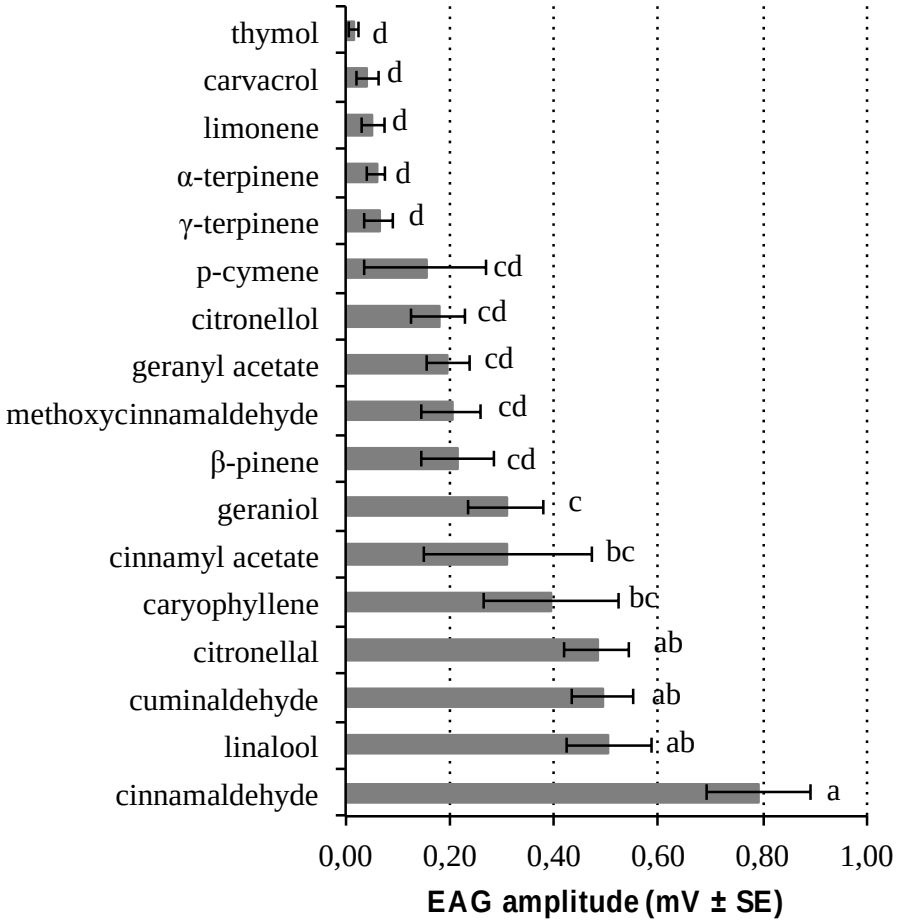


Figure 15: Réponse EAG d' ***Anopheles gambiae*** **à 17 composés majeurs provenant des huiles essentielles de cannelle, citronelle, thym et cumin.**

L'amplitude des réponses (moyenne ± ES) a été corrigée par rapport au témoin, solvant et présenté comme une réponse relative au standard, 100 µM octanal. Chaque composé a été testé à 1% (v/v) dans l'éthanol sur 28 moustiques femelles.

Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Les produits répulsifs issus des plantes qui sont identifiés aujourd'hui sont soit des alcaloïdes qui peuvent affecter les récepteurs d'acétylcholinestérase au niveau du système nerveux (Flattum et Shankland, 1971) ou les canaux membranaires des nerfs (Amar *et al.*, 1991), soit des phénols, particulièrement le groupe des flavonoïdes et des terpènes qui est le groupe comportant le plus de composés répulsifs identifiés à ce jour. L'étude de l'effet répulsif-expulsif du cinnamaldéhyde et du cuminaldéhyde, deux aldéhydes, a montré un effet plus important sur la souche résistante KdrKis que sur la souche sensible Kis (ou l'autre souche résistante AcerKis). Par ailleurs ces deux composés ont montré une réponse EAG importante. Cela suggère une interaction possible entre l'effet répulsif-expulsif et les canaux sodium voltage-dépendant. Aujourd'hui peu d'études traitent des mécanismes d'action des répulsifs sur le système nerveux. La plupart des études portent sur les effets de l'inhibition de l'acétylcholinestérase comme par exemple le cuminaldéhyde, le géraniol ou le carvacrol qui seraient des inhibiteurs réversibles de l'acétylcholinestérase, en plus d'avoir des propriétés insecticides importantes (Lopez et Pascual-Villabos, 2010 ; Abdelgail *et al.*, 2009 ; Jukic *et al.*, 2007 ; Anderson et Coats, 2012). Notre étude suggère également un effet du géraniol et du carvacrol, deux alcools, sur l'acétylcholinestérase, puisque la souche résistante AcerKis a été moins sensible à leur effet répulsif-expulsif. Mais d'autres études traitent aussi de l'effet sur les canaux TRP qui seraient impliqués dans la photoréception, la thermosensibilité, la mécanoréception, la perception des phéromones, du goût et de la douleur (Moran *et al.*, 2004). Le cinnamaldéhyde et le carvacrol ont montré un effet sur ces mêmes récepteurs (Nagata, 2007 ; Parnas *et al.*, 2007). Il a également été montré que le récepteur TRPA1 était requis pour le comportement de répulsion des insectes vis à vis du citronellal. Chez les drosophiles, le citronellal interagit avec le TRPA1 en modifiant l'activité du canal potassium activé par Ca^{2+} mais chez *Anopheles gambiae* TRPA1 est directement activé par le citronellal (Kwon *et al.*, 2010).

Le mécanisme d'action le plus probable d'un composé répulsif 'expulsif' serait donc que les insectes aient des récepteurs olfactifs spécifiques avec des glomérules associés spécifiques ce qui favoriserait une réponse rapide de l'insecte (Figure 16). Par exemple, le DEET activerait les neurones I40a+ chez *Drosophila melanogaster* et lorsque ces neurones sont désactivés les mouches perdent leur répulsion-expulsion vis-à-vis du DEET (Kain *et al.*, 2014). Les composés volatils attractifs et expulsifs seraient reconnus par des protéines de liaison et des récepteurs olfactifs différents. Ainsi les neurones activés et les glomérules activés seraient également différents. Certains glomérules seraient activés par des composés attractifs et enverraient un signal d'attraction au cerveau de l'insecte qui coderait alors pour un vol en direction de l'odeur alors que le composé explusif enverrait un signal de répulsion au cerveau de l'insecte qui coderait alors pour un vol à l'opposé de l'odeur.

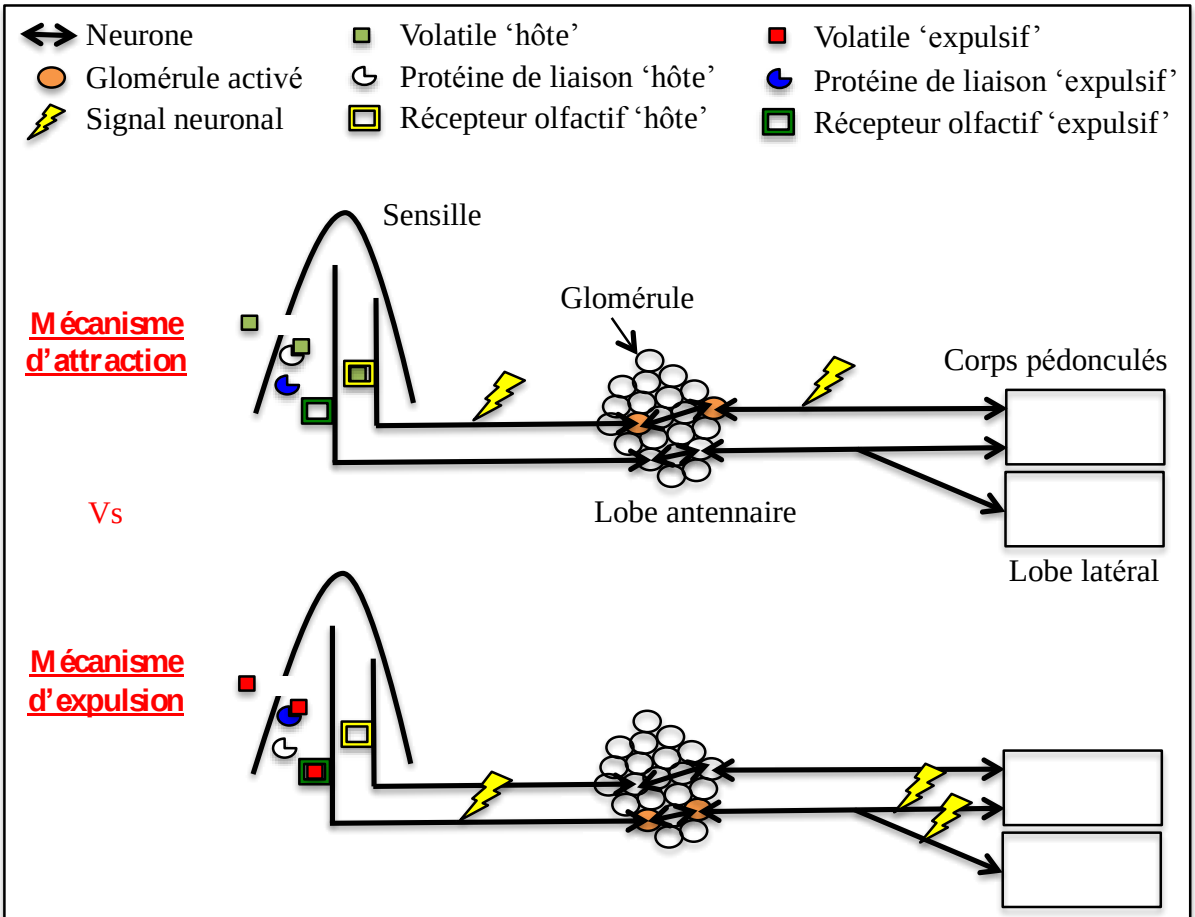
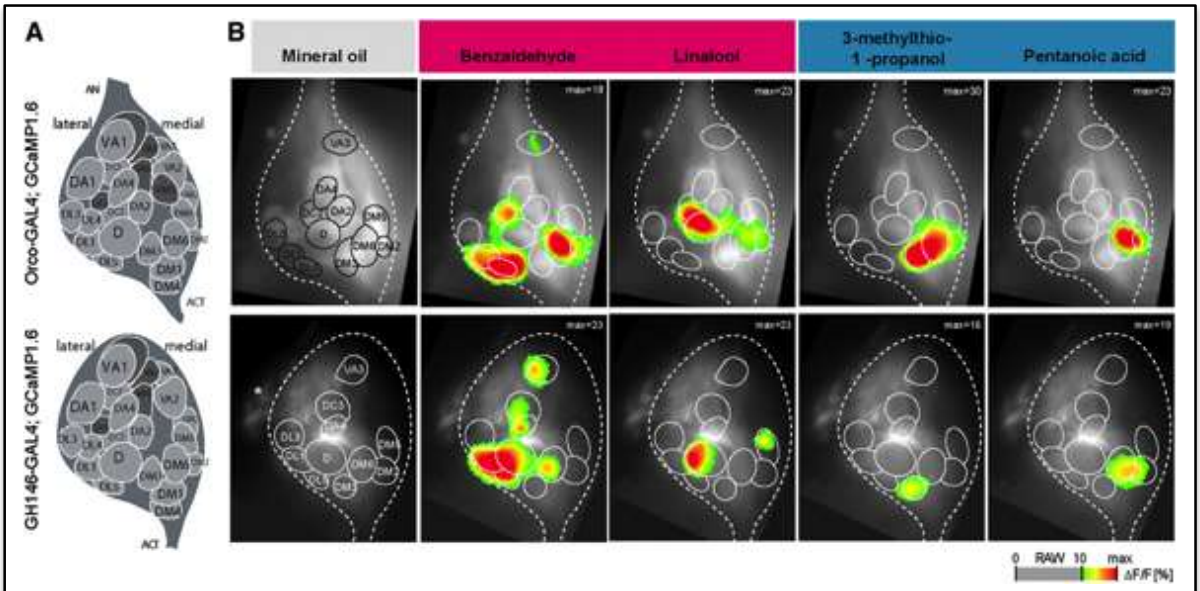


Figure 16 : Mécanisme physiologique hypothétique des répulsifs 'expulsifs'
Les répulsifs 'expulsifs' causent un mouvement de fuite de l'insecte à distance vis à vis de l'odeur quelque soit le contexte. Les insectes doivent donc avoir des récepteurs olfactifs spécialisés pour les substances également appelées 'aversives' et des glomérules associés. Les substances attractives et répulsives activeraient des récepteurs, des neurones et des glomérules différents, les messages informatifs envoyés au cerveau de l'insecte seraient différents et coderaient pour un comportement différent.
Ce schéma a été adapté de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

L'hypothèse est donc que les insectes ont des récepteurs olfactifs et des neurones olfactifs dédiés à la détection des produits répulsifs dits expulsifs. En effet, dans certains cas comme celui des phéromones, une réponse comportementale innée liée à une odeur est élicitée par un récepteur spécifique et sélectif et souvent l'intégration de l'odeur au niveau du lobe antennaire au lobe latéral se fait sans une importante activité des interneurones (Christensen & Hildebrand, 2002; Touhara & Vosshall, 2009). Par exemple, *Drosophila megalonaster* a un comportement inné de fuite au geosmin, composé produit par les champignons et les bactéries : cette molécule active le neurone ab4B spécifique seulement au geosmin et élicite une réponse de seulement deux neurones de projection (Stensmyr *et al.*, 2012). De plus, Knaden *et al.* (2012) montrent que certains glomérules sont activés distinctement soient par des composés répulsifs soient par des composés attractifs : les glomérules DA4 et DC3 chez *D. megalonaster* ont été identifiés comme des spécialistes de la répulsion (Figure 17).



Identification of Glomeruli Activated by Attractive and Aversive Odorants Using Functional Calcium Imaging.
(A) Schematized atlas of the AL representing glomeruli that have been functionally characterized. Flies expressing the genetically encoded calcium reporter G-CaMP allowed us to visualize odorant-evoked activities at the level of OSNs (top panels) and PNs (bottom panels) using the Orco-GAL4 and GH146-GAL4 line, respectively. Both lines label an overlapping set of glomeruli with the exception of glomerulus VM5, which is not labeled by the GH146 driver line. Glomeruli that were not significantly activated by any of the odorants are filled in dark gray. AN, antennal nerve; ACT, antennocerebral tract.
(B) Representative false color-coded images showing the AL after stimulation with mineral oil as a control or with aversive (magenta) and attractive (turquoise) odorants. All images are individually scaled to the strongest activated glomeruli of the entire AL (data shown only for the left AL). Values below the DF/F threshold of 10% are omitted to illustrate the specificity of the signals, as well as the glomerular arrangement as visualized by the intrinsic fluorescence. Images represent DF/F [%] superimposed onto the raw fluorescence images according to the scale below. White asterisk marks the PN soma cluster.

Figure 17 : Identification des glomeruli activés par des composés attractifs et répulsifs par imagerie calcique.
Les images ci-dessus tiré de '*Spatial representation of odorant valence in an insect brain*' (Knaden *et al.*, 2012) montrent que les composés répulsifs activent des glomérules différents que les composés attractifs.

Un des inconvénients majeurs à l'utilisation d'un répulsif-expulsif est que le produit pourrait être moins répulsif que l'hôte n'est attractif. Il faut donc faire des tests complémentaires pour montrer l'efficacité d'un répulsif-expulsif dans le cas d'une utilisation sur le terrain contre les insectes nuisibles. De plus, un composé peut être toxique par fumigation et présenter également une propriété répulsive-expulsive. Dans ce cas, ce composé pourrait être expulsif-expulsif à des doses sub-létales.

Recherche d’un répulsif-irritant

‘Je ne m’inquiétais pas de l’étrange alchimie d’odeur et de peau qui rend certains parfums plus présent sur certaine personnes que sur d’autre’ Badhika Jha, *L’Odeur*.

1. Introduction

La répulsion-irritation est un comportement de fuite de l'insecte après contact avec le composé testé. Combiné avec un filet, un produit irritant empêchera l'insecte de rester sur le filet pour éviter qu'il ne pique à travers le filet ou qu'il ne réussisse à passer à travers le filet. Notre hypothèse est que les répulsifs-irritants agissent sur les récepteurs gustatifs. Avant de tester la combinaison de la barrière physique et chimique, il faut trouver un produit irritant.

Pour tester la répulsion-irritation, il faut donc permettre à l'insecte d'être au contact du composé testé afin d'observer un comportement de fuite de l'insecte après contact voire de forcer le contact entre le produit et l'insecte. Le test doit être fait sans hôte pour ne pas risquer de biaiser l'essai. En effet, la présence de l'hôte ne nous permettrait pas de savoir si notre composé est répulsif-irritant ou répulsif-antiappétent.

Pour les insectes rampants, un bioessai classique consiste à utiliser une boite de pétri tapissée d'un papier absorbant dont une moitié a été traitée avec le produit à tester. Si le produit est irritant l'insecte évitera ou passera moins de temps sur la zone traitée. Par exemple, dans ce type de dispositif, le comportement du collembole a été suivi par video tracking pour montrer l'irritabilité d'acide gras (Nilsson & Bengtsson, 2004).

Pour les insectes volant les bioessais sont plus difficiles à imaginer puisqu'il faut obliger l'insecte à être en contact avec le produit. Par exemple, pour tester des produits répulsifs sur les moustiques, ceux-ci sont placés dans des cônes en plastique dont la base est traitée par un produit et le temps du premier envol est comparé au control (Mouchet & Cavalie, 1961; Chandre *et al.*, 2000 ; Figure 18). Un composé est d'autant plus irritant que le temps jusqu'au premier envol est court. D'autres méthodes existent en fonction de la biologie et du comportement de l'insecte.

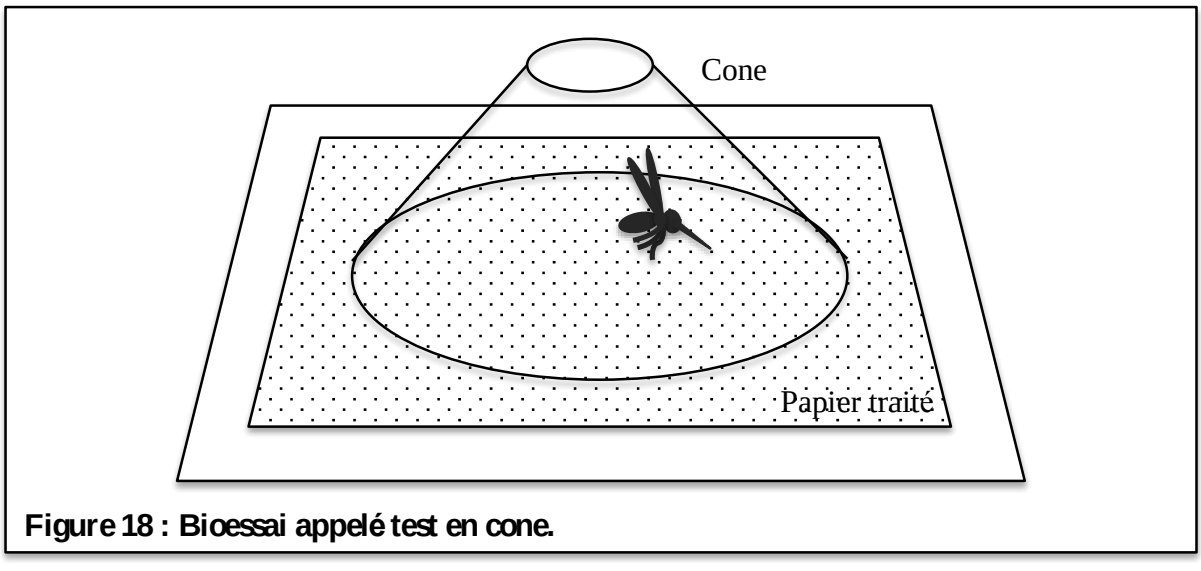


Figure 18 : Bioessai appelé test en cone.

2. Test d'irritation chez le moustique

Pour tester l'effet répulsion-irritation, le dispositif de Grieco *et al.* (2005) créé pour *Aedes aegypti* a été adapté pour *Anopheles gambiae* (Figure 19). Les moustiques sont introduits dans un tube où ils sont en contact avec le produit. Si le produit est irritant, les

moustiques peuvent s'échapper dans un autre tube, et plus le produit est irritant, plus le nombre de moustiques échappés est important dans un lapse de temps donné.

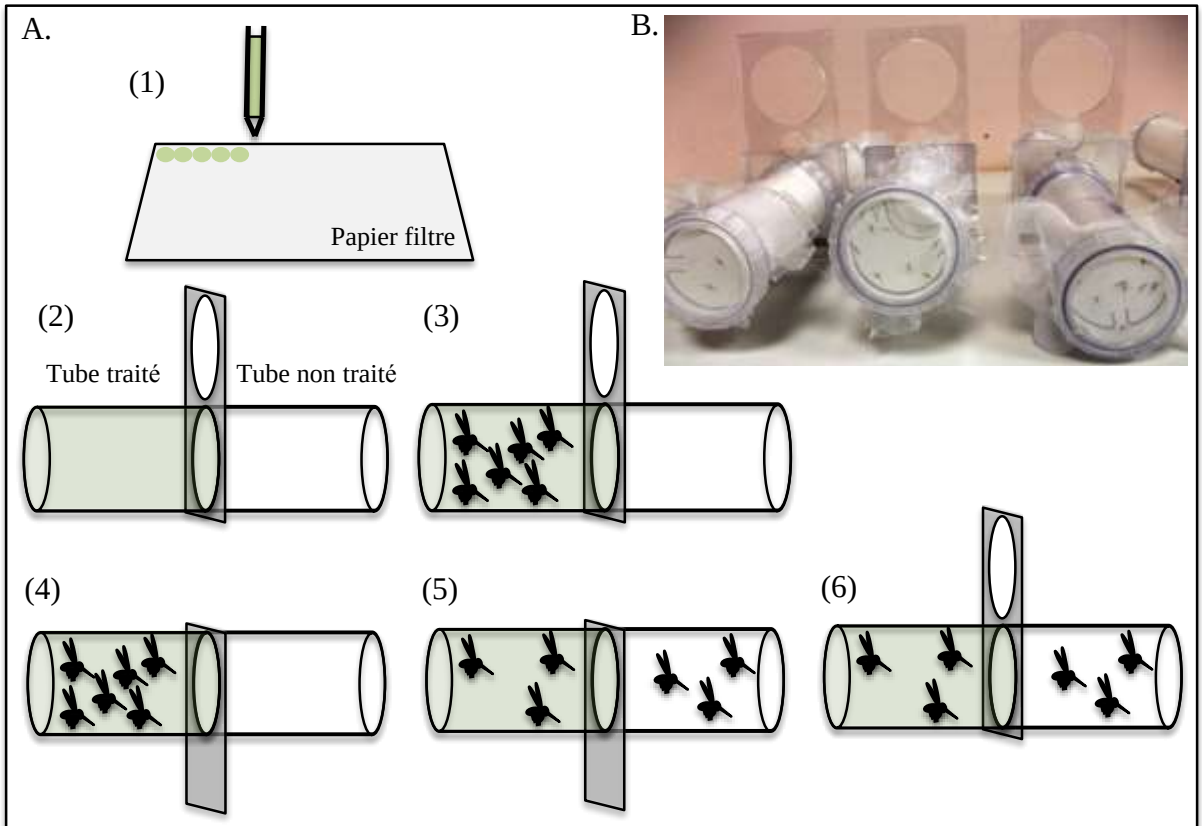


Figure 19: Test pour la répulsion-irritation pour *Anopheles gambiae*

A. Séquence de test, 1: un papier filtre (12*15cm) est traité avec 2mL de solution, 2: le papier est placé dans le tube traité, 3: les moustiques sont introduits dans le tube traité, 4: la porte est ouverte après 30s d'acclimatation, 5: les moustiques sont laissés libres pendant 10 min, 6: après fermeture de la porte, le nombre de moustiques échappés sont calculés.
B. Photo du dispositif

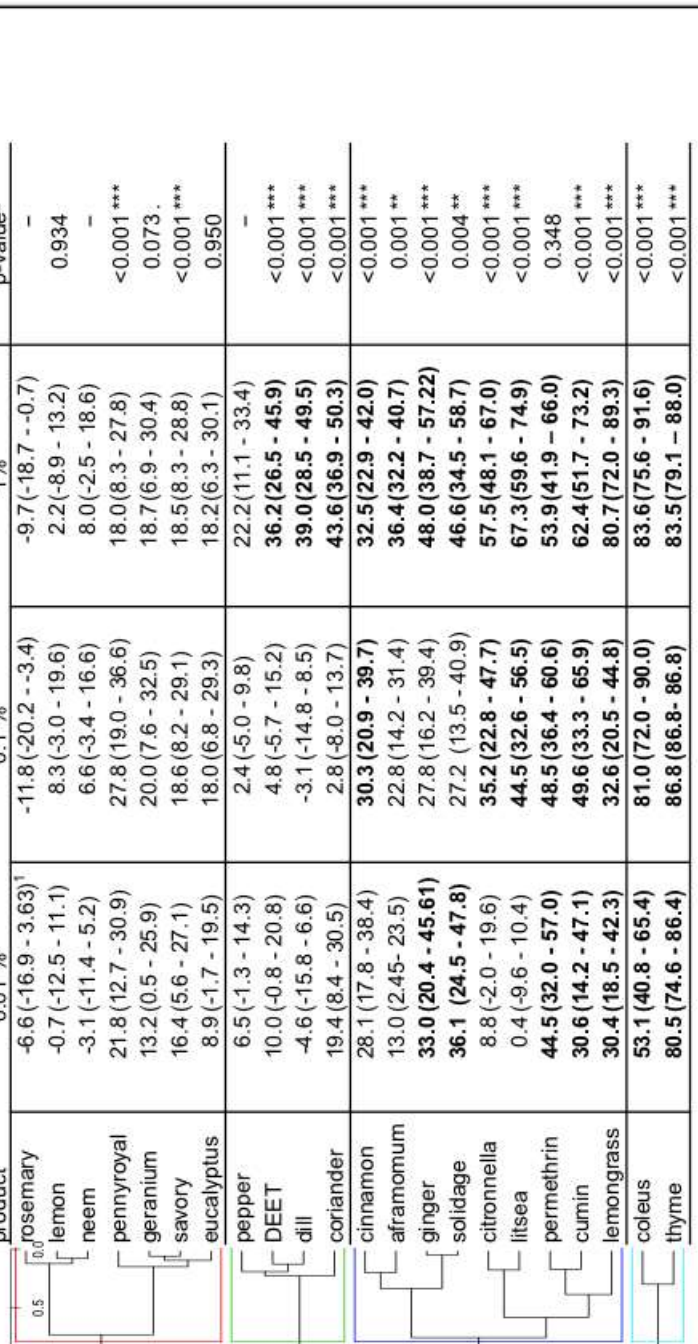
Pour ce test, 3 concentrations ont été testé: 0,01%, 0,1% et 1%. Pour chaque concentration et le témoin (éthanol pur), 3 lots de 20 moustiques ont été testé.

Les proportions du nombre de moustiques échappés ont été comparé par paire avec le témoin avec le test de Fisher et corrigées avec la méthode de Bonferroni-Holm. Un modèle linéaire généralisé (distribution binomiale) a été étudié pour connaître l'effet du produit, de la concentration et de l'interaction concentration-produit (dose-dépendance) sur l'effet irritant sur le moustique. Enfin une classification ascendante hiérarchique (Ward) a été effectué pour classer les extraits de plantes.

Pour plus de détails, cf. Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013)

Comme l'effet répulsif-expulsif, l'effet répulsif-irritant observé a été différent en fonction du produit testé et s'est révélé dose-dépendant (Figure 20). De manière générale, plus la dose était forte, plus l'effet irritant était important (GLM, $P < 0{,}001$; coefficient: 2,87). Huit extraits de plantes n'ont montré aucun effet : le romarin, le citron, le neem, le pennyroyal, le géranium, la sarriette, l'eucalyptus et le poivre. Les douze autres se sont montrés irritants à au moins une concentration, par ordre croissant: l'aneth, le coriandre, la

cannelle, l'aframomum, le gingembre, le solidage, la citronnelle, le litsea, le cumin, le lemongrass, le coléus et le thym.



	product	0.01 %	0.1 %	1%	p-value[2]
A	rosemary	-6.6 (-16.9 - 3.63)[1]	-11.8 (-20.2 - -3.4)	-9.7 (-18.7 - -0.7)	–
	lemon	-0.7 (-12.5 - 11.1)	8.3 (-3.0 - 19.6)	2.2 (-8.9 - 13.2)	0.934
	neem	-3.1 (-11.4 - 5.2)	6.6 (-3.4 - 16.6)	8.0 (-2.5 - 18.6)	–
	pennyroyal	21.8 (12.7 - 30.9)	27.8 (19.0 - 36.6)	18.0 (8.3 - 27.8)	<0.001***
	geranium	13.2 (0.5 - 25.9)	20.0 (7.6 - 32.5)	18.7 (6.9 - 30.4)	0.073 .
	savory	16.4 (5.6 - 27.1)	18.6 (8.2 - 29.1)	18.5 (8.3 - 28.8)	<0.001***
	eucalyptus	8.9 (-1.7 - 19.5)	18.0 (6.8 - 29.3)	18.2 (6.3 - 30.1)	0.950
B	pepper	6.5 (-1.3 - 14.3)	2.4 (-5.0 - 9.8)	22.2 (11.1 - 33.4)	–
	DEET	10.0 (-0.8 - 20.8)	4.8 (-5.7 - 15.2)	**36.2 (26.5 - 45.9)**	<0.001***
	dill	-4.6 (-15.8 - 6.6)	-3.1 (-14.8 - 8.5)	**39.0 (28.5 - 49.5)**	<0.001***
	coriander	19.4 (8.4 - 30.5)	2.8 (-8.0 - 13.7)	**43.6 (36.9 - 50.3)**	<0.001***
C	cinnamon	28.1 (17.8 - 38.4)	**30.3 (20.9 - 39.7)**	**32.5 (22.9 - 42.0)**	<0.001***
	aframomum	13.0 (2.45- 23.5)	22.8 (14.2 - 31.4)	**36.4 (32.2 - 40.7)**	0.001**
	ginger	**33.0 (20.4 - 45.61)**	27.8 (16.2 - 39.4)	**48.0 (38.7 - 57.22)**	<0.001***
	solidage	**36.1 (24.5 - 47.8)**	27.2 (13.5 - 40.9)	**46.6 (34.5 - 58.7)**	0.004**
	citronnella	8.8 (-2.0 - 19.6)	**35.2 (22.8 - 47.7)**	**57.5 (48.1 - 67.0)**	<0.001***
	litsea	0.4 (-9.6 - 10.4)	**44.5 (32.6 - 56.5)**	**67.3 (59.6 - 74.9)**	<0.001***
	permethrin	**44.5 (32.0 - 57.0)**	**48.5 (36.4 - 60.6)**	**53.9 (41.9 – 66.0)**	0.348
	cumin	**30.6 (14.2 - 47.1)**	**49.6 (33.3 - 65.9)**	**62.4 (51.7 - 73.2)**	<0.001***
	lemongrass	**30.4 (18.5 - 42.3)**	**32.6 (20.5 - 44.8)**	**80.7 (72.0 - 89.3)**	<0.001***
D	coleus	**53.1 (40.8 - 65.4)**	**81.0 (72.0 - 90.0)**	**83.6 (75.6 - 91.6)**	<0.001***
	thyme	**80.5 (74.6 - 86.4)**	**86.8 (86.8- 86.8)**	**83.5 (79.1 – 88.0)**	<0.001***

Figure 20: Réponse comportementale de femelles *Anopheles gambiae*[0] à l'effet répulsif-irritant du DEET, de la Perméthrine et de 20 extraits de plantes à 3 différentes concentrations (0,01; 0,1 et 1% de produit dans la solution de traitement des papiers).

A. Dendrogramme établi par classification ascendante hiérarchique

B. Proportion corrigée par la formule d'Abbot de moustiques échappés (intervalle de confiance calculé selon la méthode de Wald) par concentration

[0] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs en gras se sont montrées significativement différentes du témoin

[2] P-value du modèle linéaire généralisé de l'interaction concentration-produit (dose-dépendance) sur l'effet répulsif sur le moustique. Le coefficient a été comparé à 0 et seul les p-values des coefficients positifs ont été données.

Ces résultats sont présentés dans l'article: Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013).

Afin de mieux comprendre les mécanismes d'action des huiles essentielles, les quatre huiles essentielles les plus irritantes ont été analysées par GC et GC-MS afin d'identifier, de quantifier et de tester l'effet irritant de leurs composés. Ainsi les composés majoritaires des huiles essentielle de cannelle, de cumin, de thym et de citronnelle présentés dans le tableau 2 ainsi que les mélanges en proportion naturelle des composés majoritaires correspondants ont été testés afin d'identifier quels composés étaient actifs dans le mélange.

Les 4 mélanges des composés majeurs des huiles essentielles de cannelle, de cumin, de citronnelle et de thym se sont montrés irritants et aussi irritants que les huiles essentielles complètes aux deux concentrations testées, mis à part le cumin à forte dose qui a eu un effet irritant mais plus faible (Figure 21).

A.

Product	C1		C2	
permethrin	**37.7 (25.0 – 50.4)**[1]		**25.9 (13.8 - 38.0)**	
DEET	7.6 (-0.2 - 15.5)		**54.1 (42.6 - 65.7)**	
thyme oil	20.1 (9.5 - 30.6)		**59.0 (49.0 – 69.0)**	
thyme blend	**35.6 (23.8 - 47.4)**		**38.7 (26.6 - 50.8)**	
thymol	25.4 (13.0 - 37.7)		**34.7 (21.8 - 47.5)**	
p-cymene	9.4 (0.2 - 18.7)		-6.5 (-11.6 - -1.4)	*
carvacrol	**50.0 (38.2 - 61.8)**	*	**84.3 (77.4 - 91.2)**	
α-terpinene	1.6 (-2.4 - 5.6)		10.7 (3.3 - 18.1)	*
linalool	1.6 (-4.3 - 7.6)		10.1 (0.8 - 19.4)	*
β-caryophyllene	4.3 (-4.4 - 12.9)		20.8 (9.6 - 32)	*
citronella oil	33.0 (21.1 - 44.8)		**60.3 (50.5 - 70.2)**	
citronella blend	**34.2 (23.0 - 45.4)**		**82.2 (74.2 - 90.2)**	
citronellal	24.4 (12.5 - 36.4)		**52.2 (42.1 - 62.3)**	
geraniol	16.5 (5.2 - 27.7)		**45.9 (34.2 - 57.5)**	
citronellol	**46.4 (35.3 - 57.5)**		**68.6 (60.7 - 76.5)**	
limonene	-3.1 (-11.3 - 5.1)		-3.3 (-11.3 - 4.8)	*
geranyl-acetate	6.8 (-1.7 - 15.4)		21.7 (9.9 - 33.6)	*
cumin oil	7.9 (-1.2 – 17.0		**79.4 (72.8 – 86.0)**	
cumin blend	4.8 (-2.1 - 11.7)		**41.1 (30.1 - 52.1)**	*
cuminaldehyde	10.4 (1.1 - 19.6)		**77.5 (69.4 - 85.6)**	
β-pinene	11.2 (1.1 - 21.3)		15.6 (6.0 - 25.3)	*
γ-terpinene	8.9 (-0.4 - 18.1)		12.7 (3.2 - 22.2)	*
p-cymene	-2.7 (-9.6 -4.2)		-6.1 (-11.4 - -0.8)	*
cinnamon oil	25.8 (15.2 - 36.3)		**90.4 (85.6 - 95.2)**	
cinnamon blend	**56.7 (46.4 – 67.0)**	*	**60.2 (50.3 - 70.2)**	
cinnamaldehyde	22.0 (12.3 - 31.7)		**63.2 (52.9 - 73.4)**	
2-methoxy-cinnamaldehyde	0.3 (-9.9 - 10.6)		-1.0 (-11.1 - 9.1)	*
cinnamyl-acetate	29.2 (17.2 - 41.2)		**43.5 (32.6 - 54.4)**	*

B.

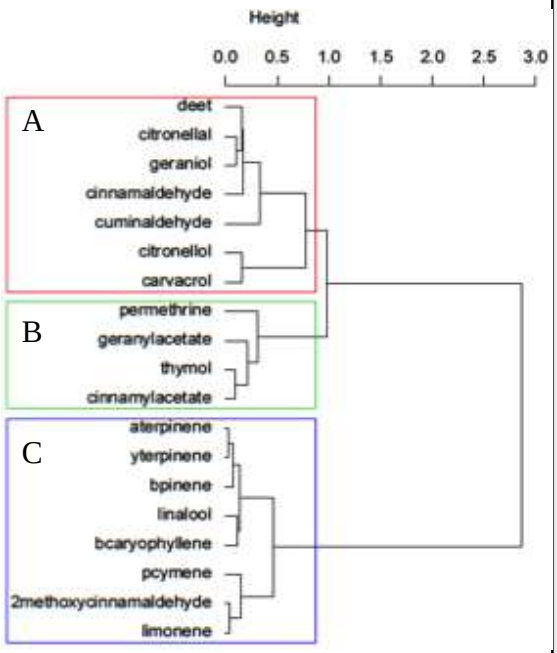


Figure 21: Réponse comportementale de femelles *Anopheles gambiae*[0] à l'effet répulsif-irritant du DEET, de la Perméthrine, des composés majoritaires des huiles essentielles de canelle, citronnelle et cumin et du mélange des composés majoritaires de chaque à 2 différentes concentrations.

A. Proportion corrigée par la formule de Sun-Sheppard de moustiques échappés (intervalle de confiance calculé selon la méthode de Wald) par concentration

B. Dendrogramme établi par classification ascendante hiérarchique

Citronella blend: 34,74% citronellal, 22,50% geraniol, 12,03% citronellol, 3,51% geranyl acetate, 3,34% limonene. Cinnamon blend: 78,51% cinnamaldehyde, 9,65% 2-metoxycinnamaldehyde, 3,15% cinnamylacetate. Cumin blend: 30,1% cuminaldehyde, 12,2% β-pinene, 11,6% γ-terpinene, 9,7% *p*-cymene. Thyme blend: 30,5% thymol, 23,7% p-cymene, 13,6% carvacrol, 8,4% α-terpinene, 4,0% linalool, 3,5% b-caryophyllene

[0] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs en gras se sont montrées significativement différentes du témoin .

Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Les composés minoritaires ne semblent donc pas jouer de rôle dans l'effet irritant des huiles essentielles. A au moins une concentration, le thymol, le carvacrol, le citronellal, le geraniol, le citronellol, le cuminaldehyde, le cinnamaldehyde et l'acétate de cinnamyl ont été irritants et ils ont tous été aussi irritants que leurs huiles essentielles correspondantes mis à part l'acétate de cinnamyle. L'effet répulsif-irritant du cumin et de la cannelle serait donc du à leur composé majoritaire, le cuminaldehyde et le cinnamaldehyde respectivement. L'effet irritant du thym serait principalement du au carvacrol même si le thymol est également irritant. L'effet irritant de la citronnelle est du au citronellol, au geraniol, au citronellal qui seul ou en mélange sont aussi irritants. Le DEET et la perméthrine se sont également montrés irritants. A forte concentration pour la population sensible, la perméthrine s'est montrée moins irritante à cause de l'effet *'Knock-down'* (30% d'individus) qui a empêché les

moustiques de s'échapper (Figure 22). Par contre, la perméthrine a été faiblement irritante pour les deux populations résistantes à fortes doses. Le DEET a été irritant à forte concentration pour toutes les populations. A forte concentration, le cinnamaldéhyde, le cuminaldéhyde, le géraniol et le carvacrol ont été irritants pour toutes les populations. A faible concentration, le carvacrol a été irritant pour la population sensible mais pas pour les populations résistantes.

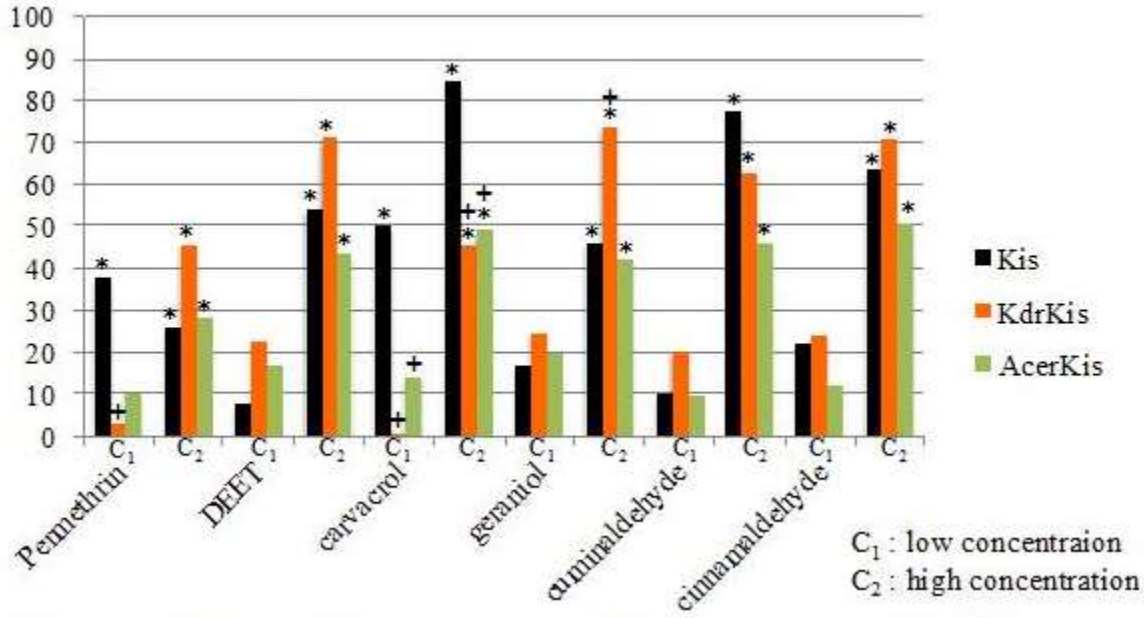


Figure 22: Réponse comportementale de femelles *Anopheles gambiae*[2] à l'effet répulsif-irritatif du DEET, de la Perméthrine, du carvacrol, du géraniol, du cuminaldéhyde et du cinnamaldéhyde à 2 différentes concentrations sur souche sensible Kis et sur 2 souches résistantes KdrKis et AcerKis.

[1] Proportion corrigée par la formule de Sun-Sheppard de moustiques échappés (intervalle de confiance calculé selon la méthode de Wald) par concentration

[2] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

* Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une * se sont montrées significativement différentes du témoin

\+ Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une + se sont montrées significativement différentes des valeurs de la souche sensible.

Ces résultats sont présentés dans l'article: Behavior modulation inducing by kdr and ace1 genes on the repellent effect of DEET, Permethrin and natural compounds on the malaria vector Anopheles gambiae (Deletre *et al.*, en préparation).

Nous avons émis l'hypothèse que les répulsifs-irritants agissent sur les récepteurs gustatifs mais Miller *et al.* (2009) ont émis l'hypothèse que l'effet irritant serait une cause d'une dose subléthale. L'effet toxique des huiles essentielles et de leurs composés a donc été testé sur des moustiques sensibles et résistants d'*Anopheles gambiae* pour tester cette hypothèse.

Le protocole de l'OMS a été utilisé pour tester leur effet toxique sur *Anopheles gambiae* (Figure 23). Les moustiques sont introduits dans un tube où ils sont en contact forcé avec le produit pendant une heure. Après avoir été transférés dans un tube non traité et conservé pendant 24h, l'effet létal du produit testé a été observé. Si un produit toxique est combiné avec un filet, cela permet d'intoxiquer l'insecte en contact avec le filet et donc de réduire la population de vecteurs. Un produit toxique serait donc intéressant à combiner avec un filet même s'il y a des risques de résitances.

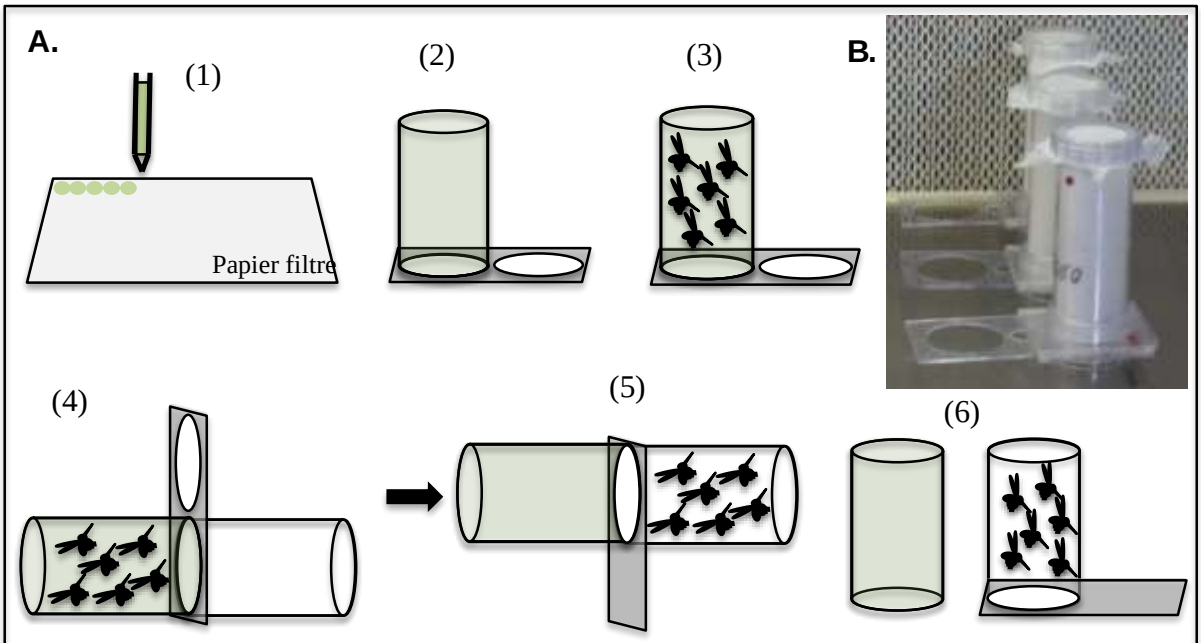


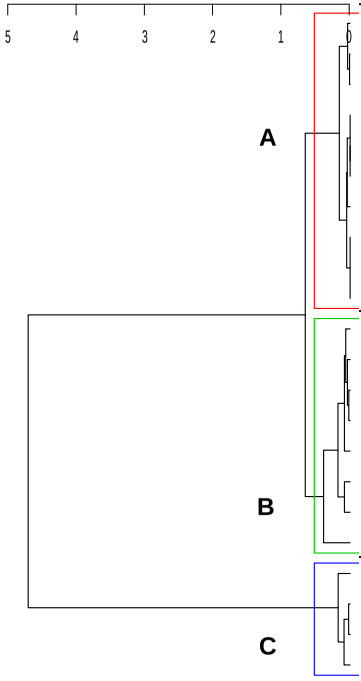
Figure 23 : Test pour la toxicité pour *Anopheles gambiae*

A. Séquence de test, 1: un papier filtre est traité avec le produit testé, 2: le papier est placé dans le tube traité, 3: les moustiques sont introduits dans le tube traité pendant 1h, 4: le tube traité est connecté à un tube non traité, 5: les moustiques sont transférés dans le tube non traité, 6: les moustiques sont nourris avec du jus sucré et gardé pendant 24h puis le nombre de moustiques morts sont calculés.
B. Photo du dispositif

Pour ce test, 3 concentrations ont été testé: 0,01%, 0,1% et 1%. Pour chaque concentration et le témoin (éthanol pur), 3 lots de 20 moustiques ont été testé.

Les proportions du nombre de moustiques morts ont été comparé par paire avec le témoin avec le test de Fisher et corrigées avec la méthode de Bonferroni-Holm. Un modèle linéaire généralisé (distribution binomiale) a été étudié pour connaître l'effet du produit, de la concentration et de l'interaction concentration-produit (dose-dépendance) sur l'effet toxique sur le moustique. Enfin une classification ascendante hiérarchique (Ward) a été effectué pour classer les extraits de plantes.

Pour plus de détails, cf. Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013)



product	0.01 %	0.1 %	1%	p-value[2]
rosemary	5.6 (-2.1 - 13.3)[1]	1.9 (-4.3 - 8.2)	-3.1 (-6.1 - -0.0)	
eucalyptus	4.8 (-1.7 - 11.2)	1.5 (-3.4 - 6.5)	2.6 (-2.7 - 7.9)	1.000
pennyroyal	4.9 (-3.0 - 12.8)	1.5 (-4.3 - 7.2)	3.7 (-4.0 - 11.3)	0.188
pepper	-1.7 (-7.1 - 3.6)	-1.8 (-7.3 - 3.6)	-1.8 (-9.8 - 6.2)	
dill	-4.1 (-8.5 - 0.4)	-1.2 (-7.0 - 4.6)	-1.1 (-6.5 - 4.3)	0.997
ginger	-3.0 (-3.0 - -3.0)	0.2 (-4.3 - 4.7)	0.3 (-4.4 – 5.0)	
neem	0 (0 - 0)	2.9 (-1.1 – 7.0)	0.0 (0.0 – 0.0)	
DEET	-4.4 (-8.6 - -0.3)	-1.1 (-6.5 - 4.4)	1.2 (-5.4 - 7.9)	0.860
geranium	-1.4 (-1.4 - -1.4)	-1.4 (-1.4 - -1.4)	2.8 (-1.8 - 7.4)	
lemon	0.2 (-3.9 - 4.3)	-2.8 (-2.8 - -2.8)	2.1 (-3.3 - 7.6)	1.000
solidage	3.1 (-2.3 - 8.5)	-1.8 (-1.8 – -1.8)	7.8 (-0.2 - 15.8)	0.934
lemongrass	-2.1 (-6.6 - 2.4)	2.3 (-4.9 - 9.6)	8.1 (-1.2 - 17.4)	0.058
litsea	0 (0 - 0)	1.6 (-1.5 - 4.6)	10.3 (3.1 - 17.5)	0.552
aframomum	0.3 (-5.1 - 5.8)	0.8 (-5.1 - 6.6)	12.0 (4.0 - 20.1)	<0.001***
coleus	0.0 (0 - 0)	7.3 (0.4 - 14.2)	9.4 (1.5 - 17.3)	0.831
coriander	7.6 (0.4 - 14.9)	6.8 (0.5 – 13.0)	13.0 (4.1 - 21.9)	<0.001***
cumin	3.7 (-1.8 - 9.1)	7.0 (0.1- 13.8)	19.3 (10.0 - 28.6)	<0.001***
permethrin	3.5 (-2.2 - 9.2)	19.3 (9.2 - 29.4)	**32.2 (20.4 – 44.00)**	<0.001***
citronnella	11.5 (2.8 - 20.2)	6.1 (-0.8 - 13.0)	**88.6 (78.5 - 98.7)**	<0.001***
savory	1.0 (-5.3 - 7.3)	1.6 (-5.2 - 8.4)	**87.3 (81.2 - 93.3)**	<0.001***
thyme	3.2 (-0.9 - 7.4)	3.3 (-0.7 - 7.3)	**87.9 (83.9 - 91.9)**	<0.001***
cinnamomum	0.7 (-4.1 - 5.6)	-1.8 (-5.3 - 1.7)	**94.5 (92.2 - 96.8)**	<0.001***

Figure 24: Réponse comportementale de femelles *Anopheles gambiae*[0] à l'effet toxique du DEET, de la Perméthrine et de 20 extraits de plantes à 3 différentes concentration (0,01; 0,1 et 1% de produit dans la solution de traitement des papiers).

A. Dendrogramme établi par classification ascendante hiérarchique

B. Proportion corrigée par la formule d'Abbot de moustiques échappés (intervalle de confiance calculé selon la méthode de Wald) par concentration

[0] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs en gras se sont montrées significativement différentes du témoin

[2] P-value du modèle linéaire généralisé de l'interaction concentration-produit (dose-dépendance) sur l'effet répulsif sur le moustique. Le coefficient a été comparé à 0 et seul les p-values des coefficients positifs ont été données.

Ces résultats sont présentés dans l'article: Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* Mosquito (Deletre *et al.*, 2013).

Les huiles essentielles ont montré des toxicités variées à la plus forte concentration (Figure 24). La mortalité a été dépendante du produit et de la concentration (GLM, p-value<0,001 pour les deux facteurs). L'effet toxique a logiquement augmenté avec la concentration estimation du modèle : 1,29). Seize huiles essentielles de plante n'ont montré aucun effet toxique : le romarin, l'eucalyptus, le pennyroyal, le poivre, l'aneth, le gingembre, le neem, le géranium, le citron, le solidage, le lemongrass, le litsea, l'aframomum, le coléus, le coriandre et le cumin. En effet, seulement quatre plantes se sont montrées toxiques à forte concentration : la cannelle, la citronnelle, la sarriette et le thym.

A.

Product	C1	C2	
permethrin	**93.8 (89.7 - 97.8)**	**96.8 (96.8 - 96.8)**	
DEET	2.6 (-3.4 - 8.6)	**98.2 (98.2 - 98.2)**	
thyme oil	5.3 (-2.5 - 13.2)	**79.5 (70.6 - 88.4)**	
thyme blend	7.5 (-0.2 - 15.2)	**83.5 (74.6 - 92.4)**	
thymol	1.2 (-5.1 - 7.5)	-1.0 (-7.0 – 5.0)	*
p-cymene	1.8 (-1.7 - 5.3)	1.4 (-1.4 - 4.2)	*
carvacrol	4.3 (-0.5 – 9.0)	0.0 (0.0 - 0.0)	*
α-terpinene	1.4 (-1.3 - 4.1)	4.5 (-0.5 - 9.6)	*
linalool	3.1 (-1.1 - 7.4)	3.2 (-1.2 - 7.5)	*
β-caryophyllene	0.1 (-3.2 - 3.4)	2.2 (-2.9 - 7.3)	*
citronella oil	5.6 (-1.0 - 12.3)	**75.1 (64.2 – 86.0)**	
citronella blend	-3.6 (-6.5 - -0.8)	-0.2 (-5.6 - 5.3)	*
citronellal	-3.2 (-3.2 - -3.2)	21.4 (10.6 - 32.2)	*
geraniol	-1.0 (-5.6 - 3.6)	-1.1 (-5.6 - 3.5)	*
citronellol	0.1 (-4.9 – 5.0)	6.3 (-1.2 - 13.7)	*
limonene	-1.8 (-4.7 - 1.2)	-1.7 (-4.8 - 1.3)	*
geranyl-acetate	3.4 (-1.2 - 8.1)	0.0 (0.0 - 0.0)	*
cumin oil	-2.9 (-2.9 - -2.9)	**34.4 (22.1 - 46.8)**	
cumin blend	-0.2 (-3.4 - 2.9)	9.4 (1.6 - 17.3)	*
cuminaldehyde	0.0 (0.0 - 0.0)	11.1 (3.4 - 18.9)	*
β-pinene	3.4 (-1.2 – 8.0)	3.9 (-1.4 - 9.2)	*
γ-terpinene	-1.6 (-1.6 - -1.6)	-1.6 (-1.6 - -1.6)	*
p-cymene	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	*
cinnamon oil	3.3 (-1.2 - 7.9)	**43.9 (32.0 - 55.9)**	
cinnamon blend	3.1 (-2.9 - 9.1)	**76.7 (66.2 - 87.3)**	*
cinnamaldehyde	-1.5 (-1.5 - -1.5)	**45.9 (32.9 - 58.8)**	*
2-methoxy-cinnamaldehyde	-1.1 (-6.9 - 4.7)	0.0 (-7.0 - 7.0)	*
cinnamyl-acetate	-3.6 (-3.6 - -3.6)	-3.6 (-3.6 - -3.6)	*

B.

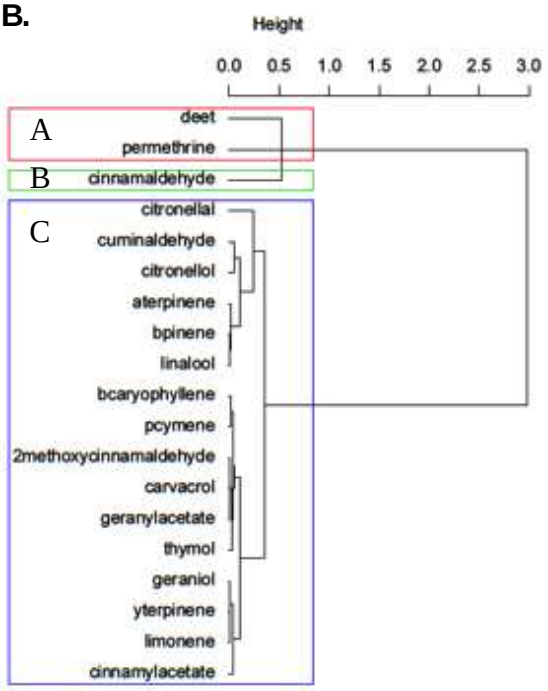


Figure 25: Réponse comportementale de femelles *Anopheles gambiae*[0] à l'effet toxique du DEET, de la Perméthrine, des composés majoritaires des huiles essentielles de cannelle, citronnelle et cumin et du mélange des composés majoritaires de chaque à 2 différentes concentrations.

A. Proportion corrigée par la formule de Sun-Sheppard de moustiques morts (intervalle de confiance calculé selon la méthode de Wald) par concentration

B. Dendrogramme établi par classification ascendante hiérarchique

Citronella blend: 34,74% citronellal, 22,50% geraniol, 12,03% citronellol, 3,51% geranyl acetate, 3,34% limonene. Cinnamon blend: 78,51% cinnamaldehyde, 9,65% 2-metoxycinnamaldehyde, 3,15% cinnamylacetate. Cumin blend: 30,1% cuminaldehyde, 12,2% β-pinene, 11,6% γ-terpinene, 9,7% *p*-cymene. Thyme blend: 30,5% thymol, 23,7% p-cymene, 13,6% carvacrol, 8,4% α-terpinene, 4,0% linalool, 3,5% b-caryophyllene

[0] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs en gras se sont montrées significativement différentes du témoin

Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Puis les composés majeurs et les 4 mélanges des composés majeurs des huiles essentielles de cannelle, de cumin, de citronnelle et de thym ont été testés.Toutes les huiles essentielles ont été toxiques à forte dose mais seuls les mélanges des composés majeurs des huiles essentielles de thym et de cannelle se sont montrés toxiques à faible dose (Figure 25). Et parmi les composés majoritaires, seul le cinnamaldéhyde s'est montré toxique. Pour conclure, la toxicité de l'huile essentielle de cannelle est du principalement à l'effet du cinnamaldéhyde. La toxicité de l'huile essentielle de thym serait du à l'effet synergique de certains de ses composés majoritaires. Enfin la toxicité des huiles essentielles de cumin et de citronnelle serait due à un effet synergique entre certains de ses composés majoritaires et minoritaires.

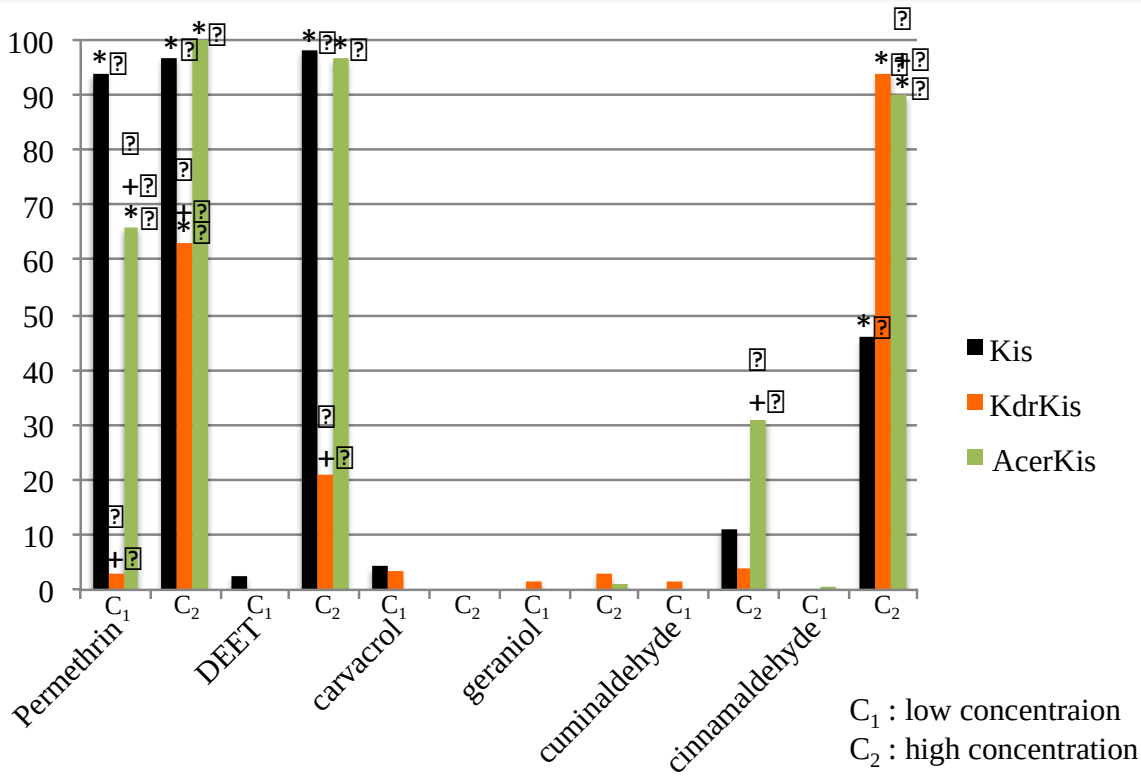


Figure 26: Réponse comportementale de femelles *Anopheles gambiae*[2] à l'effet toxique du DEET, de la Perméthrine, du carvacrol, du géraniol, du cuminaldéhyde et du cinnamaldéhyde à 2 différentes concentrations sur souche sensible Kis et sur 2 souches résistantes KdrKis et AcerKis.

[1] Proportion corrigée par la formule de Sun-Sheppard de moustiques morts (intervalle de confiance calculé selon la méthode de Wald) par concentration

[2] Moustiques âgés de 4 à 7 jours de souche KIS, non gorgés, nourris au sucre

* Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une * se sont montrées significativement différentes du témoin

+ Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une + se sont montrées significativement différentes des valeurs de la souche sensible.

Ces résultats sont présentés dans l'article: Behavior modulation inducing by kdr and ace1 genes on the repellent effect of DEET, Permethrin and natural compounds on the malaria vector Anopheles gambiae (Deletre *et al.*, en préparation).

Sur les 2 souches résistantes et la souche sensible, le carvacrol, le géraniol et le cuminaldéhyde n'ont pas montré d'effet toxique (Figure 26). Mais à forte dose, le cinnamaldéhyde a été toxique pour les 3 souches et a été deux fois plus toxique pour les souches résistantes. En ce qui concerne la perméthrine, elle a été toxique à faible dose sur Kis et AcerKis et à forte dose elle a été toxique pour les 3 souches mais de manière moins importante pour la souche KdrKis. Ce résultat concorde avec le fait que la souche KdrKis est résistante aux pyréthrinoïdes. Quant au DEET, il n'a pas montré de toxicité à faible dose pour les 3 souches. Par contre, il a été toxique pour les souches Kis et AcerKis à forte dose et très peu pour la souche kdrKis ce qui est plutôt surprenant.

5. Test d'irritation chez la mouche blanche

Pour tester l'effet répulsion-irritation sur *Bemisia tabaci*, le système utilisé était composé de deux tubes en plastique transparent séparés par un filet traité (Figure 27). Un cache a été utilisé pour couvrir le « tube sombre » et empêcher la lumière de traverser, l'autre tube étant appelé « tube clair ». Le système a été orienté horizontalement sous une source de lumière dans une chambre climatique. Cette orientation a profité de la tendance innée de l'aleurode à se déplacer vers la lumière en raison d'une phototaxie positive. Les aleurodes ont alors été introduits dans le tube sombre. Le nombre de mouches blanches dans chaque tube a été calculé après 4 h de test afin de déterminer le taux de passage à travers le filet ainsi que le taux de mortalité (Martin *et al*., 2014). Plus le produit testé est irritant, moins le taux de passage est important. Plus le produit testé est toxique, plus le taux de mortalité est important. De même que pour le moustique, l'hypothèse de Miller *et al.* (2009) que l'effet irritant serait une cause d'une dose subléthale a été testé en comparant la mortalité et l'irritabilité du produit.

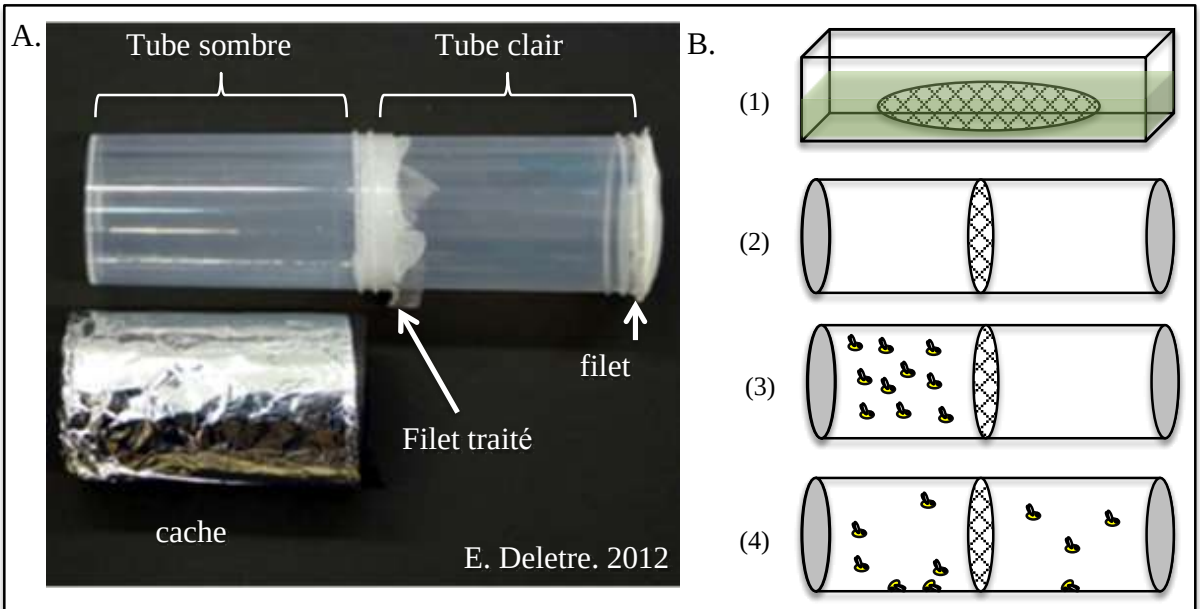


Figure 27: Test pour la répulsion-irritation pour *Bemisia tabaci*

A. Schéma du dispositif

B. Séquence de test, 1: le filet est traité avec le produit, 2: le tissu est placé entre deux cylindres, 3: les aleurodes sont introduits dans la partie du tube qui sera obscurcie avec un cache, 4: après 4 heure, le nombre d'aleurodes dans chaque tube sera comptabilisé ainsi que le nombre de morts.

Pour ce test, 3 concentrations ont été testé: 0,01%, 0,1% et 1%. Pour chaque concentration et le témoin (éthanol pur), 6 lots de 50 à 100 aleurodes ont été testé.

Les proportions du nombre d'aleurodes morts et échappés ont été comparé par paire avec le témoin avec le test de Fisher et corrigées avec la méthode de Bonferroni-Holm. Un modèle linéaire généralisé (distribution binomiale) a été étudié pour connaître l'effet du produit, de la concentration et de l'interaction concentration-produit (dose-dépendance) sur l'effet toxique sur le moustique. Enfin une classification ascendante hiérarchique (Ward) a été effectué pour classer les extraits de plantes.

Pour plus de détails, cf. Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Deletre *et al.*, soumis à Journal of Economic Entomology).

Douze des extraits de plantes ont réduit significativement, au moins à une concentration, la proportion de *B. tabaci* qui a traversé le filet traité: aframomum, cannelle, lemongrass, cumin, géranium, gingembre, citron, citronnelle, litsea, neem, solidage et sarriette (Tableau 5). Les deux produits de synthèse, le DEET et la perméthrine, ont également diminué de manière significative le taux de passage à travers le filet traité à partir de 0,01%. Après 4h de test, treize extraits de plantes ont montré un effet toxique significatif au moins à une concentration: aframomum, cannelle, citronnelle, cumin, aneth, géranium, citron, citronnelle, litsea, neem, romarin, sarriette et thym. Les deux produits de synthèse ont également eu un effet toxique. Un effet dose-dépendant a été observé sur les taux de mortalité. Les deux produits de synthèse ont également eu un effet toxique sur les aleurodes qui ont traversé le filet. Après 4 h de test, tous les aleurodes en présence d'un filet traité avec l'huile essentielle de cannelle à une concentration de 1% ont été tués avant même de traverser le filet. Les huiles essentielles d'aframomum, de cannelle, de géranium, et de sarriette ont été les plus toxiques.

Tableau 5: Réponse comportementale de *Bemisia tabaci* à l'effet répulsif-irritant du DEET, de la Perméthrine et des 20 extraits de plantes à 3 différentes concentration (0,01; 0,1 et 1% de produit dans la solution de traitement des tissus).

Nom commun	Nom latin	Taux de passage	Taux de mortalité
DEET	composé synthétique	+ + +	+ +
Permethrine	composé synthétique	+ + +	+ +
Aframomum	*Aframomum pruinosum*	+ +	+ + +
Aneth	*Anethum graveolens*	+	+
Cannellle	*Cinnamomum zeylanicum*	+	+
Citron	*Citrus limon*	**0**	**0**
Citronnelle	*Cymbopogon winterianus*	+	**0**
Coleus	*Plectranthus tenuicaulis*	+ + +	+
Coriandre	*Coriandrum sativum*	**0**	+ +
Cumin	*Cuminum cyminum*	**0**	**0**
Eucalyptus	*Eucalyptus globulus*	+	+
Geranium	*Pelargonium graveolens*	+	**0**
Gingembre	*Zingiber officinalis*	+ + +	+ + +
Lemongrass	*Cymbopogon citratus*	+ +	+ + +
Litsea	*Litsea cubeba*	+ + +	+ + +
Pennyroyal	*Mentha pulegium*	+	+
Neem	*Melia azadirachta*	**0**	**0**
Poivre	*Piper nigrum*	**0**	**0**
Romarin	*Rosmarinus officinalis*	**0**	+
Sariette	*Satureja montana*	+ + +	+ + +
Solidage	*Solidago canadensis*	+ + +	**0**
Thym	*Thymus vulgaris L.*	**0**	+

[1] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm.
+ : une concentration a eu un effet irritant, 0 : aucune concentration n'a eu d'effet irritant.
[2] Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm.
+ : une concentration a eu un effet toxique, 0 : aucune concentration n'a eu d'effet toxique.

De même que pour le moustique, les composés majoritaires des huiles essentielle de cannelle, de cumin, de lemongrass et de citronnelle présentés dans le tableau 2 ainsi que les mélanges des composés majoritaires correspondants ont été testés afin d'identifier quels composés étaient actifs dans le mélange pour mieux comprendre le mode d'action des huiles essentielles,

Le taux de passage à travers le filet a été significativement réduit pour les quatre mélanges de composés majeurs (Figure 28). Par contre les composés majoritaires du cumin ou de la citronnelle testés un à un n'ont pas été aussi efficaces que le mélange correspondant. Il devrait donc y avoir un effet additif ou synergique entre les produits. Par contre, l'effet des

mélanges de cannelle et de lemongrass semble du à un seul de leur composé majoritaire, le cinnamaldéhyde et le citral, respectivement. L'efficacité du filet imprégné (i.e. réduction du taux de passage) de l'extrait cannelle s'explique par le cinnamaldehyde, même si certains aleurodes ont réussi à traverser le filet traité, l'ensemble des aleurodes sont morts suite à l'exposition au cinnamaldéhyde. Avec cet essai, le taux de passage et la mortalité semblent très liés: à faible dose, le taux de passage est important et la mortalité est faible ; à forte dose, le taux de passage est faible mais la mortalité importante. Un autre bioessai a donc été réalisé pour mettre en évidence l'effet répulsif-irritant des composés.

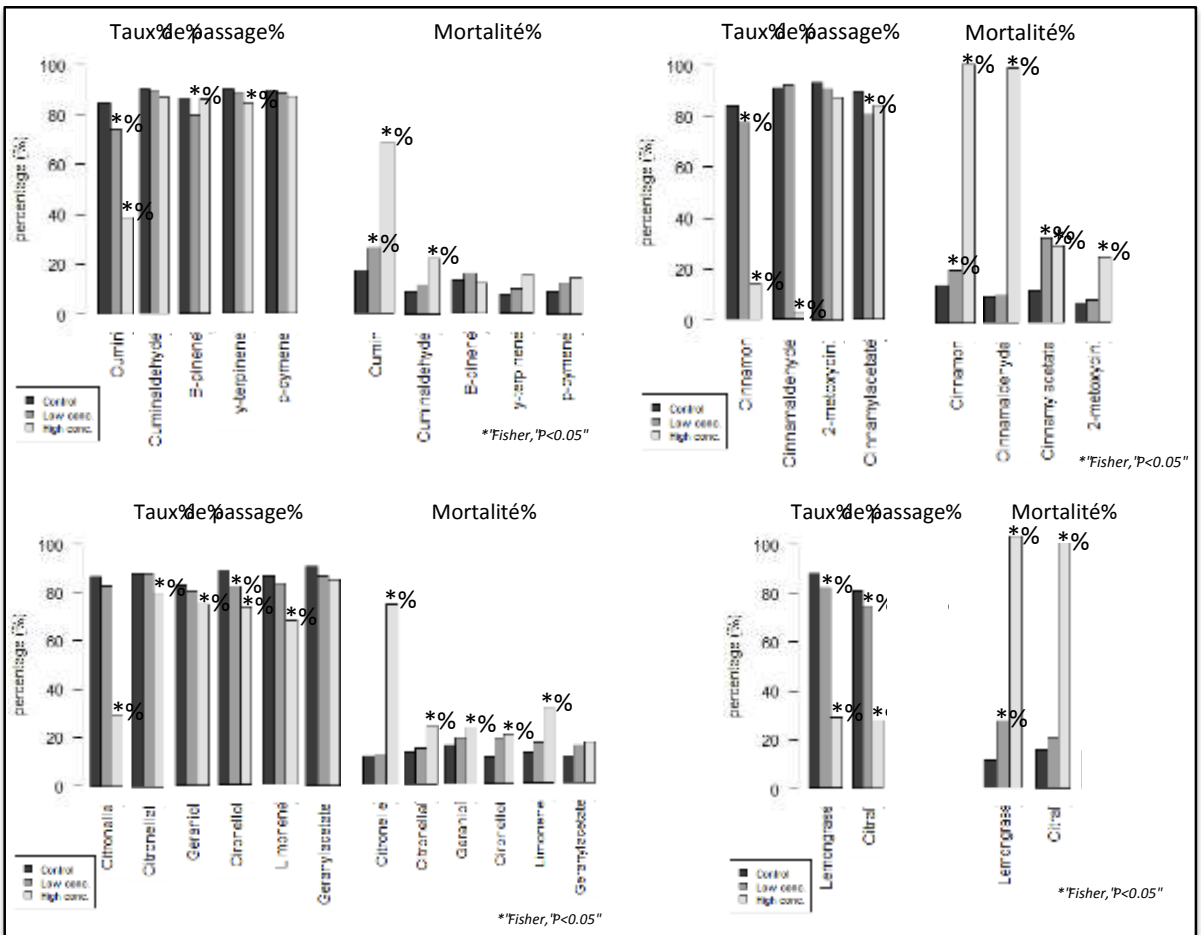


Figure 28: : Réponse comportementale de *Bemisia tabaci* à l'effet répulsif-irritant des composés majoritaires des huiles essentielles de cumin, de cannelle, de citronnelle et de lemongrass et de leur mélange associé à 2 différentes concentrations.

* Comparaisons par paire des proportions avec le test de Fisher corrigées avec la méthode de Bonferroni-Holm. Les valeurs suivies d'une * se sont montrées significativement différentes du témoin

'cumin': 30,1% cuminaldehyde, 12,2% β-pinene, 11,6% γ-terpinene, 9,7% *p*-cymene. 'cinnamon': 78,51% cinnamaldehyde, 9,65% 2-metoxycinnamaldehyde, 3,15% cinnamylacetate. 'citronnelle: 34,74% citronellal, 22,50% geraniol, 12,03% citronellol, 3,51% geranyl acetate, 3,34% limonene. 'lemongrass': 74,1% citral, 4,5% geraniol, 3,9% geranyl acetate.

Ces résultats sont présentés dans l'article: Natural occurring bioactive compounds from four repellent essential oils against *Bemisia tabaci* whiteflies (Deletre *et al.*, soumis au Journal of Chemical Ecology).

Pour ne tester que l'effet répulsif des produits, il fallait un test qui force l'insecte à être au contact du composé testé afin d'observer ou non un comportement de fuite de l'insecte après contact. Pour ce test, un papier noir a été traité avec du citronellal, de l'acétate de géranyle, ou de 2-méthoxycinnamaldéhyde à 1 %; de l'aldéhyde cinnamique ou de l'acétate de cinnamyle à 0,5% ; ainsi qu'avec les témoins positifs: DEET ou perméthrine à 1% ou l'éthanol (solvant) comme témoin négatif (Figure 29). Le dispositif était composé d'un carré de 16 cm^2 dont une moitié était traitée avec la solution (zone traitée) et l'autre avec le solvant (zone témoin). Cette zone était délimitée par un carton d'une épaisseur de 2 mm et d'un couvercle en plexiglas pour empêcher les aleurodes de s'échapper lors de l'expérience et de les forcer à marcher sur le papier au lieu de voler. Les aleurodes ont été placés un par un au centre de l'arène. Leur activité a été observé pendant 10 minutes: le temps passé à se déplacer, la vitesse moyenne lors du déplacement, la distance parcourue et le temps passé sur chaque zone.

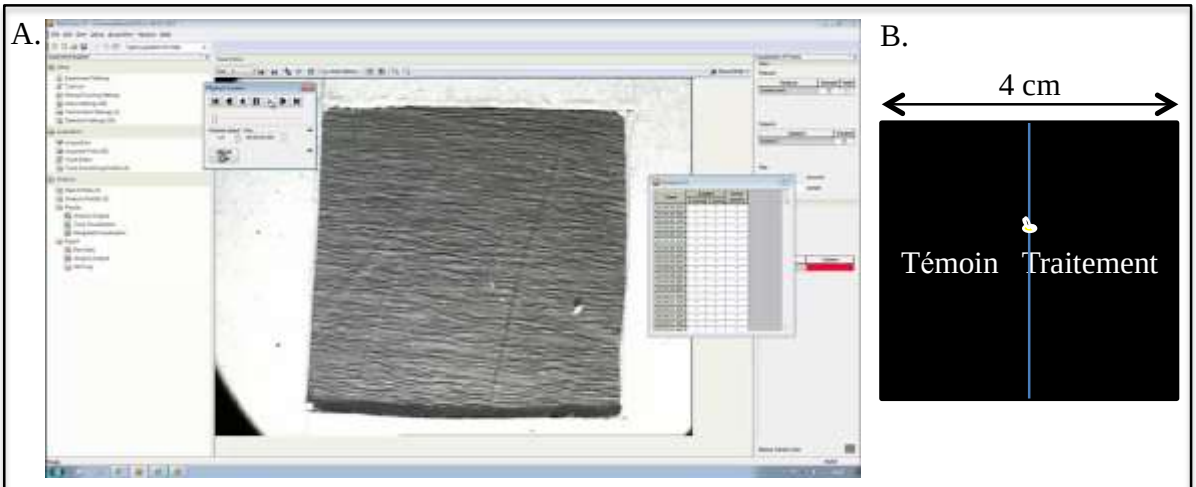


Figure 29: Test pour la répulsion-irritation pour *Bemisia tabaci*

A. Schéma du dispositif
B. La moitié d'un carré de papier filtre noir est traité avec le produit testé et l'activité de l'aleurode (distance parcourue, vitesse, temps en mouvement, temps passé sur chaque zone) sur ce papier est suivi par video-tracking pendant 10 min. Une plaque de plexiglas placé au-dessus de l'insecte l'a force à marcher sur le papier

30 individus ont été testés par produit. L'activité de l'insecte sur la partie traitée et non traitée a été comparé avec un test de student apparié ou de wilcoxon lorsque la distribution n'était pas normal.

Pour plus de détails, cf. Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Deletre *et al.*, soumis à Journal of Economic Entomology).

Dans ce test quelque soit le composé testé, le temps passé sur la zone traitée et celui passé sur la zone témoin n'ont pas été significativement différents (Figure 30). De même, l'activité des aleurodes n'a pas été différente entre les zones traitées et témoins, excepté pour l'acétate de cinnamyle où les aleurodes ont été significativement moins mobiles sur la surface traitée que sur la surface témoin et contrairement au DEET où ils ont été plus mobiles. Cet essai ne permet donc pas d'affirmer que les aleurodes *Bemisia tabaci* ne franchissent pas le filet parce qu'il est irritant.

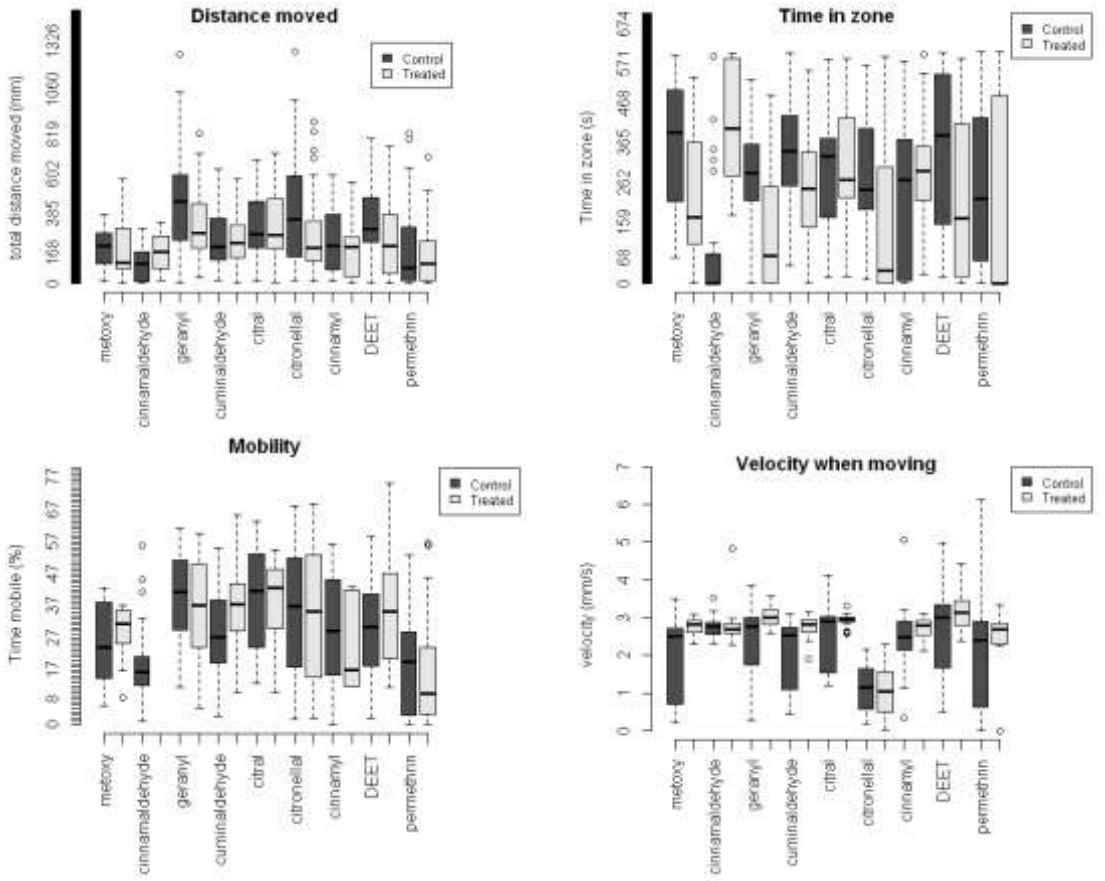


Figure 30: Activité de *Bemisia tabaci* suivi par video-tracking sur un carré de papier filtre dont la moitié a été traité.

Ces résultats sont présentés dans l'article: Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts (Deletre *et al.,* soumis à Journal of Economic Entomology).

4. Discussion

Le DEET et la perméthrine ont montré tous deux un effet irritant et toxique sur *Anopheles gambiae* et *Bemisia tabaci.* Syed et Leal (2008) ont montré que les moustiques évitaient d'atterrir sur un papier traité avec du DEET. Le DEET a été montré qu'il était un inhibiteur de l'acétylcholinestérase ce qui pourrait expliquer son effet toxique (Corbel *et al.*, 2009) et qu'il était toxique pour d'autres espèces de moustiques (Licciardi *et al*, 2006). D'autres études ont montré également un effet irritant de la perméthrine sur d'autres espèces de moustiques, même si le mode d'action exacte n'a pas été identifié (Achee *et al.*, 2009 ; Dusfour *et al.*, 2009).

De nouveau, le romarin (hydrolat biologique) n'a pas été montré d'effet sans doute parce que ses composés bio-actifs étaient en quantité trop faible. En effet, l'irritation augmente avec la concentration quelque soit l'extrait testé.

Les huiles essentielles les plus irritantes contre *Anopheles gambiae* ont été le cumin, le lemongrass, le coléus et le thym mais également la cannelle et la citronnelle. Les effets irritants de l'huile essentielle de thym, de citronnelle, de cumin et de cannelles sont dus au thymol et/ou carvacrol ; citronellal, géraniol, et/ou citronellol; au cuminaldéhyde ; et au cinnamaldéhyde respectivement. Ces composés ont été aussi irritants que leurs huiles essentielles respectives. Mais pour l'huile essentielle de cumin, il est possible qu'il y ait un

effet antagoniste entre le cuminaldéhyde et d'autres composés minoritaires. En effet, le mélange des composés majoritaires de l'huile essentielle de cumin a été moins irritant que l'huile essentielle contrairement aux autres mélanges.

Avec le premier bioessais sur *Bemisia tabaci*, il n'est pas possible de conclure à un effet irritant des huiles essentielles et de leurs composés. La réduction du taux de passage à travers le filet peut être due à l'effet toxique des produits et/ou l'effet irritant. Lorsque le produit a été testé à des doses sublétales, le traitement du filet semblait bien moins efficace. De plus, le deuxième bioessai qui aurait pu permettre de conclure à un effet irritant, n'a montré aucun effet irritant pour les composés testés.

Contrairement à l'hypothèse avancée par Miller *et al.* (2009), l'effet irritant ne serait donc pas la cause d'une dose subléthale. Les effets irritants et toxiques pourraient faire appel à des mécanismes d'action différents. En effet, certains produits irritants comme le géraniol, le cuminaldéhyde n'ont pas été toxiques. La toxicité semble avoir un mécanisme d'action plus complexe que l'irritation ce qui correspond à notre hypothèse, un récepteur spécifique et un neurone spécifique. En effet, mis à part le cinnamaldéhyde, aucun produit testé seul ne s'est montré toxique sur le moustique mais des synergies entre les produits seraient possibles.

De plus, certains produits comme le thymol, le DEET ou la perméthrine ont été irritants mais pas répulsifs. Cela conforte donc l'hypothèse que les mécanismes d'action de l'expulsion et de l'irritation sont différents : l'expulsion devrait être liée au système olfactif et l'irritation au système gustatif. Mais certains produits comme le carvacrol, le citronellal ayant été répulsifs se sont également montrés irritants. Dans le test de l'irritation, les deux effets peuvent être confondus c'est à dire que les moustiques pourraient s'échapper parce qu'ils sentent le carvacrol et non parce qu'ils le touchent. Il faudrait donc refaire un test en désactivant l'odorat des moustiques en utilisant des mutants ou en excisant les antennes pour être sûr que la fuite des moustiques ne soit pas due à la perception du carvacrol par leur odorat.

Le mécanisme d'action le plus probable d'un composé répulsif 'irritant' serait que les insectes aient des récepteurs gustatifs spécifiques qui coderaient pour un comportement de fuite, ce qui favoriserait une réponse rapide de l'insecte (Figure 31). Les insectes auraient des récepteurs gustatifs et des neurones gustatifs dédiés à la détection des produits répulsifs dits irritants. De Gennaro *et al.* (2013) ont travaillé sur des moustiques femelles mutants Orco. Bien que toujours attirés par l'odeur de l'homme, ils ne peuvent détecter le DEET par l'olfaction et pourtant ces moustiques sont repoussés au seul contact du DEET. Cela signifie que les effets répulsifs à distance et au contact s'expliqueraient par des mécanismes biologiques distincts. Ainsi les neurones activés par des répulsifs seraient différents des neurones activés par des attractifs. Certains neurones seraient activés par des composés attractifs et enverraient un signal d'attraction au cerveau de l'insecte qui coderait alors pour rester sur le composé alors que le composé expulsif enverrait un signal de répulsion au cerveau de l'insecte qui coderait alors pour un envol au contact du composé.

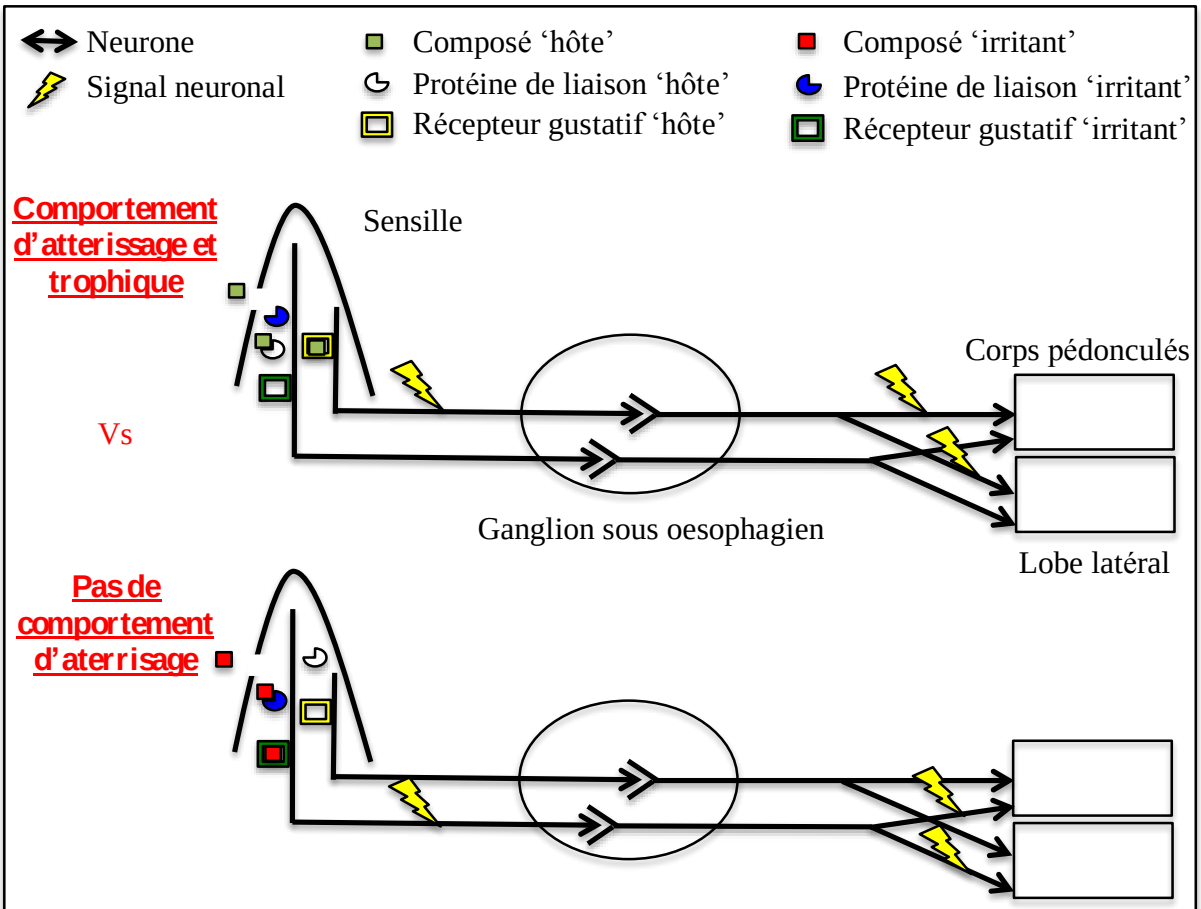


Figure 31: Mécanisme physiologique hypothétique des répulsifs 'irritant'
Les composés irritants causent un mouvement de fuite quasiment instantannée de l'insecte après un contact physique avec le composé. Les insectes doivent donc avoir des récepteurs gustatifs spécifiques sur les tarses qui codent pour un mouvement de fuite. Les substances attractives et irritantes activeraient des récepteurs et des neurones différents, les messages informatifs envoyés au cerveau de l'insecte seraient différents et coderaient pour un comportement différent.
Ce schéma a été adapté de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

En ce qui concerne la toxicité, deux mécanismes d'action ont été étudiés pour l'expliquer : (1) inhibition de l'acétylcholinestérase et des interférences avec le neuromodulateur octopamine et (2) l'action sur les canaux GABA (Isman, 2000; Isman, 2006; Regnault-Roger *et al.*, 2012). De plus, les terpènes favoriseraient la pénétration des molécules à travers la cuticule des insectes ce qui augmenterait leur biodisponibilité (Moretti *et al.*, 2013). Cette propriété est intéressante puisqu'elle permettrait de faire passer des molécules toxiques à travers la cuticule même si la surface est traitée avec un produit irritant qui ne permet pas à l'insecte de se poser longtemps sur la surface. Cette propriété pourrait également expliquer le fait que la toxicité soit principalement due à un synergisme entre molécule plutôt qu'à une seule molécule : en effet, une molécule pourrait favoriser le passage de la molécule toxique à travers la cuticule et causer un effet toxique, testées seules ces molécules ne présenteraient alors aucun effet toxique. Certains composés ont agi différemment sur les souches sensibles et résistantes ce qui suggère que l'acétylcholinestérase et les canaux GABA pourraient être des cibles potentielles de ces composés. Les mutations de ces cibles pourraient modifier l'activité de ces composés c'est à-dire modifier la sensibilité du système nerveux des

insectes résistants par rapport à celles sensibles. D'autres études sur l'affinité de ces composés avec les différents récepteurs du système nerveux pourraient permettre de découvrir de nouvelles cibles du composé répulsif et de faciliter la découverte de nouveaux composés. En outre, le mode d'action de ces composés mériterait d'être étudié afin de déterminer dans un premier temps comment ils ont pénétré dans l'insecte à savoir par ingestion, absorption respiratoire ou passage à travers la cuticule. Par exemple, la toxicité peut être due à des vapeurs toxiques et / ou application topique de produits (Regnault-Roger & Hamraoui, 1997). Certains composés comme le thymol et le linalol sont connus pour être des inhibiteurs de l'acétylcholinestérase in vitro, mais nous n'avons identifié aucun effet irritant ou toxique particulier sur *Anopheles gambiae* aux doses testées (Myazawa et al, 1997; Ryan & Byrne, 1988). Certains terpènes issus des huiles essentielles sont des inhibiteurs compétitifs de l'acétylcholinestérase in vitro (Isman 2000), mais qui ne peuvent pas être corrélés avec la toxicité comme le suggère le cas du carvacrol (Isman, 2000). Pourtant l'effet irritant du carvacrol a été plus faible sur la souche AcerKis et le carvacrol est un inhibiteur de l'acétylcholinestérase, la mutation chez la souche résistante diminuerait peut être l'effet inhibiteur du carvacrol.

L'intérêt majeur d'un produit irritant est d'empêcher les insectes de rester sur une surface traitée ce qui réduit la prise alimentaire et le risque de transmission de pathogènes. Chez *Anopheles gambiae*, la population résistante KdrKis est moins irritée par les pyréthrinoïdes que la population sensible Kis (Chandre *et al.*, 2000). Cela suggère qu'une cible possible pour l'effet irritant serait les canaux sodium et l'effet dépendrait de la dose et du temps d'exposition. Le géraniol a montré un effet irritant plus important sur la souche KdrKis, la mutation du canal sodium pourrait ainsi accentuer l'effet irritant du géraniol en modifiant son interaction. L'effet irritant du carvacrol a été plus faible sur la souche KdrKis, l'effet irritant est donc peut être lié à son action sur les canaux sodium, il pourrait avoir une action similaire aux pyréthrinoïdes.

Efficacité de la barrière physico-chimique

‘*Ne jugez pas le grain de poivre d’après sa petite taille, goûtez-le et vous sentirez comme il pique*’ Proverbe arabe.

1. Introduction

Il y a plusieurs avantages à vouloir utiliser des répulsifs : un impact sur de nombreux comportements avant, pendant et après la recherche de l'hôte, un usage possible en extérieur et en intérieur, un risque de résistance limité, un usage possible contre un nombre important d'espèces (Achee *et al.,* 2012). Ces avantages permettent donc une application aussi bien en agriculture qu'en santé publique. L'utilisation de répulsifs limiterait la transmission d'agents pathogènes par des insectes vecteurs en diminuant la probabilité de rencontre entre un vecteur et son hôte. Pour être nommé répulsif, le produit doit modifier le comportement de l'insecte vis à vis de l'interaction avec son hôte, il doit être non toxique pour l'homme et l'environnement et il ne doit pas être toxique pour l'insecte aux doses utilisées (Achee *et al.,* 2012).

Une des utilisations pour les répulsifs pour lutter contre le moustique et le paludisme est de combiner un répulsif avec une moustiquaire. Cette même stratégie pourrait être utilisée pour lutter contre les mouches blanches et la transmission des begommovirus. De plus, la combinaison de répulsifs à modes d'action différents et de type de répulsion différents pourrait être intéressante.

Nous avons préalablement sélectionné quatre répulsifs prometteurs à combiner avec une moustiquaire : le carvacrol, le géraniol, le cinnamaldéhyde, et le cuminaldéhyde. En effet, ces produits se sont montrés expulsifs, irritants et/ou toxiques sur le moustique. Pour la mouche blanche, deux extraits de plantes se sont montrés très intéressants : la cannelle et le lemongrass. L'objectif de cette partie est donc d'étudier l'efficacité de la barrière physico-chimique dans la lutte contre les insectes vecteurs d'agents pathogènes.

2. Cas du moustique

Aujourd'hui, les pyréthrinoïdes sont une des rares armes chimique recommandées pour lutter contre *Anopheles gambiae*. Les molécules de cette famille chimique présentent 4 actions sur le moustique : ils sont répulsif-expulsifs, i.e. ils réduisent l'entrée des moustiques dans une pièce contenant une moustiquaire imprégnée, ils sont irritants, i.e. ils empêchent les moustiques de rester sur une moustiquaire ou un mur traité, ils sont anti-appétants, i.e. ils inhibent la prise de repas de sang et ils sont toxiques, i.e. ils présentent un effet 'knock-down' et toxique pour les moustiques. Mais comme indiqué précédemment, il existe des populations de moustiques résistantes aux pyréthrinoïdes. De nouveaux composés répulsifs et irritants pourraient donc être utilisés pour imprégner les moustiquaires comme alternatives aux pyréthrinoïdes.

Pour tester l'efficacité des moustiquaires imprégnées, l'OMS recommande les tests en tunnel. Ce test dure 8h, il faut donc dans un premier temps vérifier que l'action des composés peut durer 8h. L'efficacité des composés a donc été vérifié en remplaçant le papier filtre des tests de répulsion et d'irritation par un filet imprégné. Il a été testé à 0, 3, 6 et 9h (Tableau 8).

L'effet irritant du géraniol perdure jusqu'à 9h mais les effets toxique et 'knock-down' n'ont été observés que jusqu'à 3h après l'imprégnation du filet. Les effets irritant, toxique et 'knock-down' du filet imprégné de cinnamaldéhyde ont perduré jusqu'à 9h après le traitement. L'effet irritant du carvacrol a perduré jusqu'à 6h contrairement à ses effets toxiques et 'knock-down' qui ont duré jusqu'à 9h. L'effet irritant du cuminaldéhyde a été observé jusqu'à 6h mais il a diminué au cours du temps et ses effets toxiques et 'knock-down'

ont uniquement été observés après le traitement. Pour le cinnamaldéhyde et le carvacrol, la majorité des moustiques (knocked down) était dans le tube non traité (cinnamaldehyde à 0 h 11.3% *vs* 0.0%; carvacrol à 3 h 14.9% *vs* 3.0%) alors que la majorité des moustiques morts était dans le tube traité (cinnamaldehyde à 0 h 64.5% *vs* 17.7%; carvacrol à 3 h 50.7% *vs* 13.4%). Mise à part le cuminaldéhyde, les autres produits ont montré un effet toxique ou irritant jusqu'à 9h même si les effets ont diminué dans le temps. Les 4 produits ont ensuite été évalués en test en tunnel, malgré leur légère perte d'efficacité dans le temps

Tableau 8: Proportion d'*Anopheles gambiae* irrités, 'knocked-down' et morts par un filet traité avec du géraniol (0,023 µL/cm ^{2}), du cinnamaldéhyde (0,079 µL/cm ^{2}), du carvacrol (0,014 µL/cm ^{2}) et du cuminaldéhyde (0,030 µL/cm ^{2}) 0, 3, 6 et 9h après son imprégnation. Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à Journal of Chemical Ecology).

traitement	temps (h)	n	irrité	'knocked-down'	mort
témoin	0	66	6.1 (0.3 - 11.9)[2]	0.0 (0.0 - 0.0)	1.5 (-1.4 - 4.4)
géraniol	0	61	**45.9 (33.4 - 58.4)**[3]	**11.5 (3.5 - 19.5)**	**16.4 (7.1 - 25.7)**
géraniol	3	65	**38.5 (26.7 - 50.3)**	**3.1 (-1.1 - 7.3)**	**12.3 (4.3 - 20.3)**
géraniol	6	66	**34.8 (23.3 - 46.3)**	3.0 (-1.1 - 7.1)	0.0 (0.0 - 0.0)
géraniol	9	60	**36.7 (24.5 - 48.9)**	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
témoin	9	65	7.7 (1.2 - 14.2)	0.0 (0.0 - 0.0)	1.5 (-1.5 - 4.5)
p-value (estimation du modèle)[4]			*0.259*	*<0.001 (-0.4)*	*<0.001 (-0.6)*
témoin	0	62	9.7 (2.3 - 17.1)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
Cinnamaldéhyde	0	62	**35.5 (23.6 - 47.4)**	**11.3 (3.4 - 19.2)**	**82.3 (72.8 - 91.8)**
cinnamaldéhyde	3	66	**40.9 (29.0 - 52.8)**	**6.1 (0.3 - 11.9)**	**68.2 (57.0 - 79.4)**
cinnamaldéhyde	6	61	**54.1 (41.6 - 66.6)**	**19.7 (9.7 - 29.7)**	**60.7 (48.4 - 73.0)**
cinnamaldéhyde	9	56	**62.5 (49.8 - 75.2)**	**16.1 (6.5 - 25.7)**	**57.1 (44.1 - 70.1)**
témoin	9	62	9.7 (2.3 - 17.1)	0.0 (0.0 - 0.0)	3.2 (-1.2 - 7.6)
p-value (estimation du modèle)			*0.001 (0.1)*	*0.006 (-0.1)*	*0.003 (-0.1)*
témoin	0	63	6.3 (0.3 - 12.3)	0.0 (0.0 - 0.0)	1.6 (-1.5 - 4.7)
carvacrol	0	61	14.8 (5.9 - 23.7)	**9.8 (2.3 - 17.3)**	**86.9 (78.4 - 95.4)**
carvacrol	3	67	**43.3 (31.4 - 55.2)**	**17.9 (8.7 - 27.1)**	**64.2 (52.7 - 75.7)**
carvacrol	6	65	**46.2 (34.1 - 58.3)**	**40.0 (28.1 - 51.9)**	**43.1 (31.1 - 55.1)**
carvacrol	9	54	20.4 (9.7 - 31.1)	**22.2 (11.1 - 33.3)**	**48.1 (34.8 - 61.4)**
témoin	9	71	18.3 (9.3 - 27.3)	0.0 (0.0 - 0.0)	2.8 (-1.0 - 6.6)
p-value (estimation du modèle)			*0.368*	*<0.001 (-0.2)*	*<0.001 (-0.2)*
témoin	0	65	7.7 (1.2 - 14.2)	0.0 (0.0 - 0.0)	1.5 (-1.5 - 4.5)
cuminaldéhyde	0	67	**52.2 (40.2 - 64.2)**	**22.4 (12.4 - 32.4)**	**38.8 (27.1 - 50.5)**
cuminaldéhyde	3	59	**61.0 (48.6 - 73.4)**	0.0 (0.0 - 0.0)	5.1 (-0.5 - 10.7)
cuminaldéhyde	6	71	**42.3 (30.8 - 53.8)**	0.0 (0.0 - 0.0)	**12.7 (5.0 - 20.4)**
cuminaldéhyde	9	63	25.4 (14.7 - 36.1)	0.0 (0.0 - 0.0)	1.6 (-1.5 - 4.7)
témoin	9	64	10.9 (3.3 - 18.5)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
p-value (estimation du modèle)			*<0.001 (-0.1)*	*1.00*	*<0.001 (-0.2)*

[1] âgées de 4 à 7jours, nourries au sucre et sans repas de sang, souche Kisumu
[2] Intervalle de confiance calculé avec la méthode de Wald

[3] Comparaison par paire des proportions entre le traitement et les témoins à 0 et 9h avec un test de Fisher. Les valeurs en gras se sont montrées significativement différentes des témoins et une correction de Bonferroni-Holm a été effectuée.
[4] P-value et estimation d'un modèle linéaire généralisé du temps sur la proportion de moustiques repoussés, 'knocked-down' et mort.

Le test en tunnel consiste à mettre un cobaye derrière une moustiquaire traitée et trouée et observer après 8h le nombre de moustiques qui ont traversé le filet, le nombre de morts et de gorgés de chaque côté du filet (Figure 32). Une ventilation est placée derrière la cage afin d'envoyer l'odeur du cobaye vers les moustiques, dans notre cas d'étude cela envoie également les volatils testés.

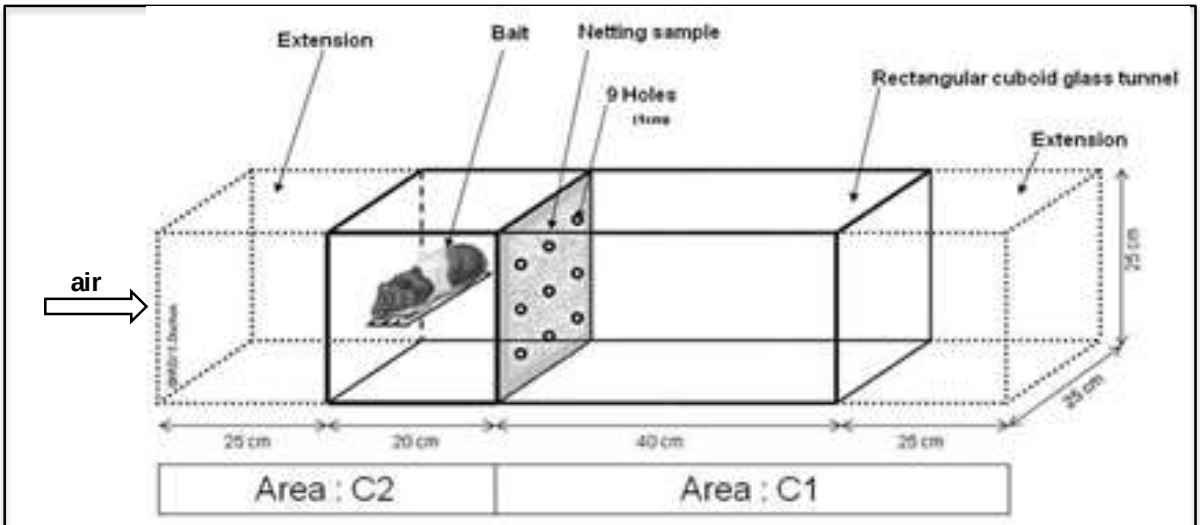


Figure 32: Tunnel utilisé pour étudier l'efficacité de moustiquaires imprégnées.
Ce tunnel respecte les consignes de l'OMS pour tester l'efficacité de moustiquaires imprégnées. Chaque produits ou mélange a été testé deux fois avec 100 moustiques femelles à chaque répétition. Les taux de passage, de gorgement et de mortalité ont été comparés avec un test de Fisher corrigé par la méthode de Bonferroni-Holm.
Pour plus de détails, cf. Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à journal of chemical ecology).

Lorsque la moustiquaire a été traitée avec de la perméthrine, le taux de passage et le taux de gorgement ont été faibles et la mortalité importante : cela caractérise un traitement efficace pour une moustiquaire (Tableau 9). A faible dose, seul le cuminaldéhyde a montré les mêmes effets. A faible dose, le cinnamaldéhyde et le carvacrol ont réduit le taux de passage mais le taux de mortalité est resté faible et le taux de gorgement important. Le géraniol n'a montré aucun effet. Aux vues de ces résultats, des tests ont été effectués à forte dose. Les résultats obtenus n'ont pas montré de différence avec les premiers.

Les huiles essentielles complètes et le mélange des quatre composés ont aussi été testés pour voir si une meilleure efficacité pouvait être obtenue. A part l'huile essentielle de thym, les autres huiles ont diminué le taux de gorgement et augmenté le taux de mortalité. Quant au mélange des 4 produits, il a seulement réduit le taux de gorgement. Le linalool a également été testé car il est détecté par les palpes du moustique mais il n'a présenté aucun effet. Pour conclure, aucun produit aux concentrations testées n'a montré une efficacité semblable à la perméthrine.

Tableau 9: Effet d'une moustiquaire imprégnée de perméthrine, géraniol, cuminaldéhyde, carvacrol, cinnamaldéhyde, de leurs mélanges, des huiles essentielles de citronnelle, cannelle, thym ou cumin ou de linalool sur le taux de passage, de gorgement et mortalité d'*Anopheles gambiae* dans un test en tunnel. Ces résultats sont présentés dans l'article: Electrophysiological and behavioral characterization of bioactive compounds of four essential oils against *Anopheles gambiae* and prospects for their use as bed net treatments (Deletre *et al.*, soumis à Journal of Chemical Ecology).

	produit	dose (µl/cm^2)	N^2	Taux de passage (%)	Taux de gorgement (%)	Taux de mortalité (%)
1	témoin	0	285	86.0	60.7	5.6
	perméthrine	0.1	362	59.1*	11.3*	64.6*
	géraniol	0.03	300	95.0*	72.0	10.0
	cinnamaldéhyde	0.08	274	80.3	46.0*	22.3*
2	témoin	0	283	86.9	68.9	10.2
	carvacrol	0.03	219	82.2	52.5*	31.1*
	cuminaldéhyde	0.05	263	57.0*	33.8*	44.9*
3	témoin	0	260	96.2	87.7	5.8
	perméthrine	0.1	263	51.0*	8.4*	64.6*
	cuminaldéhyde	0.1	259	96.5	76.4	22.0*
	cinnamaldéhyde	0.1	356	94.7	87.9	6.7
4	témoin	0	267	98.1	86.5	8.6
	géraniol	0.1	257	94.6	78.6	11.3
	carvacrol	0.1	267	91.4*	83.9	10.5
5	témoin	0	231	98.7	80.5	6.9
	Mélange[3]	0.1	235	81.3	60.4*	6.0
	HE thym	0.1	225	93.8	73.3	15.6
	HE cannelle	0.1	266	94.7	65.4*	25.9*
6	témoin	0	266	95.5	85	5.6
	HE cumin	0.1	240	93.8	74.6*	6.7
	HE citronnelle	0.1	224	95.1	62.5*	3.6
	linalol	0.1	272	93.8	77.6	4.0

[1] âgés de 7 à 9 jours, non gorgées, nourries de sucre, souche Kisumu
[2] nombre de femelles *An. gambiae* testées
[3] mélange de carvacrol, géraniol, cinnamaldéhyde, cuminaldéhyde (1:1:1:1)
* Différence significative entre le témoin et le traitement ($p<0.05$, test de Fisher corrigé par la méthode de Bonferroni-Holm.

3. Cas de la mouche blanche

Lors d'essais précédents, l'efficacité de matériaux imprégnés pour contrôler des populations de ravageurs a déjà été démontrée, par exemple sur des acariens ou des pucerons (Deletre *et al.*, 2014 ; Martin *et al.*, 2014). De plus, dans un étude précédente (Martin *et al.*, 2014), nous avons montré que les plants de tomates protégés par des filets blancs étaient moins infestés par les aleurodes comparés aux plants non protégés par un filet. De plus, les filets imprégnés d'alphacyperméthrine ont montré une meilleure efficacité à contrôler les mouches blanches. Mais comme expliqué précédemment, il existe d'ores et déjà des populations d'aleurodes résistantes aux pyréthrinoïdes. L'utilisation de produits répulsifs combinés à un filet anti-insecte pourrait donc être efficace contre les aleurodes et être une alternative à l'utilisation des pesticides et en particulier des pyréthrinoïdes.

Un essai préliminaire a été mis en place en 2013 en faisant une inter-culture lemongrass-tomate et en combinant la culture de tomate et des diffuseurs d'huile essentielle de cannelle *Zeylanicum cassia* riche en cinnamaldéhyde (Figure 33). Ces deux stratégies ont été également combinées avec l'utilisation d'un filet pour savoir s'il y avait un synergisme entre les deux moyens de lutte : une barrière physique, une barrière olfactive.

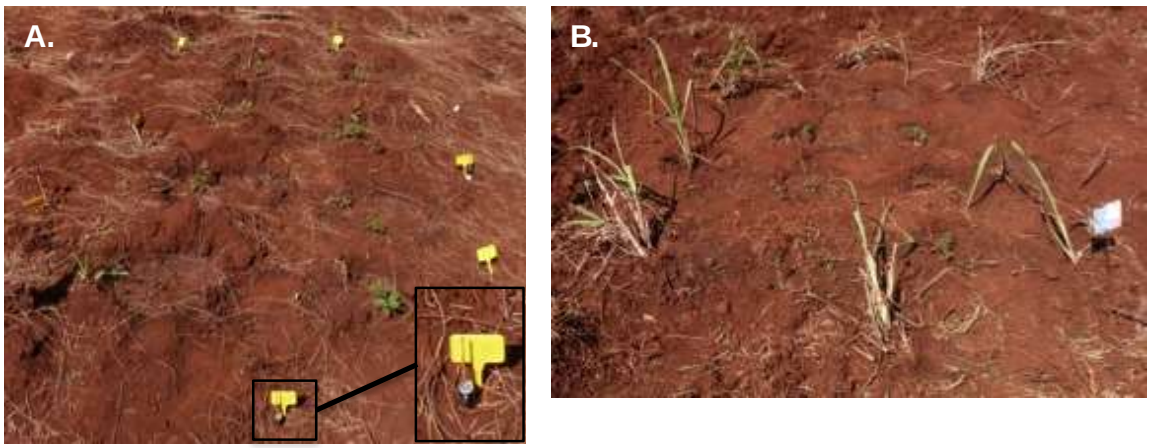


Figure 33: Photo de l'essai mis en place en 2013 au KARI, kenya pour tester l'efficacité d'une culture associée avec de l'huile essentielle de *Zeylanicum cassia* (A) et de l'inter-culture lemongrass (B).

Chaque parcelle contenait huit plants de tomate protégée ou non avec un filet anti-insecte. Les huiles essentielles de cannelle et les plants de Lemongrass ont été planté autour de la parcelle et en dehors du filet si celle-ci était protégée. Une fois par semaine, la population de mouche blanche adulte était dénombrée sur une feuille apicale et basale de chaque plant de tomate. Etant un essai préliminaire, aucune répétition n'a été effectué.

La population de *Bemisia tabaci* a été observée chaque semaine. L'infestation est restée modérée et n'a jamais dépassé les 10 individus par plant. De plus, des aleurodes ont été trouvés sur toutes les parcelles sans qu'aucune différence significative ne soit observée. Les composés volatils du lemongrass ou du diffuseur ont tenté d'être capturés au champ par fibre SPME (DVB/PDMS) avec ou non le recours à un sac avec ventilation. Aucun composé volatil n'a été retrouvé ce qui pourrait expliquer le manque d'efficacité des répulsifs.

Les composés volatils du lemongrass ont alors été capturés en laboratoire par SPME (DVB/PDMS) sous un sac en naphtalène avec une atmosphère purifiée par du charbon actif et renouvelée (Figure 34). Après analyse par GC-MS, les composés de l'huile essentielle ont été retrouvés mais uniquement quand les feuilles du lemongrass ont été froissées. La plante intacte n'émet pas ou une très faible quantité de composés volatils. Ces composés sont en effet enfermés dans des vésicules au niveau des feuilles, lorsque des insectes ou herbivores consomment les feuilles, les vésicules sont alors ouvertes et les composés sont relâchés. Ces composés émis servent à empêcher l'herbivorie, c'est pourquoi ils sont répulsifs et/ou anti-appétants.

En ce qui concerne l'huile essentielle de cannelle lorsque les composés de l'huile essentielle de cannelle ont été collectés par fibre spme en laboratoire et analysés, les composés de l'huile essentielle ont bien été retrouvés.

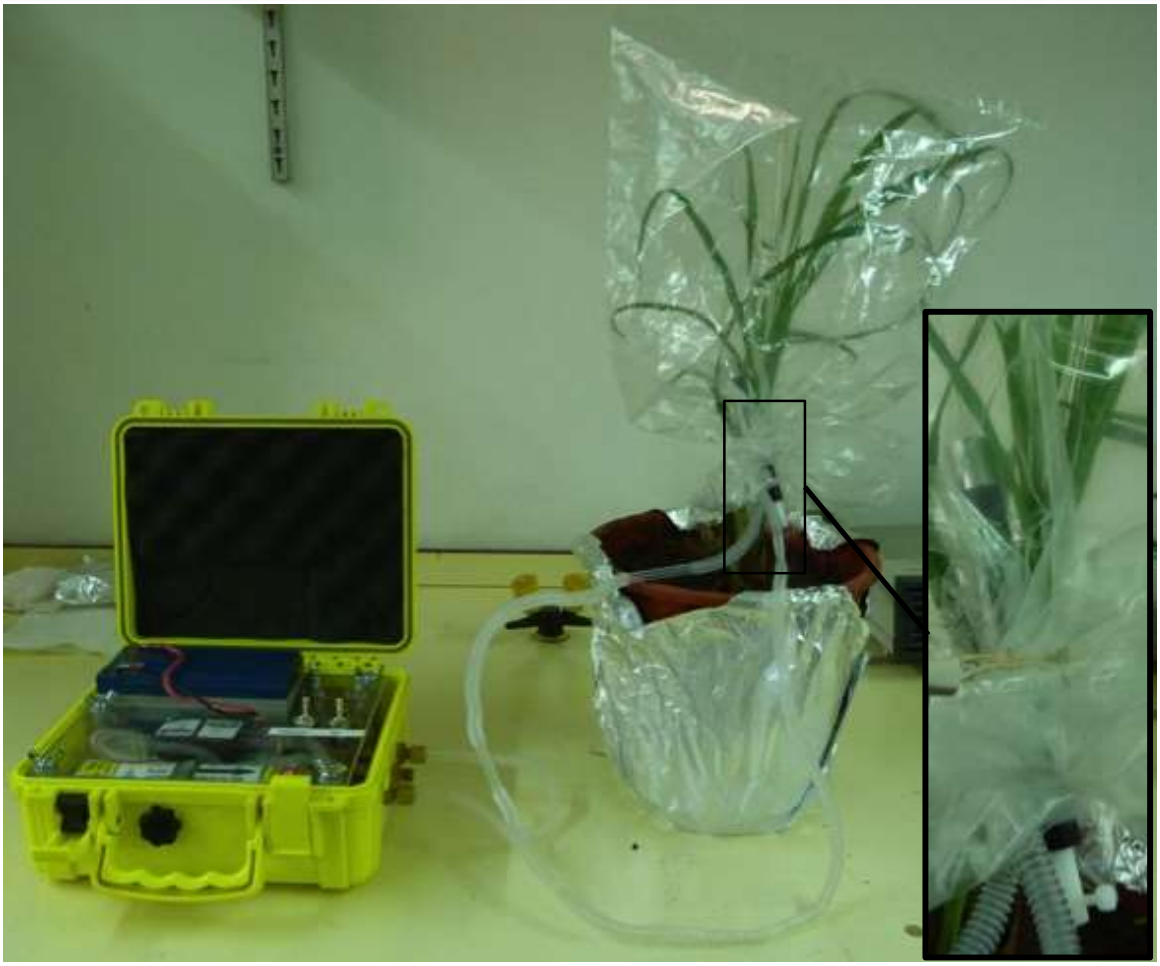


Figure 34 : Photo du piégeage des volatiles du plant de lemongrass par SPME (DVB/ PDMS).
Les volatiles ont été piégé pendant 4h avec un débit de 300mL/min puis analysé par GC-MS et GC. L'analyse de plante saine et de plante avec des feuilles froissées ont été effectués afin de comparer les quantités d'émission des volatiles. Deux plants différents ont été analysé deux fois pour chacune des modalités.

4. Discussion

Que ce soit pour le moustique et la mouche blanche, les résulltats sur le terrain ou en conditions semi-contrôlées ont été très différents de ceux obtenus au laboratoire. Comment expliquer ce manque d'efficacité ?

Après 9h de test en tube, l'efficacité des produits (irritation et toxicité) avait diminué. Cependant grâces aux nouvelles technologies, il serait possible d'augmenter la durée d'efficacité avec l'ajout de vanilline ou l'encapsulation (Picard *et al.*, 2012 ; Regnault-Roger, 1997 ; Specos *et al.,* 2010). Dans le test d'efficacité, l'efficacité des produits a diminué au cours du temps mais ce n'est pas la seule raison qui explique le manque d'efficacité des produits dans le test en tunnel. Un des désavantages à utiliser un produit anti-appétant est le phénomène d'habituation, les insectes perdent leur sensibilité au produit ou changent leur comportement alimentaire après une exposition répétée ou longue (Jermy, 1990 ; Foster et Harris, 1997). Il se pourrait que ce même phénomène existe pour les répulsifs ou les irritants. En effet dans le test d'efficacité, les insectes n'étaient en présence du produit que pendant 10

min, mais pour le test en tunnel ils sont restés en présence du produit pendant 8h et ils ont pu s'acclimater. Les récepteurs olfactifs sont peut être désensibilisés après une exposition aussi longue. Une autre explication du manque d'efficacité pourrait être que dans le test en tunnel, le cobbaye est trop près et trop visible par les moustiques ainsi ils n'utiliseraient pas ou peu leur système olfactif et gustatif mais plutôt les signaux visuels (Carnevale et Robert, 2009).

Nous avons très peu de connaissances sur la dégradation des composés volatils après leur émission par la plante. Si les composés répulsifs sont dégradés très vite dans l'environnement cela pourrait expliquer le manque d'efficacité des différentes stratégies utilisées. De plus même si certains scientifiques pensent que l'efficacité des volatils n'est pas du à leur quantité mais à leur qualité (Cunnigham, 2012), le ratio répulsif/tomate était peut être trop faible. De plus, nous avons très peu de connaissances sur le processus de diffusion des composés volatils. L'insecte détecterait les volatils par bouffées d'air et non de manière laminaire comme dans un olfactomètre, ce qui pourrait biaiser les résultats.

La technique de l'intercropping pourrait être efficace contre des insectes généralistes comme *Bemisia tabaci.* En effet, pour les insectes généralistes, choisir son hôte peut être plus difficile et plus long quand plusieurs plantes et donc plusieurs odeurs sont présentes dans l'environnement. Par contre, des insectes spécialistes n'ont pas à faire face à ce genre de dilemme, ils se concentrent sur un seul stimulus et ignorent les autres (Bernays et Funk, 1999). Donc ajouter différentes odeurs au système semble être une bonne idée pour perturber les insectes généralistes mais semble plus compliqué pour les insectes spécialistes.

Les pyréthrinoïdes actuellement utilisés en complément de la barrière physique sont des composés expulsifs et irritants. Pour cette raison, nous avons focalisé nos recherches sur des produits expulsifs et irritants. Mais il existe d'autres phénomènes de répulsions : la repulsion masquante, anti-appétante ou visuelle qui pourraient être de nouvelles pistes de recherche à exploiter pour améliorer la barrière physique.

Les autres répulsions et leurs intérêts

‘*Pour créer de nouveaux arrangements, de nouvelles formes olfactives, il suffit que vous pensiez « en odeurs », comme le peintre « en couleur », et le musicien « en sons »…*’
Edmont Roudnitzka.

1. La répulsion masquante

1.1 Introduction

La répulsion-masquante est un phénomène de répulsion où le produit masquant diminue l'attractivité de l'hôte ou interfère dans la localisation de l'hôte. Le produit n'est donc pas répulsif-expulsif de lui-même mais son effet répulsif dépend du contexte. En fonction de sa concentration et de la présence d'autres molécules, ce produit induit des comportements différents. Ce produit serait donc également intéressant à combiner avec un filet anti-insecte.

Pour tester la répulsion masquante, il faut donc permettre à l'insecte de sentir l'odeur de son hôte et l'odeur masquante, sans pouvoir entrer en contact avec le produit testé. Le produit masquant diminue l'attractivité de l'hôte ou interfère dans la localisation de l'hôte, l'insecte doit donc mettre plus de temps à trouver son hôte ou moins d'insectes doivent trouver leur hôte. La manière la plus simple de tester l'odeur masquante est d'utiliser un olfactomètre en Y avec une branche qui délivre l'odeur de l'hôte et l'autre branche avec l'odeur de l'hôte et l'odeur masquante (OMS, 2013) (Figure 35). Si le produit masquant diminue l'attractivité de l'hôte, la majorité des insectes choisiront la branche avec uniquement l'odeur de l'hôte. Ainsi Obermayr *et al.* (2012) montrent que l'homopiperazine inhibe la localisation de l'hôte chez *Aedes aegypti.* Un olfactomètre à 4 branches peut aussi être utilisé : toutes les branches délivrent l'odeur de l'hôte et 1 à 3 branches délivrent en plus l'odeur masquante. Le temps passé dans chaque branche est enregistré. Si l'insecte passe moins de temps dans les branches avec l'odeur masquante, celle-ci est bien masquante. Les olfactomètres sont très efficaces pour étudier l'effet masquant mais ces bioessais sont difficiles à mettre en place. Dans une cage, un test de choix entre un hôte seul et un hôte plus le produit masquant appliqué sur un papier filtre peut également permettre de tester l'effet masquant d'un produit. Avec cette méthode, Bruce *et al.* (2005b) ont montré que l'huile essentielle d'*Hemizygia petiolata* permet de diminuer l'attractivité du chou chinois pour *Myzus persicae*. L'effet masquant peut également être étudié avec un test de non choix en comparant le temps mis par les insectes à trouver l'hôte et avec le temps mis par les insectes à trouver l'hôte en présence du produit masquant et/ou le nombre d'insecte.

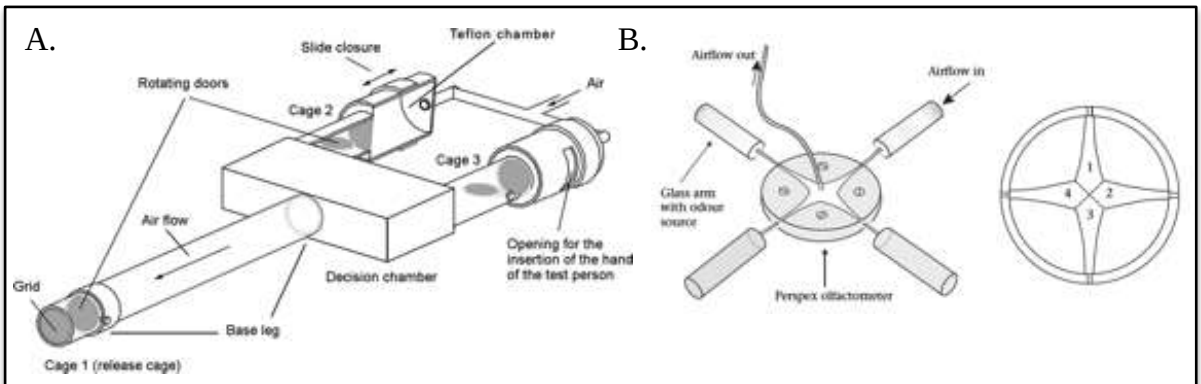


Figure 35: Différents olfactomètres pour étudier la répulsion dite masquante
A. Olfactomètre en Y tiré de '*A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours*' (Geier et Boeck, 1999).
B. Olfactomètre à 4 branches tiré de '*Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid*' (Webster *et al.*, 2010).

1.2. Test en olfactomètre sur Trialeurodes vaporarium

L'effet masquant a été étudié sur l'aleurode *Trialeurodes vaporarium*, avec un olfactomètre en Y. Dans un premier temps, l'attractivité d'un plant de tomate a été vérifiée. Une branche délivrait les volatils de la plante hôte et l'autre l'air purifié et humidifé. Dans un second temps, l'effet masquant d'un plant de géranium a été étudié. En effet, dans certains cas la présence de plantes non hôte peut affecter la localisation de l'hôte en émettant des effluves répulsives ou en masquant les caractéristiques olfactives de l'hôte (Thiéry et Visser, 1986). Dans notre bioessai, une branche délivrait les volatils de tomate et l'autre les volatils de tomate et de géranium (Figure 36). En plus du plant de tomate, un plant de géranium a été ajouté dans l'un des dessiccateurs. Si la majorité des insectes choisissait la branche avec uniquement les volatils de tomate et non le mélange de volatils, cela signifiait que les volatils de géranium diminuaient l'attractivité de la tomate. Les volatils de géranium étaient alors masquants.

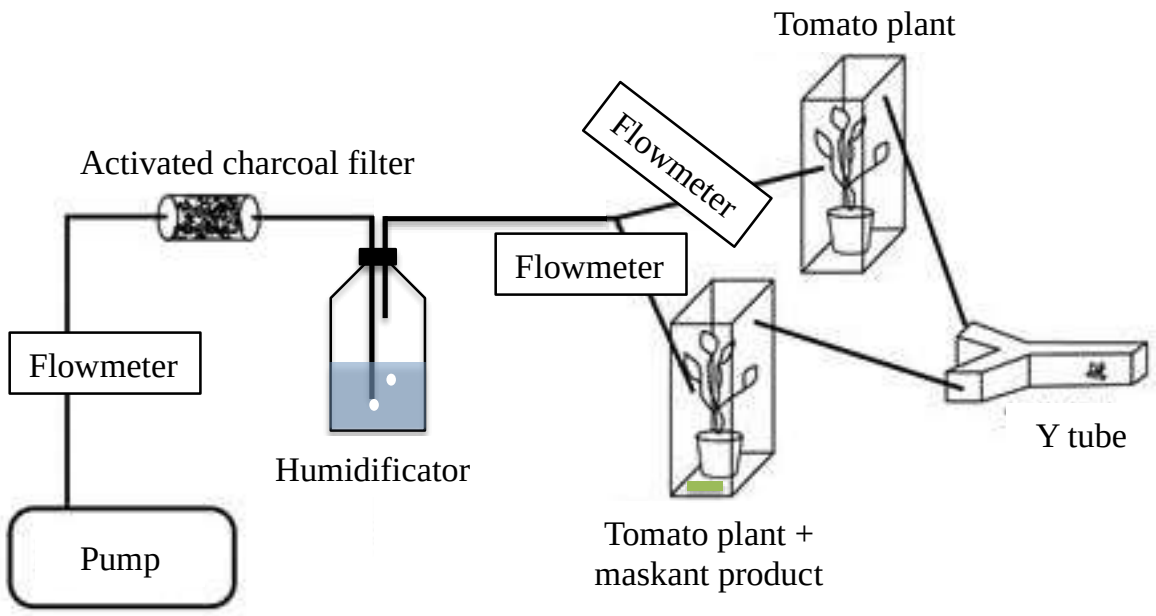


Figure 36 : Olfactomètre pour tester l'effet masquant pour *Trialeurodes vaporarium*.
L'air a été humidifié et filtré avec un débit de 300 mL/min. 3 modalités ont été testées: volatils de tomate vs air, volatils de tomates vs volatils de tomate et de géranium, volatils de tomates vs volatils de tomate et myrcène. 40 insectes ont été testés pour chaque modalité.
Le protocole est détaillé dans l'article: Preference host modulation of *Bemisia tabaci* for tomato plant in presence of geranium (Deletre *et al.*, en préparation).

L'odeur de tomate est bien attractive pour *Trialeurodes vaporarium* (Figure 37). Par contre, lorsqu'il avait le choix entre un plant de tomate seul et un plant de tomate accompagné d'un plant de géranium, l'aleurode s'est dirigé vers les volatils du plant de tomate seul. Les volatils du plant de géranium diminuent donc l'attractivité des volatils du plant de tomate et à donc un effet masquant. Aux vues de ses résultats, les volatils de tomates et de géranium ont été analysés pour essayer de comprendre les interactions entre les volatils.

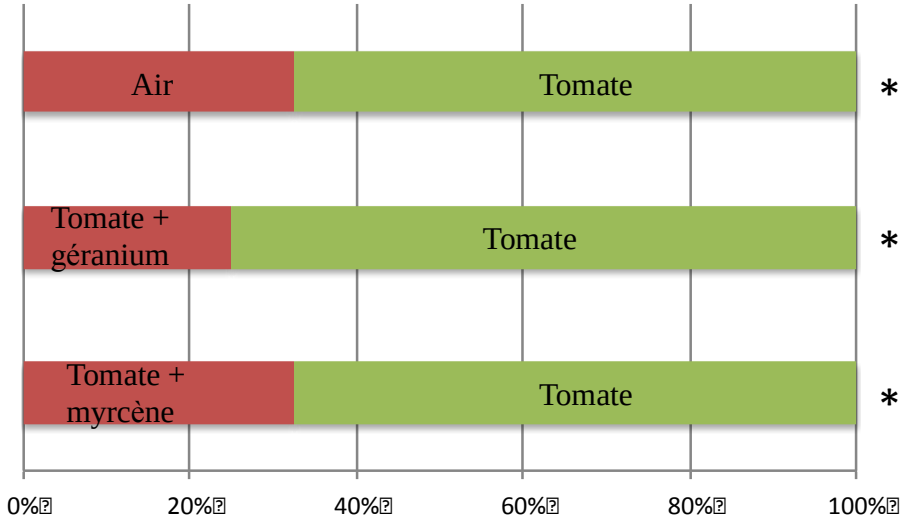


Figure 37: Choix de *Trialeurodes vaporarium* dans un olfactomètre en Y
Un test binomiale a été effectué en comparant les proportions obtenues et la proportion théorique d'une répartition équilibrée. Les proportions suivies d'une * se sont montrées significativement différentes de la proportion théorique.
Ces résultats seront présentés dans l'article: Preference host modulation of *Bemisia tabaci* for tomato plant in presence of geranium (Deletre *et al.*, en préparation).

L'analyse des volatils de tomates et de géranium a été réalisée en utilisant un polymère poreux de type Porapak Q (Figure 38). L'air entrant et sortant a été filtré par le polymère. Pour analyser les volatils, le polymère a été élué avec du dichlorométhane et la solution a été analysée par GC-MS.

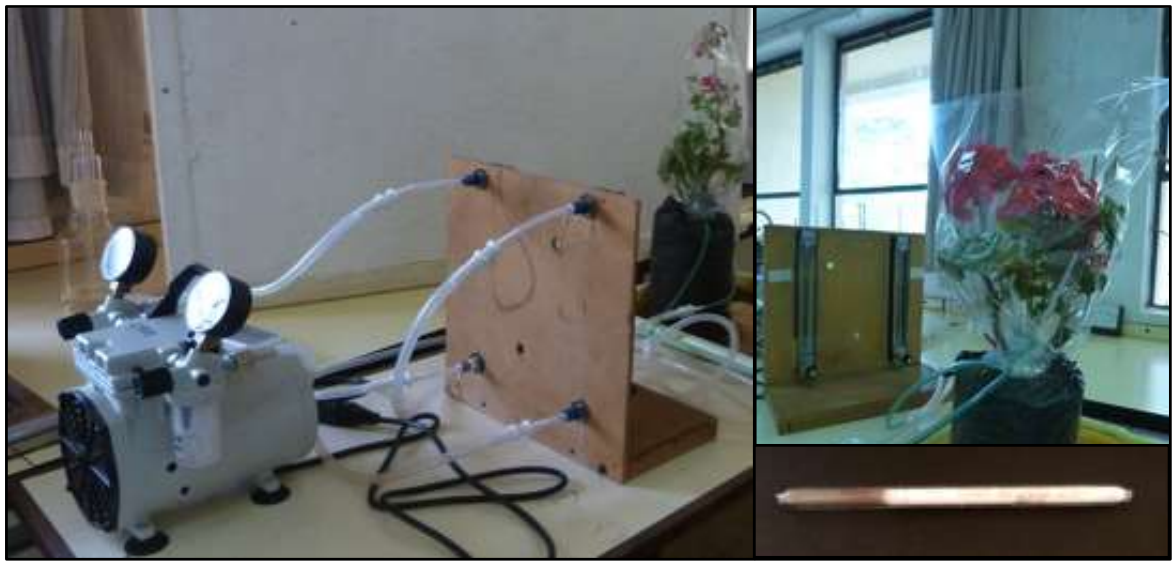


Figure 38: Photo de la méthode d'analyse des composés volatiles d'une plante.
Ce dispositif comprend une pompe reliée à des débimètres, pour contrôler les débits d'air entrant et sortant du sac, filtrés par du Porapak Q.

Certains composés volatils sont communs aux deux plantes que nous avons utilisées: l' α-pinène, le myrcène et le β –phellandrene (Tableau 6). Le β –phellandrene est le composé

majoritaire de tomate mais un composé minoritaire du géranium, l' α-pinène est un composé minoritaire des deux plantes et le myrcène est un composé minoritaire de la tomate mais il est le composé majoritaire du géranium.

Tableau 6: Composés volatils de tomate *Lycopersicum* et de géranium *Pelargonium hororum* analysés par Porapak Q et GC-MS. Ces résultats seront présentés dans l'article: Preference host modulation of *Bemisia tabaci* for tomato plant in presence of geranium (Deletre *et al.*, en préparation).

Tomate :

composé	Proportion[1]	variance
α-pinène	0,05	0,00
4-carene	0,03	0,00
β-pinène	0,01	0,00
myrcene	0,01	0,00
2-carene	0,37	0,01
α-terpinene	0,02	0,00
β -phellandrene	1,00	0,00
β-caryophyllene	0,09	0,00
α-humulene	0,04	0,00

Géranium :

composé	proportion	variance
α-pinène	0,22	0,03
β-pinène	0,12	0,01
myrcene	1,00	0,00
β -phellandrene	0,21	0,02
β-ocimene	0,04	0,00
α-ocimene	0,36	0,06
a-humulene	0,68	0,15

[1] Les proportions sont données par rapport au β-phellandrène pour la tomate et par rapport au myrcène pour le géranium qui sont les composés majoritaires.

Dans un troisième temps, le composé majoritaire du géranium, le myrcène, a été testé avec l'olfactomètre en Y pour savoir s'il était responsable de l'effet masquant du géranium. Et en effet, l'aleurode a choisi les volatils du plant de tomate seul plutot que les volatils du plant de tomate associé au myrcène. Le myrcène diminue donc l'attractivité du plant de tomate et à donc un effet masquant.

1.3. Application

Au laboratoire, les volatils de géranium ont donc présenté un effet masquant, il faut donc maintenant montrer leur efficacité sur le terrain. En 2014, un essai d'inter-culture géranium-tomate a été mis en place. De la même façon, l'inter-culture a été également combinée avec l'utilisation d'un filet pour savoir s'il y avait un synergisme entre les deux moyens de lutte : la barrière physique et la barrière olfactive (Figure 44) La population de *Bemisia tabaci* a été enregistrée chaque semaine sur une feuille apicale et une feuille basale de 10 plants de tomate sur 24 par parcelle.

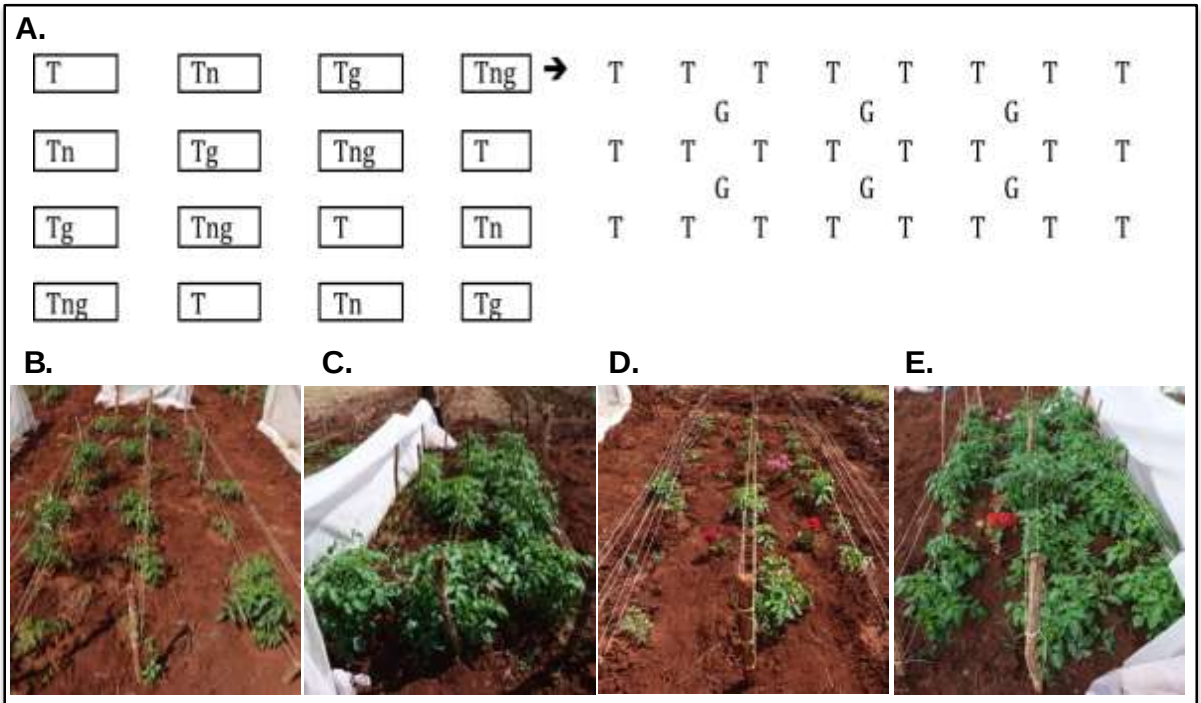


Figure 39: Plan de l'essai au champ et photos du 4 juin de l'essai

A. Schéma de disposition de l'essai et d'une parcelle de tomate et de tomates/géranium
T: parcelle de tomates; Tn: parcelle de tomates protégée par un filet; Tg: parcelle de tomate/géranium, Tng : parcelle de tomates/géranium protégée par un filet
B. parcelle de tomates
C. parcelle de tomates protégée par un filet
D. parcelle de tomate/géranium
E. parcelle de tomates/géranium protégée par un filet
24 plants de tomates par parcelle ont été planté en inter-culture avec 6 plants de géranium. Le comptage a été effectué sur les 4 parcelles de chaque modalité, 10 plantes par parcelle ont été échantillonnées avec une feuille apicale et une feuille basale.
Pour plus de détails, cf. Preference host modulation of *Bemisia tabaci* for tomato plant in presence of geranium (Deletre *et al.*, en préparation).

La présence du filet a permis un meilleur développement végétatif du plant de tomate. En effet, le filet limite la ventilation ce qui permet de garder une température et une humidité stable et cet effet de serre provoque un développement plus rapide du plant de tomate. De plus, la présence du filet retarde l'infestation des mouches blanches de par la barrière visuelle.

Par contre, il n'y a pas eu d'effet du géranium, ni de synergisme entre la tomate et le géranium (Figure 40).

Pour les parcelles de tomate en monoculture 23% des plants de tomates ont été infectés par un virus et 26% quand la parcelle était en inter-culture avec des plants de géranium. Sur les parcelles avec filet, 19% des plants ont été infectés par un virus en monoculture et 7% en inter-culture. Les plants de géranium ont été placés sous le filet pour plusieurs raisons. Le filet limite la ventilation donc nous pouvions supposer qu'en plaçant les géraniums sous le filet leurs volatils seraient concentrés. De plus, Tosh et Brogan (1998) ont étudié l'effet de l'émission de volatils d'autres plantes hôtes sur des mouches blanches se nourrissant sur des tomates et leur activité alimentaire reliée au phloème a été perturbée et retardée à cause de cette abondance de volatils. Avec les composés volatils de géranium qui diminuent l'attractivité de la tomate, nous pouvions espérer une réduction ou une cessation de l'activité alimentaire des mouches blanches. Nous pouvons d'ailleurs remarquer que le taux

de plants de tomates infestés sous filet est inférieur mais en présence de géranium le taux est encore plus faible. Nous pouvons donc émettre l'hypothèse qu'en présence des plants de géranium l'activité alimentaire de *Trialeurodes vaporarium* est perturbée.

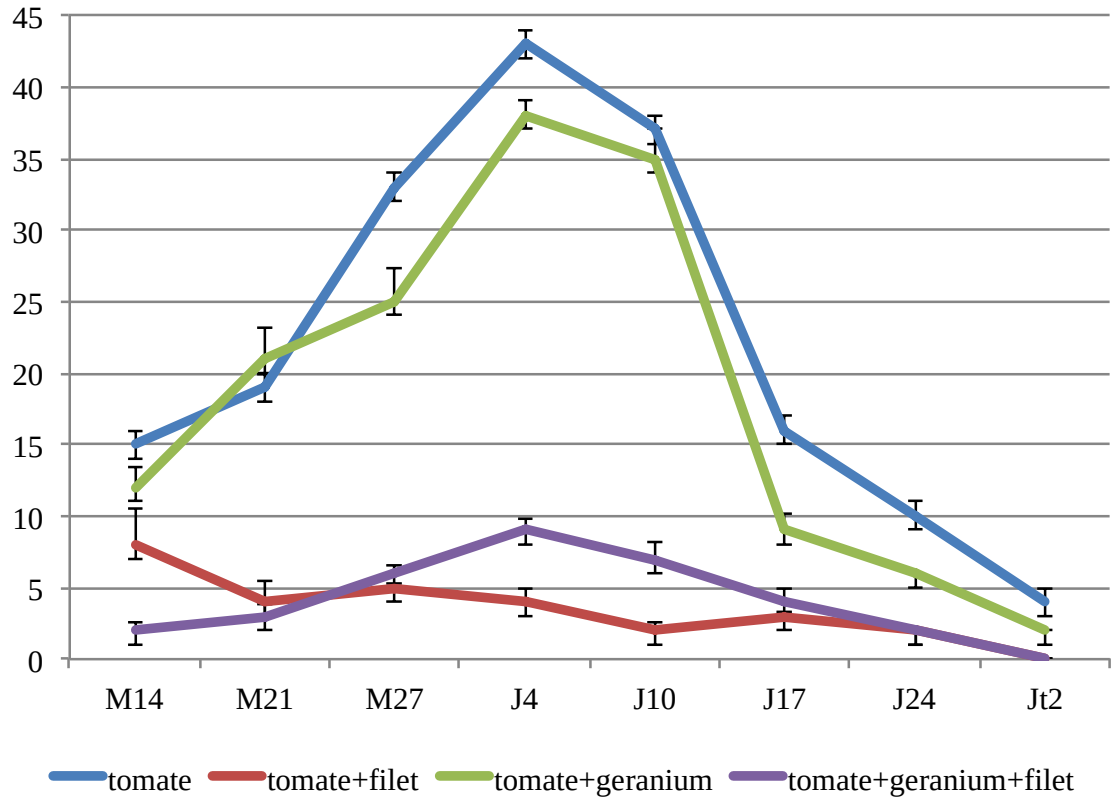


Figure 40: Nombre cumulé moyen de mouches blanches sur des parcelles de tomates, de tomates protégées par un filet, de tomates et géranium et de de tomates et géranium protégées par un filet au cours du temps.
Le comptage a été effectué sur les 4 parcelles de chaque modalité, 10 plantes par parcelle ont été échantillonnées avec une feuille apicale et une feuille basale. M: mai, J: Juin, Jt: Juillet.
Ces résultats seront présentés dans l'article: Preference host modulation of *Bemisia tabaci* for tomato plant in presence of geranium (Deletre *et al.*, en préparation).

De même qu'en 2013, les résultats obtenus au laboratoire et sur le terrain ont été très différents. Une des explications possible reste que la quantité de volatiles n'était peut être pas assez important, même si certains scientifiques pensent que l'efficacité des volatils n'est pas due à leur quantité mais à leur qualité (Cunnigham, 2012). Le ratio géranium/tomate était peut être trop faible. En effet, au laboratoire, le géranium a montré qu'il diminuait l'attractivité de la tomate avec un ratio de un pour un et des plants de taille similaire contrairement à notre essai où le ratio était de 1 pour 4 et que le plant de tomate était beaucoup plus grand que celui de géranium après un mois de culture. Si le myrcène est bien responsable de la diminution de l'attractivité de la tomate peut être que celui ci n'était pas diffusé en quantité suffisante.

1.4. Discussion

En agriculture, l'effet masquant des plantes est utilisé lors des stratégies de cultures associées ou stratégie de plantes compagnes: le but est alors de diminuer l'attractivité d'une plante cultivée en y associant une plante répulsive pour un ravageur majeur. Ainsi le mélange de leur volatil soit 'cache' le profil odorant de la culture soit en diminuera l'attractivité (Cook *et al.*, 2007). Togni *et al.* (2010) ont ainsi montré qu'une culture de tomates associée à des plants de coriandre était moins attractive qu'une culture de tomate seule.

Nous avons réussi à montrer au laboratoire qu'un insecte généraliste tel que *T. vaporarium* choisissait l'odeur de tomate plutot que l'odeur de tomate mélangée à celle du géranium. Tosh & Brogan (2014) avancent l'hypothèse que les performances d'un insecte généraliste diminuent en fonction de la diversité des plantes à laquelle il est confronté. Plus la diversité des plantes hôtes et non-hôtes est importante dans son environnement, plus il aura de difficultés à choisir sa plante hôte. Cette hypothèse semble confirmée avec les essais par olfactomètre au laboratoire.

Le composé majoritaire des composés volatils du géranium, le myrcène, semble responsable de l'effet masquant et il est également un des composés minoritaires des tomates. L'interaction plante-insecte est basée sur l'émission des volatils, elle peut être bénéfique ou dangereuse pour la plante. En effet pour une plante, l'émission des volatils peut attirer des bénéficiaires comme les pollinisateurs et nous pouvons émettre l'hypothèse que certains volatils servent à minimiser l'attaque des plantes par les herbivores. Ces volatils peuvent repousser les herbivores ou attirer leurs prédateurs et parasitoïdes. Nous pouvons supposer que le myrcène est un volatil de tomate qui peut repousser les mouches blanches ou diminuer l'attractivité des tomates pour les herbivores. De plus, Bleeker *et al.* (2009) ont montré que les plants de tomates sauvages qui contiennent plus de myrcène sont moins attractifs que les plants de tomates sélectionnés et cultivés qui contiennent moins de myrcène.

La répulsion-masquante est un phénomène de répulsion où le produit masquant diminue l'attractivité de l'hôte ou interfère dans la localisation de l'hôte. Le produit n'est donc pas répulsif-expulsif de lui-même mais son effet répulsif dépend du contexte. En fonction de sa concentration et de la présence d'autres molécules, ce produit induit des comportements différents. Nous avons émis l'hypothèse que changer le ratio des composés volatils d'une plante pouvait changer le comportement de l'insecte. Cet essai montre donc que lorsque le myrcène est en quantité plus importante, l'attractivité de la tomate diminue. De plus, tester seul le myrcène n'a pas d'effet répulsif (Bleeker *et al.*, 2009). Mais Bleeker *et al.* (2009) montre que *Bemisia tabaci* ne semble pas avoir de récepteurs olfactifs sur les antennes qui reconnaissent le myrcène. Il faudrait donc faire un EAG sur *Trialeurodes vaporarium* pour vérifier qu'il reconnaît le myrcène et ainsi montrer l'importance de la concentration du myrcène sur son comportement.

En fonction des différents contextes environnementaux, un composé répulsif 'masquant' activerait différents récepteurs olfactifs et donc différents glomérules. En effet, les récepteurs ont plus ou moins d'affinités avec les molécules. Par exemple, en plus grande concentration une molécule active plus de récepteurs. De plus, les glomérules ont plusieurs niveaux d'activation en fonction du nombre de récepteurs activés. En fonction des niveaux d'activation, le jeu des interneurones n'est pas le même, l'activation et l'inhibition des glomérules entre eux dépendent de ces niveaux d'activation. Ainsi par le jeu des inter-neurones, l'information olfactive transmise par les neurones de projections aux centres nerveux centraux coderait des comportements différents. Outre la concentration, le ratio des

molécules peut également changer la réponse comportementale par le jeu des inter-neurones et le niveau d'activation des glomérules. En effet, un même glomérule peut être activé par une molécule attractive ou répulsive mais l'information qui converge par les neurones de projection est différente grâce à l'activité des interneurones (Knaden *et al.*, 2012). Pour changer l'information, le cas le plus simple est de changer la concentration d'un produit ou d'ajouter un produit ce qui inhiberait et/ou activerait les glomérules actifs par le jeu des inter-neurones (Figure 41).

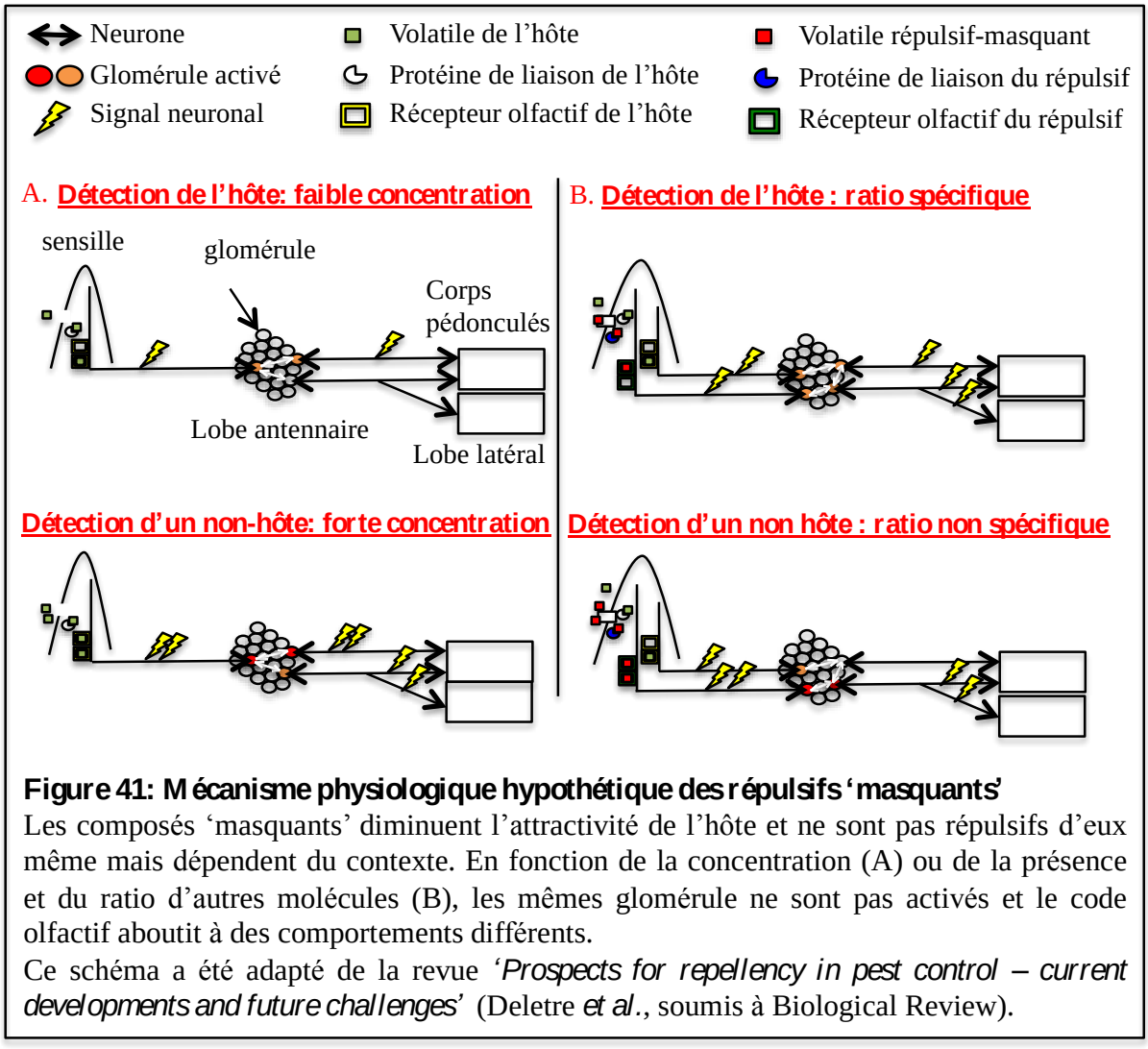


Figure 41: Mécanisme physiologique hypothétique des répulsifs 'masquants'
Les composés 'masquants' diminuent l'attractivité de l'hôte et ne sont pas répulsifs d'eux même mais dépendent du contexte. En fonction de la concentration (A) ou de la présence et du ratio d'autres molécules (B), les mêmes glomérule ne sont pas activés et le code olfactif aboutit à des comportements différents.
Ce schéma a été adapté de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

Cependant le ratio peut changer par l'augmentation du taux d'une molécule mais le changement pourrait également se faire par la réduction du taux de molécule et plus précisément par un taux faible de reconnaissance de cette molécule. Le retrait d'un composé volatil du bouquet odorant, par blocage de l'information ou le piégeage d'une molécule volatile, est aussi possible, ces répulsifs sont appelés 'inhibiteurs d'attraction' (Figure 32). Ces composés affectent l'activité des récepteurs olfactifs et/ou des protéines de liaison ce qui empêche l'activation des neurones olfactifs. Par exemple, le DEET bloquerait certains récepteurs et corécepteurs et ainsi masquerait l'odeur de l'hôte. Pour *Anopheles gambiae* et *Aedes aegypti*, il inhiberait par exemple l'attraction à l'acide lactique (Davis et Sokolove, 1976 ; Dogan *et al.*, 1999 ; Ditzen *et al.*, 2008 ; Degennaro *et al.*, 2013). L'inhibition se ferait par un changement de la perméabilité aux ions du neurones et donc une diminution de la dépolarisation et ainsi du message nerveux (Ditzen *et al.*, 2008). De plus, le DEET piègerait

le 3-octenol, un attractif, et modifierait ainsi le profil odorant de l'hôte et perturberait la recherche de l'hôte chez le moustique (Syed et Leal, 2008). Les inhibiteurs d'attraction peuvent également modifier ou bloquer la réponse des neurones olfactifs aux composés attractifs (Davis, 1985) (Figure 42). Par exemple, Bohbot *et al.* (2011) ont montré que le répulsif IR3535 inhibait la réponse des récepteurs olfactifs à l'octénol, un attractif. Les récepteurs ont différents sites d'action pour interagir avec les répulsifs, des sites allostériques et orthostériques (Dickens et Bohbot, 2013).

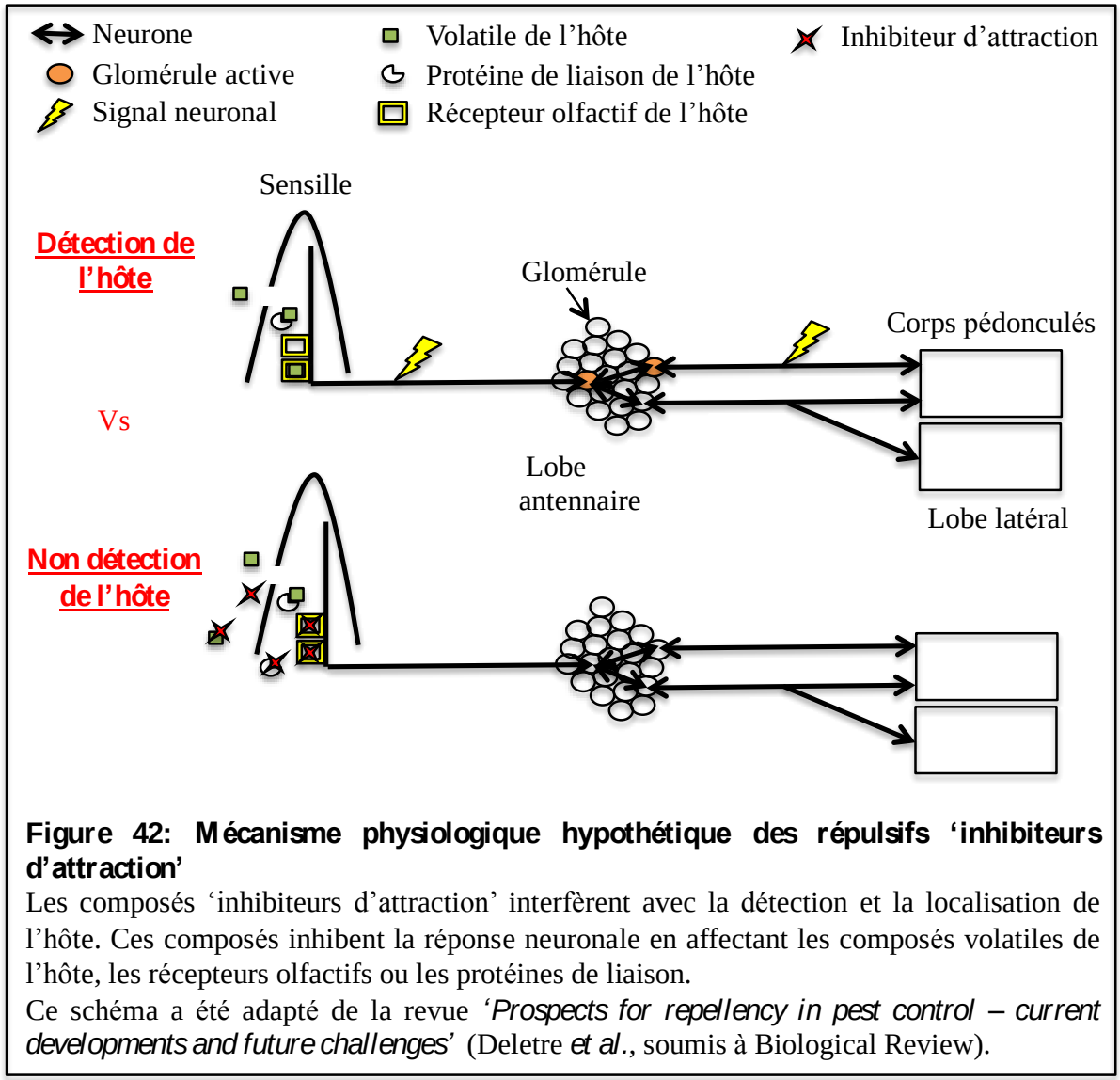


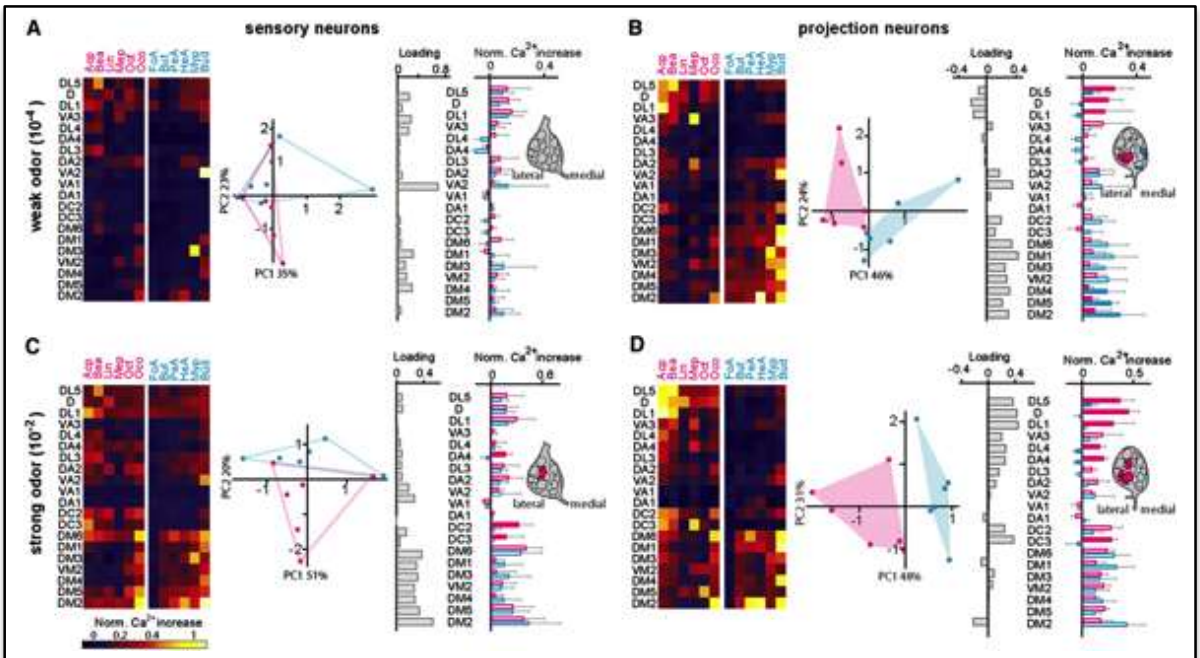
Figure 42: Mécanisme physiologique hypothétique des répulsifs 'inhibiteurs d'attraction'

Les composés 'inhibiteurs d'attraction' interfèrent avec la détection et la localisation de l'hôte. Ces composés inhibent la réponse neuronale en affectant les composés volatiles de l'hôte, les récepteurs olfactifs ou les protéines de liaison.

Ce schéma a été adapté de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

La concentration des odeurs affecte la réponse comportementale : beaucoup de composés sont répulsifs à haute dose et attractifs à faible dose (Foster & Harris, 1997 ; Hallem & Carlson, 2006). L'activation d'un plus grand nombre de glomérules par des concentrations élevées serait à l'origine de ce changement de comportement (Knaden *et al.*, 2012 ; Semmelback & Wang, 2009) (Figure 43). En effet, les récepteurs ont plus ou moins d'affinités avec les molécules odorantes, à forte concentration les molécules odorantes activent plus de récepteurs qu'à faible concentration (Hallem & Carlson, 2006). De plus, les récepteurs olfactifs et les glomérules ont différents niveaux d'activation (Cunningham, 2012). L'activation des différents glomérules entraine donc des réponses différentes des neurones de projections et donc conduit à des réponses comportementales différentes (Sasche & Galizia,

2003). De plus, la réponse augmente en amplitude et en durée quand la concentration des composés augmente jusqu'à saturation des neurones.



Representation of Odorants within the Antennal Lobe
(A and B) OSNs (A) and PNs (B) at weak stimulus concentrations. (C and D) OSNs (C) and PNs (D) at strong stimulus concentrations. Left panels, principal component analyses based on the activation patterns elicited by the 12 odorants tested .Representation of attractive odorants differed from aversive ones at the PN level (ANOSIM, Bray-Curtis, weak concentration, $p < 0.005$, strong concentration, $p < 0.002$) but not at the level of OSNs (weak concentration, $p = 0.79$, strong concentration, $p = 0.77$). Centre panels, bar graphs depicting the weight by which the activation of each glomerulus affects the first principal component. Right panels, activation of individual glomeruli by attractive (turquoise) and aversive (magenta) odorants; bar plots depict average response and standard deviation of six stimulations with attractive and with aversive odorants. Solid bars depict glomeruli that differ significantly in their response to attractive and aversive odorants ($p < 0.05$, Mann-Whitney test). Inset depicts the spatial distribution of glomeruli that discriminatively responded to attractive or aversive odorants.

Figure 43: Représentation spatiale de différentes odeurs à différentes concentrations au sein du lobe antennaire
Les images ci-dessus tirées de '*Spatial representation of odorant valence in an insect brain*' (Knaden *et al.*, 2012) montrent que les glomérules activés par un composé à faible concentration ne sont pas les mêmes que les composés activés à forte concentration. La réponse comportementale qui en découle est donc différente.

2. Le filet une barrière physique mais aussi visuelle?

La répulsion visuelle est la diminution de l'attractivité visuelle de l'hôte par un produit ou un objet qui en modifierait la couleur ou la forme. Le but est donc de perturber la reconnaissance de l'hôte. En effet pour trouver leurs hôtes, les insectes ne comptent pas uniquement sur les signaux chimiques mais également les signaux visuels comme la forme, la couleur ou la taille (Bernays er Chapman, 1994). Dans le cas de l'attraction, les signaux visuels sont déjà utilisés pour contrôler les insectes comme les pièges de couleurs combinés à des insecticides ou de la colle. Par exemple des pièges noirs et bleus sont utilisés pour contrôler et surveiller les taons (Gibson & Torr, 1999; Baldacchino *et al.*, 2013).

Un exemple de répulsif visuel est l'utilisation de haies de maïs pour dissimuler la culture (Smith & McSorley, 2000). Ils semblent difficiles de changer les propriétés visuelles d'une plante pour en diminuer l'attractivité mais par exemple, l'acide gibbérellique est utilisé pour conserver les fruits verts qui sont ainsi moins attractifs pour les mouches que les fruits

naturellement jaunes (Foster & Harris, 1997). De plus, certains plastiques absorbent les UV ce qui modifie le comportement des insectes (Raviv & Antignus, 2004). En effet, dans un environnement à haute réflectance d'UV les thrips sont repoussés des surfaces de couleurs attractives, avec un filtre d'UV les aleurodes n'arrivent plus à se disperser et l'activité de vol de *Myzus persicae* est diminuée.

Des filets de couleurs ont également montrés des propriétés intéressantes : les mouches blanches atterrissent sur les filets de couleur jaune mais ne les traversent pas pour atteindre la plante protégée et les thrips passent moins à travers des filets de couleurs bleu ou jaune (Weintraub, 2009).

Dans un premier temps, des essais au laboratoire ont été effectués pour tester l'effet de la couleur du filet : bleu, jaune et blanc combinés à un traitement sur le taux de passage et le taux de mortalité des mouches blanches à travers le filet, le protocole pour tester l'effet irritant des filets a été utilisé (Figure 27). Quelque soit la taille des mailles du filet, le taux de passage a été plus important pour le filet de couleur bleue (Tableau 7). Mais pour les filets à petites mailles, le taux de mortalité a été inférieur pour les filets de couleur. Avec le même filet traité en usine avec de l'alphacyperméthrine 1%, le taux de passage a été moins important avec les filets de couleur et avec les petites mailles, le taux de mortalité a été plus élevé.

Tableau 7: Effet de la maille, de la couleur et du traitement de filets sur le taux de passage et de mortalité de *Bemisia tabaci.*

couleur	maille	traitement	Taux de passage	p-value[1]	Taux de mortalité	p-value
Blanc	0,4	non	59,46 (55,37 - 63,54)	-	29,73 (25,93 - 33,53)	-
Jaune	0,4	non	55,85 (52,73 - 58,97)	0,18	20,23 (17,70 - 22,74)	$< 10^{-3}$
Bleu	0,4	non	74,49 (71,59 - 77,38)	$< 10^{-3}$	18,07 (15,53 - 20,63)	$< 10^{-3}$
Blanc	0,9	non	73,56 (70,83 - 76,28)	-	15,51 (13,27 - 17,74)	-
Jaune	0,9	non	74,94 (72,21 - 77,69)	0,50	16,74 (14,37 - 19,10)	0,46
Bleu	0,9	non	85,45 (82,87 - 88,03)	$< 10^{-3}$	13,56 (11,05 - 16,07)	0,27
Blanc	0,4	oui	63,36 (59,49 - 67,23)	-	14,96 (12,09 - 17,82)	-
Jaune	0,4	oui	6,99 (3,62 - 9,77)	$< 10^{-3}$	87,40 (83,32 - 91,48)	$< 10^{-3}$
Bleu	0,4	oui	4,22 (2,05 - 6,37)	$< 10^{-3}$	93,37 (90,70 - 96,05)	$< 10^{-3}$
Blanc	0,9	oui	78,21 (75,45 - 81,97)	-	29,21 (25,59 - 32,82)	-
Jaune	0,9	oui	20,75 (17,47 - 24,03)	$< 10^{-3}$	14,63 (11,77 - 17,48)	$< 10^{-3}$
Bleu	0,9	oui	26,65 (23,33 - 29,96)	$< 10^{-3}$	16,40 (13,62 - 19,18)	$< 10^{-3}$

[1] Comparaison par paires des proportions avec un test de Fisher.

Puis des essais en 2013 sur le terrain ont été mis en place pour étudier l'effet de la couleur : jaune, bleu, gris, blanc ou rayé bleu/jaune/blanc combinés à un traitement des filets sur l'infestation de *Bemisia tabaci.* Mais aucun effet significatif n'a pu être mis en évidence (Figure 44).

Figure 44: Photo de l'essai mis en place en 2013 au KARI, kenya pour tester l'influence de filets de couleurs sur le d'infestation des tomates par les mouches blanches.

4 parcelles de 16 plants ont été planté par modalité. Une fois par semaine, la population de mouche blanche adulte était dénombrée sur une feuille apicale et basale de chaque plant de tomate.

En accord avec les résultats obtenus au laboratoire, les filets de couleur ne seraient pas répulsifs, au contraire ils seraient attractifs. En effet, le taux de mortalité est plus important avec les filets de couleur puisqu'attirées par la couleur, les mouches blanches restent plus longtemps sur ces filets, mettent plus de temps à les traverser et ainsi elles s'irritent ou s'intoxiquent plus. De plus, Weintraub (2009) montre que les filets sont mêmes plus attractif que la plante puisque les mouches blanches ne traversent pas le filet une fois dessus. Les filets de couleur pourraient même augmenter la croissance de la plante et son rendement.

3. La répulsion anti-appétente

Comment un composé pourrait agir sur le système gustatif et conduire à un comportement de rejet de l'hôte? Une première réponse est que le comportement de rejet n'est pas uniquement dû au produit ingéré, mais à des récepteurs externes, car la toxicité 'interne' d'un composé n'entraine pas obligatoirement un comportement de dissuasion et un composé dissuasif n'est pas obligatoirement toxique (Koul, 2008). En combinaison d'un filet anti-insecte, un composé anti-appétent pourrait être une deuxième barrière avant la prise de nourriture des insectes.

Comme le système gustatif est assez similaire au système olfactif, les mêmes hypothèses de mode d'action physiologique peuvent être émises pour expliquer les comportements de rejet de l'hôte. Mais les modes d'action des composés anti-appétants sont encore inconnus (Koul, 2008). Tous les insectes phytophages ont des récepteurs pour les substances anti-appétantes qui réduisent voire arrêtent l'activité alimentaire (Schoonhoven & Van Loon, 2002). Les substances anti-appétantes peuvent stimuler les cellules de dissuasion par les récepteurs gustatifs et différents neurones gustatifs sont ainsi activés (Jermy, 1990) (Figure 45).

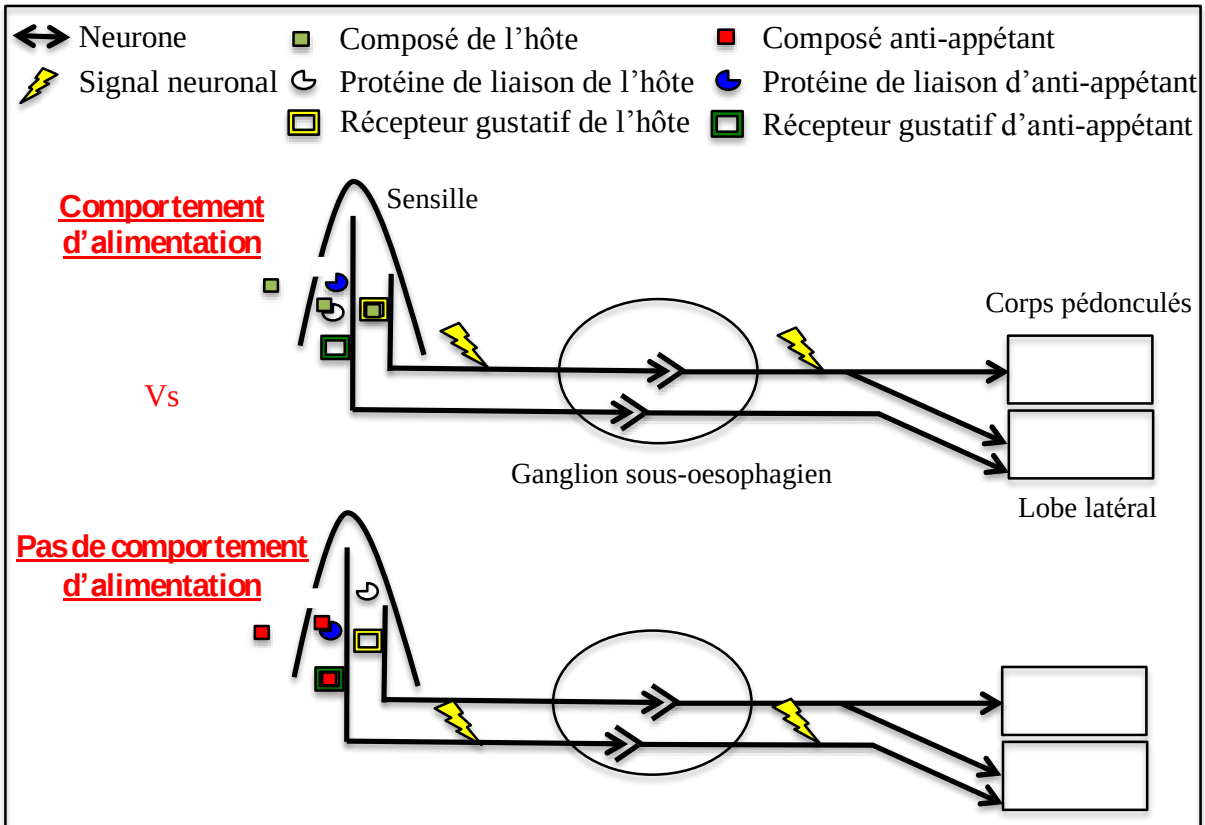


Figure 45: Mécanisme physiologique hypothétique des répulsifs 'anti-appétants'
Les composés 'anti-appétants' agissent sur le comportement d'alimentation. Ils ont des récepteurs gustatifs spécifiques qui codent pour un comportement de rejet de prise alimentaire. Ces composés activent des cellules de dissuasion qui inhibent par exemple l'extension reflexe du proboscis mais ils ne causent pas un mouvement de fuite.
Ce schéma a été adapté de la revue '*Prospects for repellency in pest control – current developments and future challenges*' (Deletre *et al.*, soumis à Biological Review).

En effet, les sucres (attractifs) et les substances amères (répulsives) activent différents récepteurs et neurones (Amrein & Thorne, 2005). Par exemple, les composés anti-appétants amères sont détectés par les cellules L2 et inhibent les cellules S et W au niveau du labellum (Montell, 2009). Plus précisément tous les composés qui activent le neurone Gr5a sont attractifs pour les drosophiles et ceux activant le neurone Gr6a sont anti-appétants (Yarmolinsky *et al.*, 2009). Comme le système olfactif, le système gustatif peut être modulé par la mémoire et l'apprentissage ce qui pourrait expliquer l'origine de ces récepteurs spécifiques (Vosshall & Stocker, 2007). De plus, les composés anti-appétants activent les cellules de dissuasion qui inhibent l'extension réflexe du proboscis. Par exemple, le DEET activerait les récepteurs des neurones responsables des gouts amères et donc dissuasifs chez

Drosophila megalonaster ce qui pourrait expliquer son efficacité à repousser les insectes (Lee *et al.*, 2010 ; Kain *et al.*, 2013).

Mais les composés anti-appétants peuvent également bloquer les récepteurs phagostimulants empêchant ainsi l'activité alimentaire. En effet, d'après Koul (2008) le code de l'information gustative peut être altéré par la stimulation de récepteurs spécialisés comme expliqué précédemment mais également par la modulation de l'activité de certains récepteurs destinés à d'autres composés. Même si un composé anti-appétant ne stimule pas de neurones, il peut diminuer ou bloquer la réponse d'un récepteur à un nutriment. Par exemple chez les larves des lépidoptères, les terpènes bloquent l'effet phagostimulant du glucose sur les cellules chimiosensorielles induisant un effet anti-appétant (Rattan, 2010). Ce type d'anti-appétant est également appelé répulsif 'inhibiteur d'alimentation' (Figure 46).

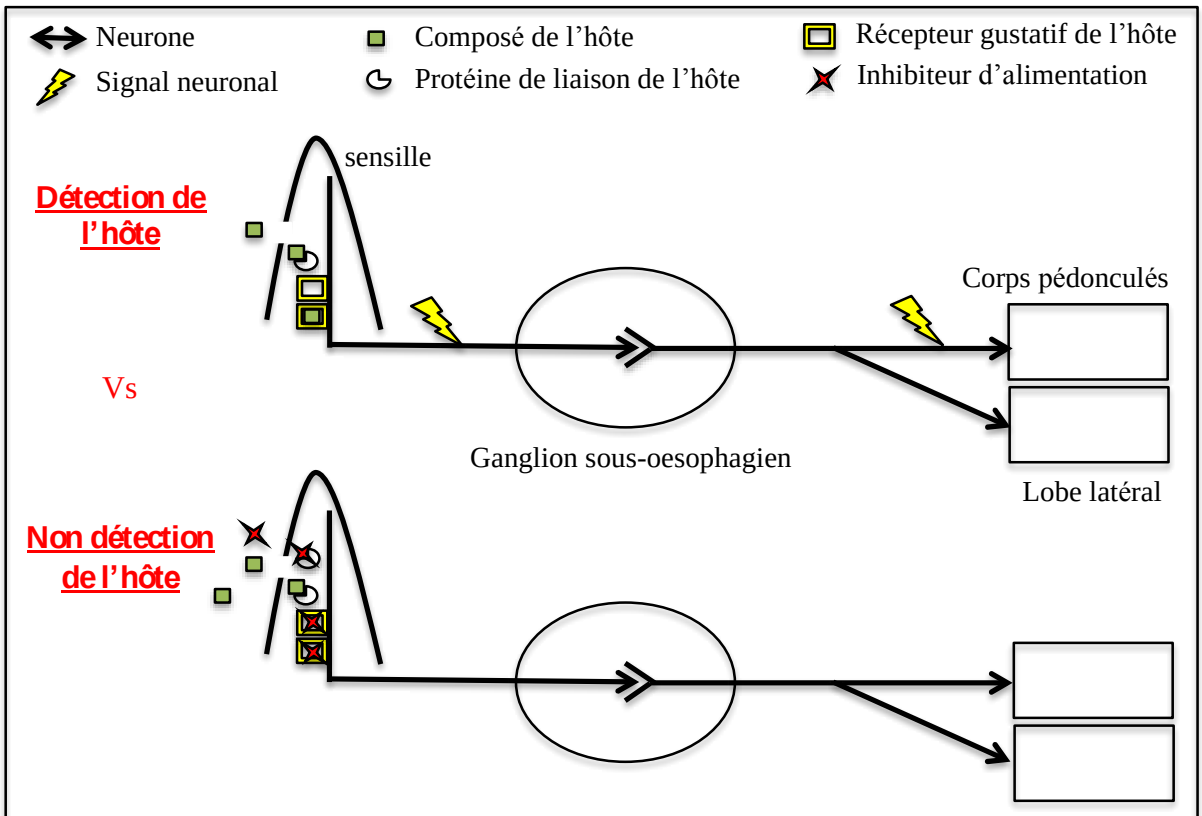


Figure 46: Mécanisme physiologique hypothétique des répulsifs 'inhibiteurs d'alimentation'

Les composés 'anti-appétants' agissent sur le comportement d'alimentation. Les inhibiteurs d'alimentation inhibent la réponse des neurones gustatifs en agissant sur les récepteurs gustatifs, les protéines de liaison ou en piégeant les phagostimulants. L'information n'est alors pas transmise au cerveau de l'insecte puisqu'aucun récepteur n'a été activé et donc aucun message neuronal n'a été envoyé.

Ce schéma a été adapté de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

Pour tester l'effet répulsif 'anti-appétant', les insectes doivent pourvoir être en contact avec l'hôte et le produit testé. Un test de choix est souvent utilisé laissant le choix à l'insecte entre son hôte et son hôte traité avec le produit à tester. Un exemple classique est de mettre des larves dans une boite de pétri avec une feuille traitée et une non traitée. Si la surface consommée de la feuille est plus importante pour la feuille non traitée alors le produit est anti-appétant (Akhtar *et al.*, 2011). Mais dans un test de non choix, il est également possible de

mesurer le poids des insects nourris sur un hôte traité ou un hôte non traité (Abdelgaleil *et al.*, 2002).

Tester l'effet anti-appétant sur des insectes broyeurs est donc assez simple mais qu'en est-il pour les insectes piqueurs-suceurs comme nos insectes modèles ? En effet pour ces insectes, il est plus difficile de voir les dommages sur l'hôte. Il est toujours possible de comparer dans un test de choix, le comportement des insectes sur l'hôte traité et sur l'hôte non traité mais cette différence pourrait également être due à un effet irritant ou masquant voire toxique. Heureusement certains traits de vie spécifique de certaines espèces permettent de mettre au point des tests plus précis.

Par exemple l'oviposition chez *Bemisia tabaci*, se déroulent normalement pendant que l'insecte se nourrit sur l'hôte. Hammad *et al.* (2001) testent ainsi l'effet anti-appétant de plants de tomates traités et non traités en comparant le nombre d'œufs sur chaque plant. Si le plant traité à moins d'œufs alors le produit est considéré comme anti-appétant. Une autre technique peut aussi être utilisée pour étudier l'effet anti-appétant des produits : l'électropénétrographie. Cette technique permet de suivre la pénétration des stylets des hémiptères dans la plante (Sauvion et Rahbe, 1999). Une des électrodes est insérée dans la plante et l'autre est placé, ainsi lorsque l'insecte se nourrit un courant électrique passe. Les différentes ondes électriques permettent de savoir par exemple si l'insecte se nourrit sur le phloème ou le xylème (Tosh, 2014). Cette technique permet ainsi de connaître les différentes phases de la prise alimentaire et de savoir à quel moment le produit anti-appétant agit.

Dans le cas des moustiques, il est facile de voir si un moustique s'est nourri ou non de sang. L'abdomen d'un moustique après un repas de sang est rouge au lieu d'être transparent. Pour tester l'effet anti-appétant de composés pour les moustiques, un test classique consiste à mettre l'avant bras traité et l'autre non traité d'un homme dans une cage de moustiques (test de choix) ou dans deux cages différentes (test de non-choix). Puis pour quantifier l'effet anti-appétant, le nombre de moustiques s'étant nourris est comparé aux nombres de moustiques ayant atterris sur l'avant bras. Cette comparaison évite de prendre en compte les effets irritants ou masquants possibles (Schrek & McGovern, 1989; WHO, 1996, Amer & Mehlhord, 2006, Baba *et al.*, 2012). Par exemple Abagli *et al.* (2012) ont montré que 42% des moustiques atterrissent sur l'avant bras traité avec 1% d'huile essentielle *Hyptis* et 22% prennent un repas de sang. Pour le témoin, les proportions ont été de 91% et de 51%, respectivement. Ceci signifie que la fréquence pour laquelle les moustiques qui ont atterris prennent un repas a été similaire sur l'avant bras traité (52%) et l'avant bras témoin (56%). Cela signifie que l'huile essentielle a été masquante ou répulsive mais n'est pas anti-appétante.

Un des désavantages à utiliser un produit anti-appétant est que les insectes peuvent perdre leur sensibilité à celui-ci et modifier leur comportement alimentaire après une exposition répétée ou prolongée (Jermy, 1990 ; Foster et Harris, 1997). Par exemple, quand les plants de riz sont traités avec de l'extrait de neem, *Nephotetix virescens* se nourrit sur le xylème au lieu du phloème (Saxena et Khan, 1985). Il y a plusieurs mécanismes possibles pour expliquer la diminution de l'efficacité comme l'habituation ou même l'adaptation sensorielle (Akhtar *et al.*, 2003). L'habituation est un phénomène d'apprentissage ou de désensibilisation temporelle alors que l'adaptation sensorielle est un changement synaptique spécifique permanent dans le système nerveux (Bernays et Chapman, 1994). L'habituation est donc réversible : elle induit une diminution de la réponse à un produit anti-appétant après une exposition prolongée en modifiant l'information sensorielle venant des récepteurs gustatifs au système nerveux (Koul, 2008). Ce phénomène est plus souvent observé lorsque les composés

sont utilisés seuls mais ce phénomène est moins observé lorsque les composés sont en mélange (Jermy, 1987). Glendinning *et al.* (2001) ont montré qu'il y avait un phénomène d'habituation à la caféine chez *Manduca sexta* après une exposition prolongée par désensibilisation des cellules gustatives.

Discussion générale

‘Pour que la composition ait une valeur artistique il faut et il suffit que ses constituants soient délibérément choisis et proportionnés de telle manière qu'ils se conjuguent significativement pour donner une forme spécifique, donc, reconnaissable, intéressante et harmonieuse. Ce sont toutes ces exigences qui satisfaites feront d'un mélange un parfum et du parfum une oeuvre d'art’ *Le Parfum,* Edmond Roudnitska.

1. Les modes d'action des répulsifs.

A travers cette thèse, nous avons vu que le DEET avait un effet irritant sur nos 2 insectes modèles. L'équipe de De Gennaro *et al.* (2013) a montré que des femelles moustiques mutantes qui ne peuvent détecter le DEET par l'odorat étaient repoussées au seul contact du DEET, il y aurait donc bien un effet de contact. L'effet répulsif-expulsif du DEET ne semble pas résolu. En effet le DEET a été répulsif-expulsif pour *Bemisia tabaci* mais pas pour *Anopheles gambiae*. Cette molécule est également peu volatile et a été testée à 25°C en laboratoire. Lorsqu'elle est utilisée en répulsif cutané, elle est appliquée sur la peau à 37°C ce qui pourrait expliquer son manque de répulsivité sur moustiques. Récemment, chez la drosophile un récepteur olfactif sensible a été découvert et il coderait pour un comportement de répulsion (Kain *et al.*, 2014). Le DEET a également présenté un effet toxique pour les 2 insectes. En effet le DEET serait un inhibiteur de l'acétylcholinestérase ce qui pourrait expliquer son effet toxique (Corbel *et al.*, 2009). Mais le DEET aurait d'autres effets. Il aurait un effet masquant en bloquant l'attraction induit par l'acide lactique et un effet anti-appétant en activant les récepteurs gustatifs dissuasifs (Dogan *et al.*, 2009; Lee *et al.*, 2010).

Que ce soit sur le moustique ou l'aleurode, la perméthrine a montré un effet irritant mais pas expulsif (Achee *et al.*, 2012). Les pyréthrinoïdes sont peu volatils ce qui peut expliquer l'absence d'effet répulsif. Une thèse est actuellement en cours pour comprendre précisément l'effet des pyréthrinoïdes sur le comportement des moustiques sensibles et résistants. La perméthrine s'est également montrée paralysante et toxique. Les pyréthrinoides empêchent les canaux sodium de se fermer créant ainsi une succession de potentiels d'action entrainant la mort et la paralysie de l'insecte (Saldago *et al.*, 2000).

Les huiles essentielles seraient-elles des alternatives possibles aux pyréthrinoïdes? En effet les huiles essentielles sont répulsives, irritantes et toxiques en laboratoire et pourraient être utilisées comme alternative aux pyréthrinoïdes avec une technique appropriée. Nous nous demandions également comment agissaient les huiles essentielles. Elles ont un effet dose-dépendant quel que soit le produit, la réponse comportementale ou l'insecte étudié. Une réponse comportementale peut être due à un produit, un effet additif ou synergique de plusieurs produits. Un seul produit n'est pas responsable des 3 effets et les effets sont indépendants ce qui conduit à penser que les cibles et mécanismes de la répulsion, de l'irritation et de la toxicité sont différentes. Nous pouvons donc conclure qu'il est difficile d'établir une relation entre la structure chimique et l'activité biologique du fait de la complexité des huiles essentielles et des effets synergiques observés dans l'action de leurs constituants. Par contre avec les essais d'électrophysiologie, nous avons remarqué que la plupart des composés expulsifs étaient réconnus par les récepteurs olfactifs, cela va dans le sens de notre hypothèse que les expulsifs agiraient sur les récepteurs olfactifs et les irritants sur les récepteurs gustatifs. Nous avons noté également qu'un composé, le cinnamaldéhyde était très actif sur les 2 insectes. Ce composé est connu pour agir sur les récepteurs TRPA1 qui sont des récepteurs de la douleur, très communs chez les insectes (Nagata, 2007). De plus, le géraniol et le le citronellol agissent sur un grand nombre d'insectes mais leur cible est inconnue. Il y aurait donc des récepteurs spécifiques aux répulsifs communs aux espèces d'insectes mais également des récepteurs spécifiques à l'espèce puisque le thymol et le carvacrol a été irritant pour le moustique et non pour l'aleurode.

2. Pourquoi les répulsifs ne sont-ils pas plus utilisés ?

Depuis plusieurs années, les études sur les répulsifs augmentent mais l'utilisation des répulsifs n'augmentent pas pour autant (Figure 47). En effet, la principale raison de leur manque d'utilisation est que leur efficacité sur le terrain n'a pas encore été démontrée clairement comme lors de cette thèse. Il faudrait donc prouver clairement, et pas uniquement avec des modèles mathématiques, qu'un produit répulsif pourrait diminuer la transmission d'agents pathogènes et l'impact de la déviation des insectes sur de nouveaux hôtes. En effet, les effets à long terme de l'utilisation des répulsifs, comme la résistance, ne sont pas connus.

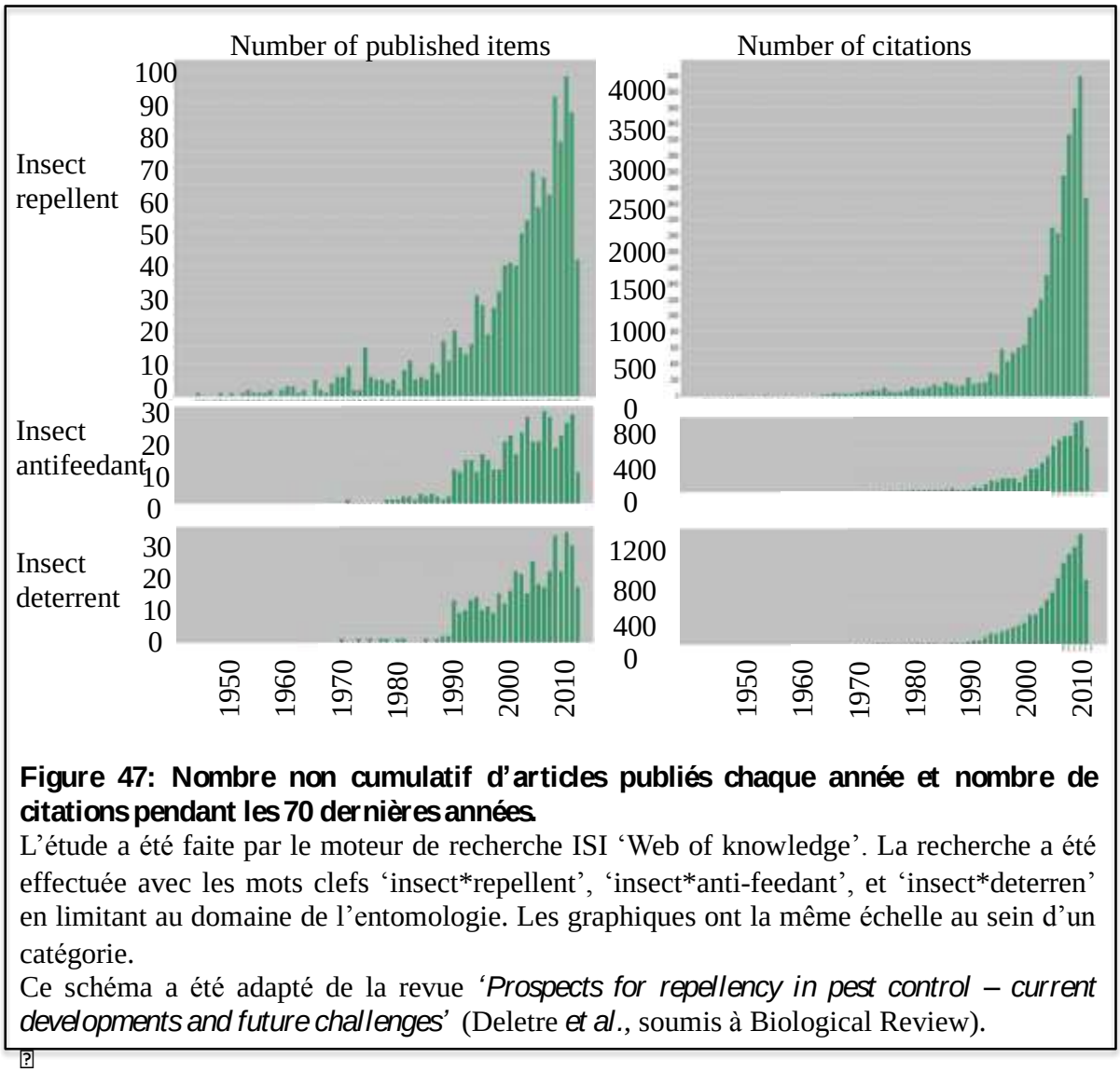


Figure 47: Nombre non cumulatif d'articles publiés chaque année et nombre de citations pendant les 70 dernières années.
L'étude a été faite par le moteur de recherche ISI 'Web of knowledge'. La recherche a été effectuée avec les mots clefs 'insect*repellent', 'insect*anti-feedant', et 'insect*deterren' en limitant au domaine de l'entomologie. Les graphiques ont la même échelle au sein d'un catégorie.
Ce schéma a été adapté de la revue *'Prospects for repellency in pest control – current developments and future challenges'* (Deletre *et al.*, soumis à Biological Review).

Plusieurs autres raisons expliquent la faible utilisation des répulsifs par rapport à l'engouement pour leur utilisation. Achee *et al.* (2012) montrent que ce manque d'utilisation est du aux faibles nombres de matières actives découvertes et disponibles et aux manques de connaissance sur leur mécanismes d'action et donc la cible de ces molécules répulsives. De plus, le manque de protocoles standardisés et donc une évaluation claire de l'efficacité des répulsifs ne favorisent pas le développement des répulsifs. En effet, dans le cas des bio-insecticides, Isman et Grieneisen (2014) montrent qu'un grand nombre d'études sont faites et

en augmentation mais également que peu d'études sont valides, il manque parfois des témoins positifs ou une absence de données sur la composition des produits ce qui explique la différence entre le nombre d'études et le nombre de produits sur le marché. Même s'il y a eu un effort de fait ces dernières années, les procédures pour mettre un répulsif sur le marché étaient également très floues.

3. Existe-t-il des résistances aux répulsifs ?

L'intérêt général pour les méthodes de lutte antiparasitaire respectueuses de l'environnement et le nombre croissant de populations résistantes aux insecticides chez les espèces nuisibles ont récemment stimulé la recherche sur les répulsifs en entomologie médicale et agricole. Mais il reste encore à confirmer sur le terrain les avantages des approches non-létales pour interrompre la transmission de maladies vectorielles. La principale explication de cet engouement pour les répulsifs est le retard potentiel de l'apparition de résistances (Achee et al, 2012).

Si les insectes sont repoussés, ils ont deux choix possibles, soient ils se retournent vers des hôtes alternatifs soient ils se nourrissent, se reproduisent et survivent moins parce que leurs hôtes sont moins accessibles (Achee *et al*., 2012). En fait, l'intensité de la pression de sélection est élevée pour un insecticide alors que la pression de sélection devrait être inférieure pour un répulsif. En effet, il modifierait le comportement des insectes et augmenterait le risque de prédation, le stress physiologique du à l'environnement ou la dépense d'énergie. Le potentiel retard pourrait aussi s'expliquer par un coût plus élevé de résistance aux répulsifs. Aucun cas de résistance à un produit répulsif n'a été rapporté, mais cela pourrait également s'expliquer par le fait qu'ils ne sont pas utilisés ou sous-utilisés en santé publique et l'hygiène domestique, et la pression de sélection peut être diluée par la multiplicité des différents ingrédients actifs, et de plus une fraction non négligeable de personnes n'utilise pas de répulsifs et sont donc des hôtes 'refuges'. De plus, il pourrait exister un retard de l'apparition de résistances s'il existait un hôte alternatif et si le coût de la résistance était plus élevé que le coût de se nourrir sur l'autre hôte. Mais la résistance pourrait être multigénique ce qui pourrait compliquer le problème.

Ils pourraient y avoir des résistances comportementales avec un évolution des récepteurs. Une équipe a récemment montré que qu'il y avait deux formes d'aedes une forme domestiques qui préfères les hôtes humains et une forme forestière qui préfère les hôtes non humains (McBride *et al.*, 2014). Cette différence est du à la surexpression d'un seul récepteur olfactif ce qui nous montre que l'evolution des comportements est possibleBien résumé par Dickens et Bohbot (2013) les modes d'action des répulsifs utilisés pour les insectes et particulièrement pour les moustiques sont activement étudiés et focalisés, entre autres, sur le DEET. Le nombre de cibles et de mécanismes d'action sont nombreux. Mais il n'est toujours pas élucidé quelle cible initie les phénomènes de répulsion. La plasticité du système sensoriel et la faculté des insectes à s'adapter rapidement à un environnement doivent être prises en compte. La réponse comportementale de répulsion peut être modulée par l'expérience et la mémorisation à court et long terme (Séjourné *et al.,* 2011). Dans le corps pédonculés, les neurones MB-V2 sont requis spécifiquement pour mémoriser les composés répulsifs et les répulsifs répriment leurs activités et la réponse induite conduit à augmenter l'évitement en augmentant les signaux du lobe latéral. Une nouvelle étude a encouragé cette idée: les populations de blattes ont évolué rapidement et ont développé un comportement adaptatif d'aversion pour le glucose, utilisé dans les pièges comme composante attractive et

phagostimulante des appâts (Wada-Katsumata *et al*., 2013). Dans les populations sauvages et adaptées de blattes, le D-fructose et le D-glucose stimulent les récepteurs gustatifs phagostimulants des sucres alors que la caféine, un anti-appétant, stimule les récepteurs gustatifs dissuasifs des composés amères. Mais dans les populations adaptées, le D-glucose active également ces mêmes récepteurs gustatifs dissuasifs des composés amères. Contrairement aux populations sauvages, leurs récepteurs inhibent l'activité des récepteurs gustatifs phagostimulants des sucres ce qui explique leur comportement de répulsion vis à vis du glucose. Nous pouvons même imaginer l'effet inverse: l'insecte adapte son comportement à un répulsif non toxique qui deviendrait attractif car il serait le signal d'un hôte potentiel. Un répulsif peut agir sur plusieurs séquences de comportement: avant, pendant et après la recherche et l'acceptation de l'hôte (Achee *et al*., 2012), de sorte qu'un seul changement de comportement pourraient être pas suffisant.

De plus, nous savons que les récepteurs olfactifs évoluent. Chez les espèces spécialistes les familles de récepteurs olfactifs évoluent rapidement et présentent un taux plus élevé de pseudogène par rapport aux espèces généralistes (Hansson et Stensmyr, 2011). Pour améliorer la détection d'une substance odorante, la stratégie est la suivante: augmenter la surface des antennes et/ou le nombre de récepteurs, et améliorer la sensibilité du récepteur (Hansson et Stensmyr, 2011). Il y a de nombreux exemples d'adaptation des insectes aux plantes, et peu de plantes qui évoluerait en s'adaptant aux attaques des insectes.

4. Les insectes généralistes et spécialistes sont-ils égaux face aux répulsifs ?

De nombreux insectes phytophages sont spécialisés sur une ou quelques espèces de plantes hôtes, ce sont des insectes spécialistes (Weenström *et al*., 2010). Les insectes spécialistes perçoivent plutôt leurs hôtes grâce à des composés spécifiques (Bernays et Chapman, 1994 ; Dudareva et Pichersky, 2006; Raguso, 2008). Comme expliqué précédemment ce qui joue sur le choix de la plante hôte n'est pas forcément le mélange d'élément type, qui ne sont pas forcément caractéristiques de la plante hôte ou de la famille, mais plutôt les proportions des composés dans ce mélange (Reissig *et al.*, 1982). Les spécialistes utilisent des signaux de contraste élevé pour améliorer l'efficacité du traitement neuronal, garantissant ainsi une reconnaissance et une discrimination rapide de leur plante hôte (Bernays, 2001). De plus, un composé commun peut avoir différentes fonctions en fonction de la plante qui l'émet et de sa perception, la réponse comportementale associée dépendra des autres composés du bouquet odorant (Dudareva et Pichersky, 2006). Ainsi la femelle doryphore est attirée par le mélange d'odeurs de pomme de terre mais sa réponse est réduite en présence d'un mélange d'odeurs de pomme de terre et de tomate (Thiéry et Visser, 1987). Les insectes spécialistes auraient également tendance à se spécialiser dans les signaux visuels vis à vis des caractéristiques des formations végétales attaquées. Même si les signaux visuels d'une plante ne sont pas assez spécifiques pour la discriminer, ils suffisent à provoquer un comportement d'attraction et avec des stimuli olfactifs, cette réponse est accentuée (Prokopy et Owens, 1978). Au contraire les insectes généralistes, de fait de l'hétérogénéité de leurs hôtes, accordent peu d'intérêts pour les signaux visuels.

Contrairement aux insectes spécialistes, les généralistes doivent faire des choix parmi un grand nombre d'options (Bernays et Minkenberg, 1997). Ainsi le choix de l'hôte par des généralistes peut être moins efficace que les insectes spécialisés car il prend plus de temps

(Bernays, 1999). L'évaluation rapide et précise entraîne donc une augmentation de leur fitness, dans certains cas, les indices visuels pourraient jouer un rôle important dans la sélection de l'hôte (Bernays, 2001). Mais chez les insectes généralistes de très faibles variations qualitatives ou quantitatives du bouquet odorant induisent des changement de comportement vis à vis de la plante hôte (Roseland *et al.,* 1992 ; Thiéry et Visser, 1986). Des modifications expérimentales du bouquet odorant de conifères ont permis de masquer le message olfactif habituel de l'hôte aux ravageurs en le rendant non attractif, répulsif, voire toxique (Dormont *et al.,* 1997 ; Nordlander, 1991 ; Smith, 1936).

5. Quelles différences entre les répulsifs d'origines animales type phéromone ou végétales ?

Certains volatils sont émis par la plante de manière spontanée et sont souvent liés à l'attraction des pollinisateurs surtout chez les plantes à fleurs (Dobson, 1994). Par contre, une autre catégorie de composés est ceux induits par le présence du ravageurs (Dicke *et al.,* 2003). Ces composés ont pour principal fonction d'attirer les parasitoïdes ou les prédateurs mais il se pourrait également que ces composés pourrait être des répulsifs (Dicke *et al.,* 2003 ; Turlings *et al.,* 1990 ; Vet et Dicke, 1992).

La sélection de la plante hôte peut être influencée par la présence d'individus de la même espèce ou d'autres espèces, voire de micro-organismes. Par exemple chez certaines espèces, les femelles perçoivent les substances végétales émises par le fruit parasité par des larves ou des substances synthétisées par les adultes ou les larves et évitent de pondre des œufs sur le même fruit. Les composés impliqués peuvent être des composés masquants comme chez la mouche de l'olive *Bactocera oleae* qui utilise le jus qui exsude du trou de ponte pour masquer les kairomones de l'olive (Propoky et Haniotakis, 1975) ou des composés répulsifs-expulsifs comme la phéromone de marquage de *R. pomonella* répandue sur le fruit après la ponte (Propoky, 1972 ; Propoky, 1976). La dégradation des plantes suite à l'attaque d'herbivores joue sur le comportement de différentes espèces d'insectes ainsi il y a également des effets inter-espèces.

Il existe des récepteurs plus ou moins spécifiques reliés donc à des neurones plus ou moins spécifiques : c'est à dire que certains peuvent être activités par un grand nombre de composés alors que les autres sont activés par un nombre restreint de composés (Hallem et Carlson, 2006). Par exemple, les récepteurs des phéromones et ceux des odeurs communes sont différents avec une spécificité et une sélectivité différentes générant ainsi un code informatif très différent dans le lobe antennaire (Christensen et Hildebrand, 2002 ; Touhara et Vosshall, 2009). Un composé odorant comme un volatil de plante peut activer un grand nombre de récepteurs et de neurones convergeant dans différents glomérules alors que les phéromones activent un récepteur et un neurone spécifique convergeant vers un glomérule spécifique (Touhara et Vosshall, 2009 ; Leal, 2013). Cela suggère que les composés de type phéromone d'alarme seraient plutôt des répulsifs de type expulsifs et les composés d'origine végétale seraient plutôt des répulsifs de type masquants. Mais certains récepteurs olfactifs impliqués dans les volatils de l'hôte et d'origine végétale peuvent être très spécifiques et sélectifs dépendant de leur concentration, de leur nombre et de leur environnement, en particulier chez les insectes spécialistes donc certains composés d'origine végétale pourrait quand même être des composés répulsifs-expulsifs (Hansson et Stensmyr, 2011).

Conclusion générale

‘Les parfums sont pleins de stratagèmes et si vous les traitez avec négligence, ils éparpillent vos secrets aux quatre vents’ Louise De Vilmorin

La répulsion chez les insectes regroupe donc tous les phénomènes qui empêchent un insecte de chercher, localiser, reconnaître et accepter son hôte : la répulsion-expulsion, la répulsion-masquante, la répulsion-irritation, la répulsion-anti-appétante et la répulsion visuelle. Faire des recherches sur des produits répulsifs est un challenge aussi bien au laboratoire qu'au champ. Mais avec des tests effectués dans un ordre précis, les différents types de phénomènes de répulsion peuvent testés, discriminés et identifiés par élimination (Figure 48). Les phénomènes de répulsion ont besoin d'être quantifiés afin que les résultats obtenus à l'échelle mondiale puissent être comparés mais jusqu'à maintenant la variabilité des méthodes utilisés : le dispositif, les paramètres, les conditions et les variables, ou un bioessai non adapté au terme utilisé ne le permettaient pas, seulement une comparaison avec les témoins positifs et négatifs le permettaient (Nerio *et al.*, 2010). Les premiers efforts de standardisation des méthodes ont été faits avec la publication des consignes de l'OMS en 2013 (WHO, 2013) pour tester des produits potentiellement répulsifs.

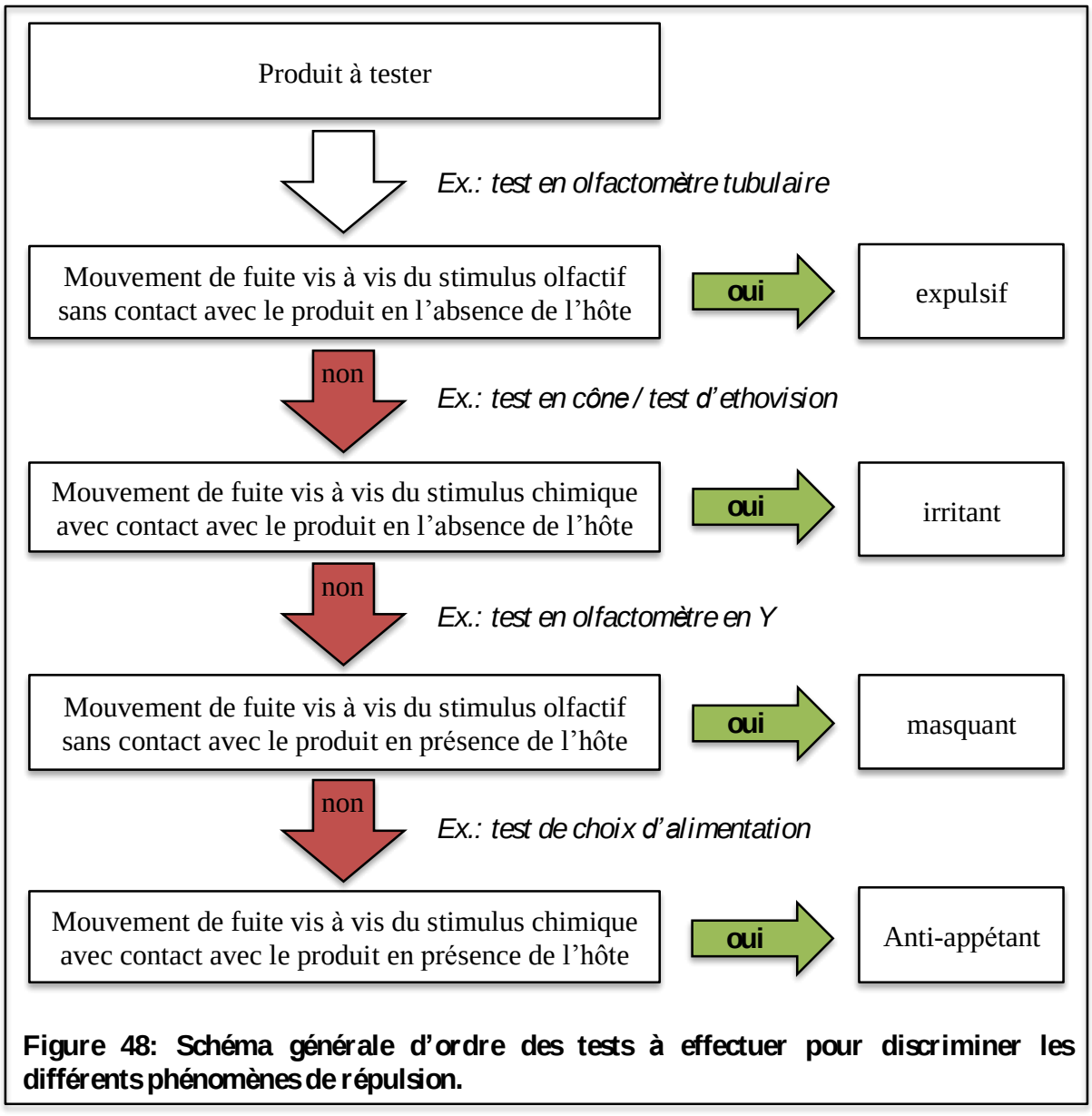


Figure 48: Schéma générale d'ordre des tests à effectuer pour discriminer les différents phénomènes de répulsion.

Les composés répulsifs agissent sur le système olfactif (antennes et palpe) et sur le système gustatif (pièces buccales, tarses, ailes, abdomen). Avec une meilleur connaissance des mécanismes d'actions de composés répulsifs, de nouvelles définitions aux phénomènes de répulsion pourrait définis en fonction de leur mode d'action et non plus sur les comportements observés (Figure 49).

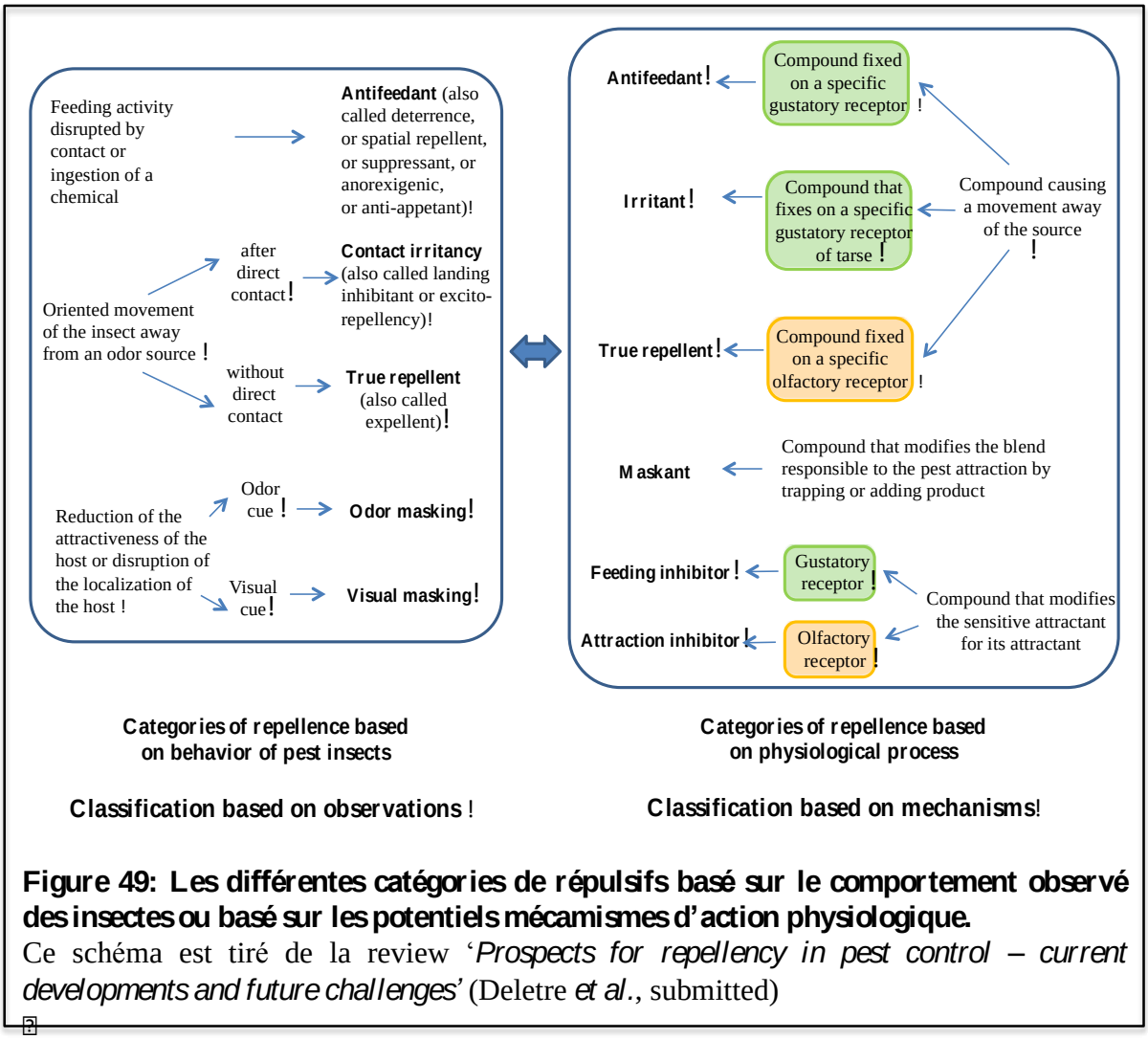


Figure 49: Les différentes catégories de répulsifs basé sur le comportement observé des insectes ou basé sur les potentiels mécamismes d'action physiologique.
Ce schéma est tiré de la review '*Prospects for repellency in pest control – current developments and future challenges*' (Deletre *et al*., submitted)

Les sources de composés répulsifs sont nombreuses et variées mais leur utilisation reste limitée. Les principaux freins au développement des répulsifs sont les couts estimés pour leur identification et le cout des analyses de leur innocuité pour l'homme et l'environnement (Kain *et al.*, 2013). Néanmoins avec les récentes avancées, la découverte et le design de nouvelles molécules répulsives pourra se faire en se basant sur la chimie moléculaire assisté par ordinateur (Leal, 2007 ; Gupta et Bhattacharjee, 2007). Kain *et al.* (2014) ont développé un modèle de criblage rapide sur l'information chimique en utilisant une approche basée sur la structure chimique pour découvrir de nouveaux répulsifs. Les récepteurs TRP, comme le récepteur TRPA1 impliqué avec le cinnamaldéhyde, pourrait être une cible des répulsifs et pourrait être utilisé avec ce type de criblae rapide pour découvrir de nouveaux répulsifs. Le besoin d'avoir un environnement sain, et les problèmes de résistances seront le moteur de ces recherches. Diverses stratégies de contrôle des insectes basés sur les composés sémiochimiques comme l'utilisation de répulsifs, de pièges attractifs, de confusion sexuelle sont les stratégies de contrôle du futur.

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‘Le mauvais goût a son droit autant que le bon gout’
Friedrich Nietzsche

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Articles associés

‘[…] L’odeur et la saveur restent encore longtemps, comme des âmes, à se rappeler, à attendre, à espérer, sur la ruine de tout le reste, à porter sans fléchir, sur leur gouttelette presque impalpable, l’édifice immense du souvenir’ Marcel Proust, *Du côté de chez Swann*

Prospects for repellency in pest control – current developments and future challenges

(Soumis à Biological Review)

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ABSTRACT

The overall interest for environmentally safe pest control methods and the rising of insecticide resistance in pest populations stimulate researches on insect repellents during the last years in medical and agricultural entomology. However, there are still several difficulties to work on repellency: 1) the different repellent phenomena are not well defined, 2) it is difficult to test and to quantify repellency, 3) the physiological mechanisms are poorly known, 4) field efficacy appears to be highly variable. Five different types of repellency have been defined: expellency, irritancy, deterrency, odor masking and visual masking. With precise and ordered bioassays where the stimuli vary it is possible to test and discriminate between them. Today these categories are defined by their behavioral response to different stimuli, but in future they could be defined by their mechanism of action. There are three main hypothesis of physiological mechanism: 1) a dose effect that modifies the behavior, 2) a repellent mechanism with specific receptors, or 3) an inhibition of the transduction of the neural information.

Key words: repellent, deterrent, anti-feeding, irritant, odorant receptor, olfaction, gustation, IPM, push-pull, pyrethroids.

CONTENTS

I. INTRODUCTION

The plant kingdom produces several hundred thousand chemical substances, which affect insect behavior (Weenström *et al.*, 2010). Secondary compounds are involved in recognition and acceptability of plant, particularly the volatil organic compounds which may be species-specific and

emitted in specific ratios (Bruce *et al.*, 2005a). Reception of host cues encompass a wide variety of stimuli: olfactory, visual, tactile, and gustatory (Thorsteinson, 1960). Host selection is believed to proceed in a hierarchical manner. The first step 'choice' is the seeking and recognition of the host using olfactory and visual cues, and the second step 'selection' is the acceptability of the host using gustatory cues (Visser, 1988).

Many phytophagous insects are specialized on one or a few species of host plant (Weenström *et al.*, 2010). Specialists use high contrast signals to improve the efficiency of neural processing, thus ensuring rapid recognition and discrimination (Bernays, 2001). Herbivores can be attracted over relatively long distance, which proves the sensitivity of its olfactory system, i.e. specialist receptors to host volatils. For example, the cabbage seed weevil *Ceutorhynchus assimilis* is attracted from >20m by the isothiocyanates of its host plant (Evans & Allen-Williams, 1993), and 30% of the olfactory neurons respond to isothyocianates (Blight *et al.*, 1989). Odors may function in behavioral efficiency after contact with the host but for some specialist gustatory cues also play a role (Bernays, 2001). For example, contact chemoreception play an important role in host selection for the cabbage root fly, *Delia radicum* which is very sensitive to a novel chemical called ' cabbage identification factor' present in leaf surface of *Brassica oleacera* (Roessingh *et al.*, 1997).

Host selection by gustatory cues is governed by the balance of phagostimulatory and deterrent inputs (Chapman, 2003). But in specific case visual cues may be even more important than odors. Specialists could home in on a particular shape, especially if their host plant has a leaf shape that is characteristic (Bernays, 2001). For example, host seeking in aphids can be based on the blend composition of the host volatil compounds and/or on the ratio of ubiquitous host volatils and/or on the color and shape of the host depending on the species and the morph (Webster, 2012). After the aphid alights on a putative host, chemotactile and gustatory cues play a key role in host acceptebility (Powell *et al.*, 2006).

In contrary to specialists, generalists must make choices among a large number of options (Bernays & Minkenberg, 1997). Rapid and accurate assessment leads to increased fitness, so in this case visual cues can play an important role in host selection (Bernays, 2001). Thus, host choosing by generalists may be less efficient than specialist insects (Bernays, 1999). Signal to noise odor contrast (Bernays, 2001), and volatil ratio appear to play key roles in recognition (Bruce *et al.*, 2005a). A knowledge gap is to understand the mechanisms of spatio-temporal resolution of olfactory signals (Bruce *et al.*, 2005a). Like phytophagous species, insects feeding on human or animal hosts use olfactory, visual,

tactile, and gustatory stimuli to find their hosts. Hence, the interaction animal-insect can share resemblance to the interaction plant-insect.

The overall interest for environmentally safe pest control methods and the increasing number of insecticide resistant populations among pest species have recently stimulated research on insect repellents in medical[1] and agricultural entomology. The main explanation for this enthusiasm for repellents is the potential delay of the onset of resistance (Achee *et al,* 2012). Actually the intensity of selection pressure is high from contact-mediated toxicity whereas the selection pressure should be lower from a repellent that would modify the insect behavior and would increase the predation risk, the physiological stressful environment or the energy expenditure (Achee *et al,* 2012). The potential delay could be also explain by a higher resistance cost for a resistance to repellent. No resistant case to repellent product have been reported, especially because they are not used or underutilized in public health and domestic hygiene and the selection pressure may be diluted by the multiplicity of different active ingredients and moreover a non-negligible fraction of people who do not use repellents. But we will still need to confirm in the field the benefits of non-lethal approaches for interrupting vector diseases transmission. Moreover, there is a delay of the onset of resistance if there is an alternative host and if the cost of resistance is higher than the cost to feed on the alternative host but the resistance could be multigenic that could complicate the problem. A new study has challenged this idea: populations of the German cockroach have rapidly evolved an adaptive behavioral aversion to glucose, the phagostimulatory component of baits (Wada-Katsumata *et al.*, 2013). We can even imagine the inverse effect: the insect will adapt their behavior to a non-toxic repellent that should become attractant. A repellent can act on several behavior sequences: pre-, during- and post-host-seeking and acceptance (Achee *et al.*, 2012), so a single behavioral change could be not enough. We know that odorant receptors (ORs) evolve. In specialist species the OR families evolve faster and show a higher rate of pseudogene compared to generalist species (Hansson & Stensmyr, 2011). To improve the detection of an odorant, the strategy is : to increase antennae surface area, number of receptors, and to improve the receptor sensitivity (Hansson & Stensmyr, 2011).

The word « repellent » has been used to characterize many different phenomenon. The word *repellent* derives from the Latin verb *repellere*, meaning « to reject ». Hence, strictly speaking, a substance

[1] [1] Here medical entomology is considered on a broad aspect including both medical and veterinary entomology

should only be considered as a repellent when it induce a movement away from its source. However, this definition is clearly too narrow. In this review, we retain a wide definition for repellency: a phenomena that prevents a pest's ability to track, locate and/or recognize its host. Hence, a repellent phenomena can be a movement away from an odor source but also an inability to find the host. A mechanism that nullifies the attraction of an insect to an odor source should also be considered a repellent (Ramirez *et al.,* 2012). Today a repellent is defined by a behavioral response to a stimulus (Miller *et al.,* 2009). The difference between behavioral responses can be very subjective. The consideration of the stimulus that produced these reactions enables to discriminate in among different kinds of repellents. The most important problem with repellent research is the use of bioassays that do not adequately test for the associated term.

Conducting research on repellents is challenging, in the lab as well as in the field. A specific test order permits discrimination of the different types of repellency by the process of elimination (Figure 1). Whereas repellent activity needs to be objectively quantified, the comparisons among results from different studies worldwide is difficult due to the variability of methods used to assess repellency, i.e. each author used different apparatus and/or conditions, variables and parameters. Consequently, results only can be compared with a positive or negative control, or to other product tested in the same assay, in order to classify the products in a grade of repellency (Nerio *et al.,* 2010). However there are some advances in methodologies; for example the World Health Organization (WHO) has recently published guidelines for testing repellent candidates product on mosquitoes (WHO, 2013).

We review here five types of repellence on the basis of the observed behavior of insect: 1) true repellence (also called expellent, spatial repellency) which corresponds to oriented movement of the insect away from an odor source without direct contact, 2) odor masking, a reduction of the attractiveness of the host or disruption of the localization of the host by the odor cue, 3) contact irritancy (also called landing inhibition or excito-repellency), an oriented movement of the insect away from a chemical after direct contact, 4) deterrence (also called antifeeding, suppressant, anorexigenic, and anti-appetant), a disruption of feeding activity by contact or ingestion of a chemical, and 5) visual masking, a reduction of the attractiveness of the host or disruption of the localization of the host by a visual cue (Figure 2). Then we reviewed the potential action mechanisms by the olfaction and gustatory pathways. To illustrate the potential of repellency in insect management to protect human or

plants, we reviewed two examples of well-known strategies: the use of impregnated bednets for disease control and the push-pull strategy for crop pest management.

II. REPELLENCE PHENOMENA

We reviewed here different phenomena of repellency by giving them a precise definition, illustrating some experimental examples, discussing the potential mode of action of the repellent compounds and summarizing the advantage/disadvantage to use a repellent in insect management.

(1) True repellent (avoidance, expellent)

A *true repellent* is a substance which can act at a distance by vapor phase causing oriented movement away from the odor source (Mathews and Mathews, 1978; Bernier *et al.*, 2007; Nerio *et al.*, 2010). For example, the aphid alarm pheromone – of which (E)-β-farnesene is the main (if not the only) component in most species – is a true repellent: aphids disperse in its presence (Cook *et al.*, 2006). In this manuscript we recognize a difference between a true repellent that prevents the host attack by creating a barrier and an antifeedant that interferes with feeding response (cf. §5). Actually a true repellent creates an odor barrier, deterring the insect from coming into a contact with the host surface (Brown & Hebert, 1997). So a true repellent compound often has high vapor toxicity to insects (Maia & Moore, 2011). Moreover, we recognize a difference between a product that is a true repellent and a product that inhibits host finding like a maskant product (cf. §2). Hence the aim of a spatial repellent is to prevent an arthropod from entering a space occupied by a potential host, as a « safe zone », and so to reduce the encounters between the insect and the host; the term of expellent is sometimes used in medical entomology (Achee *et al*, 2012). In the case of a pathogen vector the probability of pathogen transmission could be eliminated or reduced.

The behaviorial avoidance is a response caused at a distance by a compound that the insect « learned » to avoid, it is a resistant or protective behavior (White, 2007). *Avoid* connotes mental intent as part of the mechanism being employed by the insect (Kennedy, 1977) so this term can be used when the response is different within the population e.g. naive individuals *vs* exposed individuals. For example, populations of the *Bemisia tabaci* can learn to avoid tomato plants protected by the *B. tabaci* predator:

contrary to pre-exposed *B. tabaci*, the naive individual did not avoid plant hosting its predators (Nomikou *et al*, 2003). But sometimes the term « avoidance behavior » is used to describe a behavioral response of a movement away due to a combination of true repellent and irritant elements (Chareonviriyaphap *et al.*, 1997).

True repellency should be tested (i) using a bioassay that prevents contact between the insect and the test material and (ii) without the host presence that could have a masking effect, or otherwise could disturb the insect. Repellency can be studied with a tubular olfactometer oriented vertically or horizontally with a flowing or still air. For example, Zhang *et al.* (2004) used a still-air vertically oriented olfactometer to show repellency of ginger oil to *Bemisia argentifolii*. The principle was to place the product at the top end of the cylinder and in general way insects go up in a vertical cylinder or to attract the insect by the light at the cylinder top (positive phototaxis). With a repellent product insects remained at the bottom of the cylinder such as *Bemisia tabaci* in the presence of cinnamaldehyde (Deletre *et al.*, in preparation). A new system of tubular oflactometer was recently designed by Steck *et al.* (2012). In this system fifteen tubes are aligned horizontally with one insect inside (i.e. *Drosophila melanogaster*) with air flow and the insect was exposed to repeated odor pulses. The insect's position is visually tracked: repellent odors evoke decreased activity followed by downwind movement whereas attractive odor elicits directed upwind movement. The four-arm olfactometer is used to show an attractive or repellent odor. A chamber is connected to the arm to deliver the odor and prevent any visual cue and each arm is an odor source. For testing an attractive odor there is one arm which delivered the odor and the three others are control (Ukeh *et al.*, 2012). For testing a repellent odor the same arena has been used: the test odor is delivered in only one arm and the three others are control (Bruce *et al.*, 2005a; Webster *et al.*, 2010). But the opposite has been also used: the test odor was delivered in three arms with only one control arm (Togni *et al.*, 2010).

It is difficult to study repellency with a Y olfactometer because if the compound is repellent the insect will not go all the way to the choice zone. To get round the issue the insect can be put directly in the choice area. For example, Grieco *et al.* (2005) put the mosquitoes *Aedes aegypti* in the central part of a cylinder divided in three parts. The mosquitoes that want to escape have the choice between a treated and a non-treated chamber. To put a non-chemical attractant like light or heat source may provoke a faster choice by the insect. This bioassay is recommended by the WHO (WHO, 2013). If the insect is

not really active the tube can be adpated i.e. instead of using a cylinder divided in three parts the cylinder can be divided in two parts (Deletre *et al.*, 2013). In this case the insect are introduced in the chamber with the product and the proportion of mosquito that move to the second chamber was recorded to determine the repellent effect.

One disadvantage to use of a true repellent is that a product can be less repellent than a host is attractant. For example, geraniol is a repellent for *Anopheles gambiae* without host, but not with a cobbaye in a tunnel (Deletre *et al.*,in preparation).

The volatil compounds can also be toxic which is called fumigation toxicity. For example, the vapor toxicity of several monoterpenoids was showed on stored product insect: pulegone and fenchone were very toxic against the rice weevil, *Sitophilus oryzae*, the red flour beetle, *Tribolium castaneum*, the sawtoothed grain beetle, *Oryzaephilus surinamensis*, the house fly, *Musca domestica*, and the German cockroach, *B. germanica* (Lee *et al.*, 2003). Because these products have been shown to be repellent, the true repellency could occur at a sub-lethal dose of fumigant product. Moreover there is another effect between repellency and toxicity: the Knock-down effect. It is a rapid incapacitation with sometimes metabolic recovery, the early insect response to a pesticide (White, 2007). Pyrethroids are well known to have a knockdown effect on *Anopheles gambiae* (Chandre *et al.*, 2000).

(2) Odor masking (attraction-inhibition)

We call a masking odor, a compound that decreases the attractiveness of the host or interferes with its localization. Such compounds are therefore not repellent by themselves: they change the host chemical profile by modifying the ratio of compounds or by suppressing or adding another compound thereby impeding insect's host seeking. In medical entomology a spatial repellent has a wide meaning it could be an odor masking or true repellent.

Nolen *et al.* (2002) defined it as an inhibiting compound dispensed into the atmosphere that inhibits the mosquitoes to locate and track a host. It is a product that interferes with the host detection and feeding response (WHO, 2013). For example, the emission of Rutgers 612 (2-ethyl-1,3-hexanediol) inhibits the normal behavioral response of mosquitoes to an increase in the carbon dioxide gradient, that attracts the mosquito to a potential host (Simpson & Wright, 1971). This kind of compound is called an attraction-inhibitor (Bernier *et al.*, 2007). The aim of an odor masking product is to hide the host to the insect. For example, the N,N-diéthyl-3-méthylbenzamide (DEET) inhibits the acid lactic

attraction of the mosquito *Aedes aegypti* (Dogan *et al.*, 1999). DEET can also reduce the 3-octenol release hence changing the chemical host profile and disturb or prevent host seeking (Syed & Leal, 2008). In agriculture, intercropping systems use the odor masking of plants: the aim is to decrease the attractiveness of the host crop or to hide the host crop from the insect pest by another crop (Cook *et al.*, 2007). For example Togni *et al.* (2010) showed that *B. tabaci* preferred a pure tomato plot rather than tomatoes mixed with coriander. Coriander constitutive volatils were not repellent to *B. tabaci* per se but they decrease the preference for tomato constitutive volatils so it hence acting as an odor masking effect (Togni *et al.*, 2010).

To evaluate the masking effect of a product the insect should smell the host and the test product but must not be in contact with the product to avoid any eventual irritant effect. The product must also be tested alone – ie in the absence of the host – to test whether it has an expellent property. The olfactometer method for testing true repellency can be also used for assessment of masking effect if the host odor is added. For example, the four arms olfactometer can be used: all the arms have the host odor and one arm has also the repellent odor. The time spent in each arm was recorded and tested for significant differences. With this method, Bruce *et al.* (2005b) showed that *Hemizygia petiolata* essential oil had a masking effect of cabbage odor for the aphid *Myzus persicae.* Contrary to the true repellent effect the Y-tube bioassay can also be used for testing the masking effect (Nerio *et al.*, 2010). One arm contains the host – be an animal or a plant – attractant odor, the other containing the host odor and the test product. The number of insects in the two arms is scored to assess the percentage of masking activity. This test is recommended by the WHO (WHO, 2013). For example, Obermayr *et al.* (2012) showed that homopiperazine has a host seeking inhibition effect with a 95% reduction of mosquitoes in the olfactometer arm with the test compound compared with the control.

One way to study a maskant odor is to compare the attractiveness of the plant and the attractiveness of the plant with the candidate product in a choice test. The olfactometer method is a good way to study the maskant effect i.e. all the arms deliver the host odor and one arm delivers also the maskant compound. The arm with the host odor and the compound will be less choosen. The olfactometer method is not easy to prepare and easier choice tests exist to study the masking effect of an odor. The masking effect can be also studied in a choice test between the whole host vs the whole host and the candidate product diffuser, which can be either a plant, an animal, or a treated chomatography paper

(Bleeker, 2009). But the product should not be on the host because the behavioral response could be mediated by contact as an irritant and at distance as a maskant product.

The masking effect of a product may be also studied with a no choice test. Instead of comparing insects that have chosen the host against the host with the masking product, the amount of time spent to find the host may be compared. The number of insects that reach the host and the host in the presence of the masking product can also be compared. In medical entomology, the host seeking behavior includes the ability of mosquitoes to locate the target, to land on skin, and to search for a suitable site for probing (Hao *et al.*, 2008). For example, the human arm in a cage assay can be used to study the masking effect (Schrek, 1977; Nerio *et al.*, 2010). A subject (human or animal) has a treated arm and a non-treated arm in two different cages with unfed female mosquitoes (WHO, 1996). The time before the first bite (protection time) and the number of landing during a given period of time can be recorded and compared to the protective efficacy of the treatment.

For generalist insects making a host choice decision can be difficult when there are several odors from which to choose. Specialist insects do not face this dilemma, as they can focus on one important stimulus while ignoring others (Bernays & Funk, 1999). Hence, by adding different odors in the system it might be easier to disrupt the behavior of generalist rather than specialist insects.

(3) Irritability (excito-repellency, landing inhibition, contact disengagement)

An irritant product causes a movement away from the stimulus after the insect has physically contacted it (Hilje & Mora, 2006). In medical entomology, this movement away is also called landing inhibition (WHO, 2013). Several kinds of irritants exist depending on the behavioral response they induced. For instance, pyrethroids are excito-repellent because they increase the mosquito activity called hyper-locomotor activity (Miller *et al.*, 2009). The aim of an irritant product is to break the physical association between insects and hosts, and thereby reduce the probability of feeding and the risk of pathogen transmission (Achee, 2009).

By definition, the irritant effect must be tested using an experimental arena allowing contact between the insect and the tested product. The test must be performed in the absence of the host because it could disturb the insect and generate an antifeedant effect (see below). The product must be tested also without contact to reveal a potential expellent effect.

For crawling insects, Petri dish bioassays are common (Chaubey, 2007; Wang *et al.,* 2006). One half of a filter paper is treated by the studied product, the other half with the solvent for control. Because a product could be toxic, the challenge is to work at a sublethal concentration (Schrek, 1977). Insects are released at the center of the Petri dish. After a determined period of time the number of insects on the two parts are compared. The time spent on treated and on control area can also be compared (Nilsson & Bengtsson, 2004; Martin *et al.,* 2013). Video tracking facilitates the recording of insect behavior moving quickly or slowly or during long periods of time or during the night (using, in such cases, UV light). For example, behavior of collembolans was followed by video tracking and they were found to avoid the treated part in petri dishes. It was then concluded that they were irritated by endogenous free fatty acids by using this test (Nilsson & Bengtsson, 2004).

For flying insects, such as mosquitoes, a plastic cone where a treated material is placed at the base of the cone is used. After a short time of acclimation, the mosquito is landing on the the treated material and then the time before the first take off is recorded. The comparison are made for a series of mosquitoes exposed to treated or untreated material (Mouchet & Cavalie, 1961; Chandre *et al.*, 2000). Another method is the use of a cylinder divided in two parts: a chamber covered with a treated paper and a chamber covered by a non-treated paper separated by a butterfly valve (Grieco *et al.,* 2005; Deletre *et al.,* 2013). The mosquitoes are placed in the treated chamber, the valve is open and 10 min after the number of mosquitoes in the non-treated chamber is recorded for characterizing the irritant effect.

The major property of an irritant product is that insects cannot stay on the treated material thus reducing the biting, and risk of pathogen transmission. In *Anopheles gambiae*, kdr resistant populations are less irritated by pyrethroids than the susceptible population (Chandre *et al*., 2000). This suggests one target (sodium channel) with two different effects depending on dose or exposure time: irritancy and toxicity.

(4) Anti-feeding (suppressant, deterrent, anorexigenic, attraction antagonist, feeding inhibitor)

An antifeedant product acts on the feeding behavior by preventing, interrupting or otherwise disrupting the feeding activity. This can happen either once the insect enters in contact with the product (Koul, 2008) or following postgustatory effects of this product (Cook *et al*., 2006). Here, we will focus on first cause. Contrary to an irritant compound, an antifeedant may not cause the insect to

move away from the product but only inhibits feeding behavior (Kim, 2013). A suppressant inhibits the initiation of feeding, a deterrent impedes the continuation of such process and an anorexigenic causes a loss of appetite (Wharthen & Morgan, 1990). For example, the tobacco hornworm *Manduca sexta* approached the deterrent *Grindella*-treated diet but rejected it within 6 seconds of initiating biting (Glendinning *et al.,* 1998). An antifeedant is a peripheral-mediated behavior modifying substance resulting in feeding deterrence (Isman, 1994). The aim of these substances is to reduce or stop the feeding behavior (Foster & Harris, 1997). In agricultural entomology, the product is generally directly applied on the resource (Foster & Harris, 1997) and an example of a well-known antifeeding product is neem extract used against a large range of agricultural pest (Cook *et al.*, 2006). In medical entomology, a product interfering with feeding behavior is called a feeding inhibitor (WHO, 2013) and an attraction antagonist is a substance that interrupts the blood-feeding process (Bernier *et al.*, 2007).The term deterrent is used in a broader context: prevention of mosquitoes to bite or even to enter a house (White, 2007). Landing inhibition (irritant effect of the product) and feeding inhibition are characteristic to the protective efficacy of a product (WHO, 2013).

To test the anti-feeding effect of a product the insect should smell the host with the test product and be in contact with the product. Therefore, the properties of irritancy and masking effect have to be previously tested separately.

A two-choice assay is generally used to test an antifeeding product on an herbivore. For example, Abdelgaleil *et al.* (2002) placed ten snail larvae in Petri dish with treated leaves or non-treated leaves and after 6 hours the weight of leaves were compared. It is also possible to measure the weight of insect or the consumed surface of leaves. For example, Akhtar *et al.* (2011), using the same experimental set up with *Trichoplusia ni* larvae, took pictures to determine the areas of leaf discs eaten by the insects, from which a feeding deterrence index was calculated.

For piercing and sucking insects, although it is difficult to study potential anti-feeding product, it is possible to observe the damage or to calculate the number of insect settled on treated leaves. However, this assay do not discriminate irritant from antifeedant effects. Still, the specific insect behavior and life traits may be taken into account to test the antifeeding effect. For example, Hammad *et al.* (2001) tested antifeeding product with treated tomato plants and non-treated tomato plants in different cages with *B. tabaci* whitefly adults. After 3 days the number of whiteflies on each type of plants was recorded to characterize the antifeedant effect. The oviposition of *B. tabaci* occurs normally when the

insect is feeding on the plant so the different number of eggs on each type of plants enabled determination of whether the product was antifeedant or not. For testing the efficacy of feeding inhibition on mosquitoes, the human bait-technique or arm-in-cage test (Schrek & McGovern, 1989; WHO, 1996) is often use. It consists to put a forearm not treated or treated with the studied product is inserted in a cage with mosquitoes and the data are recorded for the first 3 minutes of every 1/2 hour to evaluate the protection time (Baba *et al.,* 2012). The number of landing mosquitoes on the treated area and the number of bites are recorded (Amer & Mehlhord, 2006). The proportion of bites compared to the proportion of landing permits to characterize the deterrent effect of the product. For example, Abagli *et al.* (2012) showed that 42% of mosquitoes landed on a forearm treated by 1% of *Hyptis suaveolens* essential oil and 22% of mosquitoes took a blood meal. For the control, these proportions were 91% and 51%, respectively. This means that the frequency at which landed mosquitoes took a meal was similar on the treated (52%) and control (56%) forearm. This essential oil at 1% was irritant but not antifeedant. Scientists sometimes used the number of bites received through time -providing a measure of the dose effectiveness of the repellent- but the number of bite through time did not inform on the kind of repellent. Actually if this number is low it could be due to an expellent, a maskant or an antifeedant compound. For other insects as drosophila, to study antifeeding effect it is also possible to use the proboscis extension, when antifeeding compounds are mixed with attractive compounds, such as sucrose, as this leads to a significant reduction of proboscis extension reflexes (Amrein & Thorne, 2005).

A disadvantage to use an antifeedant product is that insects can lose sensitivity to it or they can change their mode of feeding after repeated and prolonged exposure (Jermy, 1990; Foster & Harris, 1997). For example, when rice plants were treated with neem extracts, *Nephotetettix virescens* fed on xylem instead on phloem (Saxena & Khan, 1985). There are several possible mechanisms for the decrease in efficacy including sensory adaptation, motor fatigue and habituation (Akhtar *et al.*, 2003). Habituation is a phenomenon of learning or temporal desensibilisation whereas sensory adaptation can be a persistent synaptic change in specific neural pathway (Bernays & Chapman, 1994). So habituation is reversible. It is a decrease response to antifeedant after a prolonged exposure resulting from an effect of mouthpart chemosensory information on the nervous system (Koul, 2008). This phenomenon is more often observed with single product than with mixtures (Jermy, 1987). Glendinning *et al.* (2001) showed in *Manduca sexta* that its adapted aversive response (exposure induced adaptation) to caffeine

was mediated directly by the desensitized taste cells.

(5) Visual masking

A product or an object can modify the shape or the color of a crop or hide the host, hence ensuring a visual masking effect. The aim of this repellent is to disturb insect' host recognition. For example, the use of a net for protecting cabbages may delay infestations by aphids (Martin *et al.*, 2006). Indeed, to find their hosts, insects not only use odors but also visual cues like size, shape or color of the plants, particularly in the final stages of finding those plants (Bernays & Chapman, 1994). The visual stimulant may be used as an attractant-stimulant combined with insecticides or glue to trap them. For example, blue and black traps are used to control or survey different cattle flies but we could imagine that some colors could be repellent (Gibson & Torr, 1999; Baldacchino *et al.*, 2013).

It appears difficult to change the visual properties of a plant to decrease its attractiveness, but for example, gibberelic acid has been used to keep grapefruit green which is less attractive than yellow fruit to the fruit flies (Foster & Harris, 1997). The use of UV absorbing plastics as greenhouse covers may also reduce the spread of insect-borne virus diseases (Raviv & Antignus, 2004). This is because these UV absorbing plastics modify insect behavior: (i) in high UV reflectance environment anthophilous thrips are repelled from the surface of attractive colors, (ii) in *B tabaci*, adult dispersion was hindered with a filtration of UV light and (iii) in an UV deficient environment the flight activity of *Myzus persicae* was reduced. Moreover, colored shade netting may be effective against insect pests: whiteflies landed on yellow nets but did not penetrate to reach the plant and thrips were less likely to penetrate through blue and yellow nets (Weintraub, 2009). Finally, maize is often used to dissimulate some crops to their pests and can therefore be considered as a visual masking plant (Smith & McSorley, 2000).

III. POTENTIAL ACTION MECHANISMS

In the following, we consider that insect pests can detect true repellent or masking compounds through sense of smell and irritant and antifeedant compounds (i.e. non volatil compounds which deter insects at contact) through sense of taste (Kim, 2013).

(1) Olfaction pathway

Insects recognize their hosts by host' odors characterized by the blends of volatil compounds, their ratio and their diffusion in space and time (Sachse & Galizia, 2003; Bruce *et al.*, 2005a). Insects have two pairs of olfactory organs, the antennae and the maxillary palps, covered by different kind of sensilla: basiconic sensilla, trichoid sensilla, and coelonic sensilla. Why two 'noses'? These organs can serve distinct functions as the maxillary palps of *An. gambiae* mosquito that serve to detect CO_2 but palp and antennae can also show overlapping response spectra as CO_2 detection by antennae and palp in *D. megalonaster* (Hansson & Stensmyr, 2011). Palp could also play a role in taste enhancement.

Each sensillum houses 1-4 olfactory receptor neurons (ORNs), each ORN expresses a unique combination of olfactory receptors (ORs) with olfactory co-receptors (Orco) and projects axon into a single olfactory glomerulus in the antennal lobe (AL) (Kim, 2013). In the AL there are interneurons between glomeruli and projection neurons (PNs) that link the AL to the higher order processing centers: the mushroom bodies (MB) and the lateral horn (LH) (Chritensen & White, 2000). Odorant molecules pass through the sensilla pore tubule, diffuse into the endolymph by means of binding proteins (BPs) and these BPs carry the molecule to ORs (Leal, 2013). The binding of the odorant to the ORs leads to the opening of OR-associated ion channel and a subsequent depolarization of the ORNs (Kaupp, 2010). In the AL the activated glomeruli activate interneuron that modulate the activity of projection neurons (PNs) and depolarization in PNs conveys information until the MB and the LH (Ache & Young, 2005). In summary one OR can recognize multiple odorants and one odorant can be recognized by multiple ORs, but different odorants are recognized by different combinations of ORs. The olfactory system uses a combinatorial receptor coding scheme to encode odor identities. This code is conveyed to the brain that causes behavioral response.

So olfactory receptor neurons encode qualitative, quantitative, temporal, and spatial information about odors (De Bruyne *et al.*, 1999). The odor sensitivity is proportional to the number of available ORs, their specificity and their affinity to the odorant molecule (Gomez-Martin *et al.*, 2010). The same odorant can also activate some ORs and inhibit others (De Bruyne *et al.*, 1999). Moreover odorants can elicit different temporal responses so temporal coding also enhances the insect ability to recognize odors (Kaupp, 2010). The OR activation could be due to the odorant alone or to the BP-odorant complex (Leal, 2013). It is now accepted that ORs play a significant role in odorant selectivity but the

other olfactory proteins as BPs contribute to the overall odorant specifity and sensitivity of the insect olfactory system (Leal, 2013). Today the mechanisms mediating insect olfactory transduction are still controversial, Or are either ionitropic receptor or seven-transmembrane-protein with an inverse topology that formed heteromers with a well conserved coreceptor (Touhara & Vosshall, 2009; Leal, 2013). Actually the speed of G-protein-mediated transduction utilized in the vertebrate olfactory system is relatively slow versus the insect one and the use of ionotropic receptor avoids energy consumption in second messenger cascades as ATP or GTP (Ha & Smith, 2009; Touhara & Vosshall, 2009). The OR coreceptor is not involved directly in odour recognition but it takes part in the signal transduction (Hansson & Stensmyr, 2011) and assists in receptor trafficking, targeting and tuning (Kaupp, 2010). For example, De Genarro *et al.* (2013) showed in *Anopheles gambiae* orco mutant mosquitoes was less attracted to honey and did not respond to human scent in the absence of CO_2 and the spontanous activity and the odor responses of the O*rco* mutant olfactory sensory neurons were reduced.

Odor information is first coded by the distribution of several activated glomeruli corresponding to activated ORNs by the odorant molecule (Séjourné *et al.*, 2011). All ORNs expressing the same receptor converge onto one glomerula (Gomez-Martin *et al.*, 2010). The odor information is treated by local interneuron by the modulation (inhibition or activation) of ORNs to projection neurons (PNs) transformations and result of odor code in a signal-to-noise ratio improvement in PNs (Cunningham, 2012). Cortical representations of odor information created in the AL is not known (Touhara & Vosshall, 2009). The MB is probably a site for olfactory learning and memory and experience-dependent modulation of olfactory behavior whereas the LH appears to be a site for innate, experience-independent modulation of olfactory behavior (Gomez-Martin *et al.*, 2010).

How might a compound acting on this olfactory system result in a repellency behavior? The molecular target and signaling pathway involved in sensing insect repellents as well as antifeedant are poorly understood (Kim, 2013).

(a) A matter of concentration and/or ratio?

In *Drosophila,* the odor concentration affects the behavioral response: many odorants are repellents at high concentrations but attractants at low concentrations (Foster & Harris, 1997; Hallem & Carlson,

2006; Semmelhack & Wang, 2009). The recruitment of further glomeruli has been proposed as a mechanism to mediate this switch in behavioral response (Semmelhack & Wang, 2009). Individual odorants activate distincts subsets of ORs, resulting in the contruction of a glomerular activation pattern – odor map- but different concentrations result in different patterns (Touhara & Vosshall, 2009). Actually odorant receptors have different activation thresholds and glomeruli also have different levels of excitation (Cunningham, 2012). At high concentrations, most odorant molecules activated multiple receptors, whereas at low concentration fewer receptors were activated (Hallem & Carlson, 2006). De Bruyne *et al.* (1999) showed also that in the maxillary palp of *Drosophila* the pb1A neuron was broadly tuned by odorant at high dose but it was narrowly tuned by low dose. Moreover, in *Drosophila* two glomeruli DM1 and VA2 were identified as mediators of attraction to vinegar at low concentration, whereas vinegar at high concentration became aversive and activate on additional glomerulus, DM5 which mediate the decrease of attraction (Semmelhack & Wang, 2009). The different glomerular activation pattern initiates different PN response by the local interneuron activity (Sachse & Galizia, 2003). Moreover, the response increased in amplitude and duration with increasing odor concentration until a saturated maximum for the input and output neurons.

But the behavioral response did not depend only on the concentration of a compound, but also on its ratio with other compounds. Webster *et al.* (2010) show several volatils together were attractant but one by one were repellent: the black bean aphid, *Aphis fabae*, is attracted by the volatils blend of *Vicia faba* (host cue), but when the volatil were presented alone, it became non host cue. Actually insects can make the difference between host and non-host plant, healthy vs stressed plant and unripe vs ripe fruit despite similar cues thanks to the processing of odor information by interneuron in AL (Cunnigham, 2011). So a repellent phenomenon could not be due to only one compound and its concentration, the context and the presence of other compouds are also important.

We defined a maskant odor compounds that decrease the attractiveness of the host and that are not repellent by themselves. In function of their concentration and the presence of other molecule this compound could provoke different behavior, i.e. in the different context activated ORs and so activated glomeruli could be different the modulation by the interneuron of the odor information convey by the PNs would be different to higher brain resulting in different behavior (Figure 3).

(b) Specific receptors or neurons?

In certain case, olfactory behavior are elicited by dedicated receptor channels and labeled lines, one innate odorant avoidance pathway could go directly from the antennal lobes to the lateral horn. There are narrowly and broadly tuned ORs linked to specialist and generalist ORNs (Hallem & Carlson, 2006). For example the receptor for pheromone or general odorant are different with a different specificity and selectivity and the generating combinatory code in antennal lobe is different (Christensen & Hildebrand, 2002; Touhara & Vosshall, 2009). An odorant like plant volatil can activate different types of ORs in various neurons converging onto different glomeruli. For exemple in *D. melanogaster*, contrary to sex pheromone whose ORNs responding specifically to this component converging onto specialized glomeruli as for example in moth (Touhara & Vosshall, 2009; Leal, 2013). But some ORNs implied in host volatils can be also highly specialized dependent on their concentration, number, and ecological relevance (Hansson & Stensmyr, 2011). For example the scarabs have selective ORNs for the specific unripe fruit volatils, they elicit either a repellency or ignored behavior. So we can hypothesis that insect have some ORs and ORNs dedicated to the detection of repellent compound.

In the part 3.1.1 we saw at the ORN level (input), a same glomeruli can be activated by attractive or aversive odorant but at PN level (output) the subset of glomeruli activated by attractive odorant differed from those activated by aversive odorant (Knaden *et al.*, 2012). However Knaden *et al.* (2012) found some glomeruli that responded discriminately to attractive and aversive odorant: DA4 and DC3 glomeruli were identified as « aversive specific » both at the input and output levels, aversive odorant glomeruli formed a cluster at the lateral part of the antennal lobe (AL) whereas attractive odorant ones were at the medial part (Knaden *et al.*, 2012). For example, geosmin, a compound produced by fungi and bacteria, is repellent for *Drosophila melanogaster* by innate avoidance pathway (Stensmyr *et al.*, 2012). The molecule activates the ab4B neuron which is specific only to geosmin and it elicites response from only two PNs (Stensmyr *et al.*, 2012). Moreover *Drosophila* exhibited strong avoidance to odours (CO_2) released by stressed flies and CO_2 activated only a single specific glomerulus in the antennal lobe. When this specific glomerulus is inhibited the avoidance is lost (Suh *et al.*, 2004). So activation of specific ORNs innervating one glomerulus can be responsible for an innate avoidance behavior.

Another example is DEET that can activate a specific odorant receptor. Syed and Leal (2008) showed that DEET activated a specific ORN in *Culex quinquefasciatus.* Moreover DEET was detected in the sacculus (antennal structure) of *D. melanogaster* innervated by axons of Ir-40a-expressing neurons (Kain *et al.*, 2013). When the synaptic transmission in these neurons were blocked or with knock-downed Ir40a flies the repellency of DEET was decreased possibly explaining DEET repellency. We can notice Ir40a is a highly conserved receptor among insects that could explain DEET effectiveness in wide variety of species.

We defined a true repellent (expellent) as a compound that causes at distance a movement away from the odor source indepently of the context. So an expellent could be a compound that have a specific OR and glomeruli with a labeled lines that code for a movement away (Figure 4).

(c) Information blocker?

Repellent compounds could affect the OR function by modifying or blocking response of ORNs sensitive to attractant (Davis, 1985). For example Bohbot *et al.,* 2011 showed that IR3535 and DEET inhibit the response of a complex OR (AaOr8+AaOr7) to an attractant, octenol. In *Aedes aegypti* DEET decreases the sensitivity of sensitive ORNs to lactic acid, a compound of human sweat (Davis & Sokolove, 1976). OR could have different sites for interaction with repellents: allosteric and orthosteric (Dickens & Bohbot, 2013). Ditzen *et al.,* 2008 showed that DEET blocked the response of olfactory neurons to attractive odors requiring the olfactory co-receptor OR83b. DEET acts also on the olfactory system through the co-receptors of the OR83b+OR47a from the fly antennae by decreasing the current mediated by the OR due to a change in ion permeability. Actually DEET can modulate the response to food stimulus of the ORNs from the *D. megalonaster* antennae. Moreover, DEET inhibits response of mosquito olfactory neurons to the attractant 1-octen-3-ol from the maxillary palp. Moreover De Bruyne *et al.* (1999) showed in maxillary palp *Drosophila* that p2B neuron was excited by some stimuli and was inhibited by others so odor coding involves both excitatory (depolarization ion current in ORNs) and inhibitory (hyperpolarization ion current in ORNs) responses, the extent of inhibition varies with different odor stimuli. Because of this two modes of response, a same neuron could send multiple message, so in function of the concentration and the stimuli a neuron could send a different information : attraction or repellency.

We defined a masking odor as a compound that interferes with host detection and localization and can modify the host profile. A masking odor, that we called attraction inhibitor, could be a compound that inhibits ORNs response by affecting the OR, the binding protein or by trapping the attractant (Figure 5). Actually it exists another way to block the information. For exemple DEET decreased the octenol compound release that decreased octenol ORNs response which changed the chemical host profile and decreased the host attractiveness, so DEET could be an attractant trap (Syed & Leal, 2008). But this mode of action is controversial. More over our knowledge on odorant-binding proteins, sensory neuron membrane proteins, and odorant-degrading enzymes are poor, but a repellent compound could influence their function.

(2) Gustatory pathway

Insects use olfaction to find hosts but also use gustation (De Boer, 2006). Olfactory input help insects to differentiate hosts (food source) from non-host, and gustatory input would facilitate this process (Glendinning *et al.*, 1998), as well as evaluation of food quality (Vosshall & Stocker, 2007). *Drosophila* and insect in general, are attracted to sugars and low concentration of salt and are aversed by noxious and bitter compounds (Yarmolinsky *et al.*, 2009). Host plant selection depends on the balance of phagostimulatory (e.g. sugars) and deterrent (e.g. plant secondary compounds) inputs (Chapman, 2003). Taste organs are distributed over multiple body parts: mouthparts (maxillary palps, oral cavity, pharynx), legs and wings (De Boer, 2006; Vosshall & Stocker, 2007). Sensilla on these organs permit the insect to sample potential food sources without consuming them (Sturcow, 1959; Montell, 2009). The same tarsal sensilla can be involved in other behaviors such as oviposition and mating (Ozaki *et al.*, 2011). For example the gustatory receptors (e.g. Gr68a) are involved in the detection of a non-volatil pheromone which is mainly localized on the male foreleg (Isono & Morita, 2010).

External gustatory sensilla are uniporous contrary to olfactory sensilla that are multiporous (Chapman, 2003). Gustatory sensilla contained two kind of gustatory receptor neurons (GRNs) – cells responding to attractive tastant or aversive tastant) or four kind of GRNs cells responding to sugar (S cell), top water (W cell), to low concentration of salt (L1 cell), and to high salt concentration and bitter (L2 cell)). They include also one chemosensory neuron and several types of accessory cells (Vosshall & Stocker, 2007; Montell, 2009; Yarmolinsky *et al.*, 2009). Odorant binding proteins (OBPs) are also

expressed in the lymph of gustatory sensilla and have the same role as do the OBPs in the olfactory system (Amrein & Thorne, 2005). Transduction of chemical information occurs in the mouthpart by the gustatory receptor neurons when chemicals make contact with taste receptor cells, a transmembrane protein (Isono & Morita, 2010). In the proboscis, axons of the taste receptor neurons project directly without synapsing into the suboesophageal ganglion (SOG) that provides motor output to those mouthparts (Schoonhoven & Van Loon, 2002). For example in the SOG of Drosophila a motor neuron that control proboscis extension is stimulated by activity in Gr5a neurons for attractant tastant and inhibited by activity in Gr66a neurons for aversive tastant (Yarmolinsky *et al.*, 2009). The SOG contains no morphologically apparent structural subdivisions such as glomeruli in the antenna lobe (Vosshall & Stocker, 2007). But the projections of the GRNs to the SOG terminate in spatially segregated domains (Yarmolinsky *et al.*, 2009; Isono & Morita, 2010). Gustatory afferents from the pharynx, labellum, and legs traveling through different nerves terminated in distinct areas of the suboesophageal ganglion and some of these spatially distinct afferents express the same receptors, suggesting that a given tastant may trigger different behaviors depending on the site of stimulation (Vosshall & Stocker, 2007). Interneurons also link the SOG to the mushroom body (Vosshall & Stocker, 2007). Taste information is sent to higher brain centers, simple reflexes, such as proboscis extension or food ingestion, may rely on local circuitry with fairly limited processing.

Like the olfactory system, the gustatory system is coding quantitatively, qualitatively and by mixture concentration as well as by presence in space and time (Schoonhoven & Van Loon, 2002; Koul, 2008). For example, the glucose-sensitive cell in *Manduca sexta* responded differently to sucrose or glucose. The differences can be attributed to the topographical binding-site characteristic of GRs (Lam & Frazier, 1991). Many GRs are co-expressed in the same GRNs (Montell, 2009). In contrast to one receptor – one neuron in the olfactory system, different subset of GRs are expressed in different types of taste neurons. Multiple receptor expression may be able to expand the ligand spectrum but decrease the performance of discrimination (Isono & Morita, 2010). GRs were originally supposed to function as G protein-coupled receptors but recently they have be speculated to function as ligand opened ion channel like ORs (Yarmolinsky *et al.*, 2009). As ORs, GRs form heterodimers with other receptors as a common co-receptor such as Gr64f (Isono & Morita, 2010). Finally, there are three types of sensory coding: 1) each neuron conveys a specific message to the central nervous system, 2) the global message is contained in a neural activity pattern (input) transmitted by several receptors, 3) stimulus

quality affects nerve impulse patterns (output) and adaptation rate which may contain additional information (Schoonhoven *et al.*, 1992).

How might a compound acting on this gustatory system results in a repellency behavior? A first response is that behavioral rejection is not due to ingested product but to external receptors because a link does not exist between the feeding deterrence and the internal toxicity (Koul, 2008). Since the gustatory system is quite similar to the olfactory system, the same hypothesis of the mode of physiological action can be emitted to explain the repellent effects as anti-feeding and irritancy. Actually the mode of action of anti-feeding compounds is largely unknown (Koul, 2008).

(a) A matter of concentration, ratio, time?

The concentration but especially the exposure time can play a key role in stimulation or inhibition of neuronal response. For example taste cells in *Pieris brassicae* responding to phagostimulants showed a gradual decrease of sensitivity to a drimane (antifeedant) at high dose for periods of up to 30 minutes (Schoonhaven & Van Loon, 2002). But contrary to the olfactory system, the concentration effect on the behavioral response is not clear. The host plant selection depends on the balance of phagostimulatory and deterrent input that could be a dose-effect (Chapman, 2003). Actually plant secondary compounds are most often deterrent but they also stimulate phagostimulatory cells. For example, in *Leptinotarsa decemlineata* the epipharyngeal taste sensillum is innervated by five neurons: one responds to water, one to sucrose and three to two antifeedants (drimane, sinigrin) but the sucrose-sensitive cell is also strongly inhibited by drimane (Messchendorp *et al.*, 1998). One hypothesis is that the information from receptor cells sensitive to feeding stimulants and information from receptor cells sensitive to antifeedants is subtracted algebraically in the central nervous system (Schoonhoven & Van Loon, 1988).

(b) Specific receptors or neurons?

All phytophagous insects have deterrent receptors which upon stimulation reduce or fully stop feeding activity (Schoonhoven & Van Loon, 2002). Glendinning *et al.* (1998) showed that the maxillary palps

of *M. sexta* larvae are sufficient and necessary to mediate normal food rejection of an extract of gumweed, *G. glutinosa,* an unacceptable food plant for these larvae. Anti-feeding compounds stimulate the deterrent cell through chemoreceptors (Jermy, 1990) and different subset of GRNs are activated for sugar (attractive) and bitter (aversive) compounds (Amrein & Thorne, 2005). For example, in the labellum, bitter aversive compounds, are primarily detected via the L2 cell and also inhibit S cells and W cells (Montell, 2009). More precisely all compounds that activate Gr5a neurons are attractive to flies and all those activating Gr66a neurons are aversive (Yarmolinsky *et al.,* 2009). *Drosophila spp.* are attracted by low concentration of salt and averse at high concentration. Two categories of neurons respond to NaCl : Gr5a for low concentration and Gr66a for high concentration (Yarmolinsky *et al.*, 2009). But the single Gr66a did not explain the salt aversion because its ablation did not significantly affect salt avoidance (Yarmolinsky *et al.*, 2009). Moreover at the level of the labellum, pharynx and SOG, DEET can activated bitter-sensing deterrent neurons in *Drosophila megalonaster* that could explain its efficiency to repel insects (Lee *et al.*, 2010; Kain *et al.*, 2013). Like the olfactory system, the gustatory system could be modulated by learning and memory (Vosshall & Stocker, 2007) which could be the origin of specific receptor to deterrent. Deterrent cells generally show greater latency in their response than phagostimulatory cells, as well as lower adaptation rates (Schoonhoven & Van Loon, 2002).

We defined as an antifeedant a compound acting on the feeding behavior. An antifeedant could be a compound that has a specific GR that code for a non feeding behavior (Figure 6). Actually the compounds that activates the deterrent cells inhibit the proboscis extension reflex and so the feeding activity however they did not cause a movement away.

Gamma amino butyric acid (GABA) stimulates feeding and causes taste cell response among herbivorous insects and the antagonists of GABA caused feeding deterrence but also caused hyperexcitation of the central nervous system and excito-irritant effect (Rattan, 2010).

We defined as irritant a compound that causes a movement away from the source after physical contact with it. An irritant could be a compound that have a specific GR that code for movement away (Figure 7). The difference between an expellent and an irritant is that the movement away is distance-mediated behavior through specific ORs and contact-mediated behavior through specific GRs, respectively. Actually De Gennarro *et al.* (2013) showed even in the presence of DEET orco mutant female mosquitos were still attracted to human hosts, but were repelled upon contact. This indicates

that olfactory- and contact-mediated effects of DEET are mechanistically distinct that encourage our hypothese that irritancy is coded by GR on the tarse.

(c) Information blocker?

A compound can block the stimulant taste receptor. According to Koul (2008) the sensory code may be altered due to the stimulation of specialized receptors and also to the modulation of the activity of receptors tuned to other compounds. Actually even if an antifeedant compounds do not stimulate any neuron within a sensillum it may decrease the responsiveness of a cell responding to a nutrient (Schoonhoven & Van Loon, 2002). For example deterrents may inhibit sugar cell (Schoonhoven & Van Loon, 2002). In mouthparts of lepidopteran larvae, terpenes block the stimulatory effects of glucose on chemosensory cells inducing an antifeedant effect (Rattan, 2010). Azadirachtin, the main active compound of neem oil, stimulates deterrent receptor in various insects (Schoonhoven, 1988) but also appears to inhibit sugar or inositol receptors in other species (Schoonhoven, 1988). Sinigrin inhibits the inositol cell in *Heliothis virescens* (Bernays & Chapman, 2000).

We defined as an antifeedant a compound acting on the feeding behavior. An antifeedant, that we called feeding inhibitor, could be a compound that inhibit GRNs response by affecting the GR (Figure 8).

As well resumed by Dickens & Bohbot (2013) the mode of action of mosquito repellent is widely studied and particularly for the DEET, the potential molecular target and pathways are numerous. But it still not clear which target initiates the repellent phenomenon. We must also emphasize the plasticity of the sensory system and the faculty of insects to adapt to rapid environmental change. Actually the avoidance behavioral response can be modulated by experience and memorized at short and long term (Séjourné *et al.*, 2011). In the mushroom body, the MB-V2 neurons are required specifically to retrieve aversive olfactory memory (Séjourné *et al.*, 2011). Aversive odor represses their activity and the reduced response could lead to an enhanced avoidance to the aversive odor by resulting in lower inhibition of olfactory signaling mediated by the LH (Séjourné *et al.*, 2011). Other neurons provide the output for appetitive memory however the process could be the same (Séjourné *et al.*, 2011). The German cockroach has rapidly evolved an adaptive behavioral aversion to glucose, the phagostimulant component of baits. In both wild-type and glucose-averse cockroaches, D-fructose and D-glucose

stimulated sugar–gustatory receptor neurons, whereas the deterrent caffeine stimulated bitter-gustatory receptor neuron (Wada-Katsumata *et al.*, 2013). In contrast, in glucose-averse cockroaches, D-glucose also stimulated bitter-gustatory receptor neuron and suppressed the responses of sugar-gustatory receptor neuron.

Other receptors types than ORs and GRs could be involved in repellent phenomena. The transient receptor potential (TRP) channels are non selective ion channels and participate in the detection of pain, hot temperature, gravity sensation, sound sensation, visual transduction and in chemosensation (Kim, 2013). For example TRPA1 is required for avoiding the volatil insect repellent citronellal (Kim, 2013). In *Drosophilla* citronellal interacts with TRPA1 that modify the activity of the Ca2+-activated potassium channel, but in *An. gambiae* TRPA1 is directly activated by citronellal (Kwon *et al.*, 2010).

To study the mode of physiological action, electrophysiology tests are performed as electroantennography or single sensillum recording. To study the anti-feeding effect, highly sensitive and sophisticated equipment and tools are also available such as electronic feeding monitor, which provides electrical penetration waveforms or graph (EPG) when an insect feeds (Walker & Perring, 1994). Recently using single-cell recordings from sensilla on the labella of *Ae. aegypti*, Sanford *et al.* (2013) demonstrated the presence of a gustatory receptor neuron that responds to DEET and other repellents including Picaridin, IR3535, and citronellal. Another electrophysiological technique to study the effect of repellent on odorant receptor is the whole-cell current. Bohbot *et al.* (2011) recorded the current of *Xenopus laevis* oocyte that expressed insect odorant receptors exposed to odorant or a combination of odorant-repellent using the two-microelectrode voltage clam technique and they showed that some insect repellents activate specific receptor and inhibits others.

IV. TWO EXAMPLES OF REPELLENT USE

(1) Relative importance of repellent studies

Research on insect repellency is increasing steadily with nearly 150 articles published in 2012 on this topic (90 for insect repellent, 28 for insect antifeedant and 30 for insect deterrent). Consequently, the number of citations is growing explosively, with nearly 6,000 citations in 2012 (4,000 for insect repellent, 800 for insect antifeedant and 1,200 for insect deterrent) (Figure 9). Publications and

citations on repellence are more numerous and older than for antifeedant and deterrent. The numbers of articles on repellence began to increase in 1970 and increased by at least 8 times from 1990 to 2010. Concerning antifeedant and deterrent, 10 studies were published from 1990 to 2000 followed by 20-30 studies in the next decade. The first citations on repellence (antifeedant and deterrent) started in 1950 and around 1975, respectively. Their numbers have grown exponentially from 1990 until 2013 (Fig. 1). An extension of our survey using the ISI 'Web of Knowledge' from past to present shows that the number of citations is largely smaller for other terms linked to repellence (115 citations for insect*irritance, 50 for insect*spatial repellent, 17 for insect*arrestant, 14 for insect*odor masking, 13 for insect*repulsive, 10 for insect*suppressant and 3 for insect*visual masking, each refined also by 'entomology'). The number of published items on repellent, antifeedant and deterrent differs also for the different groups of insect concerned (survey done for only mosquito, fly, tick, beetle, ant, moth, cockroach and aphid): repellence is mainly studied on mosquitoes (41.7%), ants (20.4%) and ticks (9.5%), antifeedant effect are mostly documented for ants (69.2%), beetles (11.8%) and moths (7.1%) and deterrent also focused in ants (35.5%), beetles (17.7%) and in mosquitoes (11.4%) (Table 1). Lastly, the database Scifinder®, used in chemical ecology, poorly referred a repellent effect to compound with only 22 articles for natural repellent and 6 for natural deterrent.

Sources of potential repellent products are diverse (Foster & Harris, 1997) but may be classified into three categories: (i) plants sources. Plants have specialized organs to repel enemies: the nettle *Urtica* has trichomes, lemon possesses oil vacuoles in the peel, mint displays extracellular glands, pine exhibits resin canals (Hossaert-Mc key & Bagneres-Urbany, 2012). Neem extract proved to be a famous deterrent (Cook *et al.*, 2006). Plants also emit some volatil organic compounds to attract pollinators, predators and parasitoids or to repel pests like the tobacco plant that releases induced-herbivore volatils that are repellent for the female moth *Heliothis virescens* (De Moraes *et al.*, 2001; Kessler & Baldwin, 2001). The herbivore-induced plant volatils and their emission are quite well studied: they have three defensive functions, in general, to 1) attract parasitoids/predators and 2) repel pest and 3) decrease oviposition (Unsicker *et al.*, 2009). These induced secondary metabolites of plants can be antidigestive and antinutritive proteins (Baldwin *et al.*, 2001). The essential oils of the major plant families (*Myrtaceae*, *Lauraceae*, *Lamiaceae*, and *Asteraceae*) may be irritant, repellent, antifeedant, or maskant (Regnault-Roger *et al.*, 1997; Tawatsin *et al.*, 2001;Isman, 2006; Moore *et al.*, 2007; Regnault-Roger *et al.*, 2012; Nerio *et al.*, 2010). (ii) Insects sources. Insects may produce

defense secretions such as alarm pheromones that disperse the population. For example, (E)-(β)-farnesene is the major component of alarm pheromones of a number of aphid species (Pickett *et al.*, 1992). Another famous example is the ant cues that affect flight landing of the mango fruit flies *Ceratitis capitata* and *Bactocera invadens* on ant-exposed mangoes (Van Mele *et al.*, 2009). (iii) Synthetic compounds. DEET is the most famous and studied repellent however there are also IR3535, DEPA (N,N-diethyl phenylacetamide), PMD (*p*-Menthane-3,8-diol), Picaridin and some pyrethroids.

The two first classes of repellent sources underscore the need to understand the chemical ecology of pests in order to provide candidate semiochemicals necessary to develop better attractants and repellent formulations (Dickens & Bohbot, 2013). The Scifinder® database contains 25 compounds with a repellent effect 10 of them are pyrethroids. The 'ecochimiothèque' referred any repellent product.

(2) The push-pull strategy in crop protection

The push-pull strategy is a famous insect pest management developed by Pike *et al.*, (1987) in Australia. The aim of this strategy is to modify the abundance and distribution of the insect pests (Cook *et al.*, 2007). The principle is based on a combination of a repellent stimulus and attractant stimulus. This method repels the insect away from the resource making it hard to locate, unattractive, or unsuitable by modifying host location and host acceptance; and simultaneously it attracts the insect to another area (Ratnadass *et al.*, 2012). Cook *et al.* (2007) underlined that the source (push) can mask host odor or can be repellent or deterrent, it depends on the management tactics on visual or chemical cues or signals at short range: antifeedants, oviposition deterrents, deterring pheromons; and at long range: visual cues, synthetic repellents, non-host volatils, host volatils, and anti-aggregation, sex or alarm pheromones. For example the push-pull strategy is also called the stimulo-deterrent diversion (Miller & Cowles, 1990) because the pull stimulus increases the efficacy of the push one. The attractant stimulus is sometimes designed as a trap because it concentrates the pest and facilitates its elimination (Cook *et al.*, 2007). For attracting the insect we can use: visual stimulants, host volatils, aggregation and sex pheromones, oviposition stimulants and gustatory stimulants (Cook *et al.*, 2007). This strategy can also be used on beneficial insects to push them from the surrounding area and attract them to the crop infested by their preys (Cook *et al.*, 2007). The stimulus is generally plants delivering

visual and semiochemical stimuli, but it can be also synthetic blend or plant extract (Cook *et al.*, 2007).

The most famous example of push-pull strategy is the control of stem borers in maize and sorghum (Khan & Pickett, 2004). The push stimulus is an inter-cropping with a repellent non-host plant such as *Desmodium uncinatum, Desmodium intortum,* or *Molasse minutiflora*) and the pull stimulus is an attractant trap plant such as *Sorgum vulgare sudanese,* or *Pennisetum purpureum* (Khan *et al.,* 1997a). The intercrop reduces the stem borer infestation by the repellent molasses volatil: (E)-β-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene (Kimani *et al.,* 2000; Khan *et al.*, 2000) and increases the parasitism by *Cotesia sesamiae* (Khan *et al.,* 1997b). The trap crop *Pennisetum purpureum* attracts the stem borer *Chillo partellus* by attractant volatils: octanal, nonanal, napthalene, 4-allylanisole, eugenol, and (R,S)-linalool (Khan *et al.*, 2000) and it produces compounds that reduce survival of larvae (Khan *et al.,* 2006). But the principle and efficiency of intercropping is put in doubt by Finch & Collier (2012). At few metres from the source, specialist insects respond only to the volatil chemicals released by their host plants even emitted in small quantities. These insects could not be 'repelled' from landing on non-host plants. The 'intercrops' could disrupt host-plant finding by providing insects with a choice of host (appropriate) and non-host (inappropriate) plant leaves on which to land that we called masking repellency. For generalist insect, Tosh & Brogan (2014) emitted the idea that to supply insect generalist as *Bemisia tabaci* with a super-abundance of volatils by intercropping could cause a confusion effect.

Another example is the control of the Colorado potato beetle *Leptinotarsa decemlineata* in potato crop, *Solanum tuberosum* (Martel *et al.,* 2005a). The push stimulus is the use of neem-based antifeedant on the crop (Martel *et al.,* 2005a). The pull stimulus is the use of combined attractants (Z)-3-hexenyl acetate, (R,S)-linalool, and methyl-salicilate on the potato crop with release of insecticide to decrease the population (Martel *et al.*, 2005b). To improve this system the use of the beetle aggregation pheromone (S-3,7-dimethyl-2-oxo-6-octene-1,3-diol) could be use to concentrate the beetle in the trap crop (Dickens *et al.*, 2002; Dickens, 2006).

The herbivore induced plant volatils could be also a source of compounds to use in the push-pull strategies. Herbivores cause different types of damages, each one being associated to a different compound (Pierre *et al.*, 2011). Volatils are involved in plant indirect defense by attracting the herbivore specific predators or parasitoids but they could be also deterrent, repellent, and/or

oviposition deterrent. For example, (Z)-β-ocimene and linalool are some VOCs (Volatil Organic Compound) induced by fusarium infection of wheat and barley and they are repellent for the coleopteran *Oulema cyanella*, an herbivorous cereal beetle (Piesik *et al*, 2013).

The push pull strategy is used in horticulture (Miller & Cowles, 1990), forestry (Borden, 1997) and for control of veterinary and medical pests (Nalyana *et al.*, 2000). This strategy maximizes control efficacy, efficiency, sustainability, and output and minimizes the negative environmental impacts (Cook *et al.*, 2007). Due to the synergism between the pull and push stimuli some limited efficient stimulus become more efficient like antifeedant and the habituation problem (Jermy, 1990). Another advantage of this strategy is to facilitate the resistance management (Cook *et al.*, 2007). But this strategy is usually not as effective as a broad-spectrum insecticide at reducing pest numbers (Cook *et al.*, 2007) – a potential problem protection of crops with low economic thresholds. This strategy is operationally complex, requiring monitoring and decision systems, and thus induces higher operational costs than conventional system. The lack of knowledge is also a limiting factor for its is adoption by small farmers (Cook *et al.*, 2007; Ratnadass *et al.*, 2012). Another disadvantage is the cost of the semiochemical and usually their lack of commercial supplies (Cook *et al.*, 2007).

(3) The use of pyrethroids in human health protection

Pyrethroids are derived from natural pyrethrins extracted from the *Chrysanthemum cinerariaefolium* (Elliott *et al.*, 1978; Duvallet & De Gentille, 2012). Many molecules more stable to UV have been synthesized, such as permethrin, bifenthrin, deltamethrin, lambda-cyhalothrin and alphacypermthrin. Pyrethroids are considered as spatial repellent, irritant, and lethal molecules and these effects may vary depending on target and dose. Pyrethroids have four main effects on mosquitoes: (i) a dissuasive effect, i.e. deterrence of adults from entering treated rooms; (ii) an excito-repellent effect, i.e. the mosquitoes leave the room after contact with treated bednets; (iii) a toxic effect, i.e. a knockdown mortality effect (Duvallet & De Gentille, 2012). Pyrethroids are very efficient against most insects, odorless, resistant to degradation by UV, heat, hydrolysis, and low toxicity for mammals. All these properties permit the use of pyrethroids in a wide variety of situations.

Pyrethroid toxicity is due to a modification of the gating kinetics of the voltage-dependent sodium channel (Narahashi, 1971). But one question subsists: is the irritant effect a consequence of sublethal

dosages (Miller *et al.,* 2009) or is there another mode of action? In field experiments the number of mosquitoes entering huts with a treated net was usually low compared to a untreated net (Adeogun *et al.*, 2012), indicating deterrency, repellency after irritation or masking effect. But in presence of pyrethroid treated bednet fabric the observed disengagement of female mosquitoes could be explained by the loss of response to host cues rather than repellency (Siegert *et al.,* 2009). The low vapour pressure of pyrethroids (e.g. permethrin 7 x 10^6 Pa at 25°C) probably explains its lack of spatial repellency (Achee *et al.*, 2009; Deletre *et al.*, submitted). Thus the deterrent effect of pyrethroids could be explained by their irritant effect meaning that mosquitoes leave the treated huts after contact with pyrethroids.

Pyrethroids are used to treat bednets to provide physical-chemical barrier from biting mosquitoes in several ways (McCain & Leach, 2007). Pyrethroids are irritating (excito-repellency) so mosquitoes avoid landing and staying on the net preventing them to bite through the net. If mosquitoes stay longer on the net or after many landings, they are either knocked down or killed. Pyrethroid treated bednets are recommended by WHO against malaria vectors especially with indoor biters (endophagic species) such as *Anopheles gambiae* and *Anopheles funestus* (WHO, 2002). Martin *et al.* (2013) recently showed the effect of treated nets in agriculture to protect cabbage agaisnt the aphid *Myzus persicae*.

Pyrethroids are also used for impregnating clothes, mainly battle dress and hammocks (Pennetier *et al*., 2010; Hougard *et al*., 2007). The first test of pyrethroids on clothes was in 1949 with allethrin and in 1972 with permethrin (Ware & Whitacre, 2004). Permethrin (0.125 mg/cm^2) treated clothes gave 98% protection against mosquito bites (Mc Cain & Leach, 2007).

Except transfluthrine and metofluthrine, most of pyrethroids lack the volatility to function as expellents at concentration necessary to be active against mosquitoes under ambient conditions. But systems exist to heat or aerosolize them in a room to obtain a sufficient gaseous or suspended concentration causing phenomena similar to expellency (Chadwick & Lord, 1978). First, a non-lethal irritation, possibly consisting of several physiological responses, causes the insect to avoid or to leave the area. Second, the toxic action causes either reversible knockdown or death.

Nevertheless, vector management is under the threat of resistance development to pyrethroids. Indeed, *Anopheles* resistance to pyrethroids has been reported in 27 countries in sub-Saharan Africa, underscoring the urgent need to find other alternatives to these insecticides (WHO, 2011; Ranson *et al.*, 2011). Prioritization of toxic actions over spatial repellent and contact irritant actions should be

balanced with the higher risk of rapid selection for resistance to the active compounds (Achee *et al.*, 2009). Additionally, pyrethroids are commonly used in crop fields adjacent to *An. gambiae* habitat, thus contributing to development of resistant mosquitoes (Zaim *et al.*, 2000; Yadouleton *et al.*, 2011; Temu *et al.*, 2013). Pyrethroid resistance in *An. gambiae* might be due to a mutation in the sodium channel sequence (Namountougou et al., 2012).

V. CONCLUSION AND PERSPECTIVES

(1) We defined the repellency in insect as a phenomena that prevents a pest's ability to track, locate and/or recognize its host and we reviewed five repellence phenomens: true repellency, odor masking, irritability, anti-feeding and visual masking.

(2) The most important problem with repellent research is the use of bioassays that do not adequately test for the associated term. The consideration of the stimulus that produced these reactions permits to discriminate different kinds of repellents. But understanding the mode of action of insect repellents and how these chemicals interact with odorants to modulate receptor activity will allow us to design potent formulations aimed at interfering with insect sensory signaling to ultimately disrupt their cognitive processes (Dickens & Bohbot, 2013). By increasing the number of studied species and a standardization of bioassays we can compare the product effects and increase the field efficacy.

(3) Insect repellents exert their effects through interactions with olfactory (antennae and maxillary palp) and gustatory (mouthpart, tarsi and wind) receptors. With more knowledge on neural mechanisms the repellent definitions could not longer be based on behavioral response but rather on their modes of action and they could be summarized in this way mechanisms as explained in the figure 10.

(4) The source of repellent is wide but the use of them is limited. Actually the main barriers in developing improved repellents are the estimated cost for identification and the subsequent cost for safety analyses for new chemistries (Kain *et al.*, 2013). Neverthless with the future advances the discovery and design of new repellents could be done by molecular-based chemical prospecting (Leal, 2007) and by computer-aided molecular modeling (Gupta & Bhattacharjee, 2007) confirmed by behavioral assay. Kain *et al.* (2013) developed a high-troughput chemical informatics screen using a structure activity approach to discover new repellents. The new found repellent activated the same chemosensory pathways as DEET. The need to have a safe environment, the want in IPM, and the

resistance issues will be motors for this research. Diverse strategies of chemosensory-based insect control as repellent/antifeedant strategy, trap strategy, mating disruption strategy were insect pest management of the future.

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Table 1**.** Number of papers articles published, surveyed using the ISI 'Web of Knowledge'.

topic	total number	number refined by entomology
insect* arrestant	31	17
insect* spatial repellent	64	50
insect* repulsive	64	13
insect* irritant	191	115
insect* suppressant	23	10
insect* anorexigenic	4	2
insect* odor masking	31	14
insect* visual masking	16	3
insect* arresment	no response	
insect* expellent	no response	
insect* anti-appetant	no response	

topic	**Repellent**	**Antifeedant**	**Deterrent**	**%Repellent**	**%Antifeedant**	**%Deterrent**
Insect	2631	445	901	66,2%	11,2%	22,7%
Mosquito	989	13	114	41,7%	1,6%	3,8%
Fly	143	11	116	6,0%	1,4%	3,9%
Tick	226	2	18	9,5%	0,2%	0,6%
Beetle	185	95	279	7,8%	11,8%	9,3%
Mite	98	17	51	4,1%	2,1%	1,7%
Ant	483	555	2041	20,4%	69,2%	68,1%
Moth	108	57	241	4,6%	7,1%	8,0%
Cockroach	72	4	12	3,0%	0,5%	0,4%
aphid	67	48	126	2,8%	6,0%	4,2%
	2371	802	2998			

Fig. 1. Test order in function of the behavioral response to determine the properties of a candidate repellent product

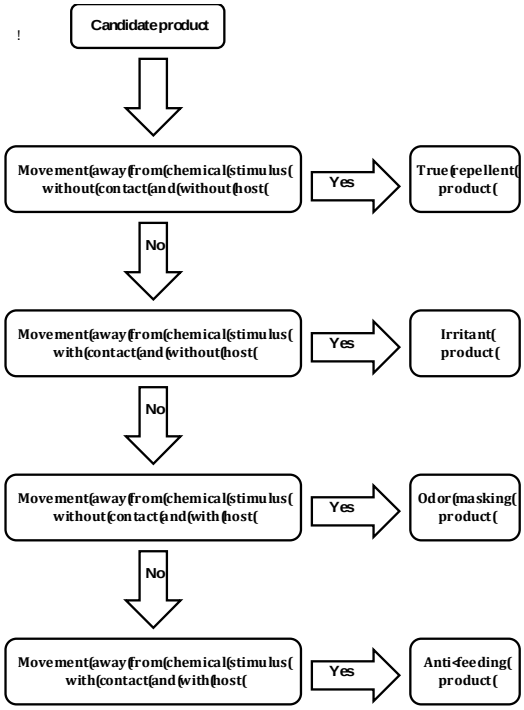


Fig. 2. Four types of repellence: 1) *expellence*: which corresponds to oriented movement of the insect away from an odor source without direct contact, 2) *odor masking*: a reduction of the attractiveness of the host or disruption of the localization of the host by the odor cue, 3) *contact irritancy:* an oriented movement of the insect away from a chemical after direct contact, 4) *deterrence*: a disruption of feeding activity by contact or ingstion of a chemical.

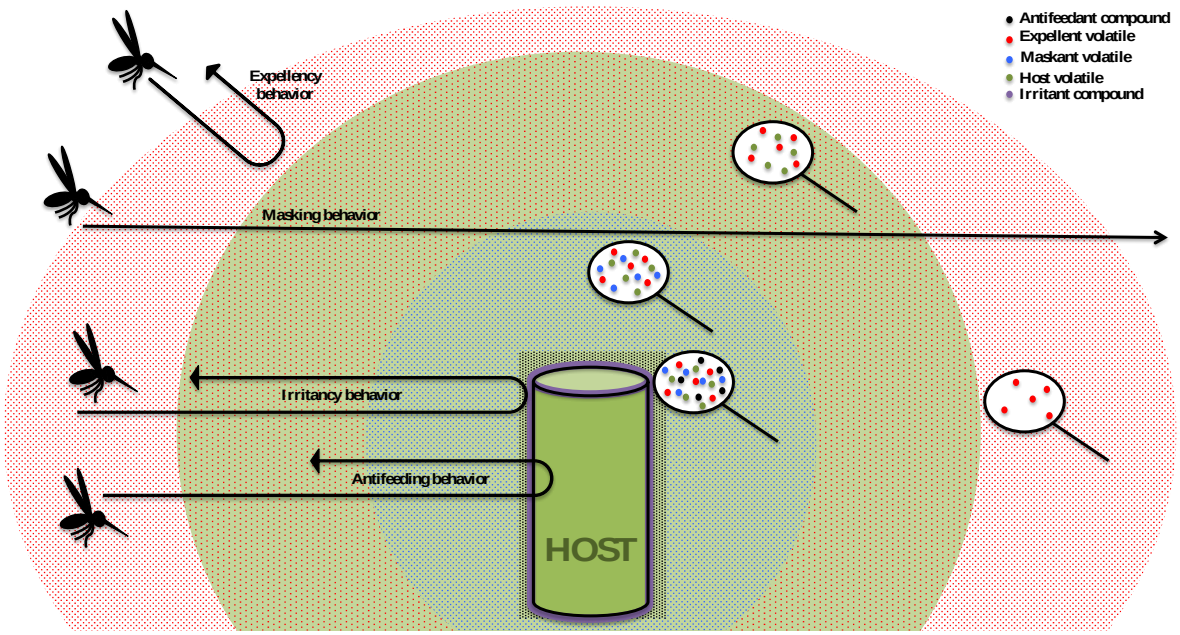


Fig. 3. Hypothetical mechanism of a maskant odor. Maskant odor compounds decrease the attractiveness of the host and are not repellent by themselves but depend of the context. In function of their concentration (A) and the presence of other molecule (B) this compound provoke different behavior, i.e. in the different context activated ORs and so activated glomeruli could be different, the modulation by the interneuron of the odor information convey by the PNs would be different to higher brain resulting in different behavior.

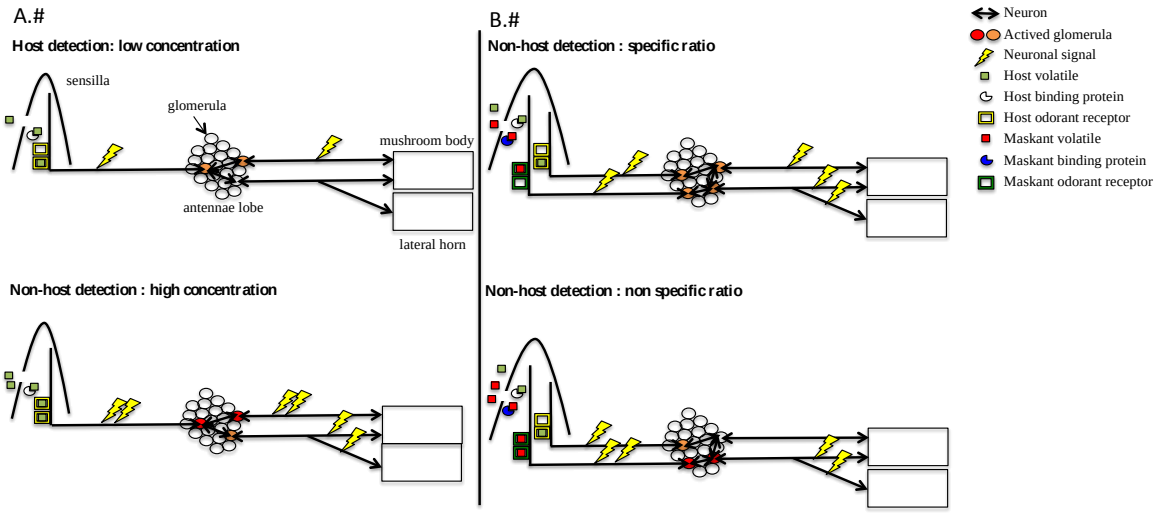


Fig. 4. Hypothetical mechanism of an expellent.True repellents (expellents) cause at distance a movement away from the odor source indepently of the context. Expellents have a specific OR and glomeruli with a labeled lines that code for a movement away from the odor source, i.e. a true repellent phenomen.

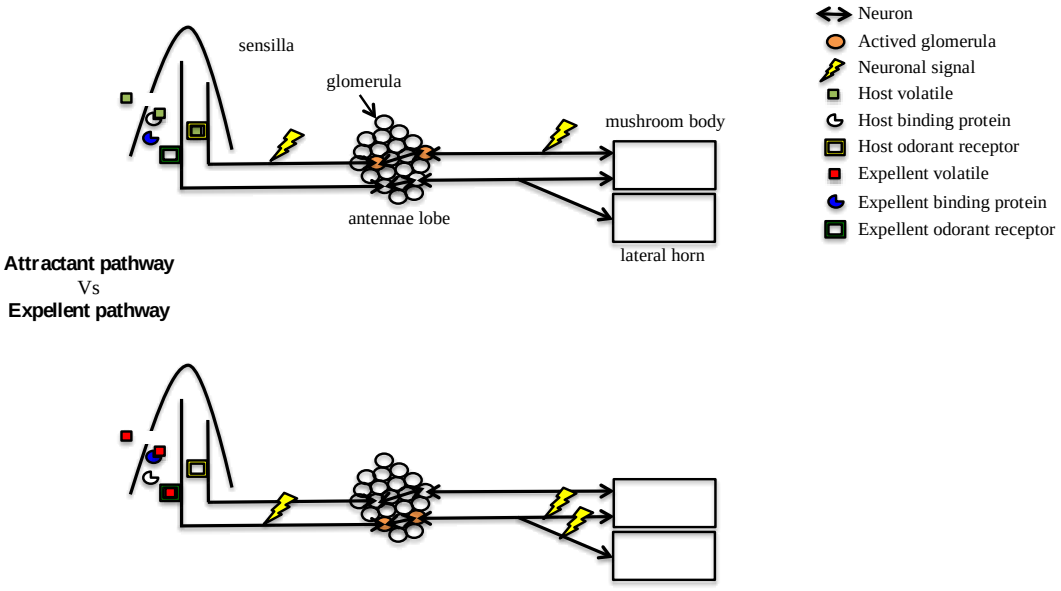


Fig. 5. Hypothetical mechanism of an attraction inhibitors. Attraction inhibitors interfere with the host detection and localization. These compound inhibit ORNs response by affecting the OR or binding proteins.

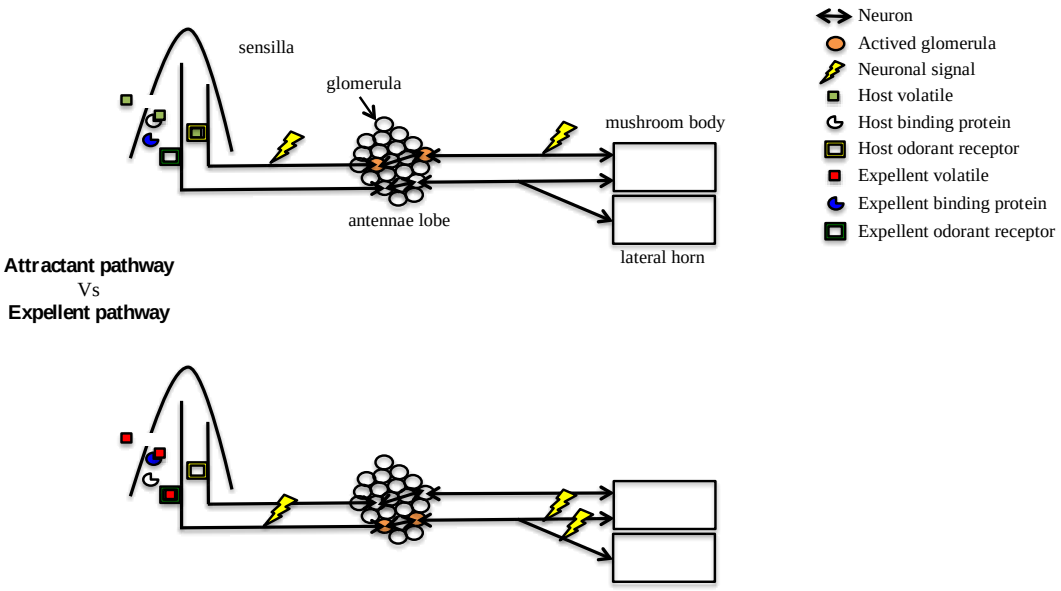


Fig. 6. Hypothetical mechanism of antifeedant. Antifeedant compounds act on the feeding behavior. Antifeedant compounds have a specific GR that code for a non-feeding behavior. Compounds activate the deterrent cell that inhibit the proboscis extension reflex and so the feeding activity but they did not cause a movement away.

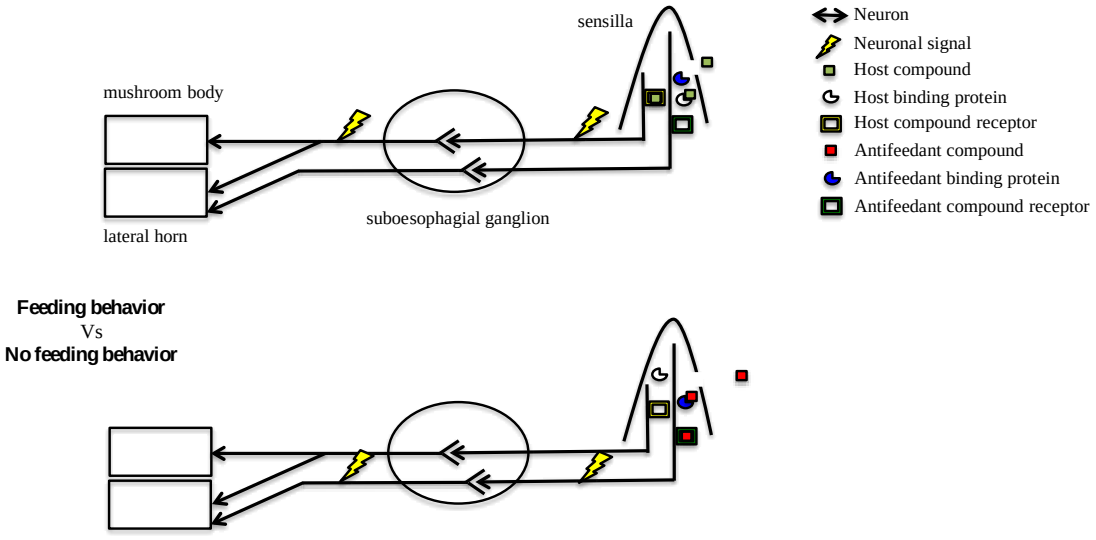


Fig. 7. Hypothetical mechanism of an irritant. Irritant compounds cause a movement away from the source after physical contact with it. They have a specific GR that code for a movement away, i.e. an irritant phenomen. The difference between an expellent and an irritant is that the movement away is a distance-mediated behavior through specific ORs and a contact-mediated behavior through specific GRs, respectively.

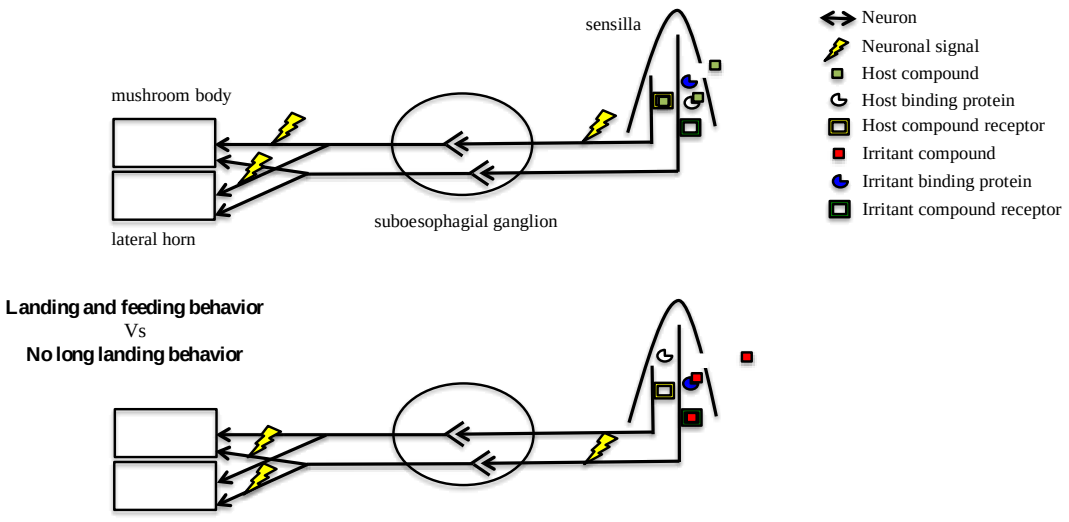


Fig. 8. Hypothetical mechanism of a feeding inhibitors. Antifeedant compounds act on the feeding behavior. Feeding inhibitors inhibit GRNs response by affecting the GR or binding proteins.

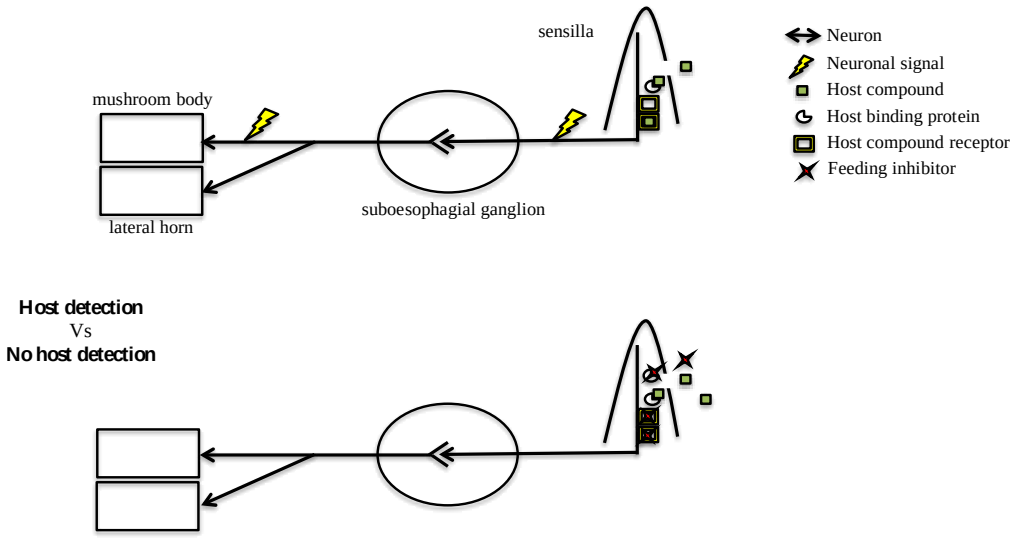


Fig. 9. Non-cumulative number of papers published yearly and the number of citations during the past 70 years, surveyed using the ISI 'Web of Knowledge'. The search included wild cards for 'insect*repellent', 'insect*repellent' and 'insect*deterrent' (each refined by 'entomology'). Height of histogram are proportional among graphs per the two categories.

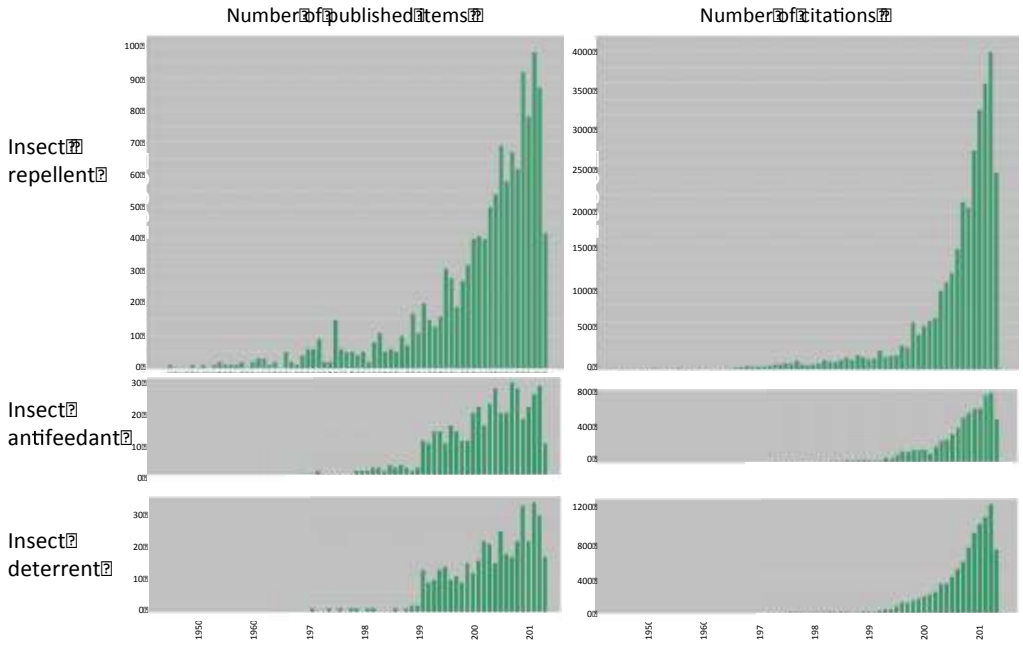
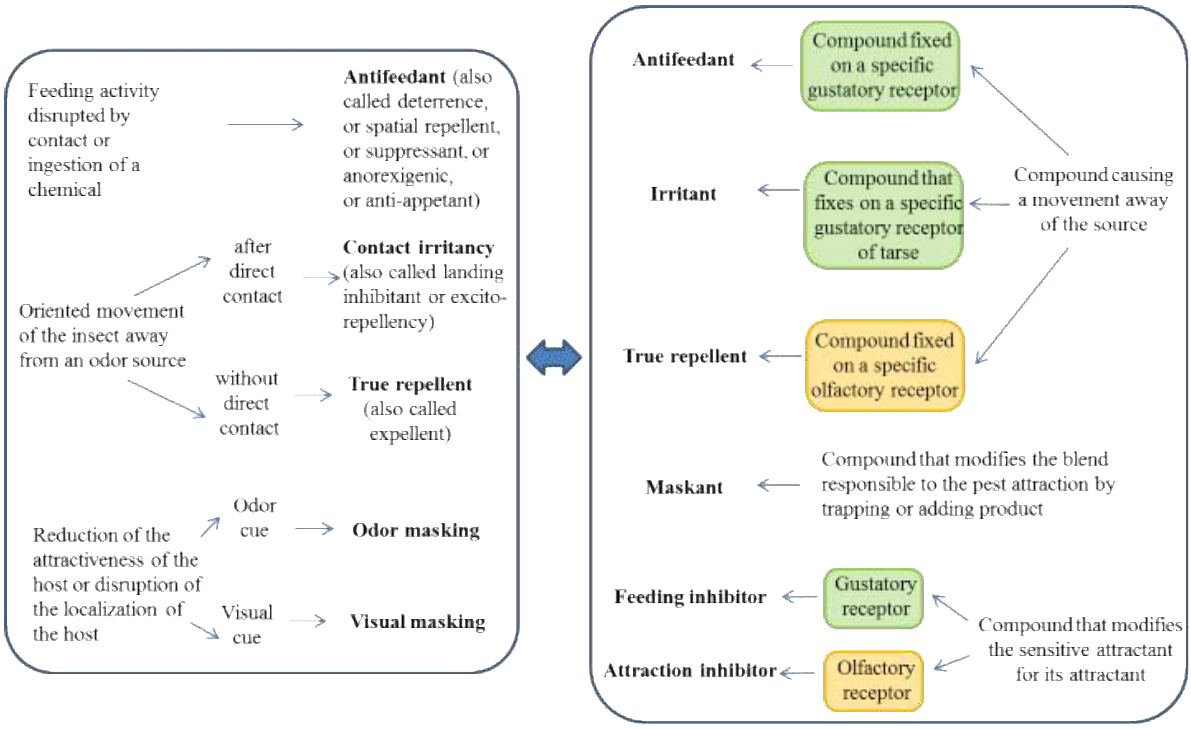


Fig. 10. From the repellent definitions based on behavioral response to definitions based on their neural mechanism.



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Repellent, Irritant and Toxic Effects of 20 Plant Extracts on Adults of the Malaria Vector *Anopheles gambiae* Mosquito

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Abstract

Pyrethroid insecticides induce an excito-repellent effect that reduces contact between humans and mosquitoes. Insecticide use is expected to lower the risk of pathogen transmission, particularly when impregnated on long-lasting treated bednets. When applied at low doses, pyrethroids have a toxic effect, however the development of pyrethroid resistance in several mosquito species may jeopardize these beneficial effects. The need to find additional compounds, either to kill disease-carrying mosquitoes or to prevent mosquito contact with humans, therefore arises. In laboratory conditions, the effects (i.e., repellent, irritant and toxic) of 20 plant extracts, mainly essential oils, were assessed on adults of *Anopheles gambiae*, a primary vector of malaria. Their effects were compared to those of DEET and permethrin, used as positive controls. Most plant extracts had irritant, repellent and/or toxic effects on *An. gambiae* adults. The most promising extracts, i.e. those combining the three types of effects, were from *Cymbopogon winterianus, Cinnamomum zeylanicum* and *Thymus vulgaris*. The irritant, repellent and toxic effects occurred apparently independently of each other, and the behavioural response of adult *An. gambiae* was significantly influenced by the concentration of the plant extracts. Mechanisms underlying repellency might, therefore, differ from those underlying irritancy and toxicity. The utility of the efficient plant extracts for vector control as an alternative to pyrethroids may thus be envisaged.

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Introduction

Anopheles gambiae (Giles, 1902) complex are major vectors responsible for the transmission of *Plasmodium* spp., particularly *Plasmodium falciparum*, which is the most hazardous protozoan parasite causing malaria infection in humans [1]. In 2010, approximately 3.3 billion people were exposed to malaria resulting in 655,000 deaths [2]. Although it still remains one of the most severe human diseases across the world, the overall incidence of malaria has fallen by 17% between 2010 and 2011. This decrease has been ascribed to an enormous progress in the control of malaria due to the use of efficient tools, such as rapid diagnostic tests in combination with treatments like artemisinin-based combination therapy (ACT) against *P. falciparum*, and control with indoor residual spraying or long-lasting insecticide-treated mosquito nets. These strategies have contributed to improved public health in many countries [3]. Nevertheless, vector management is under the threat of resistance development to pyrethroids. Indeed, resistance to pyrethroids has been reported in 27 countries in sub-Saharan Africa, underscoring the urgent need to find other alternatives to these insecticides [2,4]. Historically, the search for novel compounds to be used in vector control has focused on their lethal effects [5]. Nevertheless, other effects such as repellency or irritancy [6] may be used to reduce vector–host contact. Pyrethroids have four main effects on mosquitoes causing: (i) a spatial repellent effect, i.e. deterrence of adults from entering treated rooms; (ii) a contact irritant effect, i.e. short-lived settling of mosquitoes on treated bednets or walls; (iii) an anti-feedant effect, i.e. blood feeding inhibition of female mosquitoes and 4) toxic effect, i.e. a knock down and mortality effect [7].

According to Mathews & Mathews [8], a compound can be considered a spatial repellent, when its odour causes a shifting of animals away from the source. Spatial repellency has increasingly been given attention over the last few years since it has the potential to reduce the encounters between hosts and vectors [5]. A compound is considered irritant whenever insects move away after contact with it [9]. Compounds like pyrethroids or DDT increased insect activity because of their irritant effect [10].

Plants contain compounds such as repellents, anti-feedants, and growth regulators preventing attack from phytophagous insects, but some of these compounds are also repellent for haematophagous insects [11]. This could be an evolutionary relict from plant-feeding ancestors since many plant compounds evolved as

repellents to phytophagous insects [12]. Plants are used worldwide to protect people from haematophagous arthropods and numerous studies report repellent effects of essential oils [11,13,14,15,16]. These natural compounds are biodegradable, environmentally friendly and popular [17], and they generally have a low mammalian toxicity [18]. Moreover, traditional medicine is largely plant-based (herbs or shrubs) and is available at low cost in most tropical areas [14].

Essential oils present several interesting properties. First, they easily penetrate insect cuticle, which increases their bioavailability [16]. This property could be of interest if it resulted in shortened stay of insects on treated surfaces. Second, essential oil compounds such as acyclic or monocyclic monoterpenes are small and volatile molecules that might have spatial repellency properties. For example, insect sensilla are specialized for detecting odorants and have been shown to respond to volatile monoterpenes [16]. Finally, active compounds in essential oils may have specific mode of action, which makes them good alternatives to the use of pyrethroids.

Large screening programmes of chemicals traditionally used for vector control have aimed to generate baseline data for comparison with novel compounds. Using a high-throughput screening system (HITSS), compounds can be rapidly assayed and their effect on mosquito behaviour explored [19]. This study aimed at identifying the most promising plant extract(s) to complement the existing collection of molecules used in the control of malaria vectors. The broad aim of our study was to adapt the HITSS, originally developed for *Aedes aegypti*, [6,19,20,21] to perform assays on *An. gambiae*, to: 1) assess any spatial repellent, contact irritant and/or toxic effects of 20 plant extracts, 2) determine whether the influence of these extracts is concentration-dependent and 3) assess the potential of the selected candidates by comparing their effects with those induced by pyrethroid or neurotoxic insecticides. Among the 20 plant extracts, we identified three that could be used to augment the existing methods of malaria vector control.

Materials and Methods

Mosquitoes

Behavioural assays were performed on female *An. gambiae* originating from the insecticide susceptible reference strain "Kisumu". This strain, originally collected in Kenya in 1953, has been reared at LIN-IRD, Montpellier, France. The insecticide susceptibility of the Kisumu strain was confirmed with World Health Organization (WHO) diagnostic doses (i.e. 4% DDT, 0.75% permethrin) and is controlled every 4 months as recommended by the iso 9001 norm. The colony has been maintained in a climatic room at 27±2°C, 80±10% RH and with a photoperiod cycle of 12 h Light: 12 h Dark. Mosquito larvae were fed with fish food. Emerged adults were placed in 25×25×25 cm cages and fed with 10% honey solution. Females used in the bioassays were from batches of non-blood-fed mosquitoes (4 to 7 days after emergence). Each test was performed three times on 20 females. This was because previous experiments to determine a suitable sample size required for statistical power showed that three replications of 20 females was the smallest number of replicates with the best accuracy for visual observation and with the lowest manipulating time.

Products

A list of plant was established from the literature [9,11,13,14,15,16,22] based on the major compounds of plant extracts, plant extract effects on insects, and their non-toxicity to humans. The 20 plant extracts were selected among this list of plant for their effects on insect with a very different chemical composition described in literature and confirmed by the provider and composed by one or two major compounds (Table 1). This choice should permit the relation between the chemical composition and the behavioural response. DEET (Sigma Aldrich, France; CAS: 134-62-3) and permethrin (Sigma Aldrich, France; CAS 52645-53-1) were used as positive controls.

For each product (the 20 plant extracts, DEET [N,N-diethyl-3-méthylbenzamide] and permethrin), solutions were prepared at 0.01, 0.1 and 1% (volume/volume) diluted in a solvent constituted by 1/3 ethanol and 2/3 silicone oil in Dow Corning® 556 fluid. These three concentrations were chosen after preliminary assays and based on published data [6].

All papers used during the day were treated the morning at the same time. In spatial repellency assays, 3.3 mL of a same solution was deposited at 1.5 cm from the edge of 13×30 cm chromatography papers. Treated papers were allowed to dry at room temperature and used 1 hour later. Papers of the same size were also treated with 3.3 mL of solvent and later used in control assays. One paper is used for three replicates. For contact irritancy and toxicity assays, 2 mL of a same solution was deposited on 12×15 cm chromatography papers. Papers of same size were also treated with 2 mL of solvent, and solvent for the control assays. After drying at room temperature for 30 min, treated papers were stored at 4°C and used 2 to 4 hours later, the time to do the spatial repellency bioassays. Different papers were used in each replicate. For each plant extract, DEET, and permethrin, solutions at 0.01, 0.1 and 1% corresponded to 0.001, 0.01 and 0.1 µl of product per cm^2, respectively. For DEET (permethrin), dilutions of 0.01, 0.1 and 1% corresponded to 0.55 (0.34), 5.5 (3.4) and 55 (34) nmoles of active ingredient per cm^2 (a.i./cm^2).

Bioassays

Bioassays were conducted between 10 am and 6 pm at 24±1°C and 50±10% RH. For each product, each concentration was replicated three times, i.e. three replicates per concentration in the three types of assays: spatial repellency, contact irritancy and toxicity. For each product, all assays were performed the same day. For each type of assay, the control was first evaluated (three replicates) then the lowest concentration was evaluated (three replicates), followed by the mid-level concentration (three replicates), then the highest concentration (three replicates). The HITSS was washed at the end of each testing day and only one plant extract was tested per day. This protocol reduced the risk of contamination and interactions between volatile compounds. The HITSS was cleaned overnight in the TFD4 detergent (Franklab S.A., France) at 20% for the parts that were in contact with the treated paper (see below) and at 10% for any other parts. The material was rinsed and allowed to dry before reuse. To reduce the risk of contamination, a plastic clear film (Laser transparency films, Apli®, Spain) was placed between the treated chamber (see below) and the treated paper. A new film was used for each test.

a) Spatial repellency assays. The HITSS, originally developed for *Ae. aegypti* [6,19,20,21], was adapted for *An. gambiae*. The original HITSS is composed of three chambers in a row. The two extreme chambers correspond to the treated and untreated chambers, respectively. *Ae. Aegypti* are introduced in the third chamber, located in the middle of the HITSS [19]. During the experiment mosquitoes have the choice to stay in this middle chamber or to move, either in the treated or in the untreated chamber. Grieco *et al.* [19] used this choice test and considered a spatial activity measure. However, this choice test was not adequate in *An. gambiae* since this species exhibits much lower

Table 1. Plant extracts chosen from the literature [9,13,14,16,21,53] for their effects on insects, non-toxicity to humans and main compounds.

Common name	Scientific name	Extract form, extracted organ	Major compounds (%)[1]	Provider
Aframomum	*Aframomum pruinosum*	Essential oil, leaf	*E*-(R)-nerolidol (95%)	IBMM*, France
Cinnamon	*Cinnamomum zeylanicum*	Essential oil, bark	Cinnamaldehyde (80%)	Nactis, France
Citronella	*Cymbopogon winterianus*	Essential oil, leaf	citronellal (34%) – geraniol (22%) – citronellol (12%)	Nactis, France (Lot 4001850)
Coleus	*Plectranthus tenuicaulis*	Essential oil, leaf	Epoxyocimene (74.4%)	IBMM, France
Coriander	*Coriandrum sativum*	Essential oil, seed	(+)-linalool (72%)	Fabster, France
Cumin	*Cuminum cyminum*	Essential oil, seed	Cuminaldehyde (30%)	Ipra, France (Lot 902560)
Dill	*Anethum graveolens*	Essential oil, seed	(+)-carvone (60%) – limonene (30%)	IBMM, France
Eucalyptus	*Eucalyptus globulus*	Essential oil, leaf	1,8-cineole (81%)	Huiles & Sens, France (Lot B38037)
Geranium	*Pelargonium graveolens*	Essential oil, leaf	citronellol (41%) – geraniol (18%)	IBMM, France
Ginger	*Zingiber officinalis*	Essential oil, root	Zingiberene (30%)	Ipra, France (Lot 902724)
Lemon	*Citrus limon*	Essential oil, fruit	Limonene (95%)	Capua, Italy (Lot 20500)
Lemongrass	*Cymbopogon citratus*	Essential oil, leaf	Citral (geranial, neral) (75%)	IBMM, France
Litsea	*Litsea cubeba*	Essential oil, leaf	Geranial (45%), neral (32%)	IBMM, France
Pennyroyal	*Mentha pulegium*	Essential oil, leaf	(+)-pulegone (87%)	IBMM, France
Neem	*Melia azadirachta*	Vegetal oil, seed	azadirachtin (<1%)	Huiles & Sens, France (Lot 00028/11)
Pepper	*Piper nigrum*	Essential oil, seed	ß-caryophyllene (30%), limonene (14%), pinenes (14%)	IBMM, France
Rosemary	*Rosmarinus officinalis*	Biologic hydrolat, leaf	1,8-cineole (<1%), camphene (<1%), camphor (<1%)	Huiles & Sens, France (Lot EB815N002)
Savory	*Satureja montana*	Essential oil, leaf	Carvacrol (47%), γ-terpinene (18%), p-cymene (13%)	Huiles & Sens, France (Lot B854002)
Solidage	*Solidago canadensis*	Essential oil, leaf	Germacrene D (32%) - Limonene (13%)	Huiles & Sens, France (Lot A2)
Thyme	*Thymus vulgaris L.*	Essential oil, leaf	Thymol (35%), p-cymene (23%), carvacrol (15%)	Huiles & Sens, France (Lot A2)

[1]The percentage composition of the essential oil xas computed by the normalization method from GC/FID analyses, response factors being taken as one for all compounds.
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activity than *Aedes* spp. Furthermore, *An. gambiae* is not clearly attracted or repelled by light or by any external warm source. Hence, irrespective of the experimental condition, *An. gambiae* mosquitoes stayed in the middle chamber of the original HITSS. Consequently, the HITSS used in our experiments (Figure 1) had only two chambers, the treated (part #3) and untreated (part #5) chambers. Treated papers, with products or with only the solvent (for controls), were rolled around the inner surface of the treated chamber and maintained by means of part #4. The inner surface of the untreated chamber (part #5) was covered by a chromatograph paper, which was treated with neither product nor solvent. Thus the two chambers, treated and untreated, received an equivalent brightness. A metallic net (part #2) of 0.3 µm mesh was inserted within part #4, preventing direct mosquito contact with the treated paper. Two end caps (part #1) covered both sides of the HITSS. Part #4 contained a 'butterfly' valve that allowed mosquitoes to freely move between the untreated and treated chambers. During assays, the HITSS was held steady and parallel to the bench top by a cradle of 1.3-cm-thick Plexiglas made by Plexi d'Oc, St Gely du Fesc, France.

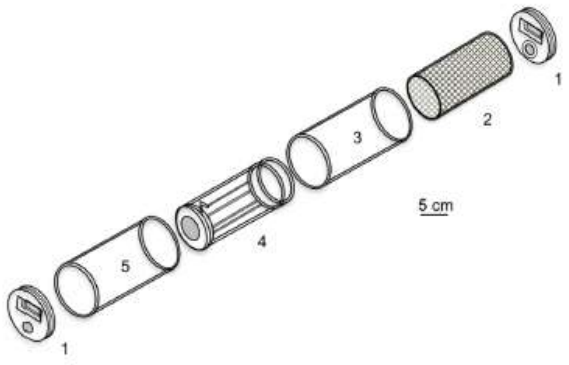


Figure 1. Schematic drawing of a modified HITSS system, used to test spatial repellency. The spatial repellency assay components are: 1, end cap; 2, metallic net; 3, treated chamber; 4, linking section (with a butterfly valve); 5, untreated chamber (adapted from Grieco et al. [18]).
doi:10.1371/journal.pone.0082103.g001

For each assay, 20 mosquitoes were transferred into the treated chamber using mechanical aspiration. After a 30-sec acclimation period, the butterfly valve was opened for 10 minutes. Mosquitoes moving from the treated chamber to the untreated chamber were referred as 'escaped'. Conversely, mosquitoes remaining in the treated chamber were referred as 'stayed'. At the end of the test, the butterfly valve was closed and the number of 'escaped' and 'stayed' mosquitoes recorded. Before running a new replicate, mosquitoes were removed from the system using CO_2 anaesthesia and the HITSS system partially disassembled in 5 minutes (chambers were disconnected and the end caps opened) to drive off any volatilized compounds. The assays for a given product were considered as valid whenever less than 20% of 'escaped' mosquitoes were in the control replicate. Spatial activity index used by Grieco *et al.* [19] for *Ae. aegypti* was not realistic for *An. gambiae* because the HITSS used in our experiments did not allow adult mosquitoes to make a choice. Thus, we decided to estimate the ability of a plant extract to repel mosquitoes by the proportion of 'escaped' mosquitoes: the higher the proportion of escaped, the stronger the spatial repellency effect.

b) Contact irritancy assays. These assays were performed with the tube used in the WHO test kit (Figure 2). A treated paper, with the diluted product or with solvent only (for controls) was put in the 'treated' tube and an untreated paper (i.e. a paper treated with neither a product nor solvent) in the 'untreated' tube. Twenty mosquitoes were initially placed inside the treated tube through the small hole of the slide unit (part #3). The untreated tube was fixed in the opposite part of the apparatus. Then, after a 30-sec acclimation period, the slide unit was opened for 10 minutes allowing the mosquitoes to freely move from tube to tube. Mosquitoes moving from the treated tube to the untreated tube were considered as 'escaped'. Conversely, mosquitoes staying in the treated tube were referred as 'stayed' mosquitoes. Once the guillotine valve was closed, the number of 'escaped' and 'stayed' mosquitoes in each tube was recorded. For each product, the assays were considered valid whenever the proportion of 'escaped' mosquitoes in the control assay (the assay performed with a paper treated with only the solvent) was lower than 50%. In case this ratio was >50%, all replicates were re-run until the ratio was <50% in the control assay. The contact irritant activity of a product was estimated based on the proportion of 'escaped' mosquitoes, a high activity translating into high proportions.

c) Toxicity assays. Toxicity assays were performed using a WHO test kit [23]. Twenty mosquitoes were exposed during 1 hour to a treated paper (with products or with the solvent only) in the treated tube used for the contact irritancy assay. Mosquitoes were then transferred into an untreated tube with 10% sucrose solution and maintained at 27°C and 80% RH. The number of dead and alive *An. gambiae* was recorded after 24 hours. The assay was considered valid whenever there were less than 10% of dead mosquitoes in the control (treated paper with the solvent) after 24 hours. The toxic effect of each product was expressed as the proportion of dead mosquitoes.

Data Analysis

We used the same method to analyse the proportion of dead mosquitoes in toxicity assays and the proportion of escaped mosquitoes in both spatial repellency and contact irritancy assays. Data analysis was carried out using the R 2.12.2 software [24]. The proportions of escaped or dead mosquitoes in control and treated assays were compared using Fisher's exact test. To take into account multiple testing, *P*-values of those tests were corrected according to Bonferroni using the Holm's sequential method [25]. Generalized linear models (GLM) were fitted to assess the effects of products and concentrations on the proportions of escaped or dead mosquitoes using a binomial distribution with a logit-link function. To assess the adequacy of the models, residuals were checked graphically using a normal quantile-quantile plot. GLM coefficients relative to the effect "concentration × product" were compared to 0 and their significance tested using multiple comparison procedures for GLMs [26].

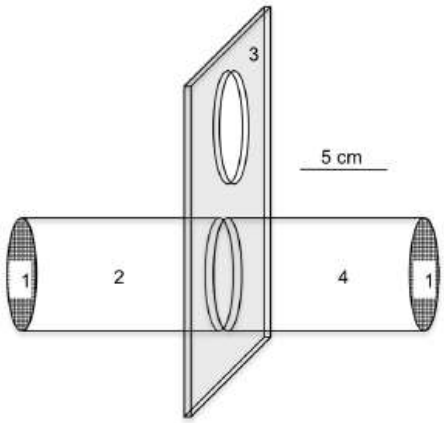


Figure 2. Schematic drawing of a simplified WHO diagnostic test kit for measuring insecticide susceptibility/resistance status in adult malaria mosquitoes, used to demonstrate contact irritancy. The contact irritancy assay components are: 1, end cap covered by net; 2, treated chamber; 3, linking section (guillotine valve); 4, untreated chamber (adapted from Grieco et al. [18]).
doi:10.1371/journal.pone.0082103.g002

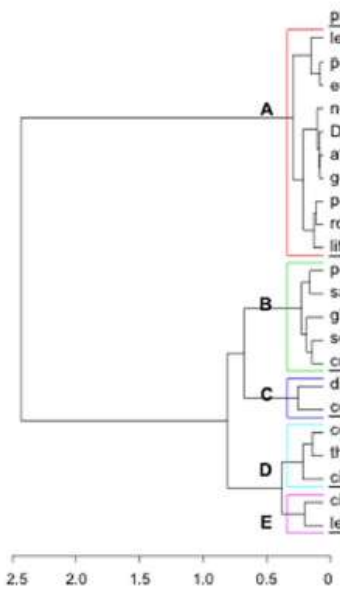
As previously described by Achee *et al.* [6], the proportions of escaped or dead mosquitoes were corrected by the control assay values using Abbot's formula [27]. For all products and concentrations, these corrected proportions were used to perform a principal component analysis (PCA). Then, a hierarchical ascendant classification (HAC) based on Ward's algorithm was used to group the plant extracts based on the similarity of their effects using PCA-axes coordinates. This process yielded a binary segmentation tree, reflecting the hierarchy of similarities between responses to plant extracts. The optimal number of classes in the tree was determined by the decrease of the interclass variance (branch height-Appendix 1).

Results

Spatial Repellency Assays

The spatial repellent effects of the different extracts significantly differed among plants (GLM, $P<0.001$) and were positively (model estimate: 0.82) associated with high concentrations of plant extracts (GLM, $P<0.001$) (Figure 3). Eight plant extracts did not exhibit a significant repellent effect at any concentration. These were lemon, eucalyptus, neem, aframomum, geranium, penny-royal, rosemary, and litsea. Twelve out of the 20 plant extracts were found to be repellent at least at one concentration. These were pepper, savory, ginger, solidage, cumin, dill, coleus, coriander, thyme, citronella, cinnamon and lemongrass. Essential oils of lemongrass and coleus had a significant repellent effect at all concentrations tested. The two synthetic chemicals, DEET and permethrin were not repellent at 1% and below.

According to the similarity of the behavioural response, the clustering procedure based on HAC yielded 5 contrasted response



	product	0.01 %	0.1 %	1%	p-value[2]
A	lemon	-5.2 (-13.4 – 3.0)[1]	13.6 (1.8 - 25.4)	14.2 (2.00 - 26.4)	0.003 **
	permethrin	5.1 (-2.7 - 13.0)	10.3 (1.0 - 19.5)	7.7 (-0.7 - 16.1)	–
	eucalyptus	2.2 (-1.8 - 6.3)	9.2 (2.3 - 16.1)	4.4 (-1.1 – 9.9)	0.300
	neem	4.1 (-2.3 - 10.4)	2.7 (-3.8 - 9.3)	10.3 (2.0 - 18.6)	–
	DEET	0.9 (-5.1 - 6.8)	2.0 (-4.3 - 8.2)	8.1 (0.3 - 15.9)	–
	aframomum	2.0 (-6.1 - 10.0)	-3.2 (-10.0 - 3.6)	10.6 (0.5 - 20.7)	1.000
	geranium	-6.9 (-13.2 - -0.6)	-1.3 (-9.2 - 6.7)	11.1 (0.6 - 21.5)	0.929
	pennyroyal	12.5 (1.2 - 23.7)	-10.6 (-21.8 - 0.6)	7.5 (-4.7 - 19.6)	<0.001 ***
	rosemary	13.2 (1.9 - 24.6)	-3.1 (-10.9 - 4.8)	13.0 (2.2 - 23.7)	0.042 *
	litsea	7.0 (0.4 - 13.7)	1.0 (-2.7 - 4.7)	16.4 (6.7 - 26.0)	–
B	pepper	17.2 (6.5 - 27.8)	5.7 (-2.6 - 13.9)	**30.0 (17.1 - 42.9)**	0.004 **
	savory	12.5 (2.6 - 22.3)	2.1 (-4.8 - 8.9)	**40.6 (27.9 - 53.3)**	<0.001 ***
	ginger	10.7 (-4.6 – 18.0)	16.0 (27.9 - 50.6)	**35.9 (-4.6 – 18.0)**	<0.001 ***
	solidage	2.2 (-6.9 - 11.3)	-4.7 (-11.7 - 2.3)	**36.9 (24.8 - 48.9)**	<0.001 ***
	cumin	2.5 (-6.9 - 11.8)	9.2 (-1.9 - 20.2)	**42.4 (30.0 - 54.8)**	<0.001 ***
C	dill	9.3 (-1.3 – 20.0)	**35.9 (23.9 - 47.8)**	**37.3 (26.0 - 48.7)**	<0.001 ***
	coleus	**32.1 (20.0 - 44.1)**	**43.9 (31.4 - 56.3)**	**26.5 (14.0 – 39.0)**	0.050 .
D	coriander	8.8 (0.8 - 16.8)	9.8 (1.7 - 17.9)	**53.1 (42.6 - 63.7)**	<0.001 ***
	thyme	6.7 (-2.4 - 15.7)	**39.2 (26.7 - 51.8)**	**63.4 (52.0 - 74.8)**	<0.001 ***
	citronnella	-3.7 (-9.3 - 1.9)	17.4 (5.9 - 28.9)	**69.5 (60.0 - 79.0)**	<0.001 ***
E	cinnamomum	**26.3 (14.9 - 37.7)**	14.8 (5.6 – 24.0)	**73.5 (64.9 - 82.1)**	<0.001 ***
	lemongrass	**26.0 (14.6 - 37.5)**	**23.5 (11.8 - 35.1)**	**60.2 (49.1 - 71.3)**	<0.001 ***

Figure 3. Response of four- to seven-day-old, non-blood-fed, sugar-fed, Kisumu strain of *Anopheles gambiae* females to the repellent effect of DEET, permethrin and 20 plant extracts at 3 concentrations (0.01, 0.1 and 1% of product in the solution on chromatographic papers): dendrogram determined by hierarchical ascendant classification and corrected proportion escaping using Abbott's formula (confidence interval calculated with the Wald method) by treatment concentration. 1) Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the control with the Holm's sequential Bonferroni correction method. 2) P-value of the generalized linear model of the interaction concentration-product (dose-dependency) on the mosquito repellency. The coefficient was compared to zero so only the P-value of positive coefficient is given.
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classes. Class A grouped products that were not repellent, irrespective of their concentration. Classes B, C, D, and E grouped products that were significantly repellent at least at one concentration. Class B grouped 5 products that were efficient at only 1%. It is noteworthy that their activities slightly increased as concentration increased. Class C included two products that were repellent at several concentrations. For instance, coleus was repellent at all concentrations. These two products appeared to have a maximum efficiency of around 40%. Class D contained products that were repellent at least at one dose. The three products might be repellent at higher concentrations in agreement with their positive coefficients relative to the effect concentration. Class E regrouped the most repellent products for which a response was observed at least at two concentrations. Among the 20 plant extracts, the essential oils of lemongrass and cinnamon were the most repellent.

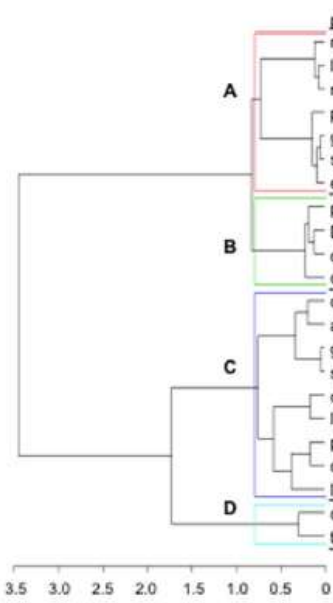
Contact Irritancy Assays

As observed in the repellency assays, the contact irritant activity of the 20 extracts significantly differed among plants (GLM, $P<0.001$) and increased with respect to the concentration of product (GLM, $P<0.001$, model estimate: 2.87) (Figure 4). Eight plant extracts had no irritant effects: rosemary, lemon, neem, pennyroyal, geranium, savory, eucalyptus and pepper. The other plant extracts, dill, coriander, cinnamon, aframomum, ginger, solidage, citronella, litsea, cumin, lemongrass, coleus and thyme, had irritant effects even at low concentrations. Similar to permethrin, cumin, lemongrass, coleus and thyme appeared irritant at all concentrations. Conversely, DEET was observed to be irritant at only 1%.

The HAC could be summarized by four response classes: Class A (8 products) containing products that were not irritant; Class B (4 products) that included products that were irritant at 1% concentration (except pepper oil), and whose interactions 'products×concentration' were significant, suggesting possible irritancy effects at higher concentrations; Class C (9 regrouped products) that were observed irritant at 2 or 3 concentrations included permethrin, which appeared to have a maximum escape threshold of around 50%; and class D (2 products) that were irritant at three concentrations and whose coefficients relative to 'product×concentration' interaction suggest that they might be irritant at lower concentrations. Among all plant extracts, coleus and thyme were the most irritant.

Toxicity Assays

Plant extracts had varied toxicity, notably at the highest concentration tested (Figure 5). Once again, mortality rates were significantly influenced by both product and concentration (GLM, $P<0.001$ in both cases). The toxic activity was, therefore, positively influenced by increase in concentration (model estimate: 1.29). Sixteen plant extracts had no toxic effect, even at the highest concentration. These were rosemary, eucalyptus, pennyroyal, pepper, dill, ginger, neem, geranium, lemon, solidage, lemongrass, litsea, aframomum, coleus, coriander and cumin. In contrast, four plant extracts exhibited a toxic effect at 1%. These were cinnamon, citronella, savory and thyme. As expected, permethrin showed a toxic effect at 1%. Conversely, whatever the concentration, DEET did not appear efficient in killing mosquitoes. Knockdown response was not observed using either the plant extracts or the synthetic compounds.



	product	0.01 %	0.1 %	1%	p-value[2]
A	rosemary	-6.6 (-16.9 - 3.63)[1]	-11.8 (-20.2 - -3.4)	-9.7 (-18.7 - -0.7)	–
	lemon	-0.7 (-12.5 - 11.1)	8.3 (-3.0 - 19.6)	2.2 (-8.9 - 13.2)	0.934
	neem	-3.1 (-11.4 - 5.2)	6.6 (-3.4 - 16.6)	8.0 (-2.5 - 18.6)	–
	pennyroyal	21.8 (12.7 - 30.9)	27.8 (19.0 - 36.6)	18.0 (8.3 - 27.8)	<0.001 ***
	geranium	13.2 (0.5 - 25.9)	20.0 (7.6 - 32.5)	18.7 (6.9 - 30.4)	0.073 .
	savory	16.4 (5.6 - 27.1)	18.6 (8.2 - 29.1)	18.5 (8.3 - 28.8)	<0.001 ***
	eucalyptus	8.9 (-1.7 - 19.5)	18.0 (6.8 - 29.3)	18.2 (6.3 - 30.1)	0.950
B	pepper	6.5 (-1.3 - 14.3)	2.4 (-5.0 - 9.8)	22.2 (11.1 - 33.4)	–
	DEET	10.0 (-0.8 - 20.8)	4.8 (-5.7 - 15.2)	**36.2 (26.5 - 45.9)**	<0.001 ***
	dill	-4.6 (-15.8 - 6.6)	-3.1 (-14.8 - 8.5)	**39.0 (28.5 - 49.5)**	<0.001 ***
	coriander	19.4 (8.4 - 30.5)	2.8 (-8.0 - 13.7)	**43.6 (36.9 - 50.3)**	<0.001 ***
C	cinnamon	28.1 (17.8 - 38.4)	**30.3 (20.9 - 39.7)**	**32.5 (22.9 - 42.0)**	<0.001 ***
	aframomum	13.0 (2.45- 23.5)	22.8 (14.2 - 31.4)	**36.4 (32.2 - 40.7)**	0.001 **
	ginger	**33.0 (20.4 - 45.61)**	27.8 (16.2 - 39.4)	**48.0 (38.7 - 57.22)**	<0.001 ***
	solidage	**36.1 (24.5 - 47.8)**	27.2 (13.5 - 40.9)	**46.6 (34.5 - 58.7)**	0.004 **
	citronnella	8.8 (-2.0 - 19.6)	**35.2 (22.8 - 47.7)**	**57.5 (48.1 - 67.0)**	<0.001 ***
	litsea	0.4 (-9.6 - 10.4)	**44.5 (32.6 - 56.5)**	**67.3 (59.6 - 74.9)**	<0.001 ***
	permethrin	**44.5 (32.0 - 57.0)**	**48.5 (36.4 - 60.6)**	**53.9 (41.9 – 66.0)**	0.348
	cumin	**30.6 (14.2 - 47.1)**	**49.6 (33.3 - 65.9)**	**62.4 (51.7 - 73.2)**	<0.001 ***
	lemongrass	**30.4 (18.5 - 42.3)**	**32.6 (20.5 - 44.8)**	**80.7 (72.0 - 89.3)**	<0.001 ***
D	coleus	**53.1 (40.8 - 65.4)**	**81.0 (72.0 - 90.0)**	**83.6 (75.6 - 91.6)**	<0.001 ***
	thyme	**80.5 (74.6 - 86.4)**	**86.8 (86.8- 86.8)**	**83.5 (79.1 – 88.0)**	<0.001 ***

Figure 4. Response of four- to seven-day-old, non-blood-fed, sugar-fed, Kisumu strain *Anopheles gambiae* females to the irritant effect of DEET, permethrin and 20 plant extracts at 3 concentrations (0.01, 0.1 and 1% of product in the solution on chromatographic papers): dendrogram determined by hierarchical ascendant classification and corrected proportion escaping using Abbott's formula (confidence interval calculated with the Wald method) by treatment concentration. 1) Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the control with the Holm's sequential Bonferroni correction method. 2) P-value of the generalized linear model of the interaction concentration-product (dose-dependency) on the mosquito irritancy. The coefficient was compared to zero so only the p-value of positive coefficient is given.
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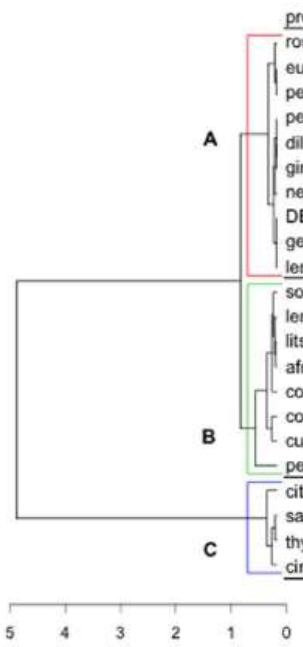
The HAC analysis yielded three response classes: Class A (10 products) containing all products that were not toxic at all to mosquitoes, even at highest concentrations; Class B (8 products) that contained products with unclear effects at all concentrations (with one exception, permethrin) although toxicity slightly increased for aframomum, coriander and cumin, suggesting a potential toxicity at higher concentrations; and Class C (4 products) that appeared to be very toxic at 1%. Among the 20 plant extracts, cinnamomum, citronella, savory and thyme were the most toxic.

Discussion

Our results showed that nearly all the 20 plant extracts tested had a significant effect on adults of the malaria vector *An. gambiae* (Table 2). Several were irritant or repellent but only a minority were toxic. For each of these three types of effects, several strong candidates were found. Some of these compounds presented interesting properties in more than one type of effect. These were cinnamon, citronella and thyme, which were shown to be repellent, irritant and toxic at the same time (Table 2). Compounds such as lemongrass, coleus, cumin and savory exhibited clear but restricted effects. Thyme is already known to have a toxic effect on Bruchidae [28], therefore, its mode of action might not be very specific. Rattan [29] showed that thymol, a natural monoterpene phenol found in oil of thyme, acts on the GABA system, reducing the neural inhibition, leading to hyper-excitation of the central nervous system, convulsions, and death. Thymol can also block the octopamine receptors that play a key role in the nervous transmission [29]. This certainly explains the irritant and toxic effects of thyme oil in our experiment.

Our results suggest that plant extracts exhibit different combinations of effects (i.e., spatial repellency, contact irritancy and/or toxicity). The magnitude of these effects differs among plant extracts and concentrations. For instance, irritancy, repellency and toxicity are, respectively, the primary, secondary and tertiary actions of thyme oil since these effects occur at low, medium and high concentrations, respectively. This contrasts with other plant extracts. The primary and secondary actions of dill oil are repellency and irritancy. This oil is not toxic on *An. gambiae* even at the highest concentration. This pattern suggests that the three effects observed here, i.e. repellency, irritancy and toxicity, involve different physiological mechanisms. Dekker *et al.* [30] showed that several repellent compounds elicit consistent electro-physiological responses in antennae of *Ae. aegypti*. The irritant effect of a product might be due to its action through tarsi on the nervous system [10]. Some individual compounds of essential oils are clearly detected and avoided by mosquitoes through their antennae. Still, the physiological influence of essential oils leading to repellency remains largely unknown [30,31]. Deciphering the mechanisms underlying repellency might be challenging since this effect may be due to a synergistic effect of several compounds contained in plant extracts. Knowing the relation between the mechanism and behaviour could be of use in finding synergistic combinations. If our hypothesis is correct, (i.e. that irritancy, repellency and toxicity have independent modes of action), there may well be no cross-resistance, i.e. the resistance to one mode of action might not confer resistance to the other two modes of action. The evaluation of the relation between the mode of action and behaviour could be useful in reducing the risk of selecting resistant individuals. For example, linalool (the major compound of *C. sativum* essential oil), which showed a toxic effect on



	product	0.01 %	0.1 %	1%	p-value[2]
A	rosemary	5.6 (-2.1 - 13.3)[1]	1.9 (-4.3 - 8.2)	-3.1 (-6.1 - -0.0)	
	eucalyptus	4.8 (-1.7 - 11.2)	1.5 (-3.4 - 6.5)	2.6 (-2.7 - 7.9)	1.000
	pennyroyal	4.9 (-3.0 - 12.8)	1.5 (-4.3 - 7.2)	3.7 (-4.0 - 11.3)	0.188
	pepper	-1.7 (-7.1 - 3.6)	-1.8 (-7.3 - 3.6)	-1.8 (-9.8 - 6.2)	
	dill	-4.1 (-8.5 - 0.4)	-1.2 (-7.0 - 4.6)	-1.1 (-6.5 - 4.3)	0.997
	ginger	-3.0 (-3.0 - -3.0)	0.2 (-4.3 - 4.7)	0.3 (-4.4 – 5.0)	
	neem	0 (0 - 0)	2.9 (-1.1 – 7.0)	0.0 (0.0 – 0.0)	
	DEET	-4.4 (-8.6 - -0.3)	-1.1 (-6.5 - 4.4)	1.2 (-5.4 - 7.9)	0.860
	geranium	-1.4 (-1.4 - -1.4)	-1.4 (-1.4 - -1.4)	2.8 (-1.8 - 7.4)	
	lemon	0.2 (-3.9 - 4.3)	-2.8 (-2.8 - -2.8)	2.1 (-3.3 - 7.6)	1.000
B	solidage	3.1 (-2.3 - 8.5)	-1.8 (-1.8 – -1.8)	7.8 (-0.2 - 15.8)	0.934
	lemongrass	-2.1 (-6.6 - 2.4)	2.3 (-4.9 - 9.6)	8.1 (-1.2 - 17.4)	0.058
	litsea	0 (0 - 0)	1.6 (-1.5 - 4.6)	10.3 (3.1 - 17.5)	0.552
	aframomum	0.3 (-5.1 - 5.8)	0.8 (-5.1 - 6.6)	12.0 (4.0 - 20.1)	<0.001***
	coleus	0.0 (0 - 0)	7.3 (0.4 - 14.2)	9.4 (1.5 - 17.3)	0.831
	coriander	7.6 (0.4 - 14.9)	6.8 (0.5 – 13.0)	13.0 (4.1 - 21.9)	<0.001***
	cumin	3.7 (-1.8 - 9.1)	7.0 (0.1- 13.8)	19.3 (10.0 - 28.6)	<0.001***
	permethrin	3.5 (-2.2 - 9.2)	19.3 (9.2 - 29.4)	**32.2 (20.4 – 44.00)**	<0.001***
C	citronnella	11.5 (2.8 - 20.2)	6.1 (-0.8 - 13.0)	**88.6 (78.5 - 98.7)**	<0.001***
	savory	1.0 (-5.3 - 7.3)	1.6 (-5.2 - 8.4)	**87.3 (81.2 - 93.3)**	<0.001***
	thyme	3.2 (-0.9 - 7.4)	3.3 (-0.7 - 7.3)	**87.9 (83.9 - 91.9)**	<0.001***
	cinnamomum	0.7 (-4.1 - 5.6)	-1.8 (-5.3 - 1.7)	**94.5 (92.2 - 96.8)**	<0.001***

Figure 5. Responses of four- to seven-day old, non-blood-fed, sugar-fed, Kisumu strain of *Anopheles gambiae* females to the toxic effect of DEET, permethrin and 20 plant extracts at 3 concentrations (0.01, 0.1 and 1% of product in the solution on chromatographic papers): dendrogram determined by hierarchical ascendant classification and corrected mortality proportion using Abbott's formula (confidence interval calculated with the Wald method) by treatment concentration. 1) Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the control with the Holm's sequential Bonferroni correction method. 2) P-value of the generalized linear model of the interaction concentration-product (dose-dependency) on the mosquito mortality. The coefficient was compared to zero so only the p-value of positive coefficient is given.
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mosquitoes, was identified as an inhibitor of acetylcholinesterase [28]. Unfortunately, the efficacy of linalool on *An. gambiae* should be limited because resistance alleles at the acetylcholinesterase gene have already selected in West African populations of this species [32,33,34,35]. The physiological mechanisms of plant extracts are largely unknown and interactions between individual compounds could be antagonistic, additive or synergistic. Since multiple resistance mechanisms could be involved, hypotheses on resistance development to essential oils are still speculative and need further investigations. Although neem oil has also been demonstrated to inhibit feeding behaviour [18,36,37,38], it was not repellent because it is not volatile. Rosemary extract did not show any effect because hydrolats contain few active ingredients.

An. gambiae females were not significantly repelled or killed by DEET. This product showed only a contact irritancy effect at 1% (55 nmol/cm^2). The vapour tension of DEET is low (0.27 Pa at 25°C) compared to other repellents such as p-menthane 3,8 diol (4.5 Pa at 25°C). Moreover, in our experiment, DEET was applied on a paper at 25°C rather than directly on skin (skin temperature is usually around 33°C), a difference that could explain the absence of repellent effect in the present investigation. Ditzen *et al.* [39] showed that DEET hides host odours (particularly 1-octen-3-ol) by inhibiting subsets of insect odorant receptors that require the OR83b co-receptor (masking effect). These olfactory receptor neurones (ORN) are involved in detecting semiochemicals that induce and facilitate host-seeking behaviour in mosquitoes [40]. However, according to Syed & Leal [41] ORN mosquitoes can detect and avoid DEET. In a sugar-feeding and surface-landing choice bioassay, mosquitoes did not land on DEET-treated paper and instead chose to land on solvent-treated paper. As a consequence, a 'repellent' may have more than one mode of action. DEET is reported as an inhibitor of acetylcholinesterase activity [42] and it was toxic on other species of mosquito at higher concentration [43] or with a different test method [44]. In our study, DEET did not showed toxic effect, that may be explained by a low concentration test or a product not enough bio-available. Our results showed that DEET is irritant but not repellent at a concentration equal to or below 1%. Indeed, we showed that without attractant bait and possible contact, adult mosquitoes did not avoid the tube containing DEET. According to Pickett *et al.* [45] a true behavioural repellent is a substance causing, at a distance, oriented movements away from the odour source. Thus, at 1% and 25°C, DEET cannot definitely be considered as a spatial repellent product.

Permethrin showed a contact irritant effect at 0.34 nmol/cm^2, toxic and irritant effects at 3.4 nmol/cm^2 and no spatial repellent effect. This corroborates the results of Achee *et al.* [6] on *Ae. aegypti*. In their experiments, permethrin was irritant and toxic at 2.5 nmoles/cm^2 but did not appear repellent. Similarly, Dusfour *et al.* [46] showed that permethrin was irritant at 25 nmol/cm^2 on *An. albimanus* but had no repellent effect. Pyrethroids are toxic because they modify the gating kinetics of the voltage-dependent sodium channel [47]. Their irritant effect might also be due to their influence on the nervous system. The low vapour pressure of permethrin (7×10^6 Pa at 25°C) probably explains its lack of spatial repellency. Although pyrethroids are considered to have repellent, irritant and toxic effects [7], the treated bednets recommended by WHO could only be irritant and toxic [48] and not spatially

Table 2. Synthesis of the behavioural response of *An. gambiae* females to DEET, permethrin and 20 plant extracts at 3 concentrations (0.01, 0.1 and 1% of product in the solution on chromatographic papers).

Common name	Scientific name	Repellent effect	Irritant effect	Toxic effect	Extract form
DEET		0	+	0	Synthetic compound
Permethrin		0	+++	+	Synthetic compound
Aframomum	*Aframomum pruinosum*	0	+	0	Essential oil
Cinnamon	*Cinnamomum zeylanicum*	++	++	+	Essential oil
Citronella	*Cymbopogon winterianus*	+	++	+	Essential oil
Coleus	*Plectranthus tenuicaulis*	+++	+++	0	Essential oil
Coriander	*Coriandrum sativum*	+	+	0	Essential oil
Cumin	*Cuminum cyminum*	+	+++	0	Essential oil
Dill	*Anethum graveolens*	++	+	0	Essential oil
Eucalyptus	*Eucalyptus globulus*	0	0	0	Essential oil
Geranium	*Pelargonium graveolens*	0	0	0	Essential oil
Ginger	*Zingiber officinalis*	+	++	0	Essential oil
Lemon	*Citrus limon*	0	0	0	Essential oil
Lemongrass	*Cymbopogon citratus*	+++	+++	0	Essential oil
Litsea	*Litsea cubeba*	+	++	0	Essential oil
Neem	*Melia azadirachta*	0	0	0	Vegetal oil
Pennyroyal	*Mentha pulegium*	0	0	0	Essential oil
Pepper	*Piper nigrum*	+	0	0	Essential oil
Rosemary	*Rosmarinus officinalis*	0	0	0	Biologic hydrolat
Savory	*Satureja montana*	0	0	+	Essential oil
Solidage	*Solidago canadensis*	+	++	0	Essential oil
Thyme	*Thymus vulgaris*	++	+++	+	Essential oil

0 = significant difference from the control with Fisher's test, + = significant difference from the control with Fisher's test at one concentration.
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repellent. In our study, the repellency of permethrin was not exhibited. However in field experiments the number of mosquitoes entering huts protected by a treated net was usually low compared to a non-treated net [49], indicating repellency. Finally, we must keep in mind that permethrin is a synthetic analogue of natural pyrethrins extracted from *C. cinerariaefolium*. Hence, we can consider that plants have already provided us good tools for managing mosquitoes [7].

Pyrethroids are widely used to control *An. gambiae* [2]. They are employed in bednet treatment, impregnation of cloths, indoor residual spraying and spatial treatments. The advantage of pyrethroids is their effectiveness at low dosages. They are also toxic, irritant, fast acting, stable and safe for humans [50]. Prioritization of toxic actions over spatial repellent and contact irritant actions should be balanced with the higher risk of rapid selection for resistance to the active compounds [6]. Additionally, the huge number of crop fields treated with pyrethroids indirectly speeds up the selection of resistant *An. gambiae* populations [50,51,52]. Pyrethroid resistance in *An. gambiae* might be due either to a mutation in the sodium channel sequence or to a higher metabolic detoxification through increase of enzyme activities [33]. Pyrethroids, like some of the plant extracts that were tested in the present study, are also irritant and toxic. Moreover, many plant extracts could have an effect on both host-seeking and -feeding behaviour [26]. Unfortunately, the knowledge gaps on repellents' mode of physiological action has made it difficult to target the search for natural compounds to replace or synergize the DEET or the pyrethroids' action [29]. Can some plant extracts be used as alternatives to pyrethroids and DEET? From our results, the most promising plant extracts are those from *C. winterianus*, *C. zeylanicum* and *T. vulgaris* because they combine the three effects. A mixture containing complementary active compounds and modes of action could reduce the selective pressure for resistance [53]. Plant extracts can be good candidates to find efficient spatial repellent, contact irritant or toxic products. They have been largely studied but their use is limited because of their volatility. Plant extracts evaporate quickly causing a rapid decline in efficacy. Fortunately, new technologies (e.g. gelatin–gum arabic microcapsules) can preserve a repellent effect for up to 30 days on treated fabric stored at room temperature (22°C) [54]. The mere addition of vanillin increases the efficacy duration of an essential oil [55]. Tawatsin *et al.* [55] showed that lemongrass oil with 5% vanillin had a repellent activity of 8 hours. Some commercialized products based on cinnamon oil, are already sold as insecticides and miticides [18]. It would be interesting to test such products against disease vectors like *An. gambiae*. Currently, it is difficult to impregnate a bednet with an essential oil that is both long-lasting and provides resistance to 20 washings as recommended by WHO [56]. Thus, the identification of compounds contained in active essential oils is a necessary step before carrying out specific technologies for material impregnation (L2I company, France, personal communication).

Consistent with their properties, essential oils might be useful for vector control. Their use will depend on their effects. The toxic effect could be useful in indoor residual spraying (IRS) or spatial spray treatment. Their irritancy effect could be suitable in IRS or

treated bednet use. As indicated by the new WHO guidelines [57], the spatial repellency effect could also be a useful tool in vector control, as well as potential use as insect repellent (after safety tests), and in treatment of clothes or bednets. IRS, spatial spray, and repellent diffusers could also be considered. For instance, impregnating bednets with an irritant and repellent compound originating from essential oils for a long-lasting efficacy would be an interesting possibility. In addition, it would be particularly interesting, economically speaking, to choose essential oils from plants that are locally cultivated or with a rapid turnover in the wild. Amer & Mehlhorn [13] showed that cinnamom, citronella and lemongrass oils are repellent for three species of mosquitoes - *Ae. aegypti, Culex quinquefasciatus, An. stephensi*. The proportion of bites on arms treated with these essential oils was very close to zero. These authors also demonstrated an additive effect when using a blend of several essential oils extracted from *Litsea cubeba, Melaleuca leucadendron, M. quinquenervia, Viola odorata,* and *Nepeta cataria*. It is likely that a mixture of these five essential oils could be a suitable option in terms of personal protection because they do not have the same effects as some are irritant, others are repellent and a few might be toxic against these mosquitoes. However, these results cannot be extended to other mosquito species because variation in vector host-feeding preferences is known to trigger differential responses to essential oils [58].

The efficacy of the major compounds from the promising plant extracts (*C. winterianus, C. zeylanicum* and *T. vulgaris*) should be investigated to identified precisely their main mode of action and to determine if their combination have synergistic effects on *An. gambiae* and could be envisaged as serious alternatives to chemical insecticides for vector control.

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Author Contributions

Conceived and designed the experiments: ED. Performed the experiments: ED AC. Analyzed the data: ED PC FC. Contributed reagents/materials/analysis tools: ED CM RB. Wrote the paper: ED TM DB.

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ELECTROPHYSIOLOGICAL AND BEHAVIORAL CHARACTERIZATION OF BIOACTIVE COMPOUNDS OF FOUR ESSENTIAL OILS AGAINST *ANOPHELES GAMBIAE* AND PROSPECTS FOR THEIR USE AS BED NET TREATMENTS

(soumis à Journal of Chemical Ecology)

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Abstract-Laboratory and field studies showed that repellent, irritant and toxic actions of common public health insecticides reduce human-vector contact and thereby interrupt disease transmission. One of the more effective strategies to reduce disease risk involves the use of long-lasting treated bednets. However, development of insecticide resistance in mosquito populations makes it imperative to find alternatives to these insecticides. Our previous study identified four essential oils as alternatives to pyrethroids: *Thymus vulgaris, Cymbopogon winterianus, Cuminum cyminum, Cinnamomum zeylanicum.* The objectives of this study were to identify active compounds of these essential oils, to characterize their biological activity, and to examine their potential as a treatment for bed nets. We evaluated the electrophysiological, behavioural (repellency, irritancy) and toxic effects of the major compounds of these oils against *Anopheles gambiae* strain 'Kisumu'. Aldehydes elicited the strongest responses and monoterpenes the weakest responses in electroantennogram (EAG) trials. However, EAG responses did not correlate consistently with results of behavioral assays. In behavioral and toxicity studies, several of the single compounds did exhibit repellency, irritancy or toxicity in *An. gambiae*; however, the activity of essential oils did not always correlate with activity expected from the major components. On the contrary, the biological activity of essential oils appeared complex, suggesting interactions between individual compounds and the insect under study. Data also indicated that the three effects appeared independent, suggesting that repellency mechanism(s) may differ from mechanisms of irritancy and toxicity. Based on the bioassays reported here, some of the compounds merit consideration as alternative bednet treatments.

Key words-Essential oil, repellency, toxicity, vector control, DEET, permethrin.

INTRODUCTION

Anopheles gambiae (Giles, 1902) complex includes major vectors responsible for the transmission of *Plasmodium* spp., particularly *Plasmodium falciparum*, which is the most hazardous protozoan parasite that

causes malaria infection in humans (WHO 1993). One strategy to reduce vector transmission of pathogens that cause malaria is the through strategies which involve protection against mosquito bites. Bed nets treated with pyrethroids act as a physical-chemical barrier and thereby disrupt the vector-host contact. Pyrethroids are used because they are relatively safe for humans and they have rapid excito-repellent, knock-down and killing effects (Zaim *et al.*, 2000). However, pyrethroid resistance has been reported in 27 countries from sub-Saharan Africa, underscoring the urgent need to find alternatives to these insecticides (WHO 2008; WHO 2011; Ranson et al. 2011).

Plant-produced compounds have demonstrated efficacy in the prevention of mosquito bites (Moore et al. 2007). Some of the better known repellents are citronellal, myrcene, geraniol, citral, limonene, pinenes, citronellol, eugenol, and linalool (Moore et al. 2007). These natural compounds are biodegradable and environmentally friendly and are well accepted by people who do not want to avoid synthetic chemicals (Regnault-Roger et al. 2012). Terpenoids are the major constituents of essential oils. Essential oils are blends comprised of 30 to 100 different compounds (or more according to their source) in various proportions. From Ipek *et al.*, (2005), two or three of the major compounds of an essential oil are usually responsible for their biological activity. With multiple bioactive compounds present in an essential oil, the oil can affect multiple targets at the same time; therefore, neither resistance nor adaptation to these products has been yet documented (Bakkali et al. 2008). Despite their wide use, it is important to improve upon our knowledge of bioactive compound(s) to better understand their full potential as repellents and/or insecticides.

In previous studies we evaluated promising essential oils from four plants: *Thymus vulgaris, Cymbopogon winterianus, Cuminum cyminum, Cinnamomum zeylanicum* for their repellent, irritant and toxic effect (Deletre et al. 2013). These oils and their constituents might function as either topical repellents for use on skin or as a treatment for bed nets, but their active compounds are still unidentified. The objectives of the present study were to identify bioactive compounds in these essential oils, and to evaluate the responses of *An. gambiae* mosquitoes to these compounds using electrophysiological and behavioural assays that will shed light on whether they are suitable candidates for bed net treatment.

MATERIALS & METHODS

Mosquitoes. Behavioural assays were performed using female *An. gambiae* originating from the insecticide susceptible reference strain Kisumu. This strain, originally collected in Kenya in 1953, has been reared at LIN-IRD, Montpellier, France. The insecticide susceptibility of the Kisumu strain was confirmed with World Health Organization (WHO) diagnostic doses (i.e. 4% DDT, 0.75% permethrin) and is controlled every 4 months as recommended by the ISO 9001 norm. The colony was maintained in a climatic controlled room at 27 ± 2°C, 80 ± 10% RH and with a photoperiod cycle of 12h Light: 12h Dark. Mosquito larvae were fed a diet of fish food (TetraMin). Emerged adults were mechanically aspirated and transferred into 25 x 25 x 25 cm cages and provided access to 10% honey-water solution.

Products. Studies were performed with four plant essential oils: citronella (leaf), *Cymbopogon winterianus* (Nactis, France, lot 40018500); cumin (seed), *Cuminum cyminum* (Ipra, France, lot 902560); cinnamon (bark), *Cinnamomum zeylanicum* (Nactis, France); and thyme (leaf), *Thymus vulgaris* (Huiles & sens, France, lot A2) and 19 chemical standards (Sigma Aldrich, St Louis, MO, USA): citronellal (≥ 95% purity), geraniol (98% purity), citronellol (≥ 95% purity), (S)-(-)-limonene (96% purity), geranyl acetate (98% purity), cuminaldehyde (98% purity), (-)-β-pinene (99% purity), γ-terpinene (≥ 97% purity), *p*-cymene (99% purity), (E)-cinnamaldehyde (99% purity), 2-methoxy-cinnamaldehyde (98% purity), cinnamyl acetate (99% purity), thymol (99.5% purity), carvacrol (≥ 98% purity), α-terpinene (85% purity), linalool (97% purity), and β-

caryophyllene (≥ 80% purity), and (N,N-diethyl-3-methylbenzamide (DEET) and permethrin (≥ 80% purity) from Sigma-Aldrich, France. For the tunnel test (see below), formulated permethrin (PERIPEL 10 EC, Bayer Crop Science) was used. The pyrethroid permethrin, mainly used in mosquito nets and the insect repellent DEET, which is effective at reducing mosquito bites (Curtis et al. 1987; Badolo et al. 2004; Costantini et al. 2004), have been used as positive controls.

Four blends were prepared, each comprised of the major compounds found within the 4 selected essential oils: citronella blend (citronellal, geraniol, citronellol, limonene and geranyl acetate), cumin blend (cuminaldehyde, β-pinene, γ-terpinene and *p*-cymene), cinnamon blend (cinnamaldehyde, 2-methoxy-cinnamaldehyde and cinnamyl acetate) and thyme blend (thymol, p-cymene, carvacrol, α-terpinene, linalool and β-caryophyllene). Each blend was prepared by diluting the major compounds in ethanol in a ratio based on their respective proportions in the essential oils. DEET, permethrin, the four essential oils, the 17 essential oils compounds and the four blends were diluted at 0.1% and 1% (v/v for liquid compound or w/w for powdered compound) in a solvent that consisted of ethanol (2/3) and silicone oil Dow Corning 556 (1/3). All major compounds were tested at the relative concentration that they are found in the essential oils (Deletre et al. 2013), at the efficient concentration (concentration C2) and 1/10 of this concentration (concentration C1) (Table 1). For instance, citronellal accounts for approximately 34.7% of the citronella essential oil. The citronella oil was efficient at 1%, so the citronellal was tested at C2= 0.35% (0.03 mg/cm²) and 10 times less at C1= 0.035% (0.003 mg/cm²). By diluting in this manner, the quantity of a compound tested was approximately the same within the essential oil, the blend and for the compound alone. Each assay with a treatment was preceded by evaluation of a negative control that consisted of the solvent ethanol-silicone oil (Table 2). In spatial repellency assays, 3.3 mL of this solution was deposited on a 13 x 30 cm chromatography paper except on a border margin of 1.5 cm width. For contact irritancy and toxicity assays, 2 mL of the solution was deposited on 12 x 15 cm chromatography paper.

We impregnated 100 denier multifilament polyester netting for the residual effect assays with WHO tests kits (17cm x 20 cm) and the tunnel tests (25 cm x 25 cm), respectively. Polyester nets were impregnated with 1.9 ml and 3.5 ml solution respectively to obtain C2 (i.e. geraniol: 0.023 µl/cm^2, cinnamaldehyde: 0.079 µl/cm^2, carvacrol: 0.014 µl/cm^2, and cuminaldehyde: 0.030 µl/cm^2. The small pieces of net closing the tubes were also impregnated with the tested product. These volumes of solution corresponded to the specific absorption capacity of the net (56 ml/m^2) previously calculated according to WHOPES procedure (WHO 1998). The nets were allowed to dry for 30 min before the first test. For the tunnel tests, we used 0.1 µl/cm^2 each of permethrin, geraniol, cinnamaldehyde, carvacrol, cuminaldehyde, blends comprised of these last four products, citronella oil, cinnamon oil, thyme oil, cumin oil or linalool. Linalool was included in these tests because it was electrophysiologically active in EAG trials.

Gas chromatography analysis. The four essential oils (citronella, cinnamon, cumin, and thyme) were analysed on a Varian gas chromatograph, model CP-3380, equipped with a flame ionisation detector (FID). The FID was operated at 220°C and separation was effected using an HP_5 J&W Agilent (5%-phenyl-95% methylpolysiloxane) capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 220 and 250°C, respectively. The oven temperature was held at 60°C for 1 min after injection, then programmed to increase at 3°C min^{-1} to 220°C and held at 220°C for 1 min. The carrier gas was N_2 set at a flow rate of 0.8 ml/min. A 1 µl solution (10% essential oil in ethyl ether) was injected manually. A blend of alkanes (C9-C22) was injected to calculate the retention index: RI= [TR(X)-TR(n)]/[TR(n+1)-TR(n)]*100+100*n where TR(X) is the retention time of a studied product, TR(n) is the retention time of the alkane with *n* carbons that eluted before X, TR(n+1) is the retention time of the alkane of *n*+1 carbons that eluted

after X. The percentage composition of the essential oil was computed by the normalization method from GC/FID analyses, response factors were assumed equal to one for all compounds.

Coupled gas chromatography mass spectrometry analysis. GC-MS analyses were performed on a Hewlett Packard 5890 II gas chromatograph, interfaced to a single quadrupole mass selective detector (Model 5972). The column was a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 □m). Helium was the carrier gas, set at a flow rate of 0.6 ml/min. Injector and MS transfer line temperatures were set at 220°C and 250°C, respectively. The oven programme temperature was identical to that used in GC-FID analysis. Diluted samples (10:100 in CH_2Cl_2, v/v) of 1 □L were injected manually and in a split mode (1:100 split ratio). The MS was operated in the electron ionization (EI) mode with the filament set at 70 eV. Data were acquired over a range *m/z* 35-300 with a scan rate of 2.96 scan s^{-1}. The electron multiplier was set to 1460 eV. The identification of the compounds was accomplished by comparison of their relative retention indices as well as comparison of mass spectra with those of standards (for main components), those found in the literature (Adams 2007) and those supplemented by the NBS75K database and Wiley 7^{th} NIST 98 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software).

Electrophysiology. The major compounds (Table 2) were tested individually as olfactory stimuli using an EAG system. For the EAG recording, each compound was diluted to 1% in absolute ethanol (Carlo-Erba Reagents, Val de Reuil, France). Stimulus applicators were prepared by pipetting 25 µl of a test solution onto a 6 cm by 0.5 cm strip of Whatman No. One filter paper (Whatman International Ltd., Maidstone, Kent, UK), after which the filter paper was placed inside a 14.5-cm long glass Pasteur pipette. Fresh stimulus applicators were prepared after 2 h of use. Three controls were used: 1) an empty pipette, 2) a pipette containing 25 µl ethanol only on filter paper, and 3) a pipette containing 25 µl 100 µM octanal in ethanol on filter paper (octanal standard).

The EAG apparatus (Syntech Ltd., Hilversum, The Netherlands) was linked to a desktop computer (with IDAC-02 data acquisition interface board) on which recording, storing, and quantifying EAG responses were performed. The recording and indifferent electrodes were silver wires enclosed in drawn glass capillary tubes filled with phosphate buffered saline (NaCl, 4g; Na_2HPO_4, 0.57g; KH_2PO_4, 0.1g; KCl, 0.1g in 500 ml distilled water; pH 7.4).

Non-blood-fed females *An. gambiae* (4 to 7 days after emergence) were cooled in refrigerator (4°C) before excising the head with a scalpel. Both antennae remained intact and the tip of one randomly chosen antenna was removed with a scalpel. The recording electrode was placed on the tip of the cut antenna. The antennal preparation was bathed continuously by a stream of charcoal-filtered and humidified air at a flow rate of 1 l/min. Air temperature and relative humidity was measured 15 cm from the antennal preparation (overall ranges for all trials: 21-25°C, 42-68% RH). EAG recording began 6 min after the antennal preparation was mounted. At this time, the following test protocol was used for each recording trial. The controls were tested in the following order (empty, ethanol, octanal, empty), after which the first nine randomly chosen chemical treatments among the possible 17 were tested, then the controls again, then the last eight randomly chosen chemical treatments, and finally the controls again. Presentation of controls throughout the recording session permitted standardization of antennal responses. Test compounds and controls were applied (0.5 s pulse) at 30 s intervals separated by a purge of filtered-humidified air via an aluminum tube ca. 5 mm from the antenna. EAGs were measured as maximum amplitude of depolarization (mV). Each chemical was tested on 28 individuals.

Maximum EAG responses were control-adjusted with the ethanol only control, and expressed as proportional responses relative to the octanal standard. These data were then square root-transformed $0.5(\sqrt{x} + \sqrt{(x + 1)})$ (Zar 1996), and analysis of variance was used to compare maximum EAG deflection between

chemicals followed by a posthoc Tukey's HSD pairwise comparison test with the R 2.12.2 software (R Development Core Team 2012).

Behavioral bioassays. Detailed descriptions of the apparatus, assay protocols, and data analysis procedures have been published previously (Deletre et al. 2013 modified from Grieco et al. (2005). To summarize, bioassays were conducted between 10 am and 6 pm local hours at 24±1°C and 50±10% RH, and for each product, all assays were performed the same day.

Spatial repellency assays. The apparatus is a cylinder divided into two chambers, one treated and one untreated. Treated papers, with products or with only the solvent for control, were rolled around the inner surface of the treated chamber, whereas the inner surface of the untreated chamber was covered by untreated chromatograph paper. A metallic screen prevented direct mosquito contact with the treated paper. Twenty non-blood-fed females (aged 4 to 7 d old) were introduced in the treated chamber and after a 30-se acclimation period, the butterfly valve that separated the two chambers was opened for 10 min. At the end of the test, the butterfly valve was closed and the number of insects in each chamber was recorded. Mosquitoes moving from the treated chamber to the untreated chamber were recorded as 'escaped'. Conversely, mosquitoes remaining in the treated chamber were considered to have 'stayed'. Tests were replicated three times for each chemical.

Contact irritancy assays. These assays were performed using the system described above for the spatial repellent assay, and consisted of two connected tubes used in the WHO test kit and a possible mosquito contact with the chemical. Ten non-blood-fed females (age 4 to 7 d old) were introduced in the treated chamber and each test was performed six times for each chemical. After a 30-s acclimation period, the guillotine valve that separated the two chambers was opened for 10 min allowing the mosquitoes to move freely throughout the arena. Once the guillotine valve was closed, the number of mosquitoes in each tube ('stayed' *vs* 'escaped') was recorded.

Toxicity assays. Toxicity assays were performed using a WHO test kit (WHO 1998). Twenty non-blood-fed females (aged 4 to 7 d old) were exposed for 1 h to a treated paper (with products or with the solvent only) in the treated tube. Mosquitoes were then transferred to an untreated tube with 10% honey solution and maintained at 27°C and 80% RH. The number of dead and alive *An. gambiae* were recorded after 24 h post-exposure. Each test was replicated three times for each chemical.

We used the same method to analyse the proportion of dead mosquitoes in toxicity assays and the proportion of escaped mosquitoes in both spatial repellency and contact irritancy assays. Data analysis was carried out using the R 2.12.2 software. Tests of treatment effects for the different behavioural assays were carried out on the proportion of escaped or dead mosquitoes in (i) control and treated assays, (ii) essential oil and their associated compounds treated assays. We used Fisher's exact test corrected according to Bonferroni using the Holm's sequential method (Holm 1979). The behavioural and mortality data were corrected using Sun-Shepard's formula (Püntener 1981). For all products and concentrations, these corrected proportions were used to perform a principal component analysis (PCA). Then, a hierarchical ascendant classification (HAC) based on Ward's algorithm was used to group the compounds of essential oils based on the similarity of their effects using PCA-axes coordinates. This process yielded a binary segmentation tree, reflecting the hierarchy of similarities between responses to plant extracts. The optimal number of classes in the tree was determined by the decrease of the interclass variance (branch height-Appendix 1).

Residual efficacy assays. To determine if carvacrol, geraniol, cuminaldehyde and cinnamaldehyde would be efficacious during the 8 h duration of the tunnel experiment, these compounds were tested for their residual toxicity on bed nets. Using the same model of the contact irritancy assay, these assays were performed with two connected tubes used in the WHO test kit which allows for possible mosquito contact with the

chemical. Instead of using treated paper, treated bed nets with products or with solvent only (controls) were rolled around the inner surface of the treated chamber, whereas the inner surface of the untreated chamber was covered by a net, which were treated with neither product nor solvent. Non-treated chromatograph paper were introduced between the bed net and the tube to obtain the same luminosity than for the previous assays. Ten non-blood-fed females (aged 4 to 7 d old) were introduced in the treated chamber at intervals of 0, 3, 6, and 9h after the treated bed net had been dried. Controls (solvent treated net) were performed at 0 and at 9h. After a 30-s acclimation period, the slide unit that separated the two chambers was opened for 10 min. Mosquitoes were allowed to move freely and partition in the tubes. Once the slide unit was closed, the number of mosquitoes that 'escaped' and 'stayed' were recorded as well as their status: alive, knocked-down, or killed. To differentiate between knocked-down *vs* killed insects, the non-mobile mosquitoes were transferred into an untreated tube with 10% sucrose solution and maintained at 27°C and 80% RH. The number of dead and knocked-down i.e. 'mobile' *An. gambiae* was recorded after 24 h. Each test was replicated six times for each chemical. Between trials, the chambers were placed in a fume hood to remove the previous treatments and avoid contamination between replicates of the same product and to avoid contamination from one product to the next.

The same method was used to analyse the proportion of repelled, knocked-down, and killed mosquitoes in the assay. The proportions of mosquitoes of each treated assays : 0, 3, 6, and 9h were compared using Fisher's exact test to the proportions of the both control : at 0h and 9h. To account for multiple testing, *P*-values of those tests were corrected according to Bonferroni using the Holm's sequential method. Generalized linear models (GLM) were fitted to assess the effect of time, i.e. persistence effect of the product, on the proportions of repelled, knocked-down, or killed mosquitoes using a binomial distribution with a logit-link function (Hothorn et al. 2008). To assess the adequacy of the models, residuals were checked graphically using a normal quantile-quantile plot.

Tunnel assays. The tunnel assay system consisted of a square glass tunnel (height 25 cm, width 25 cm, length 60 cm) with netted cage ends (25 cm x 25 cm x 25 cm), subdivided by a changeable piece of netting with 9 × 1 cm holes inserted on a cardboard frame across the tunnel.

At one end of the tunnel (bait chamber), a guinea pig was used as bait. The animal was held in a small metallic cage to prevent contact with the netting. At the other end of the tunnel, 100 unfed female mosquitoes (aged 7–9 d old) were introduced at 0900 hr local time and the apparatus was left in a dark room maintained at 28°C and 80% relative humidity. At 1700 hr local time, the numbers of mosquitoes in both compartments were counted and their mortality and blood feeding rates were scored. Tests were replicated two times for each chemical.

We used the same method to analyse the proportion of mosquitoes that passed through the net, which were blood fed, and killed in the assay. The mosquito rates in control and treated tunnels were compared using Fisher's exact test. To take into account multiple testing, *P*-values of those tests were corrected according to Bonferroni using the Holm's sequential method (Holm 1979).

RESULTS

Electrophysiology. EAG responses of *An. gambiae* females clearly revealed that the insects responded to the compounds tested ($F= 23.5$, $DF= 18$, $P<0.001$) (Figure 1). The strongest responses were elicited by the two aromatic benzaldehydes: cinnamaldehyde and cuminaldehyde, an acyclic monoterpene alcohol (linalool), and an acyclic monoterpene aldehyde (citronellal). Mosquitoes were least responsive to the two cyclic monoterpene phenols tested (carvacrol and thymol). Other compounds elicited intermediate responses (Figure 1, Appendix 1).

Repellent assays. DEET and permethrin did not exhibit a repellent effect regardless of the concentration tested (C1 and C2) compared to the control (Figure 2). On the contrary, the essential oils and the blends of the major compounds had a significant repellent effect at the high dose (C2) and at the low dose (C1), only the citronella blend was repellent. The blends were as repellent as their associated essential oils. The following products exhibited the same repellency as the essential oil from which they come from: carvacrol, citronellal, geraniol, citronellol, cuminaldehyde, γ-terpinene. Although cinnamaldehyde was repellent, it was not as repellent as the cinnamon essential oil. According to the similarity of the behavioural response, the clustering procedure based on HAC yielded four contrasted response classes: Class A comprised four products (carvacrol, citronellal, geraniol, and cinnamaldehyde) that were efficacious at C2 and were the most repellent; Class B was not as repellent and included five products (cuminaldehyde, citronellol, linalool, geranyl acetate, cinnamyl acetate); Class C contained two products (DEET, β-caryophyllene) that were not repellent at any tested concentration even if the highest tested concentration showed a higher repellency effect; Class D consisted of eight products that were not repellent, irrespective of their concentration.

Irritant assays. DEET was a significant irritant at the high dose (C2) but not at the low dose (C1) (Figure 3). Permethrin was irritant at C1 and C2 concentrations. When permethrin was tested at the C2 concentration, 28.8% of the mosquitoes were knocked down, and therefore did not escape. All essential oils and blends produced an irritant effect at C2 as did all blends except for cumin blend at C1. At C2 there was no significant difference between the thyme, citronella and cinnamon essential oils and the associated blends. At least one concentration of thymol, carvacrol, citronellal, geraniol, citronellol, cuminaldehyde, cinnamaldehyde, and cinnamyl acetate were irritant compared to the control, and except for cinnamyl acetate, these compounds were as or more irritant as the essential oil that they come from. The HAC could be summarized by three response classes: class A with highly irritant products (DEET, citronellal, geraniol, cinnamaldehyde, cuminaldehyde, citronellol, carvacrol); class B with low irritant products (permethrin, geranyl acetate, thymol, cinnamyl acetate) and class C (eight products) with no irritant product.

Toxicity assays. Permethrin was lethal at the two concentrations tested (C1 and C2) and DEET only at the high dose C2 (Figure 4). All the essential oils were toxic at C2 but only the thyme blend and the cinnamon blend were toxic at C2 and as or more toxic than their associated essential oils. Only one compound, cinnamaldehyde was toxic at C2 as the *Cinnamomum zeylanicum* essential oil from which it is derived. The HAC analysis yielded three response classes: Class A with two products corresponding to permethrin and DEET; Class B with one product, cinnamaldehyde, the only natural compound that were toxic; and Class C with 16 products that were not toxic at all to mosquitoes at the high concentrations tested (C2).

Residual efficacy assays. The irritant effect of geraniol persisted up to 9 h post bed net treatment (Table 2). However a toxic and knock-down effect was observed at 3 h after the treatment, but not later. The irritant, knock-down and toxic effects of the cinnamaldehyde were still observed 9 h after the treatment. The irritant effect of the carvacrol was still observed 6 h after the treatment, but not later and the knock-down and toxic effect were present after 9 h, with the only observed significant decrease between 0 and 3 h. The irritant effect of cuminaldehyde was still observed 6 h after the treatment, but decreased over time. The knock-down and toxic effects were observed just after the treatment but not 3 h later.

Tunnel assays. At 0.1 µl/cm^2, the permethrin-treated net reduced significantly the cross rate of mosquitoes through the net (Table 3). There were fewer engorged mosquitoes and the mortality rate was significantly higher compared to the control. At the lowest concentration tested (0.05 µl/cm^2), cuminaldehyde showed the same effects, but to a lesser extent. At the lowest concentration tested, cinnamaldehyde and carvacrol did not reduce significantly the rate of mosquitoes that passed through the net. However, there were fewer

engorged mosquitoes and the mortality rates were significantly higher compared to the control. However, at higher doses these effects were not observed except for cuminaldehyde, for this compound, the mortality rate increased. We tested the nets impregnated with four essential to evaluate if synergism would occur between active compounds. A decrease in engorged mosquitoes and an increase in mortality were observed for cumin oil (30% cuminaldehyde), cinnamon oil (78% cinnamaldehyde), but not for thyme oil (14% carvacrol). The 1:1:1:1 blend of compounds (cinnamaldehyde, cuminaldehyde, carvacrol, geraniol) was evaluated for synergistic / additive effects. This blend reduced significantly the engorged mosquito rate compared to the control but there was no reduction of mosquitoes that passed through the treated net and the mortality rate was low. Surprisingly, more mosquitoes passed through the geraniol-treated net compared to the control, but no significant effect was observed upon the engorged and mortality rates. On the contrary, the engorged rate was significantly reduced with citronella oil (22% geraniol) compared with the control. No significant effects were observed for linalool.

DISCUSSION

This study is one of the first to explore electrophysiological and behavioral responses of *An. gambiae* to essential oils and their constituents. For EAG trials, aldehydes as cinnamaldehyde generally elicited stronger responses than did monoterpenes as limonene or terpinenes. Results from the behavioural trials were not always consistent with EAG responses. For example, the EAG response to carvacrol was relatively weak, but strong behavioural responses to this compound were observed. Conversely, mosquitoes exhibited relatively strong EAG responses to cuminaldehyde and linalool, but were not repelled well by these compounds. Correlation was observed for cinnamaldehyde and citronellal, both of which elicited relatively strong EAG and behavioral responses. These results may suggest involvement of different sensory neurons or another pathway than antennal reception in the phenomena of how repellents function. Inconsistencies between electrophysiological and behavioral results have been previously reported (Wee et al. 2008, Williams et al. 2010), and underscore the value of using a variety of research approaches when studying complex behavior such as repellency at the level of the whole organism. In their study on *Aedes aegypti*, Dekker et al. (2011) examined the repellent effect of electroantennographic detection (EAD)-active compounds of the headspace extracts of crushed *Ocimum forskolei*. They discovered that not all of the EAD-active compounds of this plant were repellent and the repellent compounds were structurally dissimilar. They did not study the repellent effect of the other non-active compounds. In another repellents study, the effect of *Osmanthus fragrans* on the cabbage butterfly *Pieris Rapae*, Ômura et al. (2000) demonstrated the repellency of γ-decalactone, and this correlated well with its deterrent effect on proboscis extension reflex but not necessarily with antennal sensitivity.

We demonstrated that geraniol, cuminaldehyde, carvacrol and cinnamaldehyde produced consistent behavioural effects compared to those of the essential oils from which they were derived, despite the fact that several were not a major constituent in the respective essential oil. In the repellency assays, the major compound blends were not significantly less repellent than the associated essential oils, suggesting that the major compounds in the blend could be the main essential oils responsible for the observed repellency of the oil. The repellency of carvacrol, citronellal, geraniol, or cuminaldehyde were not significantly different than the repellency of their corresponding essential oils. Therefore, these compounds appeared to be responsible for the repellent effect of their essential oils. Cinnamaldehyde was less repellent than the cinnamon oil suggesting a synergistic or additive effect with one or more other compounds within the oil. The irritant effects of the thyme oil, citronella oil, cumin oil, and cinnamon oil could be explained by thymol and/or carvacrol; citronellal, geraniol, and/or citronellol; cuminaldehyde; and cinnamaldehyde, respectively, because there was no significant difference in irritancy between the single compounds and the associated essential oils. For cumin oil it is

possible that there was an antagonistic effect between cuminaldehyde and another constituent because the cumin blend was less irritant than the cumin essential oil. Moreover, none of the major compounds appeared to be responsible for the toxicity observed from the citronella and cumin oil. No difference was observed between cinnamon oil and the cinnamon blend, between cinnamon oil and cinnamaldehyde or between thyme blend and the thyme oil; therefore, the cinnamon oil toxicity could be due to the cinnamaldehyde and the thyme oil toxicity to the major compounds in the blend. Overall, it appeared that the effect of an active compound could be enhanced by the other major compounds and/or modulated by minor compounds to give additive or synergistic effects. For example, repellency and toxicity of cinnamaldehyde could be synergised by minor compounds, while the irritancy of carvacrol appeared to be reduced by minor compounds. Repellent and irritant effects of essential oils were usually due to one compound except for citronella oil (citronellal, geraniol and citronellol). So another phenomenon that was identified was the importance of minor compounds in the toxicity of an essential oil. When the minor compounds of citronella and cumin essential oil were not present, toxicity was reduced. This suggests different modes of action for irritancy and repellency than for toxicity. The toxicity of the two other essential oils could be due to a minor compound or a synergistic effect due to the mixture of several compounds. Until now it was assumed that the major compounds of an essential oil reflected the biological response of this essential oil and the response level depended on the concentration of the compound (Ipek et al. 2006). In previous studies, only the effect of a complete essential oil and sometimes the major compound were studied (Isman 2000; Regnault-Roger and Hamraoui 1997; Shaaya et al. 1991). However, our results showed that the bioactivity of an essential oil does not necessarily mirror the activity of the major component. Instead, activity of an essential oil is complex and depends on interactions between individual compounds and the insect under study.

After 9h, the efficacy of natural compounds, i.e. their repellency, irritancy, and toxicity, was decreased. The toxic and knock-down effect of all products decreased over time. Geraniol and carvacrol produced relatively stable irritancy, while cuminaldehyde decreased over time, and the irritancy of cinnamaldehyde increased (Appendix 2). However, for cinnamaldehyde and carvacrol, the majority of knocked-down mosquitoes (cinnamaldehyde at 0 h 11.3% *vs* 0.0%; carvacrol at 3 h 14.9% *vs* 3.0%) were in the non-treated chamber, whereas the majority of dead mosquitoes (cinnamaldehyde at 0 h 64.5% *vs* 17.7%; carvacrol at 3 h 50.7% *vs* 13.4%) were found in the treated chamber. Therefore, if we take into account the living mosquitoes that 'stayed' and 'escaped', the irritant effect of geraniol, carvacrol, and cuminaldehyde decreased over time, whereas the repellent effect of cinnamaldehyde increased over time (Appendix). The effect of compounds were higher than in the contact irritancy assay while the amount of product per unit area of bed net was the same. Indeed, a net is mainly composed of empty spaces between the polyester fibres where the compound is concentrated and where the tarsal contact occurred. Short duration of protection time is a drawback to essential oils (Nerio et al. 2010). However, with the new technologies currently available it is possible to increase their residual efficacy. The active products can be encapsulated, used with polymer resins or synergised by other compound like vanillin (Picard et al. 2012; Regnault-Roger 1997). However, this rapid decrease of efficacy cannot explain the lack of efficiency of the compound in tunnel tests. For most compounds, repellency appeared to be weaker than the attraction to the host. Cuminaldehyde and cinnamaldehyde, which were the most efficient against *An. gambiae*, were also the most efficient in the tunnel test but less than permethrin. A disadvantage to the use of antifeedant products is that insects can lose sensitivity or change their mode of feeding after repeated and prolonged exposure (Jermy 1990; Foster and Harris 1997). This could also be true for repellent/irritant compounds, so the lack of efficiency in time could be due to habituation, not volatility. After a long exposure time, mosquitoes are acclimated to the product and the sensory stimuli have a decreased effect on the nervous system leading to a

modified behavioural response. Another explanation is that in the tunnel test, the host is so close and visible that mosquitoes did not use their olfactory system, but relied instead on visual cues (Carnevale and Robert 2009).

In view of our results, the use of natural compounds to treat nets holds promise, but their use in personal protection should also be considered. The World Health Organisation (2008) provided the following definition: « for a material to be valuable as a mosquito repellent it must effectively discourage insect attack on the treated area for many hours and on many different types of surfaces, it must work in different environmental conductions, it must be environmental friendly when applied to human or animal skin, it must be cosmetically acceptable having a pleasant odour, taste and feel, it should also be harmless to clothing, it should have a relatively low cost and be effective against other common types of insects, such as flies». Carvacrol, α-terpinene, citronellal, citronellol, geraniol, cuminaldehyde, cinnamaldehyde and cinnamyl acetate were repellent and/or irritant to insect attack and so they are good candidates for personal protection. However, to be useful, skin repellents need to be innocuous (low toxicity to humans) and to provide protection at least 4 h (Duvallet and De Gentille 2012). Efficacy of essential oils is usually less than 20 min, and moreover, they can be photosensitive and allergenic even if mammalian toxicity is low (Isman 2000; Isman 2006). In this study we focused on characterization of the bioactive compounds in essential oils and so it is easier to move research forward and determine the characteristics and the target of the active compounds. For example, in cinnamon essential oil the active compound is mainly cinnamaldehyde but Smith Pease et al. (2003) showed it is allergenic and thus cannot be used as skin repellent. Compounds from citronella can be potential alternatives to repellents, especially since they are non-toxic individually and when mixed. It would be interesting to mix two or three compounds with different effects to avoid habituation behaviour from mosquitoes. The efficacy of the citronella major compounds mixed equals the one of the essential oil so the blend: citronellal-citronellol-geraniol could be interesting. Different mode(s) of action could delay resistance by mutation or insensitivity to one particular product, which has a specific target.

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TABLE 1. RATIOS AND QUANTITIES OF THE INDIVIDUAL COMPOUNDS OF THE ESSENTIAL OILS: CITRONELLA, CUMIN, THYME AND CINNAMON.

Essential oil and plant species	**Composition (%)**[1]		**Quantity tested (µl/cm²)**[2] **C1**	**C2**
Citronella *Cymbopogon winterianus*	34.7	citronellal	0.004	0.035
	22.5	geraniol	0.002	0.023
	12.0	citronellol	0.001	0.012
	3.5	geranyl-acetate	0.0003	0.003
	3.3	limonene	0.0003	0.003
	76.0	**sub-total (blend)**	**0.010**	**0.100**
	4.2	elemol	NT	NT
	2.9	citronellyl acetate	NT	NT
	2.5	β-elemene	NT	NT
	2.2	δ-cadinene	NT	NT
	0.9	linalool	NT	NT
	0.8	eugenol	NT	NT
	89.5	**total**	**NT**	**NT**
Cumin *Cuminum cyminum*	30.1	cuminaldehyde	0.003	0.030
	12.2	β-pinene	0.001	0.012
	11.6	γ-terpinene	0.001	0.012
	9.7	*p*-cymene	0.001	0.097
	63.6	**sub-total (blend)**	**0.010**	**0.100**
	16.6	p-mentha-1,3-dien-7-al	NT	NT
	8.8	p-mentha-1,4-dien-7-al	NT	NT
	0.6	α-pinene	NT	NT
	0.4	myrcene	NT	NT
	0.4	limonene	NT	NT
	90.4	**total**	**NT**	**NT**
Thyme *Thymus vulgaris*	30.5	thymol	0.003	0.031
	23.7	*p*-cymene	0.002	0.024
	13.6	carvacrol	0.001	0.014
	8.4	α-terpinene	0.001	0.008
	4.0	linalool	0.0004	0.004
	3.5	□-caryophyllene	0.0004	0.004
	83.7	**sub-total (blend)**	**0.010**	**0.100**
	1.7	myrcene	NT	NT
	1.1	borneol	NT	NT
	1.1	α-pinene	NT	NT
	1.4	γ-terpinene	NT	NT
	1.2	terpinen-4-ol	NT	NT
	0.9	limonene	NT	NT
	0.8	α-thujene	NT	NT
	91,9	**total**	**NT**	**NT**
Cinnamon *Cinnamomum zeylanicum*	78.5	(E)-cinnamaldehyde	0.008	0.079
	9.6	2-methoxy-cinnamaldehyde	0.001	0.096
	3.1	cinnamyl-acetate	0.003	0.031
	91.2	**sub-total (blend)**	**0.0100**	**0.1000**
	1.1	benzaldehyde	NT	NT
	0.9	coumarine	NT	NT
	0.7	phenyl ethyl alcohol	NT	NT
	0.4	(Z)-cinnamaldehyde	NT	NT
	94,3	**total**	**NT**	**NT**

[1] The percentage composition of the essential oil was computed by the normalization method from GC/FID analyses, response factors being taken as one for all compounds. The composition of the four essential oils was identified by gas chromatography and mass spectrometry.

[2] The used quantities are expressed in µl/cm^2 of chromatograph paper or net.

TABLE 2. PROPORTION OF *ANOPHELES GAMBIAE*[1] FEMALES THAT WERE IRRITATED, KNOCKED DOWN, AND KILLED BY GERANIOL (0.023 µL/CM^2), CINNAMALDEHYDE (0.079 µL/CM^2), CARVACROL (0.014 µL/CM^2) AND CUMINALDEHYDE (0.030 µL/CM^2) AFTER 0, 3, 6 AND 9 HOURS OF THE NET TREATMENT.

product	time (h)	n	irritated	knocked-down	killed
control	0	66	6.1 (0.3 - 11.9)[2]	0.0 (0.0 - 0.0)	1.5 (-1.4 - 4.4)
geraniol	0	61	**45.9 (33.4 - 58.4)**[3]	**11.5 (3.5 - 19.5)**	**16.4 (7.1 - 25.7)**
geraniol	3	65	**38.5 (26.7 - 50.3)**	**3.1 (-1.1 - 7.3)**	**12.3 (4.3 - 20.3)**
geraniol	6	66	**34.8 (23.3 - 46.3)**	3.0 (-1.1 - 7.1)	0.0 (0.0 - 0.0)
geraniol	9	60	**36.7 (24.5 - 48.9)**	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
control	9	65	7.7 (1.2 - 14.2)	0.0 (0.0 - 0.0)	1.5 (-1.5 - 4.5)
p-value (model estimate)[4]			*0.259*	*<0.001 (-0.4)*	*<0.001 (-0.6)*
control	0	62	9.7 (2.3 - 17.1)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
cinnamaldehyde	0	62	**35.5 (23.6 - 47.4)**	**11.3 (3.4 - 19.2)**	**82.3 (72.8 - 91.8)**
cinnamaldehyde	3	66	**40.9 (29.0 - 52.8)**	**6.1 (0.3 - 11.9)**	**68.2 (57.0 - 79.4)**
cinnamaldehyde	6	61	**54.1 (41.6 - 66.6)**	**19.7 (9.7 - 29.7)**	**60.7 (48.4 - 73.0)**
cinnamaldehyde	9	56	**62.5 (49.8 - 75.2)**	**16.1 (6.5 - 25.7)**	**57.1 (44.1 - 70.1)**
control	9	62	9.7 (2.3 - 17.1)	0.0 (0.0 - 0.0)	3.2 (-1.2 - 7.6)
p-value (model estimate)			*0.001 (0.1)*	*0.006 (-0.1)*	*0.003 (-0.1)*
control	0	63	6.3 (0.3 - 12.3)	0.0 (0.0 - 0.0)	1.6 (-1.5 - 4.7)
carvacrol	0	61	14.8 (5.9 - 23.7)	**9.8 (2.3 - 17.3)**	**86.9 (78.4 - 95.4)**
carvacrol	3	67	**43.3 (31.4 - 55.2)**	**17.9 (8.7 - 27.1)**	**64.2 (52.7 - 75.7)**
carvacrol	6	65	**46.2 (34.1 - 58.3)**	**40.0 (28.1 - 51.9)**	**43.1 (31.1 - 55.1)**
carvacrol	9	54	20.4 (9.7 - 31.1)	**22.2 (11.1 - 33.3)**	**48.1 (34.8 - 61.4)**
control	9	71	18.3 (9.3 - 27.3)	0.0 (0.0 - 0.0)	2.8 (-1.0 - 6.6)
p-value (model estimate)			*0.368*	*<0.001 (-0.2)*	*<0.001 (-0.2)*
control	0	65	7.7 (1.2 - 14.2)	0.0 (0.0 - 0.0)	1.5 (-1.5 - 4.5)
cuminaldehyde	0	67	**52.2 (40.2 - 64.2)**	**22.4 (12.4 - 32.4)**	**38.8 (27.1 - 50.5)**
cuminaldehyde	3	59	**61.0 (48.6 - 73.4)**	0.0 (0.0 - 0.0)	5.1 (-0.5 - 10.7)
cuminaldehyde	6	71	**42.3 (30.8 - 53.8)**	0.0 (0.0 - 0.0)	**12.7 (5.0 - 20.4)**
cuminaldehyde	9	63	25.4 (14.7 - 36.1)	0.0 (0.0 - 0.0)	1.6 (-1.5 - 4.7)
control	9	64	10.9 (3.3 - 18.5)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)
p-value (model estimate)			*<0.001 (-0.1)*	*1.00*	*<0.001 (-0.2)*

[1]4- to 7-day-old, non-blood-fed, sugar-fed, Kisumu strain

[2]confidence interval calculated with the Wald method

[3]Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the controls with the Holm's sequential Bonferroni correction method.

[4]P-value and model estimate of the generalized linear model of the time on the mosquito repellency, knock down effect, and mortality.

TABLE 3. EFFECT OF NETTING IMPREGNATED WITH PERMETHRIN, GERANIOL, CINNAMELDEHYDE, CARVACROL, CUMINALDEHYDE, BLEND OF THE LAST FOUR, CITRONELLA OIL, CINNAMON OIL, THYME OIL, CUMIN OIL OR LINALOOL IN TUNNEL CAGE ON *ANOPHELES GAMBIAE*[1] FEMALES.

	produit	dose (µl/cm2)	N[2]	passed through net (%)	engorged (%)	mortality (%)
1	control	0	285	86.0	60.7	5.6
	permethrin	0.1	362	59.1*	11.3*	64.6*
	geraniol	0.03	300	95.0*	72.0	10.0
	cinnamaldehyde	0.08	274	80.3	46.0*	22.3*
2	control	0	283	86.9	68.9	10.2
	carvacrol	0.03	219	82.2	52.5*	31.1*
	cuminaldehyde	0.05	263	57.0*	33.8*	44.9*
3	control	0	260	96.2	87.7	5.8
	permethrin	0.1	263	51.0*	8.4*	64.6*
	cuminaldehyde	0.1	259	96.5	76.4	22.0*
	cinnamaldehyde	0.1	356	94.7	87.9	6.7
4	control	0	267	98.1	86.5	8.6
	geraniol	0.1	257	94.6	78.6	11.3
	carvacrol	0.1	267	91.4*	83.9	10.5
5	control	0	231	98.7	80.5	6.9
	Blend[3]	0.1	235	81.3	60.4*	6.0
	thyme oil	0.1	225	93.8	73.3	15.6
	cinnamon oil	0.1	266	94.7	65.4*	25.9*
6	control	0	266	95.5	85	5.6
	cumin oil	0.1	240	93.8	74.6*	6.7
	citronella oil	0.1	224	95.1	62.5*	3.6
	linalol	0.1	272	93.8	77.6	4.0

[1] 7- to 9-day-old, non-blood-fed, sugar-fed, Kisumu strain
[2] number of *An. gambiae* female tested
[3] blend of carvacrol, geraniol, cinnamaldehyde, cuminaldehyde (1:1:1:1)
* Significant difference ($p<0.05$, fisher test with the Holm's sequential Bonferroni correction method) between values for control and treatment tunnels.

Fig. 1 EAG response of *Anopheles gambiae* to 17 synthetic compounds of four essential oils. EAG amplitudes (mean ± SE) are control-adjusted and presented as relative response to the standard, 100 µM octanal. Each compound was tested on 28 female mosquitoes at 1% (v/v) concentration in ethanol.

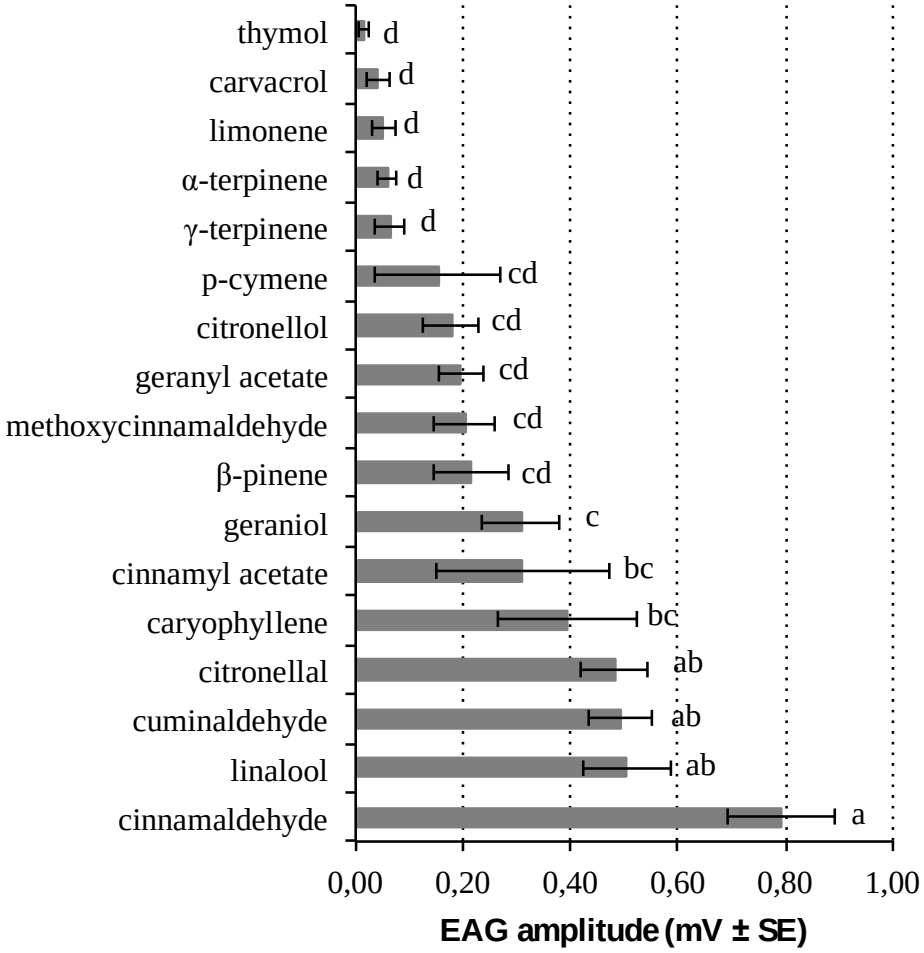
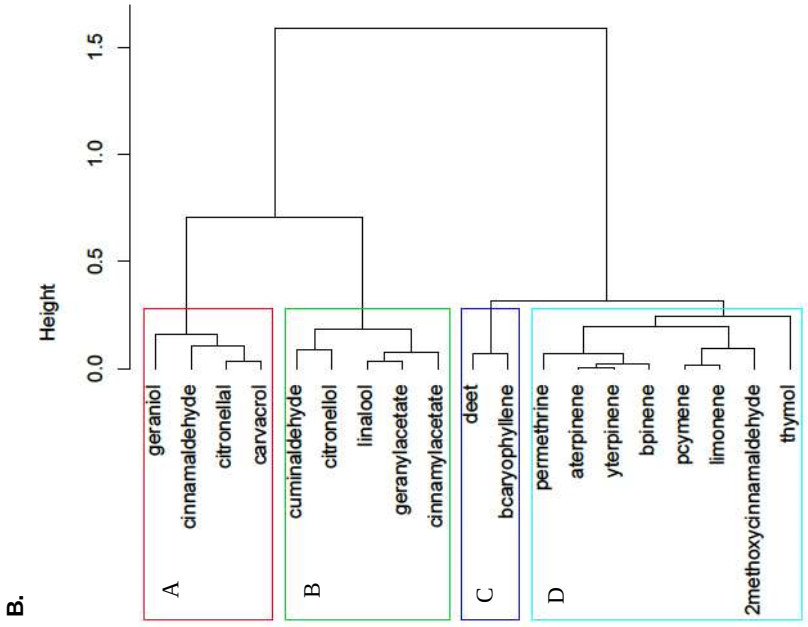


Fig. 2 Response of 4-7-day-old, non-blood-fed, sugar-fed, Kisumu strain of *Anopheles gambiae* females to the repellent effect of DEET, permethrin and four essential oils and their compounds at two different concentrations (C1 and C2 µl/cm^2 of product on chromatographic papers, refer to Table 1): A. corrected proportion escaping using Sun-Shepard's formula (confidence interval calculated with the Wald method) by treatment concentration and B. dendrogram determined by hierarchical ascendant classification.



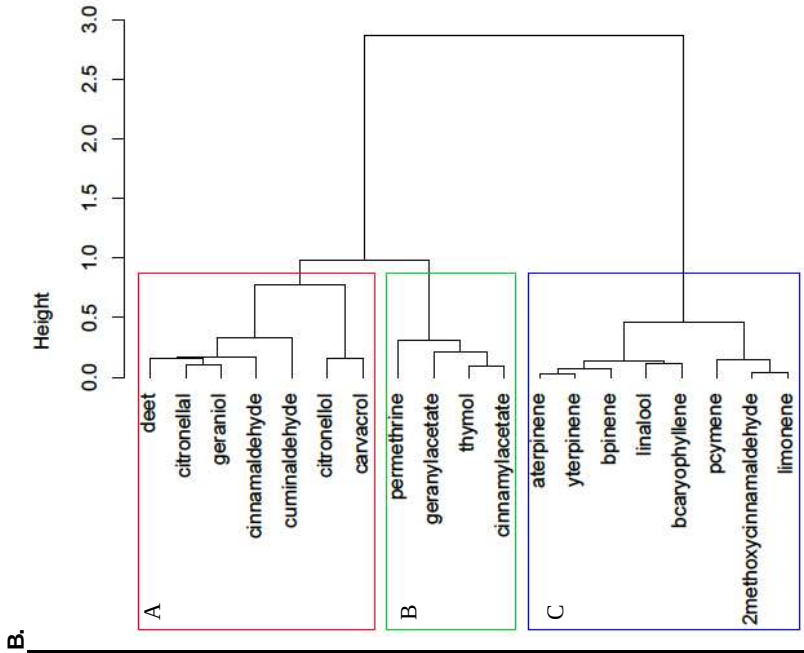
A.

Product	C1	C2
permethrin	-0.2 (-5.3 - 4.9)	6.3 (-1.5 – 14.0)
DEET	-3.3 (-8.0 - 1.4)	15.0 (5.2 - 24.7)
thyme oil	-1.3 (-5.1 - 2.5)	**51.4 (39.3 - 63.4)**
thyme blend	8.6 (-0.1 - 17.2)	**83.0 (75.2 - 90.9)**
thymol	16.6 (6.2 – 27)	-2.4 (-5.7 – 1.0) *
p-cymene	-0.1 (-4.0 - 3.7)	-1.5 (-4.3 - 1.4) *
carvacrol	9.4 (1.7 – 17.0)	**36.6 (25.0 - 48.2)**
α-terpinene	25.7 (14.4 – 37.0) *	4.3 (-2.4 – 11.1) *
linalool	12.7 (4.5 - 20.9)	17.6 (8.6 - 26.7) *
β-caryophyllene	-8.7 (-14.3 - -3.1)	10.4 (-0.2 - 21.1) *
citronella oil	-1.3 (-8.1 - 5.4)	**54.3 (42.7 - 65.8)**
citronella blend	**32.9 (21 - 44.8)** *	**76.2 (67.3 - 85.2)**
citronellal	8.9 (0.3 - 17.4)	**40.1 (28.1 - 52.2)**
geraniol	5.1 (-2.7 – 13.0)	**50.5 (38.0 - 63.1)**
citronellol	10.6 (1.1 – 20.0)	25.2 (13.8 - 36.6)
limonene	-0.1 (-4.4 - 4.2)	-3.3 (-3.3 - -3.3) *
geranyl-acetate	13.1 (4.6 - 21.6)	15.5 (7.1 - 23.9) *
cumin oil	4.7 (-0.5 - 9.9)	**37.3 (25.7 - 48.9)**
cumin blend	0.0 (0.0 - 0.0)	**30.6 (19.2 - 42.1)**
cuminaldehyde	1.6 (-1.5 - 4.7)	**25.4 (14.3 - 36.5)**
β-pinene	3.9 (-1.2 – 9.0)	2.8 (-1.9 - 7.5) *
γ-terpinene	4.5 (-1.1 - 10.1)	21.0 (11.0 – 31.0)
p-cymene	0.2 (-6.1 - 6.5)	0.2 (-6.1 - 6.5) *
cinnamon oil	8.5 (0.9 - 16.1)	**84.0 (75.7 - 92.3)**
cinnamon blend	12.4 (3.3 - 21.5)	**66.2 (54.4 – 78.0)**
cinnamaldehyde	16.5 (7.8 - 25.3)	**43.0 (32.1 – 54.0)** *
2-methoxy-cinnamaldehyde	-6.8 (-12.2 – -1.5)	-3.0 (-10.3 - 4.2) *
cinnamyl-acetate	7.6 (-0.8 - 16)	15.8 (6.0 - 25.7) *

1) Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the control with the Holm's sequential Bonferroni correction method.

* Pairwise comparison of proportion was done using Fisher's test between one compound and the essential that it comes from. Values followed by a star were significantly different from the original essential oil with the Holm's sequential Bonferroni correction method.

Fig. 3 Response of 4-7-day-old, non-blood-fed, sugar-fed, Kisumu strain of *Anopheles gambiae* females to the irritant effect of DEET, permethrin and four essential oils and their compounds at two different concentrations (C1 and C2 µl/cm^2 of product on chromatographic papers, refer to Table 1): A. corrected proportion escaping using Sun-Shepard's formula (confidence interval calculated with the Wald method) by treatment concentration and B. dendrogram determined by hierarchical ascendant classification.



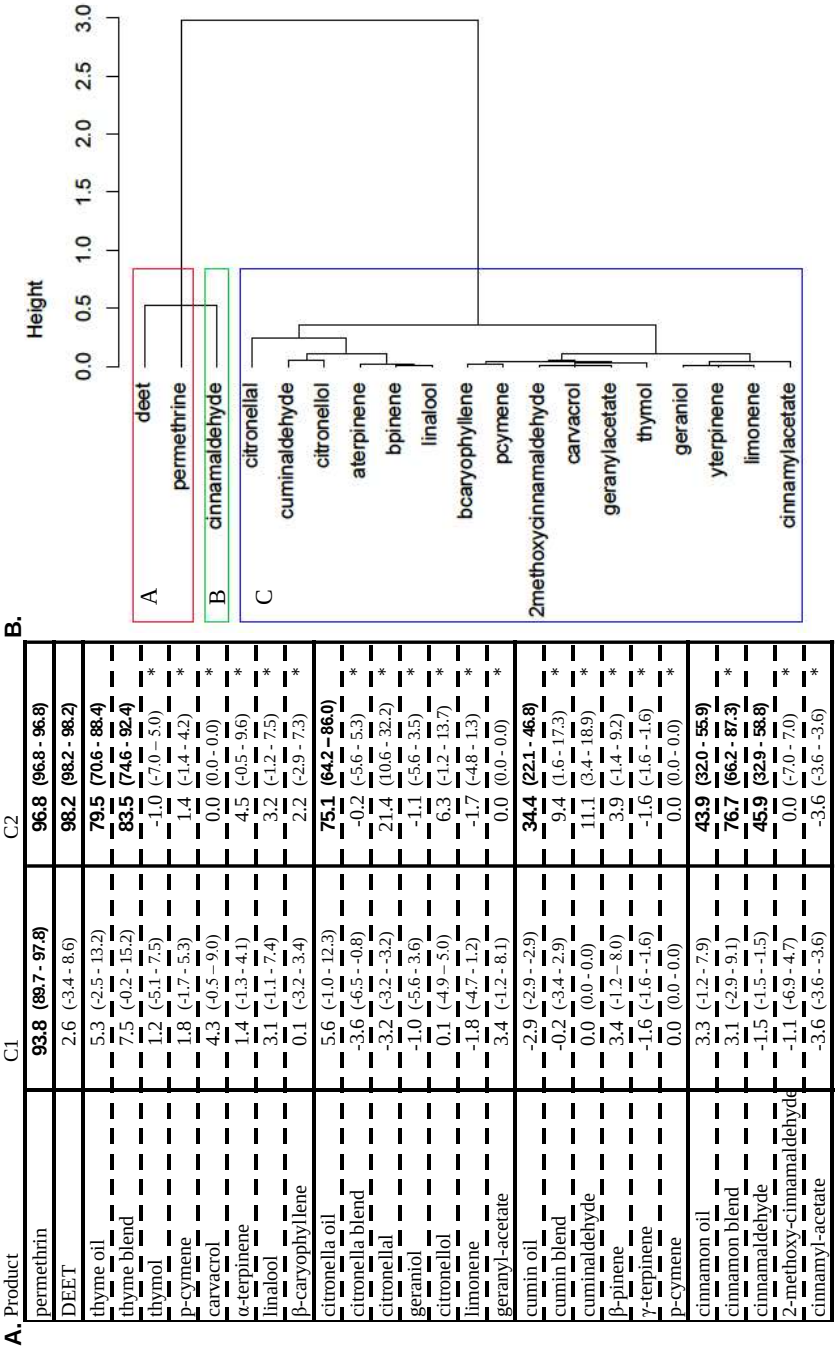
A.

Product	C1	C2
permethrin	**37.7 (25.0 – 50.4)**	**25.9 (13.8 - 38.0)**
DEET	7.6 (-0.2 - 15.5)	**54.1 (42.6 - 65.7)**
thyme oil	20.1 (9.5 - 30.6)	**59.0 (49.0 – 69.0)**
thyme blend	**35.6 (23.8 - 47.4)**	**38.7 (26.6 – 50.8)**
thymol	25.4 (13.0 - 37.7)	**34.7 (21.8 - 47.5)**
p-cymene	9.4 (0.2 - 18.7)	-6.5 (-11.6 - -1.4) *
carvacrol	**50.0 (38.2 - 61.8)** *	**84.3 (77.4 - 91.2)**
α-terpinene	1.6 (-2.4 - 5.6)	10.7 (3.3 - 18.1) *
linalool	1.6 (-4.3 - 7.6)	10.1 (0.8 - 19.4) *
β-caryophyllene	4.3 (-4.4 - 12.9)	20.8 (9.6 - 32) *
citronella oil	33.0 (21.1 - 44.8)	**60.3 (50.5 - 70.2)**
citronella blend	**34.2 (23.0 - 45.4)**	**82.2 (74.2 - 90.2)**
citronellal	24.4 (12.5 - 36.4)	**52.2 (42.1 - 62.3)**
geraniol	16.5 (5.2 - 27.7)	**45.9 (34.2 - 57.5)**
citronellol	**46.4 (35.3 - 57.5)**	**68.6 (60.7 - 76.5)**
limonene	-3.1 (-11.3 - 5.1)	-3.3 (-11.3 - 4.8) *
geranyl-acetate	6.8 (-1.7 - 15.4)	21.7 (9.9 - 33.6) *
cumin oil	7.9 (-1.2 – 17.0	**79.4 (72.8 – 86.0)**
cumin blend	4.8 (-2.1 - 11.7)	**41.1 (30.1 - 52.1)** *
cuminaldehyde	10.4 (1.1 - 19.6)	**77.5 (69.4 - 85.6)**
β-pinene	11.2 (1.1 - 21.3)	15.6 (6.0 - 25.3) *
γ-terpinene	8.9 (-0.4 - 18.1)	12.7 (3.2 - 22.2) *
p-cymene	-2.7 (-9.6 -4.2)	-6.1 (-11.4 - -0.8) *
cinnamon oil	25.8 (15.2 - 36.3)	**90.4 (85.6 - 95.2)**
cinnamon blend	**56.7 (46.4 – 67.0)** *	**60.2 (50.3 - 70.2)**
cinnamaldehyde	22.0 (12.3 - 31.7)	**63.2 (52.9 - 73.4)**
2-methoxy-cinnamaldehyde	0.3 (-9.9 - 10.6)	-1.0 (-11.1 - 9.1) *
cinnamyl-acetate	29.2 (17.2 - 41.2)	**43.5 (32.6 - 54.4)** *

1) Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the control with the Holm's sequential Bonferroni correction method.

* Pairwise comparison of proportion was done using Fisher's test between one compound and the essential that it comes from. Values followed by a star were significantly different from the original essential oil with the Holm's sequential Bonferroni correction method.

Fig. 4 Response of 4-7-day-old, non-blood-fed, sugar-fed, Kisumu strain of *Anopheles gambiae* females to the toxic effect of DEET, permethrin and four essential oils and their compounds at two different concentrations (C1 and C2 µl/cm^2 of product on chromatographic papers, refer to Table 1): A. corrected proportion escaping using Sun-Shepard's formula (confidence interval calculated with the Wald method) by treatment concentration and B. dendrogram determined by hierarchical ascendant classification.



A. Product	C1	C2
permethrin	**93.8 (89.7 - 97.8)**	**96.8 (96.8 - 96.8)**
DEET	2.6 (-3.4 - 8.6)	**98.2 (98.2 - 98.2)**
thyme oil	5.3 (-2.5 - 13.2)	**79.5 (70.6 - 88.4)**
thyme blend	7.5 (-0.2 - 15.2)	**83.5 (74.6 - 92.4)**
thymol	1.2 (-5.1 - 7.5)	-1.0 (-7.0 – 5.0) *
p-cymene	1.8 (-1.7 - 5.3)	1.4 (-1.4 - 4.2) *
carvacrol	4.3 (-0.5 – 9.0)	0.0 (0.0 - 0.0) *
α-terpinene	1.4 (-1.3 - 4.1)	4.5 (-0.5 - 9.6) *
linalool	3.1 (-1.1 - 7.4)	3.2 (-1.2 - 7.5) *
β-caryophyllene	0.1 (-3.2 - 3.4)	2.2 (-2.9 - 7.3) *
citronella oil	5.6 (-1.0 - 12.3)	**75.1 (64.2 – 86.0)**
citronella blend	-3.6 (-6.5 - -0.8)	-0.2 (-5.6 - 5.3) *
citronellal	-3.2 (-3.2 - -3.2)	21.4 (10.6 - 32.2) *
geraniol	-1.0 (-5.6 - 3.6)	-1.1 (-5.6 - 3.5) *
citronellol	0.1 (-4.9 – 5.0)	6.3 (-1.2 - 13.7) *
limonene	-1.8 (-4.7 - 1.2)	-1.7 (-4.8 - 1.3) *
geranyl-acetate	3.4 (-1.2 - 8.1)	0.0 (0.0 - 0.0) *
cumin oil	-2.9 (-2.9 - -2.9)	**34.4 (22.1 - 46.8)**
cumin blend	-0.2 (-3.4 - 2.9)	9.4 (1.6 - 17.3) *
cuminaldehyde	0.0 (0.0 - 0.0)	11.1 (3.4 - 18.9) *
β-pinene	3.4 (-1.2 – 8.0)	3.9 (-1.4 - 9.2) *
γ-terpinene	-1.6 (-1.6 - -1.6)	-1.6 (-1.6 - -1.6) *
p-cymene	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0) *
cinnamon oil	3.3 (-1.2 - 7.9)	**43.9 (32.0 - 55.9)**
cinnamon blend	3.1 (-2.9 - 9.1)	**76.7 (66.2 - 87.3)** *
cinnamaldehyde	-1.5 (-1.5 - -1.5)	**45.9 (32.9 - 58.8)**
2-methoxy-cinnamaldehyde	-1.1 (-6.9 - 4.7)	0.0 (-7.0 - 7.0) *
cinnamyl-acetate	-3.6 (-3.6 - -3.6)	-3.6 (-3.6 - -3.6) *

1) Pairwise comparison of proportion was done using Fisher's test. Values in bold lettering were significantly different from the control with the Holm's sequential Bonferroni correction method.

* Pairwise comparison of proportion was done using Fisher's test between one compound and the essential that it comes from. Values followed by a star were significantly different from the original essential oil with the Holm's sequential Bonferroni correction method.

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Behavioral Response of *Bemisia tabaci* to 20 Plant Extracts

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Abstract. In the Mediterranean region, the use of small mesh netting to protect horticultural crops is an effective sustainable tool against pests. But in tropical region, because of the high humidity under the net favouring the fungi development, net with bigger mesh has to be used, protecting crops against Lepidopteran pest but not against small pests as hemipterans, thysanopterans, mites. A combination of netting with a repellent or irritant product is one possible solution. But the need to reduce the use of chemicals and resistance issues calls for a natural alternative. The objective of this study was to evaluate the repellent, irritant and toxic effects of nets dipped in 20 different plant extracts on *Bemisia tabaci* adults. The repellent effect due to volatils compounds was evaluated thanks to a still-air olfactometer. The irritant effect and the toxicity were evaluated thanks to a no-choice test in tube separated in two parts by an impregnated net. Our results showed the four most irritant and toxic products against *B. tabaci* were aframomum, cinnamon, geranium, and savory. The most repellent were aframomum and lemongrass although cinnamon, geranium and savory were also repellent at

higher doses. The effects varied with the plant extract and the concentration and the effects were independent of one another, i.e. an essential oil can be irritant but not repellent. One can therefore presume that the repellent mechanism behind the irritant/toxic effects is not the same. The use of repellent compounds in combination with netting as new pest control strategy is discussed.

Keywords. repellent, toxic, whitefly, essential oil, pest management.

The whitefly *Bemisia tabaci* is a major pest of many crops in fields and greenhouses in both tropical and temperate regions (Jing et al. 2003). The pest can cause three kinds of damage: 1) direct damage by consuming the sap, 2) indirect damage via the development of *Cladosporium* spp. and *Alternaria* spp. on the honeydew excreted on leaves that may hinder photosynthesis and 3) transmission of viruses as for example the *Tomato Yellow Leaf Curl Virus* (TYLCV), a complex of geminiviruses which infect tomato crops worldwide (Berlinger, 1986). At present, cultivating tomato crops in open fields is a major challenge because of the viruliferous whiteflies *B. tabaci* biotype Q (Berlinger et al. 1996). In addition whiteflies are difficult to control with leaf sprays as they live on the underside of the leaves (Muniz et al. 2002, Zhang et al. 2004). Systemic insecticides, such as neonicotinoids, and insecticides which affect insect development, are currently used in the greenhouse or in open fields and resistance to these insecticides in whitefly populations has been demonstrated in Europe and Africa (Elbert and Nauen 2000, Houndete et al. 2010). Resistance can be developped rapidly because of the whitefly's high fecondity and short generation time (Perring 2001).

The development of green houses and tunnels with plastic or insect proof nets (IPN) enables the cost effective production of tomato and other vegetables particularly in the Mediterranean region (Berlinger et al. 2002). The different forms of IPN, their efficacy, and the importance of physical control methods for agriculture have been extensively reviewed (Weintraub 2009).

However, in tropical regions with high temperatures and high relative humidity, the use of fine mesh nets, i.e. higher than 50 mesh.cm^2, against whiteflies, reduces ventilation (Fatnassi et al. 2002) thus increasing the risk of the development of plant pathogens. One possible way to overcome this problem would be combining large-mesh netting and a repellent or irritant product. The term repellency includes all phenomena that prevent a pest tracking, locating, and/or recognizing its host (Deletre et al. *in prep).* Distance mediated repellents and contact mediated repellents (i.e. irritants) are two types of repellents that could be advantageously combined with an insect proof net. Indeed netting has been shown to act as a visual barrier to sucking pests, thereby delaying outbreaks on vegetables (Gogo et al. 2012, Muleke et al. 2013). A net treated with a pyrethroid has been shown to reduce the combination of aphids and whiteflies and to protect cabbage and tomato better than an untreated net (Martin et al. 2013). Because of pest resistance to pyrethroids and the low repellent and toxic effect of these chemicals on whiteflies and their photo sensiblity, new compounds need to be found. Essential oils appear to be both promising repellents against insects and non-toxic to humans (Regnault-Roger et al. 2002, Hilje and Mora 2006, Amer and Melhorn, 2006, De Boer et al. 2010, Maia and Moore 2011, Zoubiri and Baalioumer 2011, Regnault-Roger et al. 2012). As a result of worldwide interest in environmentally safe methods of control, the use of synthetic insecticides is discouraged because of risks associated with their use. In contrast, essential oils are considered to be low risk products (Regnault-Roger et al. 2012). Essential oils penetrate cuticle well, thereby increasing their own bioavailability inside insect pests and so their toxicity. Some essential oils have specific modes of action that make them good synergists, which is important in integrated pest management (IPM), including with the use of predators. Some essential oil compounds such as acyclic or monocyclic monoterpenes are small-volatil molecules which may have a spatial repellent effect. Indeed, specialized odorant binding proteins in insect sensilla respond to volatil monoterpenes (Regnault-Roger et al. 2012).

The objective of the present study was to evaluate the repellent, irritant, and toxic effects of 20 plant extracts on *B. tabaci* in the laboratory. Actually one role of the secondary compounds

of plants is to repel herbivore so we suppose that among plant extracts some could be repellent or irritant on *Bemisia tabaci* (Unsicker et al. 2009). These plant extracts were selected among a wide range of potentially insect repellent plants that has already shown repellent effect on other insect species. Chemical analyses were performed to identify the exact composition of the plant extracts. Repellent chemical compounds such as DEET (N,N-diéthyl-3-méthylbenzamide) have been shown to be effective against various insect species (Debboun et al. 2006). For this reason DEET and permethrin, a pyrethroid with toxic and excito-repellent properties were used as positive controls (Martin et al. 2013). Repellent, irritant or toxic effects are discussed with respect to the major compounds and their use in combination with netting.

Materials and methods

Insect.

The whiteflies *B. tabaci* biotype Q2 (MPL strain, Med Q2 identified by Dr Urbino, Cirad based on cytochrome oxydase gene) were reared on tomato (*Solanum lycopersicum* L.) in a climatic room at 27 °C ± 1 °C, 50 ± 10% relative humidity and a 12:12 h light:dark photoperiod.

Product.

A long list of plants was drawn up using information available in the literature (Regnault-Roger et al. 2002, Hilje and Mora 2006, Amer and Melhorn 2006, Boer et al. 2010, Maia and Moore 2011, Zoubiri and Baalioumer 2011, Regnault-Roger et al. 2012) on their effects on insects, their non-toxicity for humans and their major compounds (Table 1). The 20 plant extracts used for our study were selected from this list for their different chemical composition, and were characterized by one or two major compounds (Table 1). This choice enabled us to assess the possible relationship between the chemical composition and the behavioural response of insects. DEET (Sigma Aldrich, France; CAS: 134-62-3) and permethrin (Sigma Aldrich, France; CAS 52645-53-1) were used as positive controls. In

absence of a reference repellent against agricultural pests, DEET was chosen as the best known repellent against haematophagous arthropods (Delétré et al. 2013). Permethrin is a toxic and irritant pyrethroid insecticide used to impregnate bed netting for the control of malaria vectors (WHO 2011) and it has already shown an irritant effect on *bemisia tabaci* (Martin et al. 2013). The solvent of plant extract was the negative control.

Gas chromatography analysis.

The plant extracts were analysed on a Varian gas chromatograph, model CP-3380, with a flame ionisation detector equipped with a silica capillary column: HP5 J&W Agilent (5%-phenyl-methylpolysiloxane) (30 m x 0.25 mm i.d. x 0.25 m film); the carrier gas was N_2 at 0.8 ml/min; injection of 1 µL 1:10 dichloromethane solution, split ratio 1:100; injector temperature 220 °C, detector temperature 250 °C; temperature programme 60-220 °C at 3 °C/min, then maintained at 220 °C for 20 min. The percentage composition of the plant extract was computed from GC/FID analyses using the normalisation method, response factors for all compounds being taken as one.

Bioassays.

Bioassays were conducted in the laboratory between 10 am and 6 pm at 24 °C ± 1 °C with 50 ± 10% RH. All the assays and the different concentrations on each product were performed on the same day. For each type of assay, the three concentrations were tested starting with the lowest. Only one plant extract was assayed each day and the apparatus was thoroughly washed at the end of the day. This protocol reduced the risk of contamination as well as interaction between volatil compounds. The apparatus was immersed overnight in a highly detergent and decontaminating solution (TFD4, Franklab, France) at 20% (v/v).

Irritant bioassays. The apparatus was composed of two transparent plastic tubes (Dominique Dutscher SAS®, Ø 5 cm, L 10 cm) separated by a net (Fig. 1). The AgroNet 0.9 NT net was provided by A to Z Textile Mills Ltd, Arusha, Tanzania. The net was made of polyethylene with forty-mesh, 0.9 mm holes/cm^2 the specific absorption capacity of the net was 50 ml/m^2 calculated according to WHOPES procedure. A 36 cm^2 square of net was immersed for 10

seconds in a 0.01%, 0.1% and 1% (v/v) solution of plant extract with ethanol as solvent. The control was only treated with ethanol. The square of netting was dried for 15 min under an extractor hood. The tubes were closed at the one end by a net with very fine mesh that whiteflies cannot cross. A piece of black cardboard covered with a sheet of aluminium foil was used to cover one tube to create darkness (dark tube). The other uncovered tube was called the light tube. The apparatus was oriented horizontally under a light source in a climatic chamber (27 °C ± 1 °C, 50 ± 10% RH). This orientation took advantage of whitefly's innate tendency to move toward the light due to positive phototaxis. After 1 min in the freezer (-20 °C), between 50 and 100 adult *B. tabaci* (mixed sex and age) were poured in the dark tube. For each product, each concentration was replicated six times simultaneously. The number of whiteflies in each tube was counted after 4 h (Martin et al. 2014). A plant extract was considered as irritant when it caused the whitefly to move away from the product after contact with the product. The irritant effect was expressed as the number of insects that had crossed the net. Actually the insects have to land on the net to pass through a net hole and so they have to stay in contact with the product.

Toxic Effect. At the end of the bioassays using the contact irritant the status (living or dead) of the *B. tabaci* was recorded to obtain the mortality rate 4 h after contact. Then for each tube, the living and dead whiteflies which had crossed the net after the 4 h period were collected on tomato leaves (*Solanum lycopersicum* L.) and placed on 1% agar gel in a petri dish. The dishes were placed in the climatic chamber (27 ± 1 °C, 50 ± 10% HR) and the mortality rate was recorded 24 h later. The toxic effect of each product was expressed as the proportion of dead whiteflies after 4 h and after 24 h.

Repellent bioassays. A still-air olfactometer was used to evaluate the repellent effect of the plant extracts (Zhang et al. 2004). A glass cylinder (L: 30 cm, Ø 3 cm) provided by Legallais Montpellier, France, was closed at the top with a very fine mesh net that whiteflies cannot cross, a treated fabric and a glass stopper in that order from the bottom (Fig. 2). The net prevented contact between the product and the insect tarsi or mouthparts. The fabric was a 3-

cm square of non-woven fabric; the central 2 cm was treated with 40 µL of the plant extratct solution at different concentrations ranging from 10% to 0.01% (v/v) in function their activity. The fabrics were dried for 15 min under an extractor hood. For each concentration of each product, four replications were performed with four controls (fabric treated with 40 µL ethanol) at the same time under the extractor hood. The bottom of the cylinder was closed with a glass stopper pierced by a cylinder (L: 10 cm, Ø 0.5 cm). The cylinder was oriented vertically under a light source (two 30 cm, 8 W white light tubes). This orientation took advantage of whitefly's innate tendency to move up the cylinder toward the light due to positive phototaxis. The cylinder was divided into three parts: from 0 to 2 cm to the top of the cylinder, from 0 to 10 cm to the bottom of the cylinder, and the space between these two parts. After 1 min in the freezer, between 10 and 20 adult *B. tabaci* (mixed sex and age) were placed at the bottom of the cylinder. After 1 h, the number of whiteflies in each part was recorded. A plant extract was considered as repellent when it caused the whitefly to move away from the source of the odour. The repellent effect was expressed as the distance to the source of the plant extract.

Data analysis.

We used the same method to analyse the proportion of dead whiteflies in toxicity assays and the proportion of whiteflies which crossed the net in the irritancy assays. Data analysis was performed using R 2.12.2 software (R Development Core Team 2012). The proportions of living whiteflies which crossed the net and of dead whiteflies in the control and treatment assays were compared using Fisher's exact test. To take multiple testing into account, the *P*-values of the tests were Bonferroni corrected using Holm's sequential method (Holm 1979). The proportions of escaped or dead whiteflies were corrected by the control assay values using Sun-Shepard's formula (Püntener 1981). These corrected proportions were used to perform principal component analysis (PCA) of all products and all concentrations to separate the different plant extract in function of their activity in order to do a hierarchical ascendant classification (HAC). Then, a HAC based on Ward's algorithm was used to group the plant

extracts based on the similarity of their effects using PCA-axes coordinates. The HAC enabled to determine which plant extracts had the stronger effect or the weaker effect and so to classify the extracts in function of their activity level. This process yielded a binary segmentation tree reflecting the hierarchy of similarities between responses to plant extracts. The optimal number of classes in the tree was determined by the decrease in interclass variance (branch height-Fig S1). For the repellent bioassays, the distribution of the whiteflies in the olfactometer cylinders between control and treatment cylinders was compared using a Fisher's exact test.

RESULTS

Irritant Bioassays.

Twelve out of the 20 plant extracts were found to significantly decrease the rate of *B. tabaci* which crossed a treated net compared to the control, at least at one concentration: aframomum, cinnamon, citronella, cumin, geranium, ginger, lemon, lemongrass, litsea, neem, solidago and savory (Fig. 3). The eight other plant extracts did not have a significant effect on the net crossing rates. The two synthetic chemicals, DEET and permethrin, significantly decreased the crossing rate through a treated net from a concentration of 0.01% and with a very clear dose effect up to 1%, the highest concentration.

According to the similarity of the behavioural response, a clustering procedure based on HAC yielded three contrasted response classes. Class A grouped products which did not decrease - or only slightly decreased - the crossing rate at any concentration. It is worth noting that some products like coriander and dill appeared to increase the crossing rate. Class B grouped six products which decreased the crossing rate at 1% concentration. Class C grouped four extracts which decreased the crossing rate at least at two concentrations. Among the 20 plant extracts tested on nets, the essential oils of aframomum, cinnamon, and savory appeared to be the most irritant as they stopped more whiteflies crossing the net than the other plant extracts.

Toxic bioassays.

Plant extracts varied in toxicity, notably at the highest concentration tested (Fig. 4). Thirteen plant extracts exhibited a significant toxic effect compared with the control, at least at one concentration: aframomum, cinnamon, citronella, cumin, dill, geranium, lemon, lemongrass, litsea, neem, rosemary, savory, and thyme. Like these plant extracts, the two synthetic chemicals, DEET and permethrin, also had a toxic effect. Seven plant extracts had no toxic effect. HAC analysis yielded four response classes: Class A (11 products) containing all products with no toxic effect, i.e. mortality below 10%, even at the highest concentrations; Class B (two products) products had no clear effects although toxicity was slightly increased at 1%; Class C (products) appeared to be very toxic at 1% and sometimes displayed notable toxicity at lower concentration; and Class D (two products) appeared to be very toxic at 0.1% and 1%. Among all plant extracts, cinnamon and aframomum were the most toxic although not as toxic as the positive controls, DEET and permethrin.

After 24 h, the mortality rate differed significantly among the plants tested (Fig. 5). Nine plant extracts affected the whiteflies which crossed the treated net: aframomum, citronella, coriander, geranium, lemongrass, litsea, neem, pennyroyal, and savory. A dose dependent effect was observed on the mortality rates. After 24 h, the two synthetic chemicals, DEET and permethrin, also had a toxic effect on the whiteflies which crossed the net. After 4 h, cinnamon essential oil at a concentration of 1% killed all the whiteflies before they crossed the net thus making it impossible to calculate the mortality rate of the net crossing whiteflies after 24 h. HAC produced four response classes: Class A (14 products) containing products that were not toxic i.e. a mortality rate of less than 10%; Class B (3 products) included products which were toxic at a concentration of 1%; Class C (three products) that were toxic at two concentrations included cinnamon, which showed 100% toxicity after 4 h at 1%; and class D (two products) included products which led to a nearly 100% mortality rate at the two highest concentrations. Among the 20 plant extracts, aframomum and geranium were the most toxic to whiteflies after contact. However their toxicity was lower than DEET and permethrin.

Repellent bioassays.

The repellent effect of the 20 plant extracts differed among plants and with the concentration (Table 2). Two plant extracts: aframomum and lemongrass were significantly repellent at all three concentrations tested compared with the control. Twelve plant extracts were significantly repellent at two concentrations: cinnamon, citronella, cumin, dill, geranium, ginger, litsea, pennyroyal, savory, solidago, and thyme. Five plant extracts were significantly repellent at only one concentration: coriander, eucalyptus, lemon, pepper, and rosemary. Neem was not repellent at any concentration. DEET was significantly repellent at 0.1% and 1% concentrations whereas permethrin was not repellent at any concentration. The plant extracts and the positive controls, DEET and permethrin, caused a mortality rate of less than 2%.

Our results classified plant extracts from aframomum, cinnamon, citronella, geranium, lemongrass, and savory in class B or C and grouped repellent products at two or three concentrations. So the plant extracts had a strong behavioural effect on *Bemisia tabaci*. The plant extracts from cumin, neem, and litsea were grouped in class A or B and repellent products were grouped at two concentrations except neem which was not repellent at all. These plant extracts may thus have a slight behavioural effect on *B. tabaci*. In contrast, plant extracts from coleus, coriander, dill, eucalyptus, ginger, lemon, solidago, pennyroyal, pepper, rosemary, and thyme were classified in class A, which grouped repellent products at one concentration and some products that are repellent at two concentrations. We conclude that the second group of products has no biological effect on *B. tabaci.*

DISCUSSION

Our results showed that aframomum, cinnamon, geranium, and savory were the four most irritant and toxic products against *B. tabaci* (Table 3). The most repellent plant extracts against *B. tabaci* were aframomum and lemongrass, although cinnamon, geranium and savory were also repellent at higher doses. In most cases the main components reflect the biological

effect of the essential oil quite well (Bakkali et al. 2008). E-(R)-nerolidol is the major component of aframomum (Table 1, Nguikwie et al. 2013) and has already been shown to have a repellent effect on several insect species including the cryptomeria bark borer, *Semanotus japonicus* (Yatagai et al. 2002), and an anti-feeding effect on gypsy moth larvae from *Melaleuca leucadendron* (Doskotch et al. 1980). The toxic effect of cinnamon and savory has already been demonstrated on other insects: the rice weevil, *Sitophilus oryzae* and the mosquito, *Culex pipiens* and *Anopheles gambiae* (Lee et al. 2008, Michaelakis et al. 2007, Deletre et al. 2013). Cinnamaldehyde is the major compound of cinnamon oil (Table 1). This compound is an agonist to the temperature and pain receptor (TRPA1), which could explain both its irritant and toxic effects (Nagata 2007). Carvacrol is the major compound of savory oil (Table 1, Michaelakis et al. 2007). This compound effects the production of cAMP and calcium at cellular level, which could explain its toxic effect (Regnault et al. 2012). But savory has some compounds in common with thyme as p-cymene which appear to have no effect on *Bemisia tabaci*. This result suggests its repellent or toxic effect could be due to γ-terpinene. This compound is not found in thyme but has already been shown to have a toxic effect on ticks, which was attributed to its lipophilic nature and high vapour pressure (Cetin et al. 2010). Citronellol and geraniol are the two major components of geranium oil (Table 1). They have already been shown to be repellent to mosquitoes and ticks (Nerio et al. 2010). At the neurophysiological level, geraniol has an effect on spontaneous nervous impulses (Chen and Viljoen 2010). Lemongrass is well known to protect humans against mosquitoes thanks to their repellent effect (Oyedele et al. 2002). Citral (geranial and neral) is the major component of litsea and lemongrass (Table 1). It can influence oxygenase activity (Bakkali et al. 2008) and inhibit acetylcholinesterase (Lopez and Pascual-Villalobos 2010). In their study, Amer and Mehlhorn (2006) demonstrated the repellent effect of cinnamon and lemongrass on three species of mosquito.

Our results showed that the plant extracts we tested can have different combined effects on *B. tabaci*: spatial repellency, contact irritancy, and contact toxicity. The resulting effect appeared

to be closely linked to the nature of the product and the dose used. For example, lemongrass oil had a strong repellent effect at 0.1% whereas the toxic and irritant effects only appeared at 1%. The Neem extract was an irritant at 1% with a slightly toxic but no repellent effect. These results suggest that the mechanisms of action of each effect differ. Dekker et al. (2011) showed a relation between repellent compounds and olfactory responses by electroantennography whereas the irritant and toxic actions are probably due to action on the nervous system through tarse adsorption (White 2007). Although there is evidence that a single component of the complex mixture of essential oils can be detected by the insects' antennae and may contribute to repellency, the exact mode of action of essential oils has not been studied in great detail (Dekker et al. 2011, Ramirez et al. 2012). One question that remains unanswered is whether the irritant property of a product a consequence of sublethal dosages as suggested by Miller et al. (2009), or reflects another mode of action. Our data did not reveal an irritant effect at low doses and a toxic effect at high doses. The two actions were not differentiated: some insects did not cross the net because they were irritated but most often because they were dead. Moreover these effects could be due either to a single compound or to the combined action of several compounds. Understanding the relation between mode of action and behaviour could help identify synergistic combinations and help reduce the risk of acquired resistance. Actually, if these actions occur independently - as suggested by our results - a population that is resistant to one action would still be susceptible to another, i.e. a product could lose its toxic property but keep its repellent one. The actions could occur independently because the compounds involved are not the same or because the site of the action is not the same.

Given its ability to transmit begomoviruses, the infestation economic threshold for the management of *B. tabaci* is low. Indeed, a virus like the tomato yellow leaf curl virus can cause up to 100% losses in yield (Zhou et al. 2008). However in tropical regions, the cultivation of tomato -and other Solanaceae- under plastic tunnels or in greenhouses is usually impossible due to high temperatures and high humidity. As netting can be used to protect

tomato plants mainly against Lepidopteran pests, it may act as a visual barrier against aphids and whiteflies and netting could thus be usefully combined with a repellent compound (Gogo et al. 2012, Martin et al. 2013, Martin et al. 2014) in cases when the use of large mesh net alone is not sufficient. A treated net could increase the protection provided by netting alone against whiteflies in the same way as that recommended by WHO to protect people against malaria vectors (WHO 2011). Our results showed that DEET and permethrin kill *B. tabaci* efficiently and that DEET alone can repel them. DEET is reported to be a good repellent by acting on the ionotropic receptor in antennae (Kain et al. 2013) and as an inhibitor of acetylcholinesterase activity (Corbel et al. 2009). Like other pyrethroids, permethrin affects the gating kinetics of the voltage-dependent sodium channel (Narahashi 1971). At low doses, these insecticides are irritant, fast acting, stable and safe for humans (Zaim et al. 2000). However many *B. tabaci* populations worldwide have been shown to be resistant to pyrethroids (Houndete et al. 2010, Wang et al. 2010). Pyrethroids are irritant, toxic and maybe deterrent and some of the plant extracts tested here, including aframomum, cinnamon, geranium, and savory, are both also irritant and toxic. Moreover, any plant extracts could have an effect on the host seeking or feeding behaviour of the insect pest (Regnault-Roger 1997). But the lack of understanding of the mode of action of repellents makes it difficult to search for natural compounds to replace or to complement the action of pyrethroids (Ramirez et al. 2012). However in their recent study showing the ionotropic receptor of DEET, Kain et al. (2013) identified more than 100 natural products that could be as repellent as DEET. From our results, the most promising plant extracts to be used as an alternative to pyrethroids are *Aframomum pruinosum* and *Cinnamomum zeylanicum*, because they combine the three effects: repellency, irritation, toxicity. A combination of many active ingredients could also help reduce selective pressure for resistance. The use of essential oils is attractive since they generally have 20 to 60 compounds as monoterpenes (Bakkali et al. 2008) so the emergence of resistance would be slower than with a single product even though it would be difficult to treat a net with 20 compounds. A combination of two or more essential oils could help reduce

the selective pressure for resistance to a toxic mode of action. Essential oils are good candidates as efficient spatial repellents or contact irritants. These both effects can complete the toxic effect: the repellent product could have an action at a distance and the irritant product could act on contact or finally the product can kill the insect. Essential oils have been widely studied but not often used because of their price, the difficulty involved in registration, and their volatility (Regnault-Roger et al. 2012). But despite these drawbacks, the use of essential oils is increasing, and institutions are trying to facilitate their registration since they are considered to be less harmful for humans and environment compared with chemical insecticides.

Essential oils evaporate fast because of their volatilibity leading to a rapid decline in efficacy but with new technologies like gelatin-arabic gum microcapsules, the repellent effect could be maintained up to 30 days on treated fabric stored at room temperature (Specos et al. 2010). Nevertheless, the easiest solution would be to use plants that provide bioactive products or plants that produce similar organic volatil compounds as companion plants or as intercrops around or under an insect proof net, in which case the plants would be cheap natural diffusers. Finally some characteristics of particular netting could be combined with the use of essential oils. Weintraub (2009) reported that yellow nets protected plants from whitefly infestation i.e. an insect landed on the yellow net but did not cross it to reach the plant. If confirmed, a yellow net treated with a toxic product may be a useful strategy. UV-absorbing nets were shown to hinder the ability of whiteflies to disperse inside the net (Antignus et al. 1998, Antignus et al. 2001, Antignus and Ben Yakir 2004). A combination of a visual barrier reducing the orientation and attraction of whiteflies with repellent compounds emitted artificially or naturally would reduce the rate of *B. tabaci* crossing the net and hence the risk of virus transmission. The efficacy of the major components of the most promising plant extracts should be further investigated to identify their main mode of action and to determine if combining them would have synergistic effects on *Bemisia tabaci*, in which case they could be used in combination with insect proof nets.

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Table 1. Plant extracts chosen from the literature for their effects on insects, non-toxicity to humans, and main components (Regnault-Roger *et al*, 2002; Hilje & Mora, 2006; Amer & Melhorn, 2006; Boer *et al*, 2010; Maia & Moore, 2011; Zoubiri & Baalioumer, 2011; Regnault-Roger *et al*, 2012).

Common name	**Scientific name**	**Form of extract, extracted organ**	**Major components (%)**[1]	**Provided by**
Aframomum	*Aframomum pruinosum*	Essential oil, leaf	*(E)*-(R)-nerolidol (95%)	IBMM*, France
Cinnamon	*Cinnamomum zeylanicum*	Essential oil, bark	Cinnamaldehyde (80%)	Nactis, France
Citronella	*Cymbopogon winterianus*	Essential oil, leaf	citronellal (34%) – geraniol (22%) – citronellol (12%)	Nactis, France (Lot 4001850)
Coleus	*Plectranthus tenuicaulis*	Essential oil, leaf	Epoxyocimene (74.4%)	IBMM, France
Coriander	*Coriandrum sativum*	Essential oil, seed	(+)-linalool (72%)	Fabster, France
Cumin	*Cuminum cyminum*	Essential oil, seed	Cuminaldehyde (30%)	Ipra, France (Lot 902560)
Dill	*Anethum graveolens*	Essential oil, seed	(+)-carvone (60%) – limonene (30%)	IBMM, France
Eucalyptus	*Eucalyptus globulus*	Essential oil, leaf	1,8-cineole (81%)	Huiles & Sens, France (Lot B38037)
Geranium	*Pelargonium graveolens*	Essential oil, leaf	citronellol (41%) – geraniol (18%)	IBMM, France
Ginger	*Zingiber officinalis*	Essential oil, root	Zingiberene (30%)	Ipra, France (Lot 902724)
Lemon	*Citrus limon*	Essential oil, fruit	(D)-limonene (95%)	Capua, Italy (Lot 20500)
Lemongrass	*Cymbopogon citratus*	Essential oil, leaf	Geranial (45%), neral (30%)	IBMM, France
Litsea	*Litsea cubeba*	Essential oil, leaf	Geranial (45%), neral (32%)	IBMM, France
Pennyroyal	*Mentha pulegium*	Essential oil, leaf	(+)-pulegone (87%)	IBMM, France
Neem	*Melia azadirachta*	Vegetable oil, seed	azadirachtin (<1%)	Huiles & Sens, France (Lot 00028/11)
Pepper	*Piper nigrum*	Essential oil, seed	β-caryophyllene (30%), limonene (14%), pinenes (14%)	IBMM, France
Rosemary	*Rosmarinus officinalis*	Biological hydrolat, leaf	1,8-cineole (<1%), camphene (<1%), camphor (<1%) (eau (98%))	Huiles & Sens, France (Lot EB815N002)
Savory	*Satureja montana*	Essential oil, leaf	Carvacrol (47%), γ-terpinene (18%), p-cymene (13%)	Huiles & Sens, France (Lot B854002)
Solidago	*Solidago canadensis*	Essential oil, leaf	Germacrene-D (32%) - Limonene (13%)	Huiles & Sens, France (Lot A2)
Thyme	*Thymus vulgaris L.*	Essential oil, leaf	Thymol (35%), p-cymene (23%), carvacrol (15%)	Huiles & Sens, France (Lot A2)

[1] The percentage composition of the essential oil was computed from GC/FID analyses using the normalization method response factors for all the compounds being taken as one.

Table 2 (1/2). Distribution of *Bemisia tabaci* adults (confidence interval calculated with the Wald method) among three parts of a vertical olfactometer exposed to DEET, permethrin and 20 plant extracts (from 10% to 0.1% of product in 40 µL of solution).

			control			treated		
essential oil	conc. (%)	p-value[1]	top: % (CI)	middle: % (CI)	bottom: % (CI)	top: % (CI)	middle: % (CI)	bottom: % (CI)
aframomum	0.1	< 0.001	82.0 (71.4 - 92.7)	16.0 (5.8 - 26.2)	2.0 (-1.8 - 5.9)	9.8 (1.6 - 18)	21.6 (10.2 - 32.8)	68.6 (55.8 - 81.3)
	1	< 0.001	68.8 (55.6 - 81.9)	14.6 (4.6 - 24.5)	16.7 (6.1 - 27.2)	1.8 (-1.6 - 5.2)	15.8 (6.3 - 25.3)	84.2 (74.7 - 93.6)
	10	< 0.001	78.4 (67.1 - 89.7)	19.6 (8.7 - 30.5)	2.0 (-1.8 - 5.8)	7.1 (1.1 - 13.2)	1.4 (-1.3 - 4.2)	91.4 (84.8 - 97.9)
cinnamon	0.1	0.011	54.6 (42.5 - 66.6)	15.2 (6.5 - 23.8)	30.3 (19.2 - 41.3)	32.9 (21.8 - 43.8)	20.0 (10.6 - 29.3)	47.1 (35.4 - 58.8)
	1	< 0.001	90.8 (84.3 - 97.3)	3.9 (-0.4 - 8.3)	5.3 (0.2 - 10.3)	8.7 (0.6 - 16.8)	4.3 (-1.5 - 10.2)	87.0 (77.2 - 96.6)
	10	< 0.001	45.3 (31.9 - 58.7)	5.7 (-0.5 - 11.8)	49.1 (35.6 - 62.5)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
citronnella	0.1	0.873	77.8 (66.7 - 88.9)	16.7 (6.7 - 26.6)	5.6 (-0.5 - 11.6)	79.4 (69.3 - 89.3)	17.5 (8.1 - 26.8)	3.2 (-1.1 - 7.5)
	1	< 0.001	33.9 (22.4 - 45.4)	21.5 (11.5 - 31.5)	44.6 (32.5 - 56.7)	1.3 (-1.2 - 3.8)	1.3 (-1.2 - 3.8)	97.4 (93.9 - 100.0)
	10	< 0.001	66.0 (53.3 - 78.8)	18.9 (8.3 - 29.4)	15.1 (5.5 - 24.7)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
coleus	0.1	1	89.8 (81.3 - 98.3)	10.2 (1.7 - 18.7)	0.0 (0.0 - 0.0)	89.8 (81.3 - 98.2)	8.2 (0.5 - 15.8)	2 (-1.9 - 60.0)
	1	< 0.001	92.5 (85.3 - 99.6)	7.5 (0.4 - 14.7)	0.0 (0.0 - 0.0)	22.7 (12.6 - 32.8)	31.8 (20.5 - 43.0)	45.5 (33.4 - 57.4)
	10	< 0.001	89.3 (81.2 - 97.4)	8.9 (1.5 - 16.4)	1.8 (-1.6 - 5.3)	1.5 (-1.4 - 4.5)	0.0 (0.0 - 0.0)	98.5 (95.5 - 101.0)
coriander	1	0.288	94.8 (90.4 - 99.2)	3.1 (-0.3 - 6.6)	2.1 (-0.7 - 4.9)	88.0 (81.4 - 94.6)	7.6 (2.2 - 13.0)	4.3 (0.2 - 8.5)
	10	< 0.001	82.7 (74.5 - 91.0)	6.2 (0.9 - 11.4)	11.1 (4.3 - 18)	0.0 (0.0 - 0.0)	2.7 (-1 - 6.5)	97.3 (93.5 - 101.0)
cumin	0.1	0.894	88.0 (79.0 - 97.0)	4.0 (-1.4 - 9.4)	8.0 (0.5 - 15.5)	88.9 (81.1 - 96.6)	6.3 (0.3 - 12.4)	4.8 (-0.5 - 10)
	1	< 0.001	76.8 (66.9 - 86.8)	17.4 (8.5 - 26.3)	5.8 (0.3 - 11.3)	0.0 (0.0 - 0.0)	34.7 (21.3 - 48.0)	65.3 (51.9 - 78.6)
	10	< 0.001	70.1 (59.9 - 80.4)	15.6 (7.5 - 23.7)	14.3 (6.5 - 22.1)	0.0 (0.0 - 0.0)	13.2 (5.2 - 21.3)	86.8 (78.7 - 94.8)
dill	0.1	0.316	76.6 (64.5 - 88.7)	10.6 (1.8 - 19.5)	12.8 (3.2 - 22.3)	76.8 (65.7 - 87.8)	5.4 (-0.5 - 11.2)	17.9 (7.8 - 27.9)
	1	< 0.001	56.9 (44.9 - 69.0)	20.0 (10.2 - 29.7)	23.1 (12.8 - 33.3)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
	10	< 0.001	74.1 (62.4 - 85.8)	13.0 (4.0 - 21.9)	13.0 (4.0 - 21.9)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
eucalyptus	1	0.104	84.1 (75.4 - 92.7)	11.6 (4.0 - 19.2)	4.3 (-0.4 - 9.2)	69.2 (59.8 - 78.4)	22.3 (13.9 - 30.7)	8.5 (2.9 - 14.2)
	10	< 0.001	75.5 (63.5 - 87.6)	22.4 (10.7 - 34.1)	2.0 (-1.9 - 6.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
geranium	0.1	0.286	50.9 (38.1 - 63.6)	22.0 (11.4 - 32.6)	27.1 (15.7 - 38.4)	41.0 (28.6 - 53.3)	27.9 (16.6 - 39.1)	31.1 (19.5 - 42.7)
	1	< 0.001	72.9 (62.4 - 83.3)	15.7 (7.2 - 24.2)	11.4 (4.0 - 18.9)	11.1 (2.7 - 19.5)	44.4 (31.1 - 57.7)	44.4 (31.1 - 57.7)
	10	< 0.001	51.8 (38.7 - 64.9)	21.4 (10.6 - 32.1)	26.8 (15.1 - 38.3)	8.5 (1.4 - 15.6)	0.0 (0.0 - 0.0)	91.5 (84.4 - 98.6)
ginger	0.1	0.458	78.2 (67.3 - 89.1)	18.2 (8.0 - 28.4)	3.6 (-1.3 - 8.6)	86.0 (76.9 - 94.9)	10.5 (2.6 - 18.5)	3.5 (-1.2 - 8.3)
	1	< 0.001	86.7 (76.7 - 96.6)	13.3 (3.4 - 23.3)	0.0 (0.0 - 0.0)	25.9 (14.5 - 37.1)	58.6 (45.9 - 71.3)	15.5 (6.2 - 24.8)
	10	< 0.001	79.5 (70.5 - 88.5)	7.7 (1.8 - 13.6)	12.8 (5.4 - 20.2)	12.5 (5.3 - 19.8)	22.5 (13.3 - 31.6)	65.0 (54.5 - 75.4)

[1]Pairwise comparison of proportion performed using Fisher's test.

0-33	34-66	67-100

Table 2 (2/2). Distribution of *Bemisia tabaci* adults (confidence interval calculated with the Wald method) in three parts of a vertical olfactometer exposed to DEET, permethrin and 20 plant extracts (from 10% to 0.1% of product in 40 µL solution).

			control			treated		
essential oil	conc. (%)	p-value[1]	top: % (CI)	middle: % (CI)	bottom: % (CI)	top: % (CI)	middle: % (CI)	bottom: % (CI)
lemon	0.1	0.071	77.6 (66.9 - 88.3)	15.5 (6.2 - 24.8)	6.9 (0.4 - 13.4)	60.6 (49.1 - 71.9)	32.4 (21.5 - 43.2)	7.0 (1.1 - 1.03)
	1	1	83.8 (71.9 - 95.7)	16.2 (4.3 - 28.1)	0.0 (0.0 - 0.0)	82.8 (73.0 - 92.4)	13.8 (4.9 - 22.7)	1.7 (-1.6 - 5.1)
	10	< 0.001	63.5 (50.4 - 76.6)	26.9 (14.8 - 38.9)	9.6 (1.6 - 17.6)	10.8 (0.8 - 20.8)	10.8 (0.8 - 20.8)	78.4 (65.1 - 91.6)
lemongrass	0.01	0.122	72.7 (61.0 - 84.5)	16.4 (6.6 - 26.1)	10.9 (2.7 - 19.2)	88 (78.9 - 97.0)	12.0 (3.0 - 21.0)	0.0 (0.0 - 0.0)
	0.1	< 0.001	84.5 (76.8 - 92.3)	13.1 (5.9 - 20.3)	2.4 (-0.8 - 5.6)	32.3 (20.6 - 43.9)	16.1 (7.0 - 25.3)	51.6 (39.1 - 64.0)
	1	< 0.001	78.6 (69.0 - 88.2)	12.9 (5.0 - 20.7)	8.6 (2.0 - 15.1)	8.5 (1.4 - 15.6)	15.3 (6.1 - 24.4)	76.3 (65.4 - 87.1)
	10	< 0.001	75.8 (65.4 - 86.1)	6.1 (0.3 - 11.8)	18.2 (8.9 - 27.5)	3.2 (-1.1 - 7.6)	0.0 (0.0 - 0.0)	96.8 (92.3 - 101.0)
listea	0.1	0.365	72.6 (60.3 - 84.8)	19.6 (8.7 - 30.5)	7.8 (0.5 - 15.2)	84.9 (75.2 - 94.5)	11.3 (2.8 - 19.9)	3.8 (-1.3 - 8.9)
	1	< 0.001	56.9 (44.9 - 69.0)	13.8 (5.5 - 22.2)	29.2 (18.1 - 40.2)	1.9 (-1.7 - 5.5)	0.0 (0.0 - 0.0)	98.1 (94.5 - 101.0)
	10	< 0.001	64.7 (51.6 - 77.8)	7.8 (0.5 - 15.2)	27.5 (15.2 - 39.7)	1.6 (-1.4 - 4.6)	1.6 (-1.4 - 4.6)	96.9 (92.6 - 101.0)
neem	10	0.349	84.1 (75.1 - 93.2)	7.9 (1.3 - 14.6)	7.9 (1.3 - 14.6)	79.5 (70.5 - 88.4)	5.1 (0.2 - 10.0)	15.4 (7.4 - 23.4)
pennyroyal	0.1	0.565	87.0 (78.1 - 96.0)	11.1 (2.7 - 19.5)	1.9 (-1.7 - 5.5)	83.0 (72.2 - 93.7)	17.0 (6.3 - 27.8)	0.0 (0.0 - 0.0)
	1	< 0.001	81.3 (71.7 - 90.8)	7.8 (1.2 - 14.4)	10.9 (3.3 - 18.6)	40.4 (27.0 - 53.7)	42.3 (28.8 - 55.7)	17.3 (7.0 - 27.6)
	10	< 0.001	75.0 (64.7 - 85.3)	2.9 (-1.0 - 7.0)	22.1 (12.2 - 31.9)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
pepper	1	0.031	85.4 (75.4 - 95.4)	12.5 (3.1 - 21.9)	2.1 (-1.9 - 6.1)	64.4 (54.3 - 74.4)	29.9 (20.2 - 39.5)	5.7 (0.9 - 10.6)
	10	< 0.001	81.5 (72.1 - 91.0)	9.2 (2.2 - 16.3)	9.2 (2.2 - 16.3)	55.6 (44.0 - 67.0)	37.5 (26.3 - 48.6)	6.9 (1.1 - 12.8)
rosemary	1	< 0.001	81.8 (71.6 - 92)	14.5 (5.2 - 23.9)	3.6 (-1.3 - 8.6)	10.8 (3.7 - 17.9)	6.8 (1.0 - 12.5)	82.4 (73.7 - 91.1)
	10	0.042	94.9 (88.0 - 101.7)	5.1 (-1.7 - 12.0)	0.0 (0.0 - 0.0)	77.8 (64.2 - 91.4)	22.2 (8.6 - 35.8)	0.0 (0.0 - 0.0)
savory	0.1	1	85.7 (77.5 - 93.9)	14.3 (6.1 - 22.5)	0.0 (0.0 - 0.0)	85.4 (77.7 - 93.0)	14.6 (7.0 - 22.3)	0.0 (0.0 - 0.0)
	1	< 0.001	59.2 (45.4 - 72.9)	20.4 (9.1 - 31.7)	20.4 (9.1 - 31.7)	8.9 (0.6 - 17.2)	20.0 (8.3 - 31.7)	71.1 (57.8 - 84.3)
	10	< 0.001	55.1 (41.2 - 69.0)	16.3 (6.0 - 26.7)	28.6 (15.9 - 41.2)	0.0 (0.0 - 0.0)	10.2 (1.7 - 18.7)	89.8 (81.3 - 98.2)
solidage	0.1	0.29	83.9 (74.3 - 93.6)	10.7 (2.6 - 18.8)	5.4 (-0.5 - 11.2)	86.1 (78.1 - 94.1)	13.9 (5.9 - 21.9)	0.0 (0.0 - 0.0)
	1	< 0.001	76.9 (66.7 - 87.2)	18.5 (9.0 - 27.9)	4.6 (-0.4 - 9.7)	28.6 (18.4 - 38.6)	26.0 (16.1 - 35.7)	45.5 (34.3 - 56.5)
	10	< 0.001	81.4 (69.8 - 93.0)	9.3 (0.6 - 18.0)	9.3 (0.6 - 18.0)	18.9 (8.3 - 29.4)	50.9 (37.4 - 64.4)	30.2 (17.8 - 42.5)
thyme	0.1	0.173	87.2 (76.7 - 97.7)	10.3 (0.7 - 19.8)	2.6 (-2.4 - 7.5)	79.6 (71.1 - 87.9)	12.5 (5.6 - 19.4)	8.0 (2.3 - 13.6)
	1	< 0.001	78.8 (72.1 - 85.4)	16.4 (10.4 - 22.4)	4.8 (1.3 - 8.3)	57.3 (48.7 - 65.7)	24.4 (17.0 - 31.7)	18.3 (11.7 - 25.0)
	10	< 0.001	75.2 (68.0 - 82.4)	10.2 (5.2 - 15.3)	14.6 (8.7 - 20.5)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	100.0 (100.0 - 100.0)
DEET	0.1	< 0.001	92.9 (91.9 - 93.9)	4.7 (4.1 - 5.3)	1.2 (1.0 - 1.4)	0.0 (0.0 - 0.0)	21.8 (20.0 - 23.6)	78.2 (74.1 - 82.3)
	1	< 0.001	87.9 (84.7 - 91.1)	8.1 (7.1 - 9.1)	2.0 (1.5 - 2.5)	5.1 (4.6 - 5.6)	28.3 (27.0 - 29.6)	66.7 (65.7 - 67.7)
Permethrin	1	0.055	86.7 (84.0 - 89.4)	10.6 (9.4 - 11.8)	0.9 (0.6 - 1.2)	80.0 (79.3 - 80.7)	14.4 (13.5 - 15.3)	5.6 (4.6 - 6.6)

[1]Pairwise comparison of proportion was performed using Fisher's test.

0-33	34-66	67-100

Table 3. Summary of the behavioural response of *Bemisia tabaci* to the repellent, irritant, and toxic effects of DEET, permethrin and 20 plant extracts at different concentrations.

Common	Scientific name	Repellent	Irritant	Toxic	Form of extract
DEET		+ +	+ + +	+ +	Synthetic
Permethrin		**0**	+ + +	+ +	Synthetic
Aframomum	*Aframomum pruinosum*	+ + +	+ +	+ + +	Essential oil
Cinnamon	*Cinnamomum zeylanicum*	+ +	+	+	Essential oil
Citronella	*Cymbopogon winterianus*	+ +	+	+	Essential oil
Coleus	*Plectranthus tenuicaulis*	+ +	**0**	**0**	Essential oil
Coriander	*Coriandrum sativum*	+	**0**	**0**	Essential oil
Cumin	*Cuminum cyminum*	+ +	+ + +	+	Essential oil
Dill	*Anethum graveolens*	+ +	**0**	+ +	Essential oil
Eucalyptus	*Eucalyptus globulus*	+	**0**	**0**	Essential oil
Geranium	*Pelargonium graveolens*	+ +	+	+	Essential oil
Ginger	*Zingiber officinalis*	+ +	+	**0**	Essential oil
Lemon	*Citrus limon*	+	+ + +	+ + +	Essential oil
Lemongrass	*Cymbopogon citratus*	+ + +	+ +	+ + +	Essential oil
Litsea	*Litsea cubeba*	+ +	+ + +	+ + +	Essential oil
Neem	*Melia azadirachta*	**0**	+	+	Vegetable oil
Pennyroyal	*Mentha pulegium*	+ +	**0**	**0**	Essential oil
Pepper	*Piper nigrum*	+	**0**	**0**	Essential oil
Rosemary	*Rosmarinus officinalis*	+	**0**	+	Biologic hydrolat
Savory	*Satureja montana*	+ +	+ + +	+ + +	Essential oil
Solidago	*Solidago canadensis*	+ +	+ + +	**0**	Essential oil
Thyme	*Thymus vulgaris*	+ +	**0**	+	Essential oil

0 = differed significantly from the control according to Fisher's test, + = differed significantly from the control with Fisher's test at one concentration

Fig. 1. Apparatus used for the irritant bioassays on *B. tabaci*.

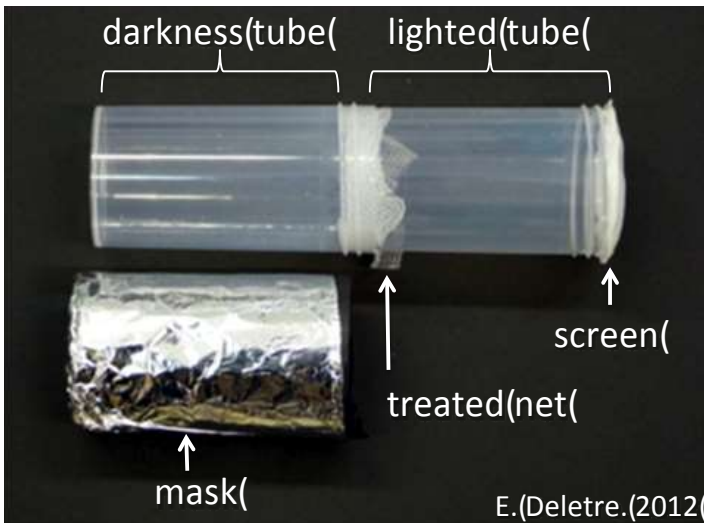


Fig. 2. Apparatus used for the spatial repellent bioassays on *B. tabaci*: vertically oriented still-air cylinder olfactometer.

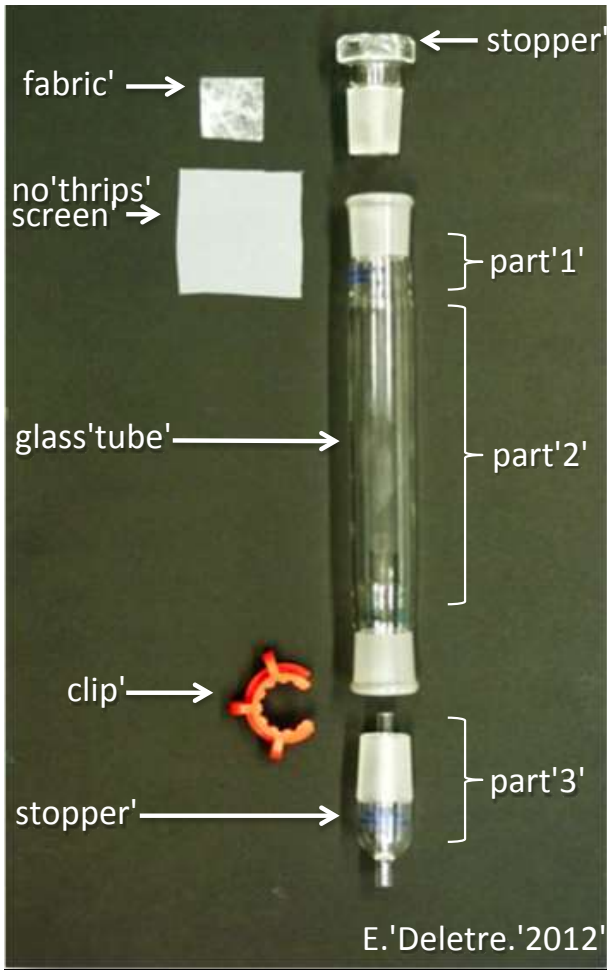
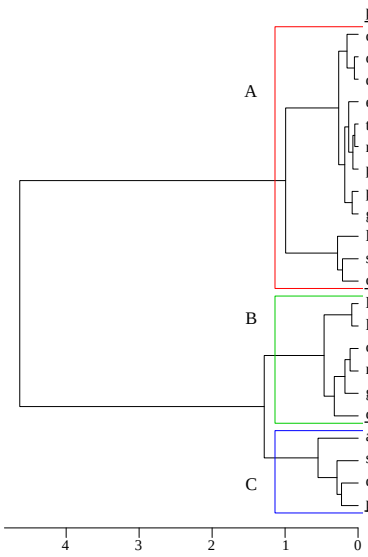


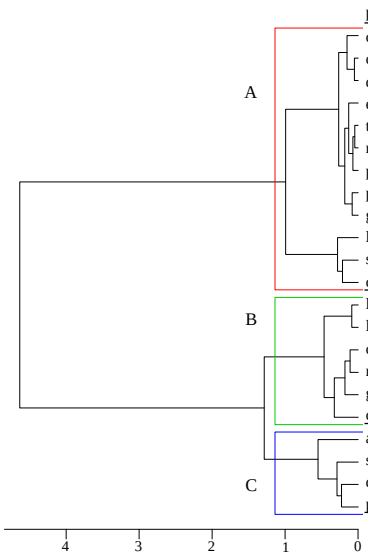
Fig. 3. The rate of *Bemisia tabaci* adults that crossed AgroNet 0.9 (polyethylene 0.9 mm mesh) nets treated with DEET, permethrin and 20 plant extracts (0.01%, 0.1% and 1% of product in the solution used to treat the net) measured after 4 h: dendrogram determined by hierarchical ascendant classification and corrected crossing proportion using Sun-Shepard's formula (confidence interval calculated with the Wald method) for each concentration used for the treatment.



	plant	0.01%	0.1%	1%	p-value[2]
A	coleus	97.5 (92.4 – 100.0) [1]	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	-
	coriander	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	-
	dill	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	100.0 (98.0 - 100.0)	0.006 **
	eucalyptus	94.8 (88.8 - 100.0)	98.0 (91.6 - 100.0)	100.0 (95.5 - 100.0)	-
	thyme	100.0 (98.7 - 100.0)	100.0 (99.0 - 100.0)	100.0 (98.9 - 100.0)	-
	rosemary	100.0 (97.6 - 100.0)	98.1 (91.1 - 100.0)	100.0 (96.2 - 100.0)	0.512
	pepper	100.0 (100.0 - 100.0)	97.2 (92.5 - 100.0)	100.0 (97.8 - 100.0)	0.994
	pennyroyal	100.0 (94.5 - 100.0)	98.4 (93.3 - 100.0)	95.9 (90.6 - 100.0)	1.000
	ginger	100.0 (96.2 - 100.0)	100.0 (98.2 - 100.0)	**88.5 (84.2 - 92.8)**	<0.001 ***
	lemon	**72.3 (65.0 - 79.6)**	**85.7 (76.2 - 95.2)**	**89.0 (82.3 - 95.7)**	0.999
	solidago	**90.8 (84.9 - 96.7)**	**74.1 (66.7 - 81.5)**	**80.2 (72.6 - 87.8)**	<0.001 ***
	cumin	**95.2 (91.1 - 99.3)**	**93.3 (88.4 - 98.2)**	**72.4 (66.0 - 78.8)**	<0.001 ***
B	lemongrass	95.8 (89.4 - 100.0)	**87.3 (82 - 92.6)**	**54.3 (47.9 - 60.7)**	<0.001 ***
	litsea	**92.7 (88.6 - 96.8)**	**95.2 (90.7 - 99.7)**	**52.1 (46.7 - 57.5)**	<0.001 ***
	citronnella	100.0 (99.8 - 100.0)	100.0 (98.1 - 100.0)	**42.9 (37.6 - 48.2)**	<0.001 ***
	neem	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	**42.5 (39.0 - 46.0)**	<0.001 ***
	geranium	100.0 (100.0 - 100.0)	93.1 (87 - 99.2)	**32.5 (27.5 - 37.5)**	<0.001 ***
	cinnamon	100.0 (100.0 - 100.0)	98.5 (94.4 - 100.0)	**13.8 (13.8 - 13.8)**	<0.001 ***
C	aframomum	**53.9 (48.2 - 59.6)**	97.8 (93.7 - 100.0)	**23.8 (20.2 - 27.4)**	<0.001 ***
	savory	**85.7 (79.3 - 92.1)**	**76.1 (68.7 - 83.5)**	**29.3 (25.5 - 33.1)**	<0.001 ***
	deet	**75.2 (70.2 - 80.2)**	**47.9 (43.7 - 52.1)**	**24.7 (21.7 - 27.7)**	<0.001 ***
	permethrin	**75.9 (70.9 - 80.9)**	**65.1 (61.2 - 69.0)**	**10.0 (9.0 - 11.0)**	<0.001 ***

[1]Pairwise comparison of proportion was performed using Fisher's test. Bold values were significantly different from the control with Holm's sequential Bonferroni correction method. Values > 100.0 were rounded down to 100.0.

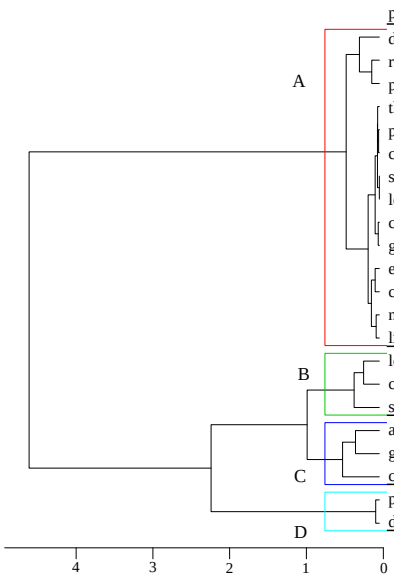
Fig. 4. Proportion of mortality of *Bemisia tabaci* adults in contact with AgroNet0.9 (polyethylene 0.9 mm mesh) nets treated with DEET, permethrin and 20 plant extracts (0.01%, 0.1% and 1% of product in the solution used to treat the net) measured after 24 h: dendrogram determined by hierarchical ascendant classification and corrected mortality proportion using Sun-Shepard's formula (confidence interval calculated with the Wald method) for each concentration used for the treatment.



plant	0.01%	0.1%	1%	p-value[2]
coleus	97.5 (92.4 – 100.0) [1]	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	-
coriander	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	-
dill	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	100.0 (98.0 - 100.0)	0.006 **
eucalyptus	94.8 (88.8 - 100.0)	98.0 (91.6 - 100.0)	100.0 (95.5 - 100.0)	-
thyme	100.0 (98.7 - 100.0)	100.0 (99.0 - 100.0)	100.0 (98.9 - 100.0)	-
rosemary	100.0 (97.6 - 100.0)	98.1 (91.1 - 100.0)	100.0 (96.2 - 100.0)	0.512
pepper	100.0 (100.0 - 100.0)	97.2 (92.5 - 100.0)	100.0 (97.8 - 100.0)	0.994
pennyroyal	100.0 (94.5 - 100.0)	98.4 (93.3 - 100.0)	95.9 (90.6 - 100.0)	1.000
ginger	100.0 (96.2 - 100.0)	100.0 (98.2 - 100.0)	**88.5 (84.2 - 92.8)**	<0.001 ***
lemon	**72.3 (65.0 - 79.6)**	**85.7 (76.2 - 95.2)**	**89.0 (82.3 - 95.7)**	0.999
solidago	**90.8 (84.9 - 96.7)**	**74.1 (66.7 - 81.5)**	**80.2 (72.6 - 87.8)**	<0.001 ***
cumin	**95.2 (91.1 - 99.3)**	**93.3 (88.4 - 98.2)**	**72.4 (66.0 - 78.8)**	<0.001 ***
lemongrass	95.8 (89.4 - 100.0)	**87.3 (82 - 92.6)**	**54.3 (47.9 - 60.7)**	<0.001 ***
litsea	**92.7 (88.6 - 96.8)**	**95.2 (90.7 - 99.7)**	**52.1 (46.7 - 57.5)**	<0.001 ***
citronnella	100.0 (99.8 - 100.0)	100.0 (98.1 - 100.0)	**42.9 (37.6 - 48.2)**	<0.001 ***
neem	100.0 (100.0 - 100.0)	100.0 (100.0 - 100.0)	**42.5 (39.0 - 46.0)**	<0.001 ***
geranium	100.0 (100.0 - 100.0)	93.1 (87 - 99.2)	**32.5 (27.5 - 37.5)**	<0.001 ***
cinnamon	100.0 (100.0 - 100.0)	98.5 (94.4 - 100.0)	**13.8 (13.8 - 13.8)**	<0.001 ***
aframomum	**53.9 (48.2 - 59.6)**	97.8 (93.7 - 100.0)	**23.8 (20.2 - 27.4)**	<0.001 ***
savory	**85.7 (79.3 - 92.1)**	**76.1 (68.7 - 83.5)**	**29.3 (25.5 - 33.1)**	<0.001 ***
deet	**75.2 (70.2 - 80.2)**	**47.9 (43.7 - 52.1)**	**24.7 (21.7 - 27.7)**	<0.001 ***
permethrin	**75.9 (70.9 - 80.9)**	**65.1 (61.2 - 69.0)**	**10.0 (9.0 - 11.0)**	<0.001 ***

[1]Pairwise comparison of proportion was performed using Fisher's test. Bold values were significantly different from the control with the Holm's sequential Bonferroni correction method. Values > 100.0 were rounded down to 100.0.

Fig. 5. Proportion of mortality of *Bemisia tabaci* adults that crossed AgroNet0.9 (polyethylene 0.9 mm mesh) nets treated with DEET, permethrin and 20 plant extracts (0.01%, 0.1% and 1% of product in the solution used to treat the net) measured after 24 h: dendrogram determined by hierarchical ascendant classification and corrected mortality proportion using Sun-Shepard's formula (confidence interval calculated with the Wald method) for each concentration used for the treatment.



plant	0.01%	0.1%	1%	p-value[2]
dill	0.0 (0.0 - 0.0)[1]	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	-
rosemary	2.6 (0.0 - 8.1)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	-
pennyroyal	**10.3 (6.2 - 14.4)**	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	-
thyme	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	-
pepper	0.0 (0.0 - 0.0)	1.1 (0.0 - 2.4)	0.0 (0.0 - 0.0)	-
coleus	0.0 (0.0 - 0.0)	0.6 (0.0 - 1.6)	0.0 (0.0 - 0.0)	-
solidago	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	-
lemon	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	-
coriander	0.0 (0.0 - 0.0)	**2.4 (1.2 - 3.6)**	0.6 (0.0 - 1.2)	-
ginger	1.3 (0.5 - 2.1)	1.7 (0.8 - 2.6)	0.8 (0.2 - 1.4)	-
eucalyptus	1.2 (0.0 - 5.4)	0.0 (0.0 - 0.0)	1.1 (0.0 - 6.3)	-
cumin	0.0 (0.1 - -3.1)	0.0 (0.0 - 0.0)	1.7 (0.0 - 3.8)	-
neem	**3.4 (2.1 - 4.7)**	1.5 (0.3 - 2.7)	**5.5 (3.3 - 7.7)**	-
litsea	0.0 (-0.4 - -0.4)	0.0 (0.0 - 0.0)	**5.6 (3.8 - 7.4)**	-
lemongrass	0.0 (0.0 - 0.0)	1.1 (0.0 - 4.1)	**20.3 (16.3 - 24.3)**	<0.001 ***
citronnella	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	**39.4 (35.7 - 43.1)**	<0.001 ***
savory	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	**54.9 (54.9 - 54.9)**	<0.001 ***
aframomum	**25.5 (21.4 - 29.6)**	**32.1 (28.3 - 35.9)**	**60.2 (57.4 - 63.0)**	<0.001 ***
geranium	**18.0 (12.0 - 24.0)**	4.0 (0.4 - 7.6)	**73.0 (67.8 - 78.2)**	<0.001 ***
cinnamon	0.0 (0.0 - 0.0)	0.3 (0.0 - 0.8)	NT	-
permethrin	2.0 (0.0 - 4.0)	**90.2 (90.2 - 90.2)**	**90.2 (90.2 - 90.2)**	<0.001 ***
deet	6.7 (4.6 - 8.8)	**90.2 (90.2 - 90.2)**	**90.2 (90.2 - 90.2)**	<0.001 ***

[1]Pairwise comparison of proportion was performed using Fisher's test. Bold values differed significantly from the control with Holm's sequential Bonferroni correction method. Values < 0.0 were rounded up to 0.0.

NATURALLY OCCURRING BIOACTIVE COMPOUNDS FROM FOUR REPELLENT ESSENTIAL OILS AGAINST *BEMISIA TABACI* WHITEFLIES

(soumis à Journal of Chemical Ecology)

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Abstract-In tropical countries, netting is an effective sustainable tool for protecting horticultural crops against Lepidoptera but not against small pests like *Bemisia tabaci*, while smaller mesh netting can be used in northern regions. A possible solution is to combine an insect-proof net with a naturally occurring repellent. In a previous study, we identified four promising repellent essential oils among 20 plant extracts: lemongrass (*Cymbopogon citratus*), cinnamon (*Cinnamomum zeylanicum*), cumin (*Cuminum cyminum*) and citronella (*Cymbopogon winternarius*). The present study was designed to identify the active compounds of these essential oils, characterize their biological activity, and examine their potential for coating insect-proof nets. We conducted a laboratory study to investigate the efficiency and toxicity of nets dipped in different solutions. We then studied the repellent effect with an olfactometer and the irritant effect by videotracking. Geraniol and citronellol were the most promising net coatings due to their repellency effect. The repellency, irritancy or toxicity varied with the product and concentration and these features were independent, indicating that the repellent mechanism and the irritant/toxic mechanism are not the same. The combined effects of these different compounds account for the bioactivity of the mixture, suggesting positive interactions between the compounds. The use of repellent compounds combined with netting as a sustainable strategy for protecting vegetable crops against whiteflies is discussed, in addition to the use of companion plants which could produce such bioactive compounds.

Key words-behavior, repellency, irritation, toxicity, DEET, permethrin

INTRODUCTION

The *Bemisia tabaci* whitefly is a serious pest of many field and greenhouse crops in tropical and temperate regions, particularly due to virus transmission (Jing *et al,* 2003; Berlinger, 1986; Cohen & Berlinger, 1986; Blackmer & Byrne, 1993; Jones, 2003). At present, cultivating tomato crops is a real challenge regarding whitefly prevention and control (Berlinger *et al*., 1996). Whiteflies are hard to control by insecticide foliar sprays as they inhabit the underside of leaves (Muniz *et al.*, 2002; Zhang *et al*, 2004). Systemic insecticides, such as neonicotinoids, or chemicals acting on insect development, are thus currently used to treat crops in greenhouses and open fields. However, most of the populations tested worldwide have been diagnosed as resistant to chemical insecticides commonly used in agriculture, such as organophosphates, pyrethroids and neonicotinoids (Denholm *et al.,* 1998; Elbert & Nauen, 2000, Palumbo *et al.,* 2001; Roditakis *et al.,* 2009; Houndete *et al.,* 2010a; Houndete *et al.,* 2010b; Wang *et al.,* 2010; Gnankiné *et al.*, 2013). Selection in favour of insecticide resistance population occurs rapidly in whiteflies because of their high birth rate, haplodiploid breeding system and short generation time (Perring, 2001; Byrne & Devonshire, 1996; Denholm *et al.,* 1998). Moreover, the way that insecticides are generally used, i.e excessive year-round use in tropical regions, increases the chance of selection of resistant populations. One way to protect plants from pest insects without using pesticides is to create a visual and physical barrier between the insect and the plant with a net (Weintraub, 2009). In Beninese fields, insect-proof nets (IPNs) have been shown to provide more effective protection against the diamondback moth (*Plutella xylostella*) and other Lepidoptera species than foliar insecticide sprays (Martin *et al*., 2006). However, in tropical regions with high temperature and humidity levels, the use of fine mesh nets increases the risk of plant pathogen development. A combination between a large-mesh screen and a repellent or irritant product could be a solution. New compounds now have to be found because of pest resistance to pyrethroids and the low repellent and toxic effects of these chemicals on whiteflies (Martin et al, 2014).

Plant-based essential oils appear promising as insect repellents/irritants (Regnault-Roger *et al*, 2002; Hilje & Mora, 2006; Amer & Melhorn, 2006; Boer *et al*, 2010; Nerio *et al.*, 2010; Maia & Moore, 2011; Zoubiri & Baalioumer, 2011; Regnault-Roger *et al*, 2012). Essential oils are blends of 10-60 molecules or even more in various proportions. Two or three of their major compounds are usually responsible for their biological activities such as repellency, irritancy or toxicity (Ipek *et al.*, 2005). Terpenoids, such as citronellal, myrcene, geraniol, citral, limonene, pinene, citronellol and linalool, are the most important chemical group to consider in terms of insect repellency (Moore *et al*., 2007). In a previous study, we identified four highly repellent essential oils among 20 plant extracts: *Cymbopogon citratus*, *Cymbopogon winterianus*, *Cuminum cyminum* and *Cinnamomum zeylanicum*. IPNs treated with 1% of these plant extracts showed the following *B. tabaci* net-crossing rates: 42.9%, 54.3%, 72.4% and 13.8%, respectively (Deletre *et al.*, submitted). A toxic effect was observed after 4 h exposure: 96.3% mortality for cinnamon oil, 64.7% for citronella oil, 61.0% for lemongrass oil and 30.0% for cumin oil. However, although these essential oils were shown to be repellent and toxic, but their active compounds are still unidentified, they could be one or several major compounds.

The objectives of this study were: (1) to identify and quantify the compounds from these essential oils, and (2) to evaluate, via behavioural assays, the bioactive effects of the major compounds alone or combined so as to shed light on the potential efficacy of essential oils or their active compounds as an appropriate supplement to the physical barrier of IPNs. We tested two hypotheses: 1) the biological effects of essential oils are only due to the effect of the majority compound, or 2) the biological effects of essential oils are the result of a synergic/additive effect of many compounds.

MATERIAL & METHODS

Insect. B. tabaci biotype Q (MPL strain) whiteflies were reared on tomato plants (*Solanum lycopersicum* L.) in a climatic room at 27 ± 1°C, 50 ± 10% relative humidity and a 12:12 h light:dark photoperiod.

Product. Studies were carried out with four plant essential oils: lemongrass (leaves), *cymbopogon citratus* (IBMM, France); citronella (bark), *Cymbopogon winterianus* (Nactis, France, lot 40018500); cumin (seeds), *Cuminum cyminum* (Ipra, France, lot 902560); and cinnamon (bark), *Cinnamomum zeylanicum* (Nactis, France) and 19 chemical standards (Sigma Aldrich, St Louis, MO, USA): β-caryophyllene (≥ 80% purity), linalool (97% purity), citral (95% purity), citronellal (≥ 95% purity), geraniol (98% purity), citronellol (≥ 95% purity), (S)-(-)-limonene (96% purity), geranyl acetate (98% purity), cuminaldehyde (98% purity), (-)-□-pinene (99% purity), □-terpinene (≥ 97% purity), p-cymene (99% purity), (E)-cinnamaldehyde (99% purity), 2-methoxy-cinnamaldehyde (98% purity), cinnamyl acetate (99% purity). DEET and permethrin were used as positive control. Indeed, DEET is a most famous insect repellent and permethrin is a toxic irritant pyrethroid that is used against most insects. DEET, permethrin, and the four mixtures of major essential oil compounds available on the market: lemongrass mixture (citral, geraniol, geranyl acetate, limonene, β-caryophyllene, linalool) citronella mixture (citronellal, geraniol, citronellol, limonene and geranyl acetate), cumin mixture (cuminaldehyde, □-pinene, □-terpinene and p-cymene), cinnamon mixture ((E)-cinnamaldehyde, 2-methoxy-cinnamaldehyde and cinnamyl acetate) and thyme mixture (thymol, p-cymene, carvacrol, □-terpinene, linalool and □-caryophyllene) were diluted at 0.1% and 1% (v/v for liquid compounds or w/w for powdered compounds) in ethanol. Each mixture was prepared by diluting the major compounds in ethanol in a ratio based on their respective proportions in the essential oils. All major compounds were tested at the relative concentration that they are found in the essential oils at 1% and 0.1% (Deletre et al. 2013) (Table 1). For instance, citronellal represents 34.7% of citronella essential oil. Citronella oil was efficient at 1%, so citronellal was tested at C2= 0.3%, i.e. 0.25 mg/mL ($d_{ethanol}$: 0.789 g/ml, $d_{citronellal}$: 0.885 g/ml) and 10-fold less: C1= 0.035% i.e. 0.025 mg/ml. Each mixture was created with the compounds in their respective proportions in the essential oils. By doing this, the quantity of a compound was the same when the essential oil, the mixture and the compound alone were tested. Each assay was preceded by a negative control in which only ethanol was tested.

Gas Chromatography Analysis. The four essential oils (citronella, cinnamon, cumin, and lemongrass) were analysed on a Varian gas chromatograph, model CP-3380, equipped with a flame ionisation detector (FID) at 220°C and using an apolar HP_5 J&W Agilent (5%-phenyl-95% methylpolysiloxane) capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 220 and 250°C, respectively. The oven temperature was maintained at 60°C for 1 min and programmed at 3°C min^{-1} to 220°C. N_2 was the carrier gas, at a 0.8 ml/min flow rate. A 1 µl solution (10% essential oil in ethyl ether) was manually injected. A mixture of alkanes (C9-C22) was injected to calculate the retention index: RI= [TR(X)-TR(n)]/[TR(n+1)-TR(n)]*100+100*n where TR(X) is the retention time of the studied product, TR(n) is the retention time of the alkane with n carbons eluted before X, TR(n+1) is the retention time of the alkane of n+1 carbons eluted after X. The percentage composition of the essential oil was computed by the normalization method from GC/FID analyses, with the response factors being taken as one for all compounds.

Coupled Gas Chromatography Mass Spectrometry Analysis. GC-MS analyses were performed using a Hewlett Packard 5890 II gas chromatograph interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25□□m). Helium was the carrier gas, at a 0.6 ml/min flow rate. Injector and MS transfer line temperatures were set at 220°C and 250°C, respectively. The

oven programme temperature was the same as that used in the GC-FID analysis. Diluted samples (10:100 in CH_2Cl_2, v/v) of 1 µL were injected manually and in split mode (1:100). MS was performed in EI mode at 70 eV, in the m/z 35-300 range; electron multiplier 1460 eV; scan rate, 2.96 scan/s. The constituents were identified on the basis of comparisons of their relative retention indices and mass spectra with those of standards (for the main components), those found in the literature (Adams, 2007) and those supplemented by the NBS75K database and Wiley 7th NIST 98 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software).

Bioassays. Detailed descriptions of the apparatus, assay protocol, and data analysis procedures were previously published (Deletre *et al.*, 2014). Bioassays were conducted between 10 am and 6 pm at 24 ± 1°C, with 50 ± 10% HR. For each product, all assays were performed the same day with only one plant extract tested per day from the lowest to highest concentration. The apparatuses were washed with a highly detergent and decontaminating solutions (TFD4, Franklab, France) at 20% (v/v).

Toxicity Bioassays. Two transparent plastic tubes (Dominique Dutscher SAS®, Ø 5 cm, L 10 cm) were separated by a polyethylene net with 40 holes/cm^2 and mesh size of about 0.9 mm (A to Z Textile Mills Ltd, Arusha, Tanzania). A 36 cm^2 net was dipped for 10 s in the different solutions and dried for 15 min under an extractor hood. Black cardboard wrapped in an aluminium sheet covered one tube to ensure darkness (dark tube). The other tube (uncovered) was called the light tube. The apparatus was oriented horizontally under a light source in a climatic chamber (27 ± 1°C, 50 ± 10% HR). After 1 min in the freezer (-20°C), between 100 and 200 *B. tabaci* adults (mixed sex and age) were placed in the dark tube. For each product, each concentration was replicated six times simultaneously. The number of whiteflies and their status (alive or dead) were recorded for each tube after 4 h to establish the whitefly net-crossing rate and the mortality (Martin *et al*, 2014). Then dead and live whiteflies that had crossed through the net after the 4 h were separately collected on tomato leaves (*Solanum lycopersicum* L.) and placed on agar gel (1%) in a petri dish. They were preserved in the climatic chamber (27 ± 1°C, 50 ± 10% HR) and then the mortality rate was determined after 24 h.

Irritant Bioassays. For each compound, the surface of a 12 x 15 cm section of black paper was treated with 2 mL of solution or 97% ethanol for the control before frying (WHO, ?). The paper was always prepared on the same day it was used, and between trials, the paper was stored at -20 °C. Only one product was tested per day to avoid contaminations. The irritant test was carried out with citronellal, geranyl acetate, cuminnaldehyde, 2-methoxycinnamaldehyde and citral at 1%, cinnamaldehyde and cinnamyl acetate at 0.5%, as well as with the positive controls DEET and permethrin at 1%. The choice test was conducted on a 16 cm^2 area (arena), where half of the surface was treated paper (treated zone) and the other half was control paper (control zone). The areas were delineated with a 2 mm thick cardboard border and a plexiglas cover to prevent whiteflies from escaping during the experiment and to force them to walk on the paper and not fly. *B. tabaci* were placed at the center of the arena, one per trial. Their activity - time spent moving, average speed when moving, distance moved and time spent in each zone for the choice assay - was monitored over a 10 min period. The experiment was repeated 30 times with different individuals, and after five recordings the arena was replaced by another one and the orientation changed. The apparatus for the no-choice test consisted of a similar set up but with two 9 cm^2 arenas, one arena was treated and the other was the control (97% ethanol). *B. tabaci* whiteflies were placed in the arena. Their activity—time spent moving, average speed when moving and distance moved—was monitored over a 10 min period. The experiment was repeated 20 times (10 for the treated arena and 10 for the control arena per test compound) with different individuals, and after five recordings the arena was replaced by another one. The monitoring was done using a video camera (25 frames/s) fixed above the arena and the images were analysed

using the Ethovision video observation system (Noldus Information Technology, Wageningen, The Netherlands) which is designed to automate animal behavior observations (Noldus *et al.*, 2002).

Expellent Bioassays. **A** still-air olfactometer was oriented vertically under a light source (two white light tubes, 30 cm, 8W) to assess the repellent effect of the essential oils (Zhang *et al*, 2004). A glass cylinder (Legallais society®, L: 30cm, Ø 3 cm) was closed at the top with very fine mesh net that whiteflies could not pass through, along with a treated filter paper and a glass stopper, in this order, and the bottom was closed with a glass stopper pierced with a cylinder (L: 10 cm, Ø 0.5 cm) (Deletre *et al.*, 2013). 40 µL of each compound or ethanol (control) were placed on the 4 cm^2 piece of non-woven fabric filter paper. The filter papers were dried for 5 min under an extractor hood. The concentrations of each compound were tested in different trials and four replications per concentration were carried out simultaneously with four controls under an extractor hood. The cylinder was divided in three parts: the top part from 0 to 2 cm to the top of the cylinder, the bottom part from 0 to 10 cm to the bottom of the cylinder, and the middle part between these two parts (Deletre *et al*, submitted). After 1 min in the freezer, 10 to 20 *B. tabaci* adults (mixed sex and age) were placed at the bottom of the cylinder. After 1 h, the number of whiteflies was recorded in each part along with the number of dead individuals.

Data Analysis. We used the same method to analyse the proportion of dead whiteflies in the toxicity assays and whitefly net-crossing rate in the irritancy assays. The data analysis was carried out using the R 2.12.2 software package (R Development Core Team, 2012). To compare the proportions of escaped or dead whiteflies in the control and treatment assays, we used Fisher's exact test corrected according to Bonferroni using the Holm's sequential method (Holm 1979). Then the proportions of escaped or dead whiteflies were corrected on the basis of the control assay values using Sun-Shepard's formula (Püntener, 1981). For all products and concentrations, these corrected proportions were used to perform a principal component analysis (PCA). Then a hierarchical ascendant classification (HAC) based on Ward's algorithm was used to group the plant extracts based on the similarity of their effects using PCA-axis coordinates. This process yielded a binary segmentation tree, reflecting the hierarchy of similarities between responses to plant extracts. The optimal number of classes in the tree was determined by the decrease in the interclass variance. For the repellent bioassays, whitefly distributions within the olfactometer cylinders were compared between control and treatment cylinders using Fisher's exact test. The choice irritant assay data were analyzed using a paired t-test or a Wilcoxon test in the case of non-normally distributed data. For the no-choice irritant assay, the data were analyzed using an unpaired t-test or a Wilcoxon test in the case of non-normally distributed data.

RESULTS

Toxicity Bioassays. The whitefly net-crossing rate was significantly reduced by DEET, permethrin, the four compound mixtures and the following pure compounds: cinnamaldehyde, cinnamyl acetate, β-pinene, γ-terpinene, citronellal, geraniol, citronellol, limonene and citral (Figure 1). After 4 h, the percentage mortality had significantly increased for cinnamaldehyde, cinnamyl acetate, γ-terpinene, citronellal, geraniol, citronellol, limonene and citral (Figure 2). After 24 h, the mortality significantly increased for the cumin and lemongrass mixtures and the cinnamyl acetate, cuminaldehyde, p-cymene, geraniol and citronellol compounds (Figure 3). According to the HAC, the most promising compounds to prevent *B. tabaci* from cross through the net are cinnamaldehyde, citronellal and limonene. The effect of the cinnamon mixture appeared to be due to cinnamaldehyde, i.e. at high concentration, all whiteflies were dead after 4 h even though some whiteflies succeeded in crossing through the treated net before dying. At high concentration, the effect of the cumin mixture limited the whitefly net-crossing rate by killing them, but when these pests did succeed in escaping, their

mortality was low after 24 h. Although cuminaldehyde showed a toxic effect, it could not explain the effect observed with the mixture, suggesting synergism between the compounds. At high concentration, the citronella mixture, like the cumin mixture, reduced the whitefly net-crossing rate by killing them, but when they succeeded in escaping, their mortality was low after 24 h. Citronellol, citronellal, geraniol and limonene also showed toxic effects and decreased the whitefly net-crossing rate, but to a lesser extent than the citronella mixture, suggesting a synergic or additive effect between these four products. With the lemongrass mixture, all whiteflies were dead after 4 h, although some of them succeeded in crossing through the treated net before dying. Citral alone could not explain the effect of the lemongrass mixture. Thus geraniol and limonene could play a role in the toxic effect of the lemongrass mixture. The two positive controls, i.e. DEET and permethrin, showed a very efficient toxic property that prevented whiteflies from crossing through the treated net.

Irritant Bioassays. In the choice assay, the time spent on the treated zone and the time spent on the control zone did not differ for all of the tested compounds (Table 2). There was no movement away from the treated zone. The whitefly activity, i.e. mobility, velocity and distance moved, did not differ between the treated areas and the control, excepted for areas treated with cinnamyl acetate and geranyl acetate where whiteflies were significantly less mobile than on the control, and for DEET where they were more mobile. In the no-choice assay, there was no difference in activity between the treated and the non-treated areas for all compounds, excepted for citral where there was less distance covered and mobility than on the treated arena (Table 3). These bioassays showed that it was not because the product was irritant that *B. tabaci* did not cross though the net.

Expellent Bioassays. DEET was repellent at 0.1% and 1%, whereas permethrin was not repellent at 1% (Table 4). The cinnamon mixture was repellent at 0.1% and caused a high vapour mortality at 1% and, among the compounds, only cinnamaldehyde was repellent irrespective of the dose, with 30% mortality. The cumin mixture was repellent at 0.1% and 1% and, among the pure compounds, only cuminaldehyde was repellent at 0.3%. The citronella mixture was repellent at 0.1% and 1%, with high vapour toxicity and, among the pure compounds, geraniol, citronellol and geranyl acetate were repellent at their highest concentration, and citronellal showed high vapour toxicity at 0.34%. The lemongrass mixture was repellent at 0.1% and caused high vapour mortality at 1% and, among the pure compounds, only geraniol was repellent at the highest concentration and citral caused high vapour toxicity at 0.8%.

DISCUSSION

We showed that cinnamaldehyde, cuminaldehyde, geraniol, citronellol, geranyl acetate were repellent compounds (Table 5). This effect depended on the concentration used. We have already shown a repellent effect of these compounds against another insect species such as *Anopheles gambiae* (Deletre *et al.*, 2014). In many studies, particularly with mosquitoes, geraniol and citronellol showed repellent properties (Amer & Mehlhorn, 2006; Muller *et al.*, 2009; Nerio *et al.*, 2010; Revay *et al.*, 2012). A recent study showed the deterrence and toxicity effects of citronellol and geraniol on *Bemisia tabaci* (Baldin *et al.*, 2014). Moreover, geraniol and citronellol from the essential oil *Dianthus caryophyllum* also showed repellent effects against *Ixodes ricinus* ticks (Tunon *et al.*, 2006). A cinnamon mixture, citronella mixture, lemongrass mixture, citronellal and citral showed high vapor toxicity, and cinnamaldehyde, geraniol, citronellol and geranyl acetate also showed vapour toxicity, but lower. The essential oils of *Cymbopogon nardus* (citronellal (33.8%), geraniol (21.6%), citronellol (9.2%), geranyl acetate (3.2%)) and *Cinnamomum verum* (cinnamaldehyde (90%)) also showed vapour toxicity against the bean weevil *Acanthoscelides obtectus*, along with the essential oil of *Cuminum cyminum* (cuminaldehyde (42.5%), pinene (11.8%), terpinene (11.4%)), which had no vapour toxicity against *Bemisia tabaci* (Regnault-Roger *et al.*, 2000). The four mixtures and cinnamaldehyde showed high toxicity, and

cinnamyl acetate, cuminaldehyde, γ-terpinene, citronellal, citronellol, geraniol, limonene and citral also showed a toxic effect, but lower. Huang and Ho (1998) also highlighted the toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects *Tribolium castaneum* and *Sitophilus zeamai*s. We noted that aldehydes and alcohols were the active components of the essential oils. Many articles have been published on essential oil effects but few have focused on the behavioral effects of their compounds, thus illustrating the lack of knowledge on the action mechanisms of essential oils. As specialized odorant binding proteins in the sensilla of insects respond to volatil monoterpenes, repellents could actually function by activating olfactory receptor neurons (Dekker *et al.*, 2011), while irritants could activate gustatory receptor neurons on tarse***(tarsi?)*** (Vosshall & Stocker, 2007). Two pathways regarding their toxic properties have been studied: the inhibition of cholinesterase and interference with the neuromodulator octopamine and with GABA-gated chloride channels (Isman, 2000; Isman, 2006; Regnault-Roger *et al.*, 2012). It is thus essential gain further insight into the bioactive compound(s) and their biological actions so as to be able to consider their overall repellent and/or insecticide potential.

Essential oils are complex blends of several molecules. This is one the first studies that links the behavioral effects of essential oils, mixtures of major compounds and single major compounds. In our previous study, *Cymbopogon citratus*, *Cymbopogon winterianus*, *Cuminum cyminum*, *Cinnamomum zeylanicum* were repellent at 1-10% and they also showed toxic effects at 1%, i.e. 96.3% mortality for cinnamon oil, 64.7% for citronella oil, 61.0% for lemongrass oil and 30.0% for cumin oil (Deletre *et al.*, submitted). In the repellency assay, the four mixtures of major compounds were repellent, like their associated essential oils. Among the cinnamon mixture compounds, only cinnamaldehyde was repellent, so the repellent effect of cinnamon essential oils would certainly be due to cinnamaldehyde. In the same way, cuminaldehyde could be responsible for the repellent effect of the cumin mixture. Among the citronella mixture compounds, geraniol, citronellol and geranyl acetate were repellent. This finding suggests that the repellent effect of the citronella mixture was due to an additive effect of all of these compounds. Moreover, citral, geraniol and geranyl acetate could be responsible for the repellency effect of the lemongrass mixture. The repellent effect of essential oils resulted to one major compounds or several major compounds in function of the essential oils. In the toxicity assay, cinnamaldehyde alone was as toxic as the cinnamon mixture, contrary to the other major compounds, so the cinnamon toxicity was certainly due to cinnamaldehyde. Cuminaldehyde and γ-terpinene were also toxic, but they were less toxic than the cumin mixture, so a synergetic effect between these two compounds or other minor compounds could explain the toxic effect. Citronellol, citronellal and geraniol were also toxic, but not as efficient as the citronella mixture. In addition, citral and geraniol were toxic but not as toxic as the lemongrass mixture. A synergistic effect between compounds could explain these results.

Except for cinnamaldehyde, the whitefly net-crossing rate of the single compounds was higher than that of their associated mixtures, i.e. a single compound was less efficient as an olfactive barrier than its associated mixture. Moreover, after 4 h, the toxicity of these single compounds was lower than that of their associated mixtures, except for cinnamaldehyde. This suggested that the major compounds of cumin, citronella and lemongrass essential oils had synergistic/additive effects. Conversely, the repellency of the mixtures appeared to be due to one or several major compounds. For example, the cinnamon mixture was repellent and only cinnamaldehyde was repellent alone and as repellent as the mixture. We obtained the same result with the cumin mixture and cuminaldehyde, but this was not always the case as all the major compounds of citronella and lemongrass were repellent at higher concentration. In many studies, the activity of an essential oil against insects is explained by the major compounds (Ipek *et al.*, 2005). However, the activity of the main compounds could be modulated by other minor molecules (Franzios et al., 1997; Santana-Rios et al., 2001; Hoet et al., 2006). Many

of the essential oil compounds are actually involved in cell penetration, lipophilic or hydrophilic attraction and fixation on cell walls and membranes, with the cellular distribution determining the different types of radical reactions produced (Bakkali *et al.*, 2010). Our results showed that the biological effect of an essential oil is not always due to the activity of the major compound alone. Indeed, synergistic effects may occur between the major or minor compounds.

This study also aimed to determine the most promising compounds of four essential oils (cumin, cinnamon, citronella and lemongrass) for pest control applications. The most promising compounds in net treatments were cinnamaldehyde, limonene, citronellol, citronellal, citral and geraniol because their associated whitefly net-crossing rates were low. Repellency includes every phenomenon that prevents a pest from tracking, locating and/or recognizing its host (Deletre *et al.*, in prep). Repellents (at a distance or expellent, i.e. olfactory-mediated effect) and repellents (contact or irritant, i.e. contact-mediated effect) are two different phenomena that could be usefully combined with insect-proof netting. Three compounds, i.e. cinnamaldehyde, citronellol and geraniol, were expellent according to the expellent bioassays, but no compounds showed any irritant effects. Among these compounds, cinnamaldehyde showed the highest toxicity (100%). Geraniol and citronellol could thus be the most promising compound in combination with netting, although these compounds caused 32.1% and 17.1% mortality after 24 h.

The laboratory findings only indicated a direction and a screening method on which products could potentially be used in the field to control whitefly thanks to their repellent and toxic effects. These products : geraniol and citronellol could be used in insect proof net treatment and this strategy has already shown good results in the field with chemical products. For example, Martin *et al.* (2013) showed that a net treated with alphacypermethrin blocked the pest *Myzus persicae* on cabbage crops in field conditions because of the irritant and repellent effects of this chemical. Moreover, a net treated with alphacypermethrin was shown to reduce the whitefly net-crossing rate and improve tomato crop protection as compared to untreated netting (Martin *et al*., 2014). However, there are also approaches other than applying chemicals on nets to repel whiteflies, e.g. intercropping. Indeed, a repellent plant - as a natural diffusor of repellent volatil - can be combined with a net to obtain a repellent effect or to confuse the pest, thereby reducing the amount of chemicals needed for crop protection. Our findings suggested that plants that produce relatively high amounts of geraniol and/or citronellol volatils could be useful for investigating in intercropping strategy. But the plants have to match the requirements for intercropping as for exemple cultural conditions of the crop. Mansour *et al.* (2012) has already shown that intercropping tomato plants with okra and brinjal decreased the whitefly infestation rate as compared to monocropped tomato. One potential limit of this concept is that the exact mode of repellency and irritancy action was not studied. Togni *et al.* (2010) also showed that coriander volatils masked tomato volatils in host plant selection. Tosh and Brogan (2014) also put forward the idea of supplying whiteflies with a super-abundance of volatils to confuse this generalist insect. In conclusion, this study identified volatil candidates that may be emitted by companion plants or by diffusor e.g. the net to repel whiteflies. These compounds could be used alone or in mixtures to establish an olfactive barrier as a supplement to the visual and physical barrier of an insect-proof nets in order to protect vegetables.

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TABLE 1. RATIOS AND QUANTITIES OF CITRONELLA, CUMIN, CINNAMON AND LEMONGRASS ESSENTIAL OIL COMPOUNDS.

Essential oil	**Composition (%)[1]**		**Quantity tested (mg/ml)[2]**	
			C1	**C2**
Citronella *Cymbopogon winterianus*	34.7	citronellal	0.291	2.91
	22.5	geraniol	0.205	2.05
	12.0	citronellol	0.102	1.02
	3.5	geranyl acetate	0.037	0.37
	3.3	limonene	0.025	0.25
	76.0	**sub-total (mixture)**		
	4.2	elemol	NT	NT
	2.9	citronellyl acetate	NT	NT
	2.5	β-elemene	NT	NT
	2.2	δ-cadinene	NT	NT
	0.9	linalol	NT	NT
	0.8	eugenol	NT	NT
	89.5	**total**		
Cumin *Cuminum cyminum*	30.1	cuminaldehyde	0.293	2.93
	12.2	β-pinene	0.087	0.87
	11.6	γ-terpinene	0.085	0.85
	9.7	*p*-cymene	0.086	0.86
	63.6	**sub-total (mixture)**		
	16.6	p-mentha-1,3-dien-7-al	NT	NT
	8.8	p-mentha-1,4-dien-7-al	NT	NT
	0.6	α-pinene	NT	NT
	0.4	myrcene	NT	NT
	0.4	limonene	NT	NT
	90.4	**total**		
Cinnamon *Cinnamomum zeylanicum*	78.5	(E)-cinnamaldehyde	0.840	8.40
	9.6	2-methoxy-cinnamaldehyde	0.090	0.90
	3.1	Cinnamyl acetate	0.032	0.32
	91.2	**sub-total (mixture)**		
	1.1	benzaldehyde	NT	NT
	0.9	coumarine	NT	NT
	0.7	phenyl ethyl alcohol	NT	NT
	0.4	(Z)-cinnamaldehyde	NT	NT
	94,3	**total**		
Lemongrass *Cymbopogon citratus*	74.1	citral	0.714	7.14
	4.5	geraniol	NT	NT
	3.9	geranyl acetate	0.037	0.37
	1.9	limonene	NT	NT
	84.4	**sub-total (mixture)**		
	1.8	β-caryophyllene	NT	NT
	0.7	linalool	NT	NT
	1.5	borneol	NT	NT
	0.6	nerol	NT	NT
	89.0	**total**		

[1] The percentage composition of the essential oil was computed by the normalization method from GC/FID analyses, with response factors being taken as one for all compounds. The composition of the four essential oils was identified by gas chromatography and mass spectrometry.

[2] The used quantities are expressed in mg/ml of solution in which the nets were dipped.

TABLE 2 . DISTRIBUTION AND MORTALITY RATE OF *BEMISIA TABACI* ADULTS (CONFIDENCE INTERVAL CALCULATED WITH THE WALD METHOD) AMONG THREE PARTS OF A VERTICAL OLFACTOMETER EXPOSED TO DEET, PERMETHRIN, NATURAL COMPOUNDS AND FOUR MIXTURES AT DIFFERENT CONCENTRATIONS.

Product	Concentration % (mg/L)	*p*-value*	Top: % (IC)	Middle: % (IC)	Bottom: % (IC)	Dead: % (IC)	Top: % (IC)	Middle: % (IC)	Bottom: % (IC)	Dead: % (IC)
DEET	0.1 (0.998)	<0,001	92.9 (87.5 - 98.3)	4.7 (0.2 - 9.2)	1.2 (0.0 - 3.5)	1.2 (0.0 - 3.5)	0.0 (0.0 - 0.0)	21.8 (12.6 - 31)	78.2 (69 - 87.4)	0.0 (0.0 - 0.0)
	1 (9.98)	<0,001	87.9 (81.5 - 94.3)	8.1 (2.7 - 13.5)	2.0 (-0.8 - 4.8)	2.0 (0.0 - 4.8)	5.1 (0.8 - 9.4)	28.3 (19.4 - 37.2)	66.7 (57.4 - 76.0)	0.0 (0.0 - 0.0)
Permethrin	1 (11.9)	0.055	86.7 (80.4 - 93.0)	10.6 (4.9 - 16.3)	0.9 (0.0 - 2.6)	1.8 (0.0 - 4.2)	73.5 (66.1 - 80.9)	21.3 (14.4 - 28.2)	5.1 (1.4 - 8.8)	0.0 (0.0 - 0.0)
Cinnamon[1]	0.1	<0,001	84.2 (76.0 - 92.4)	13.2 (5.6 - 20.8)	1.3 (0.0 - 3.9)	1.3 (0.0 - 3.9)	0.0 (0.0 - 0.0)	6.5 (1.0 - 12.0)	76.6 (67.1 - 86.1)	16.9 (8.5 - 25.3)
	1	<0,001	82.5 (74.2 - 90.8)	12.5 (5.3 - 19.7)	3.8 (0.0 - 8.0)	1.3 (0.0 - 3.7)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	7.2 (1.6 - 12.8)	92.8 (87.2 - 98.4)
Cinnamaldehyde	0.008 (0.08)	<0,001	73.2 (63.9 - 82.5)	15.9 (6.8 - 25.0)	9.8 (1.2 - 18.4)	1.2 (0.0 - 3.5)	0.0 (0.0 - 0.0)	7.3 (1.2 - 13.4)	59.8 (47.0 - 72.6)	32.9 (20.6 - 45.2)
	0.08 (0.84)	<0,001	73.5 (64.0 - 83.0)	18.1 (9.8 - 26.4)	7.2 (1.6 - 12.8)	1.2 (0.0 - 3.5)	0.0 (0.0 - 0.0)	4.3 (0.0 - 10.2)	65.2 (51.4 - 79.0)	30.4 (17.1 - 43.7)
	0.8 (8.40)	<0,001	76.1 (66.2 - 86.0)	11.3 (3.9 - 18.7)	12.7 (5.0 - 20.4)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	1.8 (0.0 - 5.3)	66.1 (53.7 - 78.5)	32.1 (19.9 - 44.3)
Cinnamyl acet.	0.003 (0.03)	0,145	73.4 (62.6 - 84.2)	15.6 (6.7 - 24.5)	4.7 (0.0 - 9.9)	6.3 (0.4 - 12.2)	88.9 (80.5 - 97.3)	9.3 (1.6 - 17.0)	0.0 (0.0 - 0.0)	1.9 (0.0 - 5.5)
	0.03 (0.32)	0,986	67.3 (54.9 - 79.7)	14.5 (5.2 - 23.8)	10.9 (2.7 - 19.1)	7.3 (0.4 - 14.2)	68.3 (56.5 - 80.1)	15.0 (6.0 - 24.0)	11.7 (3.6 - 19.8)	5.0 (0.0 - 10.5)
2-metoxycin.	0.009 (0.09)	0.823	87.3 (80.6 - 94.0)	6.4 (0.4 - 12.4)	2.7 (0.0 - 6.4)	3.6 (0.0 - 8.5)	89.5 (82.0 - 97.0)	4.8 (0.0 - 10.1)	3.8 (0.0 - 7.9)	1.9 (0.0 - 5.5)
	0.09 (0.90)	0.780	89.8 (82.3 - 97.3)	5.1 (0.1 - 10.1)	4.1 (0.0 - 8.1)	1.0 (0.0 - 3.5)	86.0 (77.0 - 95.0)	5.6 (0.2 - 11.0)	5.6 (0.2 - 11.0)	2.8 (0.0 - 6.8)
Cumin[2]	0.1	<0,001	77.0 (67.4 - 86.6)	16.2 (7.8 - 24.6)	5.4 (0.2 - 10.6)	1.4 (0.0 - 4.0)	0.0 (0.0 - 0.0)	13.2 (5.1 - 21.3)	75.0 (64.7 - 85.3)	11.8 (4.1 - 19.5)
	1	<0,001	79.5 (70.2 - 88.8)	11.0 (3.8 - 18.2)	6.8 (1.0 - 12.6)	2.7 (0.0 - 6.4)	0.0 (0.0 - 0.0)	5.2 (0.2 - 10.2)	80.5 (71.7 - 89.3)	14.3 (6.5 - 22.1)
Cuminaldehyde	0.003 (0.03)	0,095	85.7 (76.9 - 94.5)	7.1 (1.5 - 12.6)	5.7 (0.3 - 11.1)	1.4 (0.0 - 3.8)	70.6 (58.7 - 82.5)	15.7 (6.6 - 24.5)	13.7 (6.4 - 21.0)	0.0 (0.0 - 0.0)
	0.03 (0.29)	0,009	70.0 (58.4 - 81.6)	23.3 (12.6 - 34.0)	5.0 (0.0 - 10.5)	1.7 (0.0 - 4.9)	80.0 (70.6 - 89.4)	10.0 (3.0 - 17.0)	0.0 (0.0 - 0.0)	10.0 (3.0 - 17.0)
	0.3 (2.93)	<0,001	82.0 (72.4 - 91.6)	11.5 (3.5 - 19.5)	0.0 (0.0 - 0.0)	6.6 (0.4 - 12.8)	46.1 (35.7 - 56.5)	22.5 (13.8 - 31.2)	28.1 (18.8 - 37.4)	3.4 (0.0 - 7.1)
β-pinene	0.01 (0.09)	0,986	80.0 (70.9 - 89.1)	8.0 (1.9 - 14.1)	6.7 (1.1 - 12.3)	5.3 (0.2 - 10.4)	85.2 (76.3 - 94.1)	8.2 (1.3 - 15.1)	1.6 (0.0 - 4.8)	4.9 (0.0 - 10.3)
	0.10 (0.87)	0,679	76.2 (67.1 - 85.3)	13.1 (5.9 - 20.3)	8.3 (2.4 - 14.2)	2.4 (0.0 - 5.7)	74.7 (65.3 - 84.1)	13.3 (6.0 - 20.6)	9.6 (3.3 - 15.9)	2.4 (0.0 - 5.7)
γ-terpinene	0.01 (0.09)	0,429	78.5 (69.4 - 87.6)	10.1 (3.4 - 16.8)	8.9 (2.6 - 15.2)	2.5 (0.0 - 6.0)	90.2 (82.7 - 97.7)	1.6 (0.0 - 4.8)	8.2 (1.3 - 15.1)	0.0 (0.0 - 0.0)
	0.10 (0.85)	0,105	90.5 (83.8 - 97.2)	5.4 (0.2 - 10.6)	4.1 (0.0 - 8.6)	0.0 (0.0 - 0.0)	82.6 (73.7 - 91.5)	8.7 (2.1 - 15.3)	5.8 (0.3 - 11.3)	2.9 (0.0 - 6.9)
ρ-cymene	0.01 (0.09)	0,126	75.8 (67.0 - 84.6)	9.9 (3.8 - 16.0)	12.1 (5.4 - 18.8)	2.2 (0.0 - 5.2)	82.9 (74.4 - 91.4)	5.3 (0.3 - 10.3)	10.5 (3.6 - 17.4)	1.3 (0.0 - 3.9)
	0.10 (0.86)	0,679	69.2 (580.0 - 80.4)	18.5 (9.1 - 27.9)	12.3 (4.3 - 20.3)	0.0 (0.0 - 0.0)	56.0 (44.8 - 67.2)	18.7 (9.9 - 27.5)	25.3 (15.5 - 35.1)	0.0 (0.0 - 0.0)
Citronella[3]	0.1	<0,001	73.4 (63.7 - 83.1)	17.7 (9.3 - 26.1)	7.6 (1.8 - 13.4)	1.3 (0.0 - 3.8)	0.0 (0.0 - 0.0)	7.1 (0.4 - 13.8)	73.2 (61.6 - 84.8)	19.6 (9.2 - 30.0)
	1	<0,001	80.3 (71.0 - 89.6)	9.9 (3.0 - 16.8)	9.9 (3.0 - 16.8)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	2.9 (0.0 - 6.8)	48.6 (36.9 - 60.3)	48.6 (36.9 - 60.3)
Citronellal	0.034 (0.29)	0,852	78.0 (66.5 - 89.5)	10.0 (1.7 - 18.3)	6.0 (-0.6 - 12.6)	6.0 (0.0 - 12.6)	78.0 (66.5 - 89.5)	6.0 (0.0 - 12.6)	6.0 (-0.6 - 12.6)	10.0 (1.7 - 18.3)
	0.34 (2.91)	<0,001	71.7 (59.6 - 83.8)	9.4 (1.5 - 17.3)	11.3 (2.8 - 19.8)	7.5 (0.4 - 14.6)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	17.5 (7.6 - 27.4)	82.5 (72.6 - 92.4)
Geraniol	0.002 (0.02)	0,269	79.0 (69.8 - 88.2)	13.6 (6.4 - 20.8)	7.4 (0.3 - 14.5)	0.0 (0.0 - 0.0)	80.8 (71.3 - 90.3)	9.1 (1.4 - 16.8)	6.1 (0.0 - 12.6)	4.0 (0.0 - 8.1)
	0.023 (0.20)	0,005	54.3 (43.5 - 65.1)	12.3 (5.1 - 19.5)	30.9 (20.8 - 41.0)	2.5 (0.0 - 5.9)	55.9 (44.1 - 67.7)	10.3 (3.1 - 17.5)	16.2 (7.4 - 25.0)	17.6 (8.5 - 26.7)
	0.23 (2.05)	<0,001	63.9 (52.8 - 75.0)	9.7 (2.9 - 16.5)	18.1 (9.2 - 27.0)	8.3 (1.9 - 14.7)	0.0 (0.0 - 0.0)	3.0 (0.0 - 7.1)	86.4 (78.1 - 94.7)	10.6 (3.2 - 18)
Citronellol	0.012 (0.10)	0,427	73.6 (63.4 - 83.8)	12.5 (4.9 - 20.1)	8.3 (1.9 - 14.7)	5.6 (0.3 - 10.9)	81.5 (72.1 - 90.9)	4.6 (0.0 - 9.7)	7.7 (1.2 - 14.2)	6.2 (0.4 - 12.0)
	0.12 (1.03)	<0,001	69.8 (58.5 - 81.1)	17.5 (8.1 - 26.9)	7.9 (1.2 - 14.6)	4.8 (0.0 - 10.1)	0.0 (0.0 - 0.0)	11.3 (2.8 - 19.8)	62.3 (49.2 - 75.4)	26.4 (14.5 - 38.3)
Geranyl acetate	0.04 (0.37)	0,182	83.9 (74.7 - 93.1)	6.5 (0.4 - 12.6)	6.5 (0.4 - 12.6)	3.2 (0.0 - 7.6)	83.0 (72.9 - 93.1)	15.1 (5.5 - 24.7)	1.9 (0.0 - 5.6)	0.0 (0.0 - 0.0)
	0.40 (3.66)	<0,001	86.0 (75.6 - 96.4)	9.3 (0.6 - 18.0)	4.7 (0.0 - 11.0)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	6.7 (0.4 - 13.0)	68.3 (56.5 - 80.1)	25.0 (14.0 - 36.0)
Limonene	0.003 (0.03)	0,793	84.4 (76.0 - 92.8)	9.1 (1.4 - 16.8)	3.9 (0.0 - 8.0)	2.6 (0.0 - 6.2)	79.8 (70.4 - 89.2)	8.9 (2.6 - 15.2)	5.1 (0.0 - 10.9)	6.3 (0.4 - 12.2)
	0.03 (0.25)	0,011	70.9 (58.9 - 82.9)	7.3 (0.4 - 14.2)	7.3 (0.4 - 14.2)	14.5 (5.2 - 23.8)	74.1 (64.8 - 83.4)	9.4 (3.2 - 15.6)	15.3 (7.6 - 23)	1.2 (0.0 - 3.5)
	0.3 (2.52)	0,045	91.1 (83.6 - 98.6)	5.4 (0.0 - 11.3)	0.0 (0.0 - 0.0)	3.6 (0.0 - 8.5)	82.9 (74.1 - 91.7)	10.0 (3.0 - 17.0)	7.1 (1.1 - 13.1)	0.0 (0.0 - 0.0)
Lemongrass[4]	0.1	<0,001	78.7 (69.4 - 88.0)	17.3 (8.7 - 25.9)	1.3 (0.0 - 3.9)	2.7 (0.0 - 6.3)	0.0 (0.0 - 0.0)	7.1 (1.1 - 13.1)	84.3 (75.8 - 92.8)	8.6 (2 - 15.2)
	1	<0,001	81.3 (72.5 - 90.1)	10.7 (3.7 - 17.7)	6.7 (1.1 - 12.3)	1.3 (0.0 - 3.9)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	6.5 (1.0 - 12.0)	93.5 (88 - 99)
Citral	0.08 (0.71)	0.772	70.3 (59.1 - 81.5)	7.8 (1.2 - 14.4)	17.2 (8.0 - 26.4)	4.7 (0.0 - 9.9)	77.0 (67.4 - 86.6)	5.4 (0.2 - 10.6)	12.2 (4.8 - 19.6)	5.4 (0.2 - 10.6)
	0.8 (7.14)	<0,001	86.0 (77.0 - 95.0)	1.8 (0.0 - 5.2)	7.0 (0.4 - 13.6)	5.3 (0.0 - 11.1)	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	5.4 (0.0 - 11.3)	94.6 (88.7 - 100.5)

1

TABLE 3. RESULTS OF THE ETHOVISION CHOICE IRRITANCY TEST. FOR ALL TESTED COMPOUNDS THE AVERAGE RESULTS OF ALL REPLICATES AND *P*-VALUES (PAIRED T-TEST[1] OR WILCOXON'S TEST[2]) ARE GIVEN FOR THE DISTANCE MOVED, TIME SPENT MOVING (MOBILITY), AVERAGE VELOCITY AND THE TIME SPENT IN EACH ZONE

Compound		**Total distance moved (mm)**		**Mobility (%)**		**Average velocity (mm/s)**		**Time spent in zones**	
		Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**	**Treated**	**Control**
2-Methoxycinn.	Average *p*-value	202.69 0.643 [2]	202.94	27.03 0.527 [1]	24.13	2.81 0.7685 [2]	2.79	251.47 0.176 [1]	348.59
Cinnamaldehyde	Average *p*-value	146.10 0.459 [2]	127.81	17.64 0.782 [2]	18.87	2.80 0.263 [2]	2.76	358.68 0.393 [1]	241.40
Cinnamyl acetate	Average *p*-value	200.37 0.581 [1]	226.17	24.80 0.024 [2]	32.61	2.71 0.73 [2]	2.82	176.70 0.832 [1]	305.37
Citral	Average *p*-value	343.40 0.564 [1]	316.05	37.86 0.833 [2]	38.41	3.01 0.29 [2]	2.97	300.35 0.991 [1]	299.73
Citronellal	Average *p*-value	298.55 0.968 [2]	307.96	34.21 0.503 [2]	35.44	1.07 0.428 [2]	1.14	312.74 0.627 [1]	287.34
Cuminaldehyde	Average *p*-value	216.57 0.395 [1]	240.72	31.85 0.173 [2]	27.14	2.78 0.062 [1]	2.71	254.92 0.117 [1]	344.60
Geranyl acetate	Average *p*-value	318.19 0.385 [1]	359.67	35.89 0.005 [2]	40.77	2.96 0.971 [1]	2.96	314.06 0.543 [1]	286.02
DEET	Average *p*-value	256.63 0.269 [2]	337.40	35.24 0.043 [2]	30.05	3.19 0.515 [2]	3.19	247.04 0.157 [1]	353.05
Permethrin	Average *p*-value	153.84 0.570 [2]	154.92	15.80 0.184 [2]	19.55	2.63 0.39 [2]	2.48	351.15 0.205 [2]	248.93

[1] Tested with a paired t-test.

[2] Tested with Wilcoxon's test.

TABLE 4. RESULTS OF THE ETHOVISION NO CHOICE IRRITANCY TEST. FOR ALL TESTED COMPOUNDS THE AVERAGE RESULTS OF ALL REPLICATES AND *P*-VALUES (UNPAIRED T-TEST[1] OR WILCOXON'S TEST[2]) ARE GIVEN FOR THE DISTANCE MOVED, TIME SPENT.

Compound		**Total distance moved (mm)**		**Mobility (%)**		**Average velocity (mm/s)**	
		Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**
2-Methoxycinn.	Average	472.04	450.43	27.50	24.18	2.84	2.97
	p-value	0.870 [1]		0.84 [2]		0.44 [1]	
(E)-Cinnamaldehyde	Average	571.44	478.43	28.51	24.64	3.18	3.27
	p-value	0.50 [1]		0.50 [2]		0.97 [2]	
Cinnamyl acetate	Average	273.80	290.20	0.24	0.26	1.86	1.32
	p-value	0.83 [1]		0.72 [2]		0.21 [1]	
Citral	Average	152.66	345.06	0.14	0.30	1.52	1.87
	p-value	0.02 [1]		0.04 [2]		0.15 [2]	
Citronellal	Average	253.38	393.89	0.22	0.34	0.42	0.66
	p-value	0.11 [1]		0.14 [2]		0.11 [1]	
Cuminaldehyde	Average	249.67	179.26	0.22	0.16	1.58	1.82
	p-value	0.21 [1]		0.19 [2]		0.41 [2]	
Geranyl acetate	Average	246.34	364.05	0.23	0.32	1.91	1.63
	p-value	0.11 [1]		0.08 [2]		0.39 [2]	
DEET	Average	186.10	195.79	0.16	0.17	1.92	1.34
	p-value	0.86 [1]		0.90 [2]		0.31 [2]	
Permethrin	Average	435.33	446.80	24.83	24.36	2.90	3.02
	p-value	0.91 [1]		0.97 [2]		0.25 [1]	

[1] Tested with an unparied t-test.

[2] Tested with Wilcoxon's

TABLE 5. SYNTHESIS OF BEHAVIORAL BIOASSAYS

			Property:	
Product	Repellent	Irritant	Toxic (4h)	Toxic (24h)
			Property:	
Product	Repellent	Irritant	Toxic (4h)	Toxic (24h)
citronellal	+*	0	+	+
geraniol	+++	NT	+	++
citronellol	+	NT	+	++
geranyl acetate	+	0	0	0
limonene	++	NT	+	0
citronella blend[1]	+	NT	+	0
cuminaldehyde	+	0	+	++
β-pinene	0	NT	0	0
γ-terpinene	0	NT	+	0
p-cymene	0	NT	0	+
cumin blend[2]	++	NT	+	+
(E)-cinnamaldehyde	+++	0	+	0
2-methoxy-cinnamaldehyde	0	0	0	0
cinnamylacetate	0	0	++	+
cinnamon blend[3]	++	NT	+	0
citral	+	0	+	0
geraniol	+++	NT	+	++
geranyl acetate	+	NT	0	0
limonene	++	NT	+	0
lemongrass blend[4]	++	NT	+	+

+ = one *p*-values determined with Fisher's exact-test was significant, 0 = no *p*-values determined with Fisher's exact-test was significant, NT = non tested.

[1] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.

[2] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 p-cymene.

[3] Cinnamon mixture: 78.51% (E)-cinnamaldehyde, 9.65% 2-methoxycinnamaldehyde, 3.15% cinnamyl acetate.

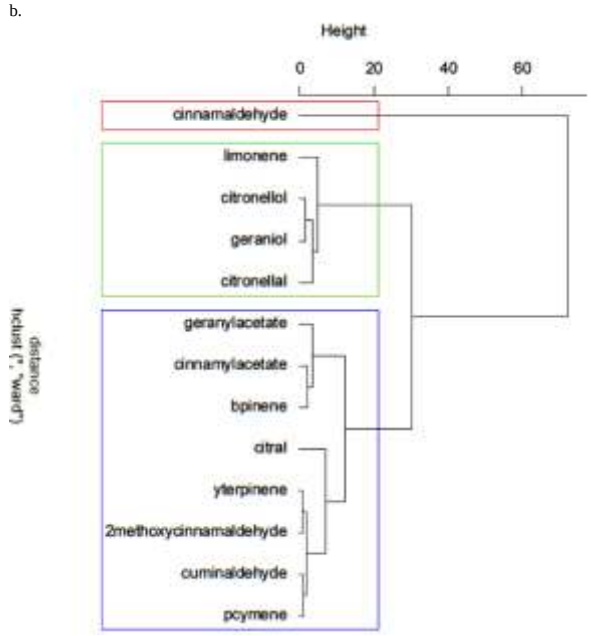
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 3.9% geranyl acetate, 1.9% limonene.

Fig. 1 The whitefly net-crossing rate measured after 4 h through a net treated with DEET, permethrin, the major compounds of four essential oils and their mixture at two different concentrations (C1 and C2 mg/mL^2 of product, refer to Table 1): A. corrected proportion escaping using Sun-Shepard's formula (confidence interval calculated with the Wald method) by treatment concentration and B. dendrogram determined by hierarchical ascendant classification.

a.

Compound	C1		C2	
DEET	**64.5***	**(60.2 - 68.8)**	**47.3**	**(44.0 - 50.6)**
Permethrin	**56.7**	**(53.4 – 60.0)**	**1.6**	**(1.3 – 1.9)**
Cinnamon[1]	**87.5**	**(85.7 - 89.3)**	**53.3**	**(51.5 - 55.1)**
Cinnamaldehyde	96.2	(94.2 - 98.2)	**48.9**	**(47.8 - 50.0)**
Cinnamyl acetate	**90.1**	**(87.0 - 93.2)**	92.1	(89.1 - 95.1)
2-methoxycinnamaldehyde	95.8	(94.0 - 97.6)	93.8	(91.4 - 96.2)
Cumin[2]	**85.9**	**(83.7 - 88.1)**	**66.8**	**(64.0 - 69.6)**
Cuminaldehyde	96.1	(93.9 - 98.3)	94.6	(92.4 - 96.8)
β-pinene	**90.4**	**(86.8 - 94.0)**	94.1	(91.3 - 96.9)
γ-terpinene	95.4	(93.2 - 97.6)	**93.2**	**(90.3 - 96.1)**
ρ-cymene	95.3	(92.7 - 97.9)	94.8	(92.1 - 97.5)
Citronella[3]	90.5	(87.2 - 93.8)	**61.8**	**(59.7 - 63.9)**
Citronellal	92.0	(87.7 - 96.3)	**87.8**	**(83.0 - 92.6)**
Geraniol	89.1	(84.0 - 94.2)	**86.0**	**(83.0 - 89.0)**
Citronellol	**90.6**	**(87.3 - 93.9)**	**85.9**	**(82.6 - 89.2)**
Geranyl acetate	93.0	(89.4 - 96.6)	92.1	(88.3 - 95.9)
Limonene	91.0	(86.1 - 95.9)	**82.8**	**(78.4 - 87.2)**
Lemongrass[4]	**84.4**	**(82.4 - 86.4)**	**49.2**	**(46.8 - 51.6)**
Citral	96.5	(94.7 - 98.3)	**89.5**	**(86.7 - 92.3)**

b.



*p-values determined with Fisher's exact-test.

[1] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate.

[2] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 p-cymene.

[3] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.

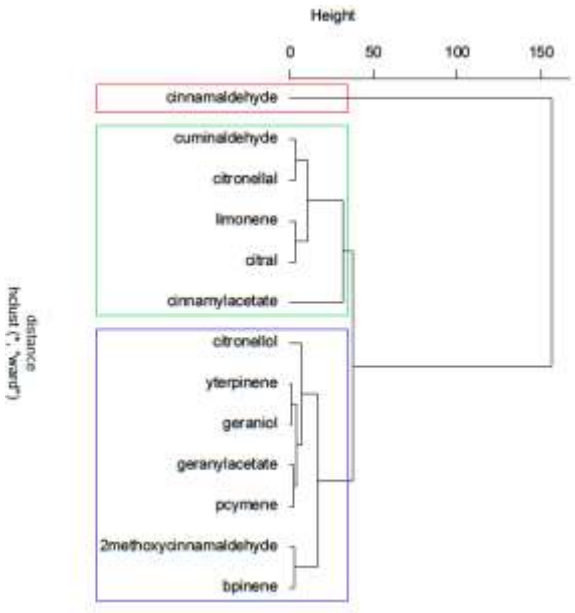
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 3.9% geranyl acetate, 1.9% limonene.

Fig. 4 The whitefly mortality rate measured after 4 h through a net treated with DEET, permethrin, the major compounds of four essential oils and their mixture at two different concentrations (C1 and C2 mg/mL[2] of product, refer to Table 1): A. corrected proportion using Sun-Shepard's formula (confidence interval calculated with the Wald method) by treatment concentration and B. dendrogram determined by hierarchical ascendant classification.

a.

Compound	C1	C2
DEET	**97.6*** **(96 - 99.2)**	**100.0 (100.0 - 100.0)**
Permethrin	**94.6 (93.4 – 95.8)**	**100.0 (100.0 - 100.0)**
Cinnamon[1]	6.5 (4.8 - 8.2)	**100.0 (98.2 - 100.0)**
Cinnamaldehyde	0.5 (0.0 - 2.0)	**100.0 (100.0 - 100.0)**
Cinnamyl acetate	**23.9 (20.6 - 27.2)**	**20.1 (16.6 - 23.6)**
2-methoxycinnamaldehyde	1.1 (0.5 - 1.7)	2.1 (0.9 - 3.3)
Cumin[2]	10.1 (8 - 12.2)	**57.0 (55.1 - 58.9)**
Cuminaldehyde	2.9 (1.2 - 4.6)	**14.7 (12.2 - 17.2)**
β-pinene	3.0 (1.4 - 4.6)	0.0 (0.0 - 1.4)
γ-terpinene	2.6 (1.4 - 3.8)	**8.5 (6.7 - 10.3)**
ρ-cymene	3.8 (1.9 - 5.7)	6.3 (4.3 - 8.3)
Citronella[3]	0.7 (0.0 - 2.2)	**65.8 (63.8 - 67.8)**
Citronellal	1.4 (0.0 - 4.1)	**11.5 (6.2 - 16.8)**
Geraniol	3.3 (0.7 - 5.9)	**8.1 (6.2 - 10.0)**
Citronellol	**8.1 (6.1 - 10.1)**	**10.0 (8.0 - 12.0)**
Geranyl acetate	5.6 (3.9 - 7.3)	7.1 (5.2 - 9.0)
Limonene	4.7 (0.6 - 8.8)	**19.7 (15.3 - 24.1)**
Lemongrass[4]	**17.6 (16.4 - 18.8)**	**100.0 (100.0 - 100.0)**
Citral	1.3 (0.0 - 2.7)	**19.5 (18.1 - 20.9)**

b.



*p-values determined with Fisher's exact-test.

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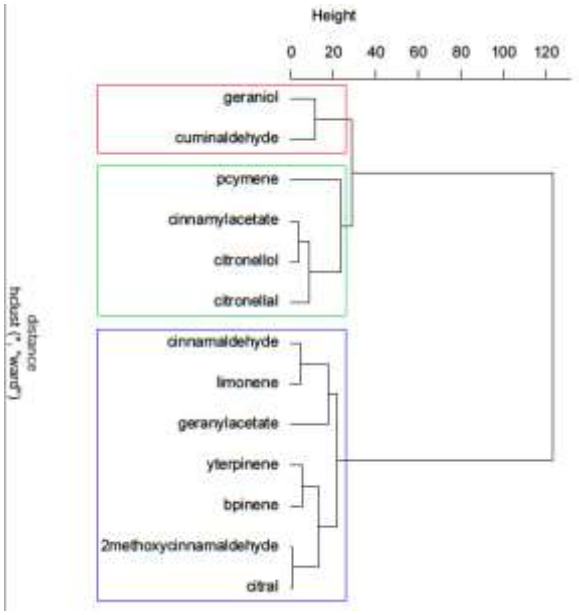
Fig. 5 The whitefly mortality rate measured after 24 h through a net treated with DEET, permethrin, the major compounds of four essential oils and their mixture at two different concentrations (C1 and C2 mg/mL[2] of product, refer to Table 1): A. corrected proportion using Sun-Shepard's formula (confidence interval calculated with the Wald method) by treatment concentration and B. dendrogram determined by hierarchical ascendant classification.

a.

Compound	C1		C2	
DEET	0.0*	(0.0 - 0.6)	0.0	(0.0 - 0.0)
Permethrin	2.1	(0.0 - 6.0)	0.0	(0.0 - 0.0)
Cinnamon[1]	3.3	(0.1 - 6.5)	0.0	(0.0 - 0.0)
Cinnamaldehyde	4.0	(0.4 - 7.6)	0.0	(0.0 - 0.0)
Cinnamyl acetate	**18.7**	**(14.2 - 23.2)**	**13.8**	**(9.2 - 18.4)**
2-methoxycinnamaldehyde	0.0	(0.0 - 0.0)	0.0	(0.0 - 0.0)
Cumin[2]	15.0	(9.8 - 20.2)	**26.7**	**(22.4 - 31.0)**
Cuminaldehyde	**20.9**	**(15.2 - 26.6)**	**23.5**	**(19.3 - 27.7)**
β-pinene	1.3	(0.0 - 7.3)	1.5	(0.0 - 7.2)
γ-terpinene	3.6	(0.0 - 8.1)	6.2	(0.6 - 11.8)
ρ-cymene	2.5	(0.0 - 8.3)	**18.5**	**(11.9 - 25.1)**
Citronella[3]	2.4	(0.0 - 6.5)	9.9	(3.8 - 16.0)
Citronellal	**21.3**	**(11.9 - 30.7)**	8.7	(0.0 - 17.7)
Geraniol	**28.3**	**(19.9 - 36.7)**	**32.1**	**(25.7 - 38.5)**
Citronellol	**17.2**	**(10.6 - 23.8)**	**17.1**	**(10.8 - 23.4)**
Geranyl acetate	0.0	(0.0 - 8.7)	0.0	(0.0 - 7.3)
Limonene	8.6	(0.5 - 16.7)	0.0	(0.0 - 7.5)
Lemongrass[4]	**24.8**	**(18.8 - 30.8)**	0.0	(0.0 - 0.0)
Citral	0.0	(0.0 - 2.8)	0.0	(0.0 - 0.0)

b.



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Repellent Effect of Alphacypermethrin-Treated Netting Against *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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ABSTRACT For >20 yr, *Bemisia tabaci* Gennadius persists as a begomovirus vector and is a serious problem in tomato production in many parts of the world. In tropical countries, the use of netting to protect horticultural crops has proven to be an effective and sustainable tool against Lepidoptera but not against small insects. This study evaluated the repellent effect of AgroNet 0.9T, a 0.9-mm pore diameter and 40-mesh size netting treated with alphacypermethrin insecticide against *B. tabaci*. This pyrethroid insecticide is known to have toxic and repellent effects against mosquitoes and has been used for treatment of mosquito nets. Two nontreated netting materials were used as control: AgroNet 0.9NT with 0.9-mm pore diameter and 40-mesh size and AgroNet 0.4NT with 0.4-mm pore diameter and 80-mesh size. The behavior of *B. tabaci* and its parasitoid *Encarsia formosa* Gahan as they progressed through the treated netting was studied in the laboratory in choice and no-choice tests. The development of wild *B. tabaci* population on tomato plants protected by the same nets was followed in two field trials implemented in Njoro, Kenya. Results obtained with the no-choice tests showed a significant reduction of movement on the treated net with 40-mesh (19%) compared with nontreated netting (35 and 46% with 80- and 40-mesh, respectively). The mortality of *B. tabaci* was significantly higher (two-fold) in the test tube containing only the treated netting compared with the nontreated one. The repellent effect of the treated netting was also demonstrated against *E. formosa*, but it did not have this toxic effect. Unlike for *B. tabaci*, the treated and nontreated nets appeared to have a similar repellent effect on *E. formosa* in the choice test, which suggests a learning behavior of the parasitoid. In both field tests, *B. tabaci* population was significantly lower on tomato protected by the treated net compared with the same nontreated net. However there was no significant difference in *B. tabaci* population between the treated 0.9-pore diameter and the nontreated 0.4-pore diameter. We discussed these findings and their implications for the use of repellent netting in integrated pest management in horticulture and more specifically in vegetable production.

KEY WORDS physical barrier, net, *Bemisia tabaci*, tomato, *Encarsia formosa*

The whitefly *Bemisia tabaci* Gennadius is an important pest of many crops in tropical and temperate regions, both in greenhouses and the field (Jing et al. 2003). This pest can cause the following three kinds of damage: 1) direct damage (by sucking the sap from the plant), 2) indirect damage (in causing development of a sooty mold on the honeydew it excretes on plant leaves), and 3) transmission of plant viruses (such as tomato yellow leaf curl virus, a complex of geminiviruses infecting tomato cultures worldwide; Berlinger 1986). Currently, it is difficult to establish tomato crops in open fields because of invasion by virus-bearing *B. tabaci* whiteflies (Berlinger et al. 1996). Moreover, whiteflies are difficult to control by insecticide foliar spray because they live on the underside of the leaves (Muniz et al. 2002, Zhang et al. 2004). Systemic insecticides, such as neonicotinoids or other chemicals acting on insect development, are currently used in greenhouses or in open fields. As a consequence, whitefly resistance to these insecticides has emerged because of the high reproductive rate of this insect (Elbert and Nauen 2000, Perring 2001).

The development of insect-proof netting has permitted the cost-effective production of tomato and other vegetables, particularly in the Mediterranean region (Berlinger et al. 2002). Various types of insect-proof netting, and their efficacy and importance as physical control methods in agriculture have been extensively reviewed (Weintraub and Berlinger 2004, Weintraub 2009). In tropical regions with high temperature and humidity levels, the use of netting with fine mesh smaller than 0.5 mm against whiteflies re-

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duces ventilation (Fatnassi et al. 2002) and tends to increase the risk of plant pathogen development.

However, promising results have been obtained in Benin by using pyrethroid-treated nets with 24-mesh size to protect cabbage against the aphid *Lipaphis erysimi* (Kaltenbach) (Martin et al. 2006). Recently an alphacypermethrin-treated net with 40-mesh size was shown to be very effective for protecting a cabbage crop against aphids in France (Martin et al. 2013). Long-lasting pyrethroid-treated nets have been widely used for personal protection against mosquitoes, and their use is recommended by the World Health Organization as a key preventive measure against malaria (Anonymous 2010). Indeed, pyrethroid-treated nets have four main modes of action against mosquitoes: 1) physical barrier, 2) excito-repellency (contact with the insecticide causes mosquitoes to leave without biting), 3) knockdown, and 4) killing effect, all resulting in a dramatic reduction of mosquito bites (Anonymous 2007).

The objective of this study was to evaluate the repellent effect of netting treated with alphacypermethrin against the whitefly *B. tabaci*. The treated netting had 0.9-mm pore diameter and a 40-mesh size. In comparison, the two nontreated netting materials had 0.9-mm pore diameter and 40-mesh size and 0.4-mm pore diameter and 80 mesh size. The repellent and toxic effects of the treated netting were investigated in the laboratory against *B. tabaci* and its parasitoid *Encarsia formosa* Gahan. The development of wild *B. tabaci* populations on tomato plants protected by the same nets was followed in two field trials implemented in central Kenya.

Materials and Methods

Insect. The biotype *Q* of the whitefly *B. tabaci* (MPL strain) was reared on tomato (*Solanum lycopersicum* L.) plants in a climatic chamber at 25 ± 1°C, 50 ± 10% relative humidity (RH), and a photoperiod of 12:12 h. In field test, the numbers of adult *B. tabaci* on each of the 14 middle plants in each experimental unit were counted once every week and the population recorded. Counting was done early in the morning (6:00–7:00 a.m.) when the pest is mostly inactive.

Netting. Netting, brand-named "AgroNets," was provided by A to Z Textile Mills Company (Arusha, Tanzania). The netting is made of knitted high-density polyethylene monofilament yarn: The AgroNet 0.9NT (treated and nontreated) with 0.9-mm pore diameter and 40-mesh size and the AgroNet 0.4NT (nontreated) with 0.4 mm pore diameter and 80-mesh. AgroNet 0.9T was treated by incorporation of 1% alphacypermethrin into high-density polyethylene polymer before extrusion of the yarn (97% purity active ingredient from Tagros Chemicals, Chennai, India), a treatment process similar to that of the long-lasting treated mosquito net Olyset, a patented product from Sumitomo Chemical Company (Tokyo, Japan) in which 98% of the total active ingredient is incorporated into the polyethylene yarn.

Tube Test. The ability of *B. tabaci* adults to cross the three AgroNet 0.9T, AgroNet 0.9NT, and AgroNet 0.4NT, was initially investigated in a no-choice test. The no-choice test device consisted of two transparent plastic tubes (Ø 5 cm, L 10 cm) separated by one AgroNet. The tubes were closed using a fine mesh AgroNet that whiteflies could not cross. An aluminum foil, to block out the light, covered one tube (darkened tube). To evaluate the repellent and toxic effects, 20 *B. tabaci* adults (of mixed sex and age) were released in the darkened tube. There were 5 replicates. The number of whiteflies crossing the net was recorded after 2, 4, and 6 h. After 6 h, dead adults were counted in each tube to determine initial mortality. Whiteflies found alive were placed separately on a tomato leaf, which was placed on agar gel (1%) in a petri dish, to evaluate delayed mortality 24 h after exposure.

The choice test device consisted of three plastic tubes (Ø 5 cm, L 10 cm) separated by two AgroNet. The tubes at both ends were closed using the same netting as described above. An aluminum foil was wrapped around the middle tube (darkened tube). Three different combinations were tested as follows: 1) AgroNet 0.9T vs. AgroNet 0.9T, 2) AgroNet 0.9T vs. AgroNet 0.9NT, and 3) AgroNet 0.9NT vs. AgroNet 0.9NT. For each test, 20 *B. tabaci* adults (of mixed age and sex) were released in the middle darkened tube. There were seven replications. The number of whiteflies crossing the AgroNet was counted in each lateral tube after 2, 4, and 6 h. The biological effect of AgroNet on the parasitoid *E. formosa* was also investigated using the same device with six replications of 20 *E. formosa* released in the middle tube for each combination of treatments.

Cage Test. Three cages were used, in the same manner as the choice test device described above. Large cages (30 by 40 by 60 cm) were used to allow *B. tabaci* to fly and to introduce a young tomato plant. These cages had a lateral shape to increase their attractiveness. The outer cages were separated from the middle one by two pieces of netting of similar mesh, that is, AgroNet 0.9T vs. AgroNet 0.9NT. The middle cage in which insects were released had a black cardboard cover. All cages were also made of fine mesh netting that whiteflies could not cross. In all, 50–100 *B. tabaci* adults (of mixed age and sex) were released every day in the dark central cage. The number of whiteflies on the tomato plant that crossed the treated or the nontreated nets was counted in each cage every 24 h for 10 consecutive days and removed using a mouth aspirator. Each day the middle cage was emptied before introducing new *B. tabaci*.

Bioassay. To test pyrethroid susceptibility of the whitefly strain used (MPL), a toxicity test was carried out. Tomato leaves were dipped in various concentrations of cypermethrin formulation (Cypermethrin 25 EC) provided by Arysta Life Science Corporation, Pau, France. Cypermethrin (a mixture of isomers) was preferred to alphacypermethrin, as it allows comparison of results with other assessments. Leaves were dipped for 10 s in aqueous solutions of insecticide formulation (11 concentrations) as per Houndété et

Table 1. Percentage of *B. tabaci* adults (±SE) that crossed through the alphacypermethrin-treated net (AgroNet 0.9T) and the nontreated nets (AgroNet 0.9NT and AgroNet 0.4NT) after 2, 4, and 6 h in no-choice test

Net	*n*	% of *B. tabaci* crossing the nets ±SE			% mortality ±SE at 6 h	% mortality ±SE at 24 h
		2 h	4 h	6 h		
AgroNet 0.9T	100	3 ± 2.0	11 ± 1.9	19 ± 2.9a	39 ± 6.8a	50.0 ± 4.6a
AgroNet 0.9NT	100	10 ± 2.2	30 ± 9.4	46 ± 5.8b	14 ± 2.5b	20.0 ± 4.1b
AgroNet 0.4NT	100	8 ± 3.4	18 ± 3.7	35 ± 2.2b	18 ± 2.6b	26.3 ± 3.1b
P value		<0.1	<0.1	<0.05	<0.05	<0.05

Values in the same column with the same letter are not significantly different.
Mortality was observed after 6 and 24 h.

al. (2010) but the control leaves were dipped in distilled water. Afterwards, leaves were air-dried for 20 min at room temperature. Leaf discs were cut (20 mm in diameter) and positioned on agar-coated petri dishes (55 mm in diameter). Adults of *B. tabaci* (20–30 whiteflies of mixed gender) from tomato plants were introduced into small plastic vials, maintained for 80 s at −20°C and then placed on the leaf discs. Each petri dish was closed with a transparent lid. Dishes were stored upside down at 24°C, 50% RH and a photoperiod of 12:12 (L:D) h, and mortality recorded after 48 h. Three replicates were made for each concentration and for the control. Mortality in the control was consistently lower than 10%. Mortality on treated discs were corrected using Abbott's formula (Abbott 1925).

Field Bioassay. Two field bioassays were conducted at the Horticulture Research and Teaching Field of Egerton University, Njoro, Kenya, from May to October 2012 (Season 1) and October 2012 to March 2013 (Season 2). The field lies at a latitude of 0° 23′ S and longitude 35° 35′ E in the Lower Highland Agro Ecological Zone and at an altitude of ≈2,238 m above sea level. The average maximum and minimum temperatures range from 19 to 22°C and 5–8°C, respectively, with a mean total annual rainfall of 1,200–1,400 mm. 'Rio Grande' tomato transplants were used as the planting material in the study. Seedlings used were started under an AgroNet 0.4NT covered nursery to ensure that they were of superior quality and virus free. Four-week-old tomato seedlings were transplanted in one row 8 m in length and at 50 cm within the row giving 16 tomato plants per experimental unit. The experiment was laid in a randomized complete block design with five replications. Transplants were established under five different treatments as follows: 1) open, unsprayed (untreated control), 2) open, sprayed with alphacypermethrin-based insecticides sprayed at 25 mg/201 on a weekly basis (treated control), 3) AgroNet 0.4NT, 4) AgroNet 0.9NT, and 5) AgroNet 0.9T. Within each block, individual experimental units measured 8 by 1 m separated by a 0.5-m buffer. In every plot, three posts 1.2 m in length were placed 4 m apart along the 8-m bed to serve as a support system for the cover and the crop. Binding wire was then pinned at 30-cm interval from the ground to the top of the posts to complete the crop support system. In addition, for the covered treatments, ordinary mild steel pieces 1 m in length were mounted on top of each post, fastened using U-nail and bent to provide a tunnel shape for dressing the covers.

Statistical Analysis. The software Minitab 12.2 was used for statistical analysis. A Kruskal–Wallis test was used to analyze the results (crossing rate and mortality) of the no-choice test. Then, if there was a significant effect of the nets a pairwise comparison of the different nets were done with a Wilcoxon Mann–Whitney test. In the choice test with tubes or cages, a Kolmogorov–Smirnov test was used as a normality test to determine whether our data set was distributed according to a normal distribution. A Student's paired *t*-test was used to compare the crossing rate in each tube or cage. Then at each observation time, the different crossing rates were compared between tests with a Student's unpaired *t*-test. The concentration killing 50% of the population (LC_{50} value) was calculated by global optimization by simulated annealing (GOSA, Bio-Log, Ramonville, France), available at http://bio-log.biz.

The Proc univariate procedure of SAS (version 9.1; SAS Institute, Cary, NC) was used to check for normality of the field data before analysis. Data were then subjected to analysis of variance using the GLM at $P \leq 0.05$. Means for significant treatments, at the *F*-test, were separated using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

Results

No-Choice Test. The results showed a repellent effect of the netting treated with alphacypermethrin, AgroNet 0.9T, against whitefly adults compared with the nontreated net (Table 1). After 6 h, the rate of *B. tabaci* adults that passed through the treated netting was significantly lower with AgroNet 0.9T sample (treated) than nontreated AgroNet 0.9NT and AgroNet 0.4NT samples (2.4- and 1.8-fold, respectively). However, passing-through rates did not differ significantly for both control samples despite the difference in mesh size. No mortality of whiteflies was observed after 4 h irrespective of the net used. After 6 h, mortality of whiteflies that passed through the treated netting was significantly higher (two-fold) than for the nontreated nets. Mortality rates did not differ significantly for both nontreated netting samples. Twenty-four hours after the no-choice test, mortality of whiteflies that crossed the treated netting was significantly higher (50%) than the nontreated sample (20% for AgroNet 0.9NT and 26% for AgroNet 0.4NT). Mortality rates observed in both control samples did not differ significantly.

Table 2. Percentage of *B. tabaci* adults (±SE) that crossed through the alphacypermethrin-treated net (AgroNet 0.9T) or the nontreated nets (AgroNet 0.9NT) after 2, 4, and 6 h in choice test

Net vs. Net	Test	*n*	*B. tabaci* adults crossing the nets (% ± SE)		
			2 h	4 h	6 h
AgroNet 0.9T vs. AgroNet 0.9T	1	140	0.0 ± 0.00	1.8 ± 0.10A	5.7 ± 0.27A
			0.0 ± 0.00	1.8 ± 0.10A	5.0 ± 0.17A
AgroNet 0.9T vs. AgroNet 0.9NT	2	140	0.0 ± 0.00	4.3 ± 0.16aB	7.9 ± 0.19aB
			1.1 ± 0.11	8.9 ± 0.29bB	17.1 ± 0.40bB
AgroNet 0.9NT vs. AgroNet 0.9NT	3	140	3.6 ± 0.30	13.2 ± 0.46C	23.6 ± 0.61C
			2.5 ± 0.17	12.9 ± 0.45C	22.9 ± 0.68C

Values in the same column with the same letter are not significantly different; Small letters indicate within test comparison and capital letters indicate between tests comparison.

Choice Test. The number of *B. tabaci* adults that crossed the netting toward the well-lit tubes was recorded after 2, 4, and 6 h (Table 2). After 4 and 6 h the passing-through rates were significantly higher for nontreated samples (AgroNet 0.9NT, 17.1%) than treated AgroNet 0.9T (7.9%). Furthermore, the crossing rate was significantly higher with two similar nontreated nets (23%) at both outer tubes than with the two similar treated nets (5%). These results confirmed the repellent effect of the alphacypermethrin-treated AgroNet 0.9T net.

For the parasitoid *E. formosa*, the number of adults that passed through the netting was counted in each well-lit tube after 2, 4, and 6 h, respectively (Table 3). The results showed a repellent effect of the treated netting AgroNet 0.9T. After 4 and 6 h, the passing-through rates of *E. formosa* were significantly lower for treated netting than untreated netting AgroNet 0.9NT when they had the choice between netting samples of the same mesh size, either treated or untreated. In contrast to *B. tabaci* (Table 2), when choice was given to *E. formosa* to come through a treated netting or a nontreated netting (Test 2), the passing-through rates were almost identical between both netting materials (Table 3). The crossing rates through both nontreated netting samples AgroNet 0.9NT were significantly higher. On the contrary, the crossing rates through the both treated netting samples AgroNet 0.9T were significantly lower. No mortality of *E. formosa* was observed in that test after 6 h.

Cage Test. The number of whiteflies that passed daily through the netting was significantly lower for treated netting (P value <0.05; Student paired t-test) than for the nontreated sample. The passing rates were very low when the middle cage was not covered compared with when the middle cage was darkened. When the middle cage was not covered, the passing rate through the netting was 3.0 ± 0.4% for AgroNet 0.9T vs. 6.4 ± 1.3% for AgroNet 0.9NT in average per day. The passing-through rates increased when the middle cage was darkened, reaching 29.5 ± 10.8% and 48.5 ± 17.2% for AgroNet 0.9T and AgroNet 0.9NT, respectively, in average per day.

Bioassay. The laboratory strain of *B. tabaci* (MPL) used for the study appeared to be resistant to cypermethrin (Table 4). The LC_{50} of MPL strain was significantly higher (4- to42-fold) than the LC_{50} of the susceptible reference strain (SUD-S) obtained by El Kady and Devine (2003), Roditakis et al. (2005), and Ma et al. (2007), but not by Kranthi et al. (2002).

Field Tests. In both seasons, *B. tabaci* population density varied from 1.6 to 8.3 adults per plant in average on nontreated and noncovered tomato plants (Table 5). The population density of *B. tabaci* was always significantly lower on all other treatments with AgroNets at each observation date. In contrast to noncovered tomato, the population density of *B. tabaci* on covered tomatoes did not increase, always staying at a low level. However, in most of the observation dates, the population density of *B. tabaci* was significantly lower on treated net AgroNet 0.9T compared with the same nontreated net AgroNet 0.9NT, particularly in the beginning of the Season 1 and during the entire Season 2. The same result was observed with the nontreated net with smaller pore AgroNet 0.4NT, where the number of *B. tabaci* per plant was significantly lower compared with the nontreated net with bigger pore AgroNet 0.9NT. Indeed, the population density of *B. tabaci* on tomato plants protected by the treated net AgroNet 0.9T was not significantly different from

Table 3. Percentage *E. formosa* adults which crossed the alphacypermethrin-treated net (AgroNet 0.9T) or the nontreated net (AgroNet 0.9NT) after 2, 4, and 6 h

Net	Test	*n*	*E. formosa* adults crossing the nets (% ± SE)		
			2 h	4 h	6 h
AgroNet 0.9T vs. AgroNet 0.9T	1	120	4.2 ± 0.34A	8.3 ± 0.47A	14.2 ± 0.34A
			2.5 ± 0.25A	5.8 ± 0.34A	13.3 ± 0.37A
AgroNet 0.9T vs. AgroNet 0.9NT	2	120	5.0 ± 0.50AB	15.8 ± 0.67B	22.5 ± 0.48B
			5.8 ± 0.34AB	15.8 ± 0.73B	23.3 ± 0.47B
AgroNet 0.9NT vs. AgroNet 0.9NT	3	120	12.5 ± 0.69BC	23.3 ± 0.69C	35.8 ± 0.61C
			13.3 ± 0.24C	28.3 ± 0.47C	37.5 ± 0.48C

Values in the same column with the same letter are not significantly different.

Table 4. Toxicity of cypermerthrin against the laboratory strain (MPL) of *B. tabaci* adults compared with the reference susceptible strain (SUD-S) from various studies

Strain	*N*	LC_{50} (mg L^{-1})	Confidence limits 95%	Slope (±SE)	References
MPL	660	23.36b	9.39–43.13	0.97 (±0.1)	–
SUD-S[1]	619	5.23a	2.50–6.87	1.9	El Kady and Devine (2003)
SUD-S[2]	575	7.0ab	5.7–10.1	2.0 (±0.4)	Kranthi et al. (2002)
SUD-S[3]	598	1.61a	1.05–2.47	0.79	Roditakis et al. (2005)
SUD-S[4]	258	0.55a	0.02–2.11	0.69	Ma et al. (2007)

N, no. of whiteflies tested; LC_{50} values with the same letter are not significantly different.

the population density observed on tomato plants protected by the nontreated net AgroNet 0.4NT.

Discussion

Our laboratory and field bioassays showed a repellent effect of the alphacypermethrin-treated netting AgroNet 0.9T against *B. tabaci* adults. We confirmed here the results obtained on cabbage aphids with AgroNet 0.9T (Martin et al. 2013). The physical barrier of the AgroNet 0.9NT was already very effective to protect tomato plants in both field bioassays as the level of infestation was ≈10-fold lower than in noncovered plants. Moreover, the populations of *B. tabaci* on covered tomatoes by AgroNet did not increase as observed in noncovered tomatoes. This result suggests that *B. tabaci* adults leave the covered-plants after emergence and before mating. It could be because of a migratory behavior of *B. tabaci* as observed by Mound (1962), which was not observed with cabbage aphids (Martin et al. 2013).

AgroNet 0.9T showed the same effectiveness as AgroNet 0.4NT with lower mesh size, as the difference of *B. tabaci* adults on tomato plants was not significant between both nets. Under laboratory conditions, whatever the test performed, the number of *B. tabaci* that crossed the treated netting AgroNet 0.9T was significantly lower than the number passing through the nontreated netting AgroNet 0.9NT. *B. tabaci* adults were likely in direct contact with the netting so the repellence mechanism could not be accurately characterized (e.g., vapor effect vs. contact effect). However, given the very low vapor pressure of alphacypermethrin ($3.85\ 10^{-5}$ mPa at 20°C), a distance-effect through, for example, vapor was unlikely to occur. Although the repellent effect was not assessed separately, a repulsion contact was frequently observed with pyrethroids, for example, irritability or excito-repellency. Narashi (1971) showed that pyrethroids act on the neuromuscular system by blocking the nerve influx; treated insects quickly develop hyperexcitation and tremor. With the cage test, we confirmed the repellent effect of the treated netting AgroNet 0.9T. This effect is mainly due to the excito-repellent effect of alphacypermethrin on *B. tabaci* after contact with the treated netting. Because of its low vapor pressure, alphacypermethrin had no or low masking effect with respect to the attractive volatile compounds of tomato plants. However, some volatile products from companion plants, such as coriander, could add an odor-masking effect (Togni et al. 2010). Moreover, Bleeker et al. (2009) showed that whiteflies choose their host plants via odor and color-driven mechanisms and noticed that a net could mask the visual attraction exerted by a plant (shape and color). Our results also showed a toxic effect of the treated netting AgroNet 0.9T against *B. tabaci* adults compared with the nontreated netting. The relatively high

Table 5. *B. tabaci* adults' pop (number per plant) on tomato plants under alphacypermethrin-treated net (AgroNet 0.9T) or the nontreated nets (AgroNet 0.9NT and AgroNet 0.4NT) during field production in Kenya (seasons, May to Oct. 2012 and October 2012 to March 2013)

Treatments	Days after transplanting												
	Season 1												
	30	37	44	51	58	65	72	79	86	93	100	107	114
Untreated control[a]	2.89a	3.70a	4.17a	5.39a	8.33a	1.6a	2.61a	2.30a	8.30a	4.89a	4.86a	4.24a	7.99a
Treated control[b]	0.37c	0.29b	0.70c	0.34c	0.23c	0.16c	0.20c	0.21c	0.60b	0.33c	0.13b	0.21b	0.26b
AgroNet 0.9T	0.34c	0.27b	0.36c	0.83c	0.30c	0.21c	0.26bc	0.26bc	0.49b	0.44c	0.19b	0.21b	0.11b
AgroNet 0.9NT	1.09b	0.61b	0.99b	2.31b	1.13b	1.00b	0.71b	0.47b	0.94b	1.05b	0.43b	0.34b	0.41b
AgroNet 0.4NT	0.19c	0.21b	0.18c	0.19c	0.23c	0.17c	0.17c	0.24c	0.37b	0.36c	0.12b	0.23b	0.04b
	Season 2												
Untreated control[a]	2.80a	3.48a	3.61a	4.71a	8.87a	9.41a	8.86a	8.66a	6.61a	7.84a	6.07a	5.24a	6.69a
Treated control[b]	0.59c	0.67c	0.74c	0.71c	0.80c	0.74bc	0.76b	0.66c	0.73b	0.51c	0.39c	0.37bc	0.43b
AgroNet 0.9T	0.30d	0.41d	0.36d	0.49c	0.46d	0.49cd	0.40c	0.40d	0.49c	0.29d	0.21d	0.20c	0.21b
AgroNet 0.9NT	1.33b	1.39b	1.53b	1.73c	1.43b	0.91b	0.91b	0.86b	0.84b	0.97b	0.56b	0.47b	0.51b
AgroNet 0.4NT	0.18d	0.28d	0.30d	0.51c	0.43d	0.28d	0.33c	0.39d	0.34c	0.26d	0.11d	0.18c	0.12c

[a] Untreated control had no pesticide applied.

[b] Treated control was sprayed with alphacypermethrin-based insecticides on a weekly basis; Means followed by the same letter within a column and a season are not significantly different according to Tukey's HSD at $P \leq 0.05$.

mortality rates observed with nontreated netting (≈20% after 24 h) could be because of the mixed age of whiteflies used for the tests, desiccation of insects, and possible injury when whiteflies pushed through netting parts with irregular smaller pore. The toxic effect of the treated netting enhanced the protection provided by both the repellent and physical barrier effects. The repellent as well as the toxic effects were observed despite the presence of a 4- to 40-fold pyrethroid resistance condition of the laboratory MPL strain used in this study and probably also in populations from Kenya as the invasive and pyrethroid-resistant *Q* biotype has been recorded in East Africa (De Barro et al. 2011) and the local biotype ASL was showed to be pyrethroid-resistant in West Africa (Houndété et al. 2010). The treated netting AgroNet 0.9T could possibly be even more effective against susceptible populations of *B. tabaci*. It could also be less effective against populations of *B. tabaci* with higher resistance levels such as that observed in many part of the world (Kranthi et al. 2002, El Kady and Devine 2003, Roditakis et al. 2005, Ma et al. 2007, Wang et al. 2010, Houndété et al. 2010). For example, a reduced efficacy of lambdacyhalothrin (pyrethroid)-treated mosquito nets against pyrethroid-resistant strains of the mosquito *Anopheles gambiae* Giles has already been observed (N'Guessan et al. 2007). Of interest, the deterrent effect of *A. gambiae* in treated huts (fitted with treated mosquito net inside or sprayed with insecticide) observed in the area with resistant mosquitoes was stronger than the one observed with susceptible ones. This result raises the issue of mechanisms for repellence and toxicity that could be driven by different genes. Other products, such as natural or synthetic repellents, could also be used for treatment of agricultural netting instead of pyrethroids (Zhang et al. 2004) with lower risk for plant and soil contamination as well as insecticide residues in food.

The repellent effect of the AgroNet 0.9T was also observed with the parasitoid *E. formosa*. But unlike the whiteflies in the choice test, it appeared that *E. formosa* responded in the same manner to treated and nontreated netting. *E. formosa* apparently showed a learning behavior in approaching the netting. After first contact with treated sample, *E. formosa* perceived both samples (treated and nontreated) as repellent. The absence of significant mortality of *E. formosa* observed in the presence of treated netting AgroNet 0.9T confirmed the repellent effect of pyrethroid at sublethal dose and suggested a learning behavior. This result also suggested that *E. formosa* released under a tunnel or a greenhouse protected with a repellent treated net would stay inside and not be killed by the product, thus improving protection of the crop through biological control (van Lenteren et al. 1996).

In conclusion, the alphacypermethrin-treated netting AgroNet 0.9T showed a repellent effect against *B. tabaci* compared with the nontreated AgroNet 0.9NT. However, the physical barrier of the AgroNet 0.9NT was already very effective to protect tomato plants in our field bioassays as *B. tabaci* population stayed at low level on covered tomato plants. By treating netting with contact repellent products it should be possible to use nets with bigger mesh size than recommended for whitefly protection. In warm climate, netting with bigger mesh provides better ventilation to the crop and is cheaper to produce than netting with smaller mesh size. Indeed, the use of netting with fine mesh may induce a significant rise in temperature and relative humidity (Bartzanas et al. 2009). On the contrary, in cold climate, the AgroNet 0.4NT improved the environmental conditions on covered tomatoes as compared with the AgroNet 0.9NT (Saidi et al. 2013). Treated netting with contact repellent properties can be used to protect plastic tunnel apertures or greenhouses. As survival of biocontrol auxiliaries was observed in our study, the use of repellent-treated netting would offer an attractive solution in the context of integrated pest management. These netting materials would be able to combine visual, physical, and chemical protection against major vector pests, such as whiteflies, while allowing effective control by auxiliaries and giving the possibility to create favorable microclimatic conditions under the protective netting (Gogo et al. 2012, Muleke et al. 2013).

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A Repellent Net as a New Technology to Protect Cabbage Crops

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ABSTRACT Floating row covers or insect-proof nets with fine mesh are effective at protecting vegetable crops against aphids but negatively impact plant health, especially under warm conditions. Furthermore, in control of cabbage insect pests, aphid parasitoids cannot enter the fine-mesh nets, leading to frequent aphid outbreaks. To surmount these difficulties, a 40-mesh-size repellent net treated with alphacypermethrin was studied in laboratory and field tests. Results showed both irritant and repellent effects of the alphacypermethrin-treated net on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and its parasitoid *Aphidius colemani* (Haliday) (Hymenoptera: Braconidae). Under field conditions, there were no pests on cabbage protected with the repellent net. The repellent net allowed combining a visual and repellent barrier against aphids. Because of this additive effect, repellent nets allowed covering cabbage permanently with adequate protection against all pests.

KEY WORDS repellent netting, alphacypermethrin, *Myzus persicae*, *Aphidius colemani*, cabbage

Floating row cover or insect-proof nets with very fine mesh are effective at protecting vegetable crops against aphids, but under warm conditions, they may injure crops by increasing air temperature beyond optimal levels. Insect-proof nets reduce the use of insecticide treatments and may constitute a better protection than chemical sprays, particularly against plant pathogen vectors, as they do not affect populations of beneficial insects (Weintraub and Berlinger 2004, Weintraub 2009). Efficacy of physical protection increases as mesh size reduces; unfortunately, air ventilation also reduces in response to reduced mesh size (Fatnassi et al. 2002). In tropical areas, Martin et al. (2006) showed that the use of a mosquito net with 25-mesh size protected cabbage effectively against the lepidopteran pests *Plutella xylostella* (L.) and *Hellula undalis* (F.) (Lepidoptera: Pyralididae). The study also showed that opening the nets during the day improved the protection of cabbage against pests, particularly the aphid *Lipaphis erysimi* (Kaltenbach), probably because of the regulatory effect of beneficial insects. These nets could also delay the outbreak of aphids, as shown by Martin et al. (2006), confirming the potential efficacy of a visual barrier against insect pest (Summers et al. 2004). However, under permanent nets, populations of aphids increase rapidly because the net is also a physical and visual barrier against predators and parasitoids. Another field study in a farmer's field in Benin showed the effectiveness of a long-lasting insecticidal-treated mosquito net (Permanet 2.0, Vestergard Frandsen, Lausanne, Switzerland) treated with a pyrethroid on a cabbage nursery against the aphid *L. erysimi* compared with a nontreated net and a nonprotected control (Licciardi et al. 2008).

The objective of this study was to test the concept of a net treated with alphacypermethrin as a repellent device for protection of cabbage against the Lepidoptera *P. xylostella* and the aphids *Brevicoryne Brassicae* (L.), *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and *L. erysimi*, both in the laboratory and the field for *M. persicae* and *L. erysimi*. The alphacypermethrin-treated net was compared with two nontreated nets with lower or similar mesh size. In addition, the impact of these nets on aphid parasitism was also investigated.

Materials and Methods

In the laboratory, we assessed the irritant, repellent, and toxicological effects of the AgroNet 0.9α alphacypermethrin-treated net on a field population of the aphid *M. persicae* and its parasitic wasp *Aphidius colemani* (Haliday) (Hymenoptera: Braconidae). Two nontreated nets (AgroNet 0.9NT and AgroNet 0.4NT), with similar and smaller mesh size, respectively, were used for comparison. In a field test, we measured the effectiveness of these nets at protecting cabbage plots covered permanently against natural pest populations of a generalist feeding aphid *M. persicae*.

Insects. An aphid population of *M. persicae* collected in 2011 on nontreated cabbage in Montferrier, France,

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was used in all experiments. These aphids were bred on fava beans (*Vicia faba* L. variety *faba*) provided by Gautier, France, in plastic boxes in climatic chambers (Binder KBWF 240, a photoperiod of 16:8 [L:D] h, 24°C, 75% relative humidity [RH]). Field-collected aphids of *L. erysimi* were also used for leaf-dip bioassay. The aphid parasitoid *A. colemani*, provided by Koppert, France, was used in cage experiments.

Nets. A to Z Textile Mills Ltd., Arusha, Tanzania, provided the AgroNets in knitted polyethylene. The AgroNet 0.9α with 0.9 mm pore diameter and 40-mesh size was treated with alphacypermethrin at 2% (w:w), according to the Olyset process patented by Sumitomo Chemical Company. In the treated net, 98% of the active ingredient is bound to the polyethylene fiber and just 2% of the active ingredient should be bioavailable. Two nontreated nets were used for comparison—AgroNet 0.9NT with the same mesh size (40-mesh) and 0.9 mm pore diameter and AgroNet 0.4NT with smaller mesh size (80-mesh) and 0.4 mm pore diameter.

Bioassay. The toxic effect of alphacypermethrin-treated net was evaluated on winged *M. persicae* and *L. erysimi* adults from field populations. Modified Munger cells (Munger 1942) were used to maintain aphids in contact with the net. Mortality was observed 24 h later. Ten winged aphids were placed on a piece of treated or nontreated net in Munger cells with five replications.

The response to pyrethroid insecticide was tested by a leaf-dip bioassay (Sawicki and Rice 1978). The test was performed with fava beans leaves dipped in various solutions of cypermethrin 25% EC provided by Arysta LifeScience (Nogueres, France). Cypermethrin (mixed isomers) was preferred to alphacypermethrin to compare results with other toxicological studies. The leaves were dipped for 10 s in five aqueous solutions of insecticide formulation at 0.01, 0.1, 1, 10, and 100 g/l and distilled water for the control. Leaves were air dried for 20 min at room temperature. The leaves were then placed on an agar-coated (7 g/l) petri dish (55 mm diameter). Ten winged aphid adults were placed onto the treated leaves and maintained at 24°C, 50% RH, and a photoperiod of 12:12 (L:D) h. Mortality was recorded 48 h later. Three replicates were carried out for each concentration of insecticide and untreated control. Mortality in the control was always lower than 10%, and data from all bioassays were corrected for control mortality by using Abbott's formula (Abbott 1925).

Video Tracking. The video tracking technique was used to evaluate the effect of the alphacypermethrin-treated net on winged *M. persicae* adults. The behavior of *M. persicae* was evaluated simultaneously on a piece of alphacypermethrin-treated net and a piece of nontreated net. The test arena was a white cardboard (10 by 10 cm) with 1 mm thickness. Two separate 2 by 2-cm squares, positioned 1 cm apart, were cut in the cardboard. The bottom of each square was covered by a piece of netting (2.5 by 2.5 cm), treated and untreated, and stapled to the cardboard. For each of the 20 replications, one adult *M. persicae* was placed on each square. A Plexiglas plate covered the cardboard to prevent insect escape. The speed, mobility (S >0.2 mm/s), and distance traveled were recorded during the 15-min video tracking.

The repellent effect of the alphacypermethrin-treated net on *M. persicae* was evaluated with the same material but with only one square (3 by 3 cm) in the cardboard. Half of the square (1.5 by 3 cm) was covered by a piece of nontreated net and the other half by a piece of the alphacypermethrin-treated net. Both nets were stapled to the cardboard. For each test of the 40 replications, one *M. persicae* adult was placed in the middle of the square. A Plexiglas plate covered the cardboard to prevent insect escape. The speed, mobility (S >0.2 mm/s), distance traveled, and exposure time on each area were recorded during the 15-min video tracking.

A closed-circuit video camera giving black and white images (Ikegami digital video camera ICD-49E, Lens Std IR 4.5–12.5 mm) was suspended 15 cm above the center of the test arena. An image analyzer (EthoVision XT 7.0 from Noldus, Wageningen, The Netherlands) acquired input from the video camera and converted the analog signal to digital data. The resolution was 768 by 576 pixels, and the acquisition and processing speed was 25 ± 1 frames per second. The presence or absence of *M. persicae* in each arena, or part of the arena, was determined by visual contrast between the insect (dark) and arena background (white), scored as the number of pixels. The data were analyzed by using EthoVision XT 7.0 from Noldus software, which recorded the movement of multiple objects in multiple zones. Each set of data were exported to and handled with Minitab.

Cage Assay. Three rectangular cages (30 by 30 by 50 cm) were fastened one to the other from the smaller side. They were separated by AgroNet 0.9α and AgroNet 0.9NT. The cages were covered on each side by a net with very fine mesh through which the aphids could not cross. A young cabbage plant was placed in each cage. The middle cage was covered by cardboard (dark cage). In total, 50–100 *M. persicae* winged adults (mixed age and sex) were placed on a cabbage plant in the central dark cage. The number of aphids that crossed the treated or the nontreated nets were counted in each cage after 24 h and collected with a mouth aspirator. The assay was replicated 10 times with a rotation of 180° of the apparatus after 24 h.

With the same material, 500 "mummies" containing *A. colemani* (mixed sex) were placed in the central dark cage. After emergence that occurred during 5 d, *A. colemani* were attracted by light in the side cages. The *A. colemani* that crossed the treated or the nontreated nets were daily collected in each cage with a mouth aspirator and counted during 5 d. The device was rotated by 180 degrees every day after observation.

Field Experiment. Cabbage seeds (*Brassica oleracea* variety *capitata*) from Vilmorin, France, were sown in cell trays placed in the greenhouse. Cabbage plants with four to five leaves were planted-out in 18 plots of 3 m^2 (1.2 by 2.5 m) in a randomized complete block design with four replicates. Weeds were re-

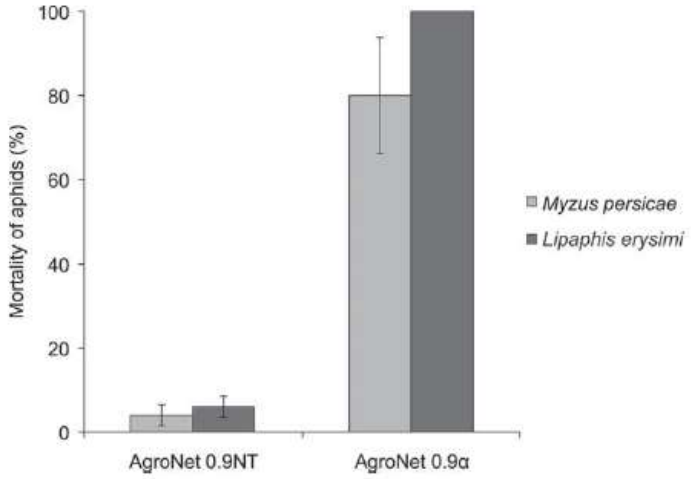

Fig. 1. Contact toxicity of the alphacypermethrin-treated net (AgroNet 0.9α) on winged aphids. Aphids from *Myzus persicae* and *Lipaphis erysimi* were compared with the nontreated net (AgroNet 0.9NT). Percentage of mortality with 95% CIs.

moved before transplanting, and soil was covered with green plastic mulch. Plots were three rows of seven plants each (21 plants per plot) produced in a climatic room (25°C, 70% RH, a photoperiod of 12:12 [L:D] h). Treatments included nontreated nets (AgroNet 0.4NT and AgroNet 0.9NT), the alphacypermethrin-treated net (AgroNet 0.9α), and the nonprotected control. The cabbage plots were covered immediately after transplant. In protected plots, the nets covered the cabbage permanently by using a light iron frame at 30 cm above the crop. Drip irrigation was applied twice a day at 0800 and 1800 hours for 5 min. The number of insects such as the Lepidoptera *P. xylostella*, the gray aphid *B. brassicae*, and the green aphids gathering *M. persicae* and *L. erysimi* was recorded weekly on the leaves of all cabbage plants. To reduce the risk of contamination, as the nets were opened for counting the insects under the leaves, we observed the plots treatment-after-treatment as follows: AgroNet 0.9α, AgroNet 0.4NT, AgroNet 0.9NT, control. The number of aphid mummies was recorded to estimate the rate of parasitized aphids. At harvest, cabbage heads were separated into marketable and nonmarketable.

Statistical Analysis. A Kolmogorov–Smirnov test was used as normality test to determine whether the data set was well modeled by a normal distribution. For analysis of laboratory test data, a paired *t*-test was used for choice assay and an unpaired *t*-test to compare no-choice assays. CIs (95%) were calculated for comparing proportions in toxicity tests. For field data analysis, analysis of variance (ANOVA) was used on log $(x + 1)$-transformed data, where x was the mean number of cumulative pests observed over the 10 wk. Fisher and Mann–Whitney tests were used for comparison of means with 0.05 error.

Results

Toxic Effect. Contact toxicity test in Munger cells (Fig. 1) showed a significant toxicity ($P = 0.047$) of the alphacypermethrin-treated net (80% mortality) on winged *M. persicae* and *L. erysimi* compared with the nontreated net (10% mortality).

The leaf-dip assays results showed the highest susceptibility of *L. erysimi* to cypermethrin compared with *M. persicae* (Fig. 2). At the lower dose tested (0.01 g/l), there was 100% mortality for *L. erysimi* compared with only 8% mortality for *M. persicae*. At 1 g/l of cypermethrin, the mortality of *M. persicae* increased to 88%. There was no mortality in the control.

Irritating Effect. The treated net appeared to have an exciting effect on the behavior of *M. persicae* winged adults. Indeed, *M. persicae* had significantly more mobility on the treated net than on the nontreated net (Table 1). They also had a higher average speed and traveled a longer distance on the treated net.

Repellent Effect. The treated net also appeared to have a repellent effect on the behavior of *M. persicae* winged adults. Indeed, when *M. persicae* had a choice between walking on a treated or a nontreated net, they significantly spent more time on nontreated than on treated net (Table 2). Moreover, *M. persicae* walked a

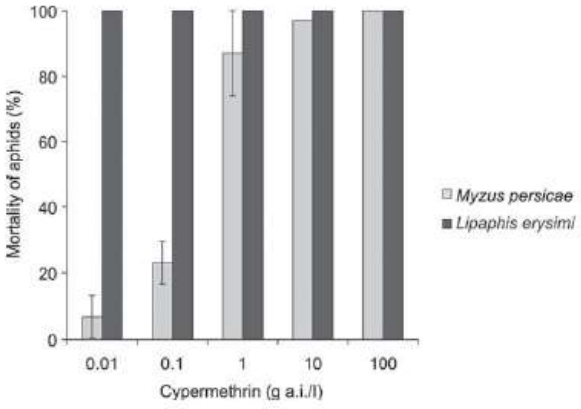

Fig. 2. Contact toxicity of cypermethrin on winged *Myzus persicae* and *Lipaphis erysimi* from field populations. Percentage of mortality with 95% CIs.

Table 1. In a no-choice test, average behavior of 20 winged *Myzus persicae* placed one by one for 15 min on a piece of alphacypermethrin-treated net (AgroNet 0.9α), compared with a nontreated net (AgroNet 0.9 NT)

Nets	N	Mobility (s ± SE)	Distance (mm ± SE)	Speed (mm/s ± SE)
AgroNet 0.9α	20	517 (±27)a[a]	245 (±20)a	0.32 (±0.03)a
AgroNet 0.9NT	20	342 (±41)b	172 (±16)b	0.22 (±0.02)b
P-value*		0.0005	0.008	0.0008

[a] Different letters represent statistically significant differences at 0.01.

* Statistical analysis with Student *t*-test for normal data (distance and speed) and Mann–Whitney test for not normal data (mobility).

longer distance on the nontreated net. There was no significant difference on the average speed between treated and nontreated nets. The crossing rate of *M. persicae* and *A. colemani* through the treated or nontreated nets was studied in a large cage assay where winged *M. persicae* were forced to go through a treated or a nontreated net when attracted by light and cabbage plants. Results showed a significantly lower crossing rate of winged aphids through the treated net than the nontreated net (Table 3). There was no mortality of aphids after 24 h. The repellent effect of the treated net for the parasitoid *A. colemani* was not really apparent with the choice cage assay. The lower crossing rate of *A. colemani* through the treated net than the nontreated net was significant in the cage assay but with a 10% *P* value (Table 3). After 24 h, the mortality of *A. colemani* that crossed the nets varied from 13.5 to 18.7% for AgroNet 0.9NT and AgroNet 0.9α, respectively, but the difference was not significant.

Field Assay. The effectiveness of the alphacypermethrin-treated net to protect cabbage plots against natural pest populations was compared with the nontreated nets. Nonprotected plots were used for control. There was a low infestation of the diamondback moth *P. xylostella*. Actually, *P. xylostella* larvae were observed only on nonprotected cabbage at all sampling dates, indicating an excellent physical protection by the nets. No *P. xylostella* were observed on cabbage protected with AgroNet 0.9α and AgroNet 0.9NT (Table 4). In addition, few *P. xylostella* were observed on cabbage protected by the AgroNet 0.4NT. Variance analysis on cumulated data showed a significant difference between all nets and the control.

Table 2. In a choice test, average behavior of 34 winged *Myzus persicae* placed one by one for 15 min in the middle of two joined pieces of net: one alphacypermethrin-treated net (AgroNet 0.9α) and one nontreated net (AgroNet 0.9NT)

Nets	N	Time (s ± SE)	Speed (mm/s ± SE)	Distance (mm ± SE)
AgroNet 0.9α	34	338 (±49)a[a]	0.37 (±0.04)	104 (±14)a
AgroNet 0.9NT	34	562 (±49)b	0.33 (±0.03)	139 (±15)b
P value*		0.011	0.252	0.029

[a] Different letters represent statistically significant differences at 0.05.

* Statistical analysis with Mann–Whitney test.

Table 3. Crossing rate of winged *Myzus persicae* and *Aphidius colemani* through a treated net (AgroNet 0.9α) or a nontreated net (AgroNet 0.9NT) in choice assays with cages after 24 h

Nets	N	*M. persicae* (n ± SE)	*A. colemani* (n ± SE)
AgroNet 0.9α	500	6.00% (±0.09)a	0.88% (±0.07)
AgroNet 0.9NT	500	16.00% (±0.27)b	1.40% (±0.11)
P value*		0.017	0.098

* Different letters represent statistically significant differences with paired *t*-test at 0.05.

The aphid *B. brassicae* was observed mainly on cabbage in nonprotected plots (control). The infestation of *B. brassicae* began 2 wk after planting out and increased to a maximum of 300 aphids per 21 cabbages, observed the first of July. Then the infestation decreased gradually until harvest. The differences of aphid numbers between no net in the control and the three AgroNets were statistically significant (Table 4). Few *B. brassicae* (0.2/plant) were observed on cabbage protected by the AgroNet 0.9NT. However, no *B. brassicae* was observed on cabbage under the AgroNet 0.9α or AgroNet 0.4NT.

The green aphids, *M. persicae* and *L. erysimi*, were observed 2 wk after planting on the nonprotected cabbage and on cabbage protected with the nontreated nets (Fig. 3). No green aphid was observed on cabbage protected with the treated net. The outbreak increased on cabbage under the nontreated nets compared with the nonprotected cabbage. In addition, as the net mesh size increased, so did the aphid outbreak. The ANOVA on the cumulated data of 10 sampling dates showed a significant difference of green aphids between the treatments. From the highest to the lowest green aphid infestations, the treatments were ranked as follows: AgroNet 0.9NT, AgroNet 0.4NT, nonprotected control, and AgroNet 0.9α (Table 4). The outbreak of green aphids disappeared 2 wk before harvest in all treatments.

Parasitized aphids observed as aphid mummies were mainly observed on nonprotected cabbage (control), suggesting an exclusion of beneficial insects by nets, especially with finer mesh (Table 4). The maximum proportion of parasitized aphids (mummies) was 25.6%, observed at the peak of aphid outbreak. A few parasitized aphids were also observed on cabbage protected by the nontreated larger-mesh-size net, with a maximum of 4.6% aphid mummies. Practically no parasitized aphids were observed on cabbage protected by the nontreated lower-size mesh. As there was no aphid on cabbage protected by AgroNet 0.9α, there were no aphid mummies. The statistical analysis on the mean of parasitized aphids showed a higher parasitism with the control than with the nets (Table 4). The average of parasitized aphids was also significantly higher with the AgroNet 0.9NT than with the AgroNet 0.4NT.

Cabbage were harvested per plot, counted, and weighted individually. The low yield in all treatments was because of small snails coming from under the plastic mulch, which damaged the plants in all plots 7 d

Table 4. Cumulative data of *Plutella xylostella* larva, *Brevicoryne brassicae*, green aphids (gathering *Myzus persicae* and *Lipaphis erysimi*) and mean rate of parasitized aphids over the 10 sampling dates on cabbage covered permanently with an alphacypermethrin-treated net (AgroNet 0.9α) or nontreated nets (AgroNet 0.9NT and AgroNet 0.4NT) in comparison with a nonprotected control

Treatments	*P. xylostella* N/plant (±SE)	*B. brassicae* N/plant (±SE)	Green aphids (N/plant ± SE)	Parasitized aphids (% ± SE)	Marketable cabbage[a] (% ± SE)
Control	1.04 (0.38)a[b]	49.3 (9.53)a	19.8 (12.8)b	8.42 (1.47)a	14.3 (11.3)a
AgroNet 0.4NT	0.02 (0.02)b	0.0b	55.4 (17.7)ab	0.02 (0.01)c	33.3 (6.4)ab
AgroNet 0.9NT	0.0b	0.18 (0.05)b	114.8 (59.4)a	1.35 (0.34)b	27.4 (9.0)ab
AgroNet 0.9α	0.0b	0.0b	0.0c	0.0c	56.0 (6.3)b
F	4.12	15.25	3.46	9.84	4.09
P value	0.03	<0.001	0.05	0.001	0.03

[a] Percentage of marketable cabbage at harvest time.
[b] Different letters represent statistically significant differences.

after planting out. The ANOVA on the percentage of marketable cabbage showed a significantly higher yield with the treated net compared with the control (Table 4). This result could be explained by the highest infestation of *P. xylostella* and *B. brassicae* in the control. There was no significant difference of yield among the nets.

Discussion

The AgroNet 0.9α net was effective in protecting cabbage against all pests and particularly against the green aphids *M. persicae* and *L. erysimi*, which easily cross the net when it is untreated. Like its nontreated counterpart, the AgroNet 0.9α was also effective against the much bigger aphid *B. brassicae* and the lepidopteran *P. xylostella*, working as a visual barrier but mainly as a physical barrier (Martin et al. 2006, Licciardi et al. 2008). Actually, the AgroNet 0.9α added three effects: a visual barrier, a physical barrier, and a chemical barrier preventing the aphids *M. persicae* and *L. erysimi* from passing through the net. This result confirmed preliminary findings by Martin et al. (2006) showing the effectiveness of a pyrethroid-treated net with larger mesh in protecting a cabbage nursery against *L. erysimi* in Benin. The repellent effect of AgroNet 0.9α was demonstrated with a video tracking system in a choice situation where *M. persicae* spent 1.7-fold more time on the nontreated net than on the treated one. It was also demonstrated with a cage assay, as the rate of crossing through the nontreated net was twofold higher than through the treated net. The repellent effect of AgroNet 0.9α is because of the well-documented neurotoxicological effects of pyrethroids (Vijverberg and Bercken 1990). Pyrethroids have a common mechanism of action on the voltage-gated membrane sodium channel (Chinn and Narahashi 1986). Their toxicity is dominated by acute pharmacological actions on excitability originating from this common mechanism (Ray 2001). However, cypermethrin was also found to effectively suppress the open state of voltage-gated chloride channels (Forshaw et al. 1993, Ray et al. 1997). All pyrethroids induce repetitive activity in sense organs depending on specific pyrethroid structure (Vijverberg and Bercken 1990). However, repetitive activity of cypermethrin occurs on motor nerve endings and muscle fibers.

In the field test, the AgroNet 0.9α showed a repellent effect on *M. persicae* and undoubtedly on *L. erysimi*, preventing theses aphids from crossing the net. After 24 h contact with AgroNet 0.9α, lower toxicity of the treated net was observed on *M. persicae* compared with *L. erysimi*, with 85 and 100% mortality,

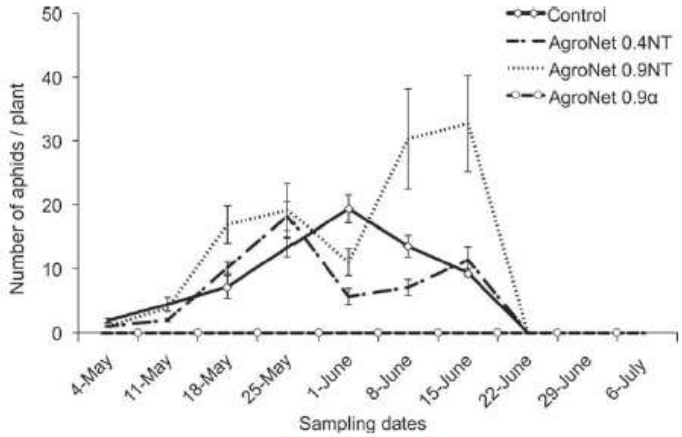

Fig. 3. Dynamic of aphid infestation on cabbage. Number of aphids (±SE) of *Brevicoryne brassicae*, *Myzus persicae*, and *Lipaphis erysimi* observed on 21 cabbages covered permanently with an alphacypermethrin-treated net (AgroNet 0.9α) or a nontreated net (AgroNet 0.9NT or AgroNet 0.4NT) in comparison with a nonprotected control.

respectively. The pyrethroid resistance level of the field-collected *M. persicae* population explains this (Guillemaud et al. 2003). Indeed, our results showed that this field population was ≈100-fold more resistant to cypermethrin than the susceptible strain of *M. persicae* named US1L (LC_{50} = 2.9 mg/l), as shown by Barber et al. (1999). In comparison, *L. erysimi* should be susceptible to cypermethrin with an LC_{50} <10 mg/l. The *kdr* mutation confers resistance to pyrethroid insecticides in *M. persicae* (Martinez–Torres et al. 1999). For *M. persicae*, esterase overproduction confers a low level of resistance to pyrethroid insecticides, but the combination of both resistance mechanisms confers a high level of resistance to this class of insecticide. In French sexually reproducing populations of *M. persicae*, *kdr* resistance mutations were present at high frequencies, with more than half of the aphids studied being *kdr*/*kds* and one-third *kdr*/*kdr* (Guillemaud et al. 2003). However, the excitorepellent effect of pyrethroids, and especially alphacypermethrin, was still working on *M. persicae*, despite the pyrethroid resistance. But this effect should depend on the resistance level (resistance ratio) and on the resistance mechanism involved in insect field populations. The AgroNet 0.9α showed a repellent effect more than a killing effect. Desneux et al. (2004) emphasized that the sublethal effect of pyrethroids could be temporary, and useful insects such as parasitoids could recover after a period without exposure. Rieth and Levin (1988) showed that the repellent effect of cypermethrin used at a sublethal dose on *Apis mellifera* (L.) should be reversible after 24 h without any side effect. However, several authors report increases in mobility of natural enemies in contact with pyrethroid (Rieth and Levin 1988, Salerno et al. 2002, Desneux et al. 2004). The video tracking system demonstrated the irritating effect of the AgroNet 0.9α in a no-choice assay. The mobility and average speed of winged *M. persicae* only in contact with the AgroNet 0.9α were 1.5-fold higher compared with the nontreated net. The irritant effect of pyrethroids may induce movement of the insects away from the treated area. Consequently, increased mobility could not be associated with increased parasitoid activity crossing the nets, particularly the alphacypermethrin-treated net.

In our field trial, all the nets appeared to be effective barriers for the parasitoids. Indeed, in our laboratory device, fewer *A. colemani* came through treated (0.88%) or nontreated (1.40%) AgroNets than *M. persicae*, but they were just attracted by light and not by aphids infesting plant. These results suggest that these AgroNets might be effective visual barriers for the parasitoids, confirming the low rate of parasitized aphids observed as mummies on cabbage protected with the two nontreated AgroNets. Moreover, the rate of parasitized aphids in the field trial appeared to be linked to the size of mesh. With the lower mesh size, AgroNet 0.4NT appeared as a total barrier for the parasitoids, as practically no parasitized aphids were observed on cabbage, despite the high aphid outbreak. These results confirmed the observations of Berlinger et al. (2002) about the reduction of crossing rate of useful insects through physical barriers. The ability of parasitoids to detect host-induced plant odors (synomones) is crucial because these odors are used to detect host patches at long range (Vet and Dicke 1992). However, in life-table experiments, parasitoids may be so close to the hosts that they detect their hosts without using long-range cues. This result suggests that in the field, a net with fine mesh might be also a barrier for plant odors. The low level of parasitized aphids under the nontreated AgroNets may be explained by the positive effect of opening AgroNets during the day, as shown on cabbage in Benin (Martin et al. 2006, Licciardi et al. 2008).

The landing approach of *M. persicae* in the field occurs preferentially on leaves reflecting a greater proportion of long-wave energy, with little or no regard for the host status of the plants (Kennedy et al. 1961). The color attraction of such "yellow-sensitive" aphids serves rather to bias their alignment toward plants of the appropriate physiological type. That could explain the delay of aphid infestation that was sometimes observed on cabbage protected with nontreated nets in Benin compared with a nonprotected control (Martin et al. 2006). Evolutionary contacts have created mechanisms that enable insects to detect and select their favorite plant hosts for feeding and oviposition (Raviv and Antignus 2004). Vision (color, shape, and size) and olfaction (host odor) are the primary cues used by insects to orient to their host plants, and sometimes the two cues work in concert (Jaycox et al. 1974, Jervis and Copland 1996). Insects' visual behavior is linked to a chain of events, which begins with their orientation to the plant from a distance and ends with their establishment on plants for feeding (Haynes 1988, Cole 1997). By interfering with different links along this pathway, we may prevent a contact between the pest and the plant, thereby preventing plant infestation and viral infection (Desneux et al. 2007).

Finally, the visual barrier and the chemical barrier appeared to have additive effects. Moreover, the net could reduce the diffusion of the volatile compounds produced by the plant, thus reducing the attractiveness of protected plants. We expect that more thorough consideration of potential additive effects in the future will help to optimize integrated pest management programs involving use of both physical and chemical barriers against pests in a kind of "push–pull" strategy. As shown by us in the semifield test, the AgroNet as a physical barrier alone protects cabbage against lepidopterans but may increase aphid outbreaks, as it appeared to represent a serious barrier for natural enemies. For that reason, the nontreated AgroNet should be removed sometimes during the day for natural enemies to do their job (Martin et al. 2006). Actually, the nontreated AgroNet 0.4NT has been used permanently with success in Kenya for protecting horticultural nurseries (Gogo et al. 2012, Muleke et al. in press). However, because the removal of AgroNets on larger plots is time-consuming for farmers, the use of a repellent net such as AgroNet 0.9α

could be a solution for protecting a cabbage crop permanently in combination with plastic mulch and drip irrigation. Moreover, as aphids are known to be major vectors of plant viruses (Ng and Perry 2004, Katis et al. 2007), a repellent net suppressing contact between the vector and host plant should reduce significantly the impact of viral diseases in vegetable crop production. Nevertheless, nontoxic and sustainable alternatives to pyrethroids should be pursued for at least two reasons: 1) the observed pyrethroid resistance in *M. persicae* (Devonshire 1989, Field et al. 1997, Field and Foster 2002) and in most vegetable sucking pests, such as *Bemisia tabaci* (Hemiptera: Aleyrodidae), another plant virus vector (Alon et al. 2006, Houndete et al. 2010); and 2) the risk of pesticide residues in rainwater-soaked plants and in soil, despite the use of a long-lasting treated net (Msangi et al. 2008, Kayedi et al. 2008, Dev et al. 2010). Because the repellent AgroNet is easy to use on small vegetable plots, protecting them against lepidopterans, aphids, and other pests such as whiteflies and thrips, they could be an eco-friendly alternative to insecticide spray, particularly for small-scale farmers in tropical countries to protect their vegetables in urban and peri-urban areas (Ahouangninou et al. 2012).

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Evaluation of acaricide-treated string curtains for control of two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) on greenhouse roses and impact of the string curtain on the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae)

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ABSTRACT

Foliar sprays of pesticides are frequently used to control two-spotted mites on rose flowers, but these also destroy predatory mites and pose a high risk of contamination for humans and the environment. Using a novel approach to avoid spray applications on rose plants, modified acaricide-treated string curtains were adapted to control the pest. Two main aims of this study were: (i) to identify the lethal concentration of string curtains treated with propargite, dicofol, flufenoxuron, acrinathrin or tau-fluvalinate (τ-fluvalinate) on *Tetranychus urticae*, and (ii) to test the design (feasibility) and efficiency of the string curtains to trap the phytophagous mites on the plants during their circadian migration. Bioassay results in the lab confirmed *T. urticae* circadian migration, toxicity of the five kinds of treated string curtains and a concentration-dependent repellent effect of each chemical on *T. urticae* females. Of the two products tested in the field, dicofol was more effective than acrinathrin in controlling *T. urticae* compared to an acaricidal spray of the same products. The effect of acaricide-treated string curtain use on *Phytoseiulus persimilis* did not differ significantly from the control. The string curtain technique proved to be effective, but because of some biotic and abiotic constraints and the low economic threshold of mites, even for the commercialized high canopy stem roses, the present device could be more appropriate for another horticultural crop, e.g. tomato.

1. Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a phytophagous mite recognized as one of the major pests on roses (Reid, 2008). In greenhouse horticulture, the intensive use of chemicals, i.e. up to 20 or more miticide sprays annually, has led to the selection of resistant populations (Nicetic et al., 2001; Van Leeuwen et al., 2010). The rapid population growth, short developmental time, high birth rate and long adult survival lead to a high risk of outbreaks (Zhang, 2003) and coupled with male haploidy, which exposes recessive resistance genes to selection, result in to a high rate of development of resistance to acaricides. Today, increasing numbers of rose growers are using Integrated Pest Management (IPM) programs to control the two-spotted spider mite. They release predators like *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) regularly but still need chemicals (Blindeman and Van Labeke, 2003). For marketing reasons, the tolerance level against *T. urticae* is close to zero because mites feed on cell chloroplasts by pumping their contents up to the surface causing the leaves to develop unaesthetic-looking whitish or yellowish stippling (Zhang, 2003). The foliar sprays containing pesticides that are frequently used to fight two-spotted mites particularly on flowers also destroy predatory mites and pose a high risk of contamination with possible adverse effect for human health and the environment through residues on the flowers and water pollution. To reduce the use of chemicals and their negative impacts, a new technique entailing covering of plants with a dicofol-impregnated net was used against the broad mite *Polyphagotarsonemus latus* (Ewing) on *Solanum macrocarpon* (Martin et al., 2010). These nets were used only at night, three times a

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week, to prevent or control *P. latus* outbreaks. The net killed mites by contact when they walked on it. Pralavorio et al. (1989) showed that *T. urticae* migrates to the top of the French dwarf bean between 10 a.m. and 2 p.m. Clotuche et al. (2011) reported that this daily migratory behaviour was a strategy to allow the mites to be dispersed by the wind or by animals. Martin et al. (2010) reported that the mites could be intoxicated by miticide sprayed on nets during their migration and exploration. The major advantage of using acaricidal-treated nets was to reduce the risk of residues on the plant and in the soil. That technique was also reported to be safer for the user than chemical sprays. It is an improved integrated pest management (IPM) strategy due to the absence of chemical residues on the plant using a combination of treated nets with predator releases. IPM is problematic in roses because the economic threshold for two-spotted mites is very low for marketing reasons. Currently, the release of predatory mites needs to be alternated with chemical sprays even though these have a negative impact on predators (Malézieux et al., 1992). The economic threshold for the low canopy rose is lower than for the commercialized stem (high canopy) rose; consequently, the low canopy rose could be protected by predatory mites (Zhang, 2003) and a treated net could protect the commercialized stem rose. However, the design of the treated net described by Martin et al. (2010) was not really suitable for the upright shape and remontant flowers of the commercialized stem rose.

In the current study we sought to find a better design for the treated net and test it on rose, an important commercial ornamental. First we needed to confirm the migratory behaviour of *T. urticae* mites and then to evaluate the efficacy of a string curtain impregnated with various chemicals. The toxic and repellent effect of five chemical compounds was evaluated on two-spotted spider mites in the laboratory. The efficiency of two treated string curtains in protecting roses was evaluated in a producer's greenhouse in Kenya. The potentially negative impact of the string curtain on predatory mites was evaluated concurrently.

2. Material and methods

2.1. Mites

The reference strain of the two-spotted spider mite, *T. urticae*, was bred on French dwarf bean *Phaseolus vulgaris* in the laboratory of SupAgro, Montpellier, France, at 28 ± 1.5 °C, 70 ± 15% R.H. and 16L: 8D photoperiod.

2.2. Products

The acaricides used in the experiments were commercial formulations of propargite (Omite 30W®, 30 g kg^{-1}), dicofol (Kelthane®, 50WSP, 50 g kg^{-1}), flufenoxuron (Cascade®, DC, 10 g kg^{-1}), acrinathrine (Orytis®, EW, 7.5 g kg^{-1}) and tau-fluvalinate (Klartan®, 240 g L^{-1}). All are contact acaricides efficient against motile stages except flufenoxuron, which acts on eggs (Tomlin, 2003).

During the field trial other pesticides were used to fight against other pests: tri-siloxilane (Silwet®, 995 g kg^{-1}), or acetamiprid (Golan®, SP, 20 g kg^{-1}) against *Planococcus citri* (Risso); oil surfactant (Cropgold®) against *T. urticae* and *P. citri* (Risso); Mg SO_4, or Octylammonium methanearsonate/Dodecyl ammonium methanearsonate (Methatox®, 8 g kg^{-1}; 8 g kg^{-1}) against *Sphaerotheca pannosa* (Wallr.); Abamectin (Dynamec®, EC, 1.8 g kg^{-1}) or Cymoxanil (Selva®, 30 g L^{-1}) against *Thrips* spp.; and Bt insecticides (Dipel®) against *Duponchelia fovealis* (Zeller) and *Helicoverpa armigera* (Hubner).

2.3. Materials

String curtains in polyethylene were manufactured and provided by A to Z Textile Mills Ltd Arusha, Tanzania. Each string curtain was impregnated with a chemical binder, according to manufacturer protocols, and measured 1.5 × 5 m with 500 strings of 1.5 mm and weighed 500 g. For the bioassays only 1 g of string was cut and impregnated. For the field tests all the string curtains were manually impregnated. Each string curtain was dipped in a bowl with an acaricide solution or distilled water for the control and mixed with gloved hands for 1 min until it was thoroughly and evenly impregnated (Martin et al., 2010). The curtain was then taken out of the bowl, spread-out and put flat to dry with low ventilation under shade at ambient temperature (20 °C) for 24 h until completely dry. Holes were driven in with four 2-m bamboo sticks in the row of roses in contact with the leaves (Fig. 1).

2.4. Bioassays

The bioassay for toxicological studies was adapted from the method used by Auger et al. (2003) with *Panonychus ulmi* (Koch). For each product, samples of string (1 g) were treated with a range of 3–5 concentrations and 3 replications to select the most promising. For each selected product, to determine the LC_{50} and LC_{90} of samples of string, 1 g was treated with 6 concentrations (except for flufenoxuron with 8 concentrations tested) and 3 replications. Distilled water was used for the controls to correct the experimental values for the natural mortality (Abbott, 1925). Twenty string samples were placed next to each other in Munger (Huffaker, 1948) cells in contact with a homogeneous set of mites (30–36 adults/concentration; 10–12 females/replication). Mites were placed on strings for a duration of 1 h after which the string sample was removed. The mortality of mites was evaluated 48 h later. The mortality criterion was the inability to walk one body length when lightly prodded (Kabir et al., 1996). The moribund mites were scored as dead (Kabir et al., 1993). The concentrations that killed 50 and 90% of the population (LC_{50} and LC_{90}) were calculated using the WindDL® software from Cirad using the log-probit method of Finney (1970). A X^2 test was realized to check that the data were well represented by the probit model.

Fig. 1. Acaricide-treated string curtains for protecting roses against red spider mite in a greenhouse in Kenya (photo E. Deletre).

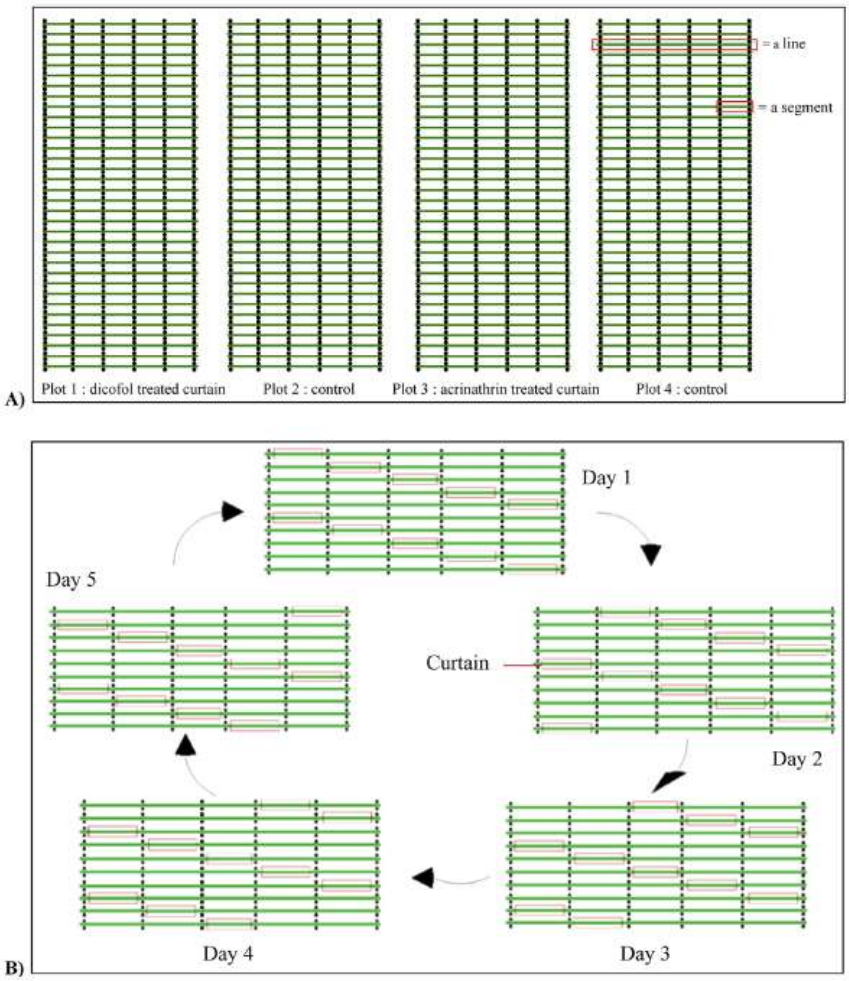


Fig. 2. Map of the greenhouse (A) with four plots: two control plots, one protected by dicofol (6 g/l) treated curtain and one protected by acrinathrin (15 g/l) treated curtain and rotation planning (B) of the treated curtains for the mite control: a curtain stayed one day on one segment of the crop and moved to the following segment the day after (a sector was protected every five days).

2.5. Behavioural study

Two tests were used to evaluate the effect of treated curtain on mite behaviour: a no-choice test and a choice test. For the no-choice test two separated squares of curtain (1.5 × 1.5 cm), one non-treated and one treated with LC_{90} of propargite, tau-fluvalinate, flufenoxuron, dicofol or acrinathrin, were fixed with glue separately in the bottom of a Petri dish. Using a hairbrush (size 00), 16 adult female mites were placed on each square under a digital camera (12.5 frames/s) with an infrared light. Ethovision® software was used to record the speed and number of moving mites ($v > 0.1$ mm/s) during 2 h (Noldus et al., 2002). A Wilcoxon–Mann–Whitney test was done with the software R 2.12.0 to compare the activity of mites on the treated and non-treated curtain samples. For the choice test, one sample of curtain (1 × 0.5 cm) was treated with the LC_{90} of propargite, tau-fluvalinate, flufenoxuron, dicofol or acrinathrin and stuck with glue side-by-side with a non-treated sample of curtain (1 × 0.5 cm) on a Petri dish. Using a hairbrush one adult female mite was placed at the centre of the device (1 × 1 cm) with 20 replications for each chemical. Ethovision® software was used to record the covered distance, speed, mobility ($v > 0.1$ mm/s) and immobility ($v < 0.09$ mm/s) time and presence on treated and non-treated part of the curtain for a duration of 15 min. A Kolmogorov–Smirnov test was done with the software R version 2.12.0 to determine whether the continuous data were normally distributed (Schüder et al., 2004). Then paired *t*-tests were used to compare mite activity on treated and non-treated curtain. The Wilcoxon signed-ranks test, a non-parametric analogue to the paired *t*-test, was used when the data did not follow a normal distribution. For checking the repellent effect (Penman et al., 1986), leaf discs (diameter 10 mm) of French dwarf bean were dipped in chemical solution (LC_{90} of propargite, tau-fluvalinate, flufenoxuron, dicofol or acrinathrin) and in distilled water for the control. Then the treated leaves were placed on a wet ball of cotton wool soaked in cold water. After 2 h, the time it took for the chemical to dry, a female adult mite was put on one leaf disc

using a thin hairbrush (50 leaf discs per chemical). After 24 h the location (leaf or cotton wool) of living or dead individuals was noted. A X^2 test was done with the software R version 2.12.0 following treatments, to see any location-dependent influence. If the variables were dependent, a linear generalized model (binomial model, logit link function) was used to determine the repellent effect. To check the migratory movement a black paper strip (1 × 20 cm) was attached to a 12 cm French dwarf bean with one leaf. Levels of infestation tested on the plant included 100, 300 or 500 mites with 3 replications. Under infrared light the Ethovision® software recorded the rise and fall of mite numbers.

2.6. Field test

The field test was carried out in a 1 ha rose (*Rosa* sp. hybrid) greenhouse of a production farm near Nairobi city in Kenya in July and August 2011. Using a rotation of treated string curtains on five days, a crop line was divided in five segments with a curtain protecting one in five segments (Fig. 2). Every day at 9.00 a.m. the curtain was moved to the following segment. The curtains were removed in the afternoon at 3 p.m. in case there was a chemical spray. In that experiment the efficiency of string curtains treated with LC_{90} of dicofol or acrinathrin on their own was compared to the farmer practice without curtain. There were 34 crop lines for each treated curtain and 68 for the control. The greenhouse was divided into four plots, including two for the control separating the two plots with treated curtains.

Actually, the treated string curtain protection was added to the usual IPM program of the producer *P. persimilis* predating mites released when the ratio of predator/pest was lower than 1/5 and a miticide sprayed when two-spotted spider mites were observed in the middle of the marketable rose stem (Fig. 3). During the experiment in the greenhouse (Day 0–21) the producer decided, after scouting, to spray chemicals on the bush or to release predators on the stem as follows: three spot treatments against *P. citri* (Risso) (D0: Cropgold®, D9: Silwet®, D16: Golan® + Cropgold®) infestations, five treatments against *S. pannosa* (Wallr.) infestation (D7 and D11: Mg SO_4, D14 and D20: Methatox®), three spot treatments against *Thrips* spp. infestations (D5: Synamec®, D9: Selva®; D12 and D19: *Neoseiulus cucumeris* [Oudemans]), one spot treatment against *D. fovealis* (Zeller) and *H. armigera* (Hubner) infestations (D11: Bipel®), five spot treatments against *T. urticae* infestations (D4, D12 and D15: Cropgold®; and D3, D12 and D19 *P. persimilis*).

The mite populations (*T. urticae* and *P. persimilis*) was evaluated twice a week in each segment of crop lines: 2 low- and 2 high-canopy leaves were observed at random and 4 other leaves were sampled on the last observed hotspots. Adults were counted and a score (0, 1, 2 or 3) assigned for the nymphs (all motile stages) and eggs: 0 for 0 individuals, 1 for 1–5 individuals, 2 for 6–10 individuals and 3 for more than 10 individuals. A Kruskal–Wallis test was done with the software R version 2.12.0 to determine if the median levels between treatments were different. If the test was significant a Siegel–Castellan test was used to compare the pairs of treatment effects. If this test did not show any difference a pairwise ranked Wilcoxon test adjusted by the Bonferroni method was done.

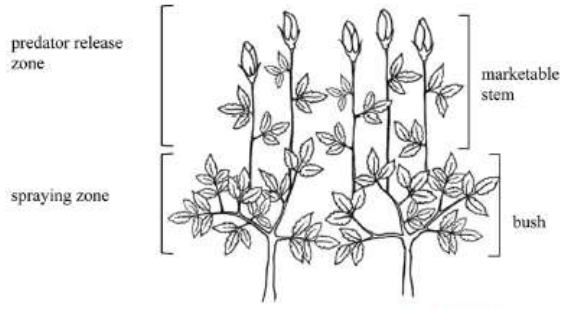


Fig. 3. Structure of a rose plant (adapted from Martin, 2000).

3. Results

3.1. Bioassays

Five of seven acaricides were pre-selected for their high toxicity to adults (>50% mortality at 12.5 g a.i./l). Their LC_{50} and LC_{90} value are presented in Fig. 4. Observing the confidence intervals of their LC_{50}, propargite, dicofol and acrinathrin were significantly more efficient on two-spotted spider mite adults than tau-fluvalinate or flufenoxuron (Table 1).

3.2. Behavioural study

In the choice and no choice tests, the mites explored the area then stopped moving or were trapped by the glue. The activity of adults on the treated and non-treated strings was recorded by video tracking simultaneously. Only the data obtained during the first 15–20 min were analysed because after that period there was more than 20% mortality in the control.

In the no choice test, the number of motile mites was significantly higher on the strings treated with propargite, acrinathrin or tau-fluvalinate compared with the non-treated strings (Table 2). In contrast, the number of motile mites was significantly lower on the strings treated with dicofol or flufenoxuron compared with the non-treated strings. The speed and distance covered by mites were significantly higher on the propargite treated strings compared to the control. But the mean speed of motile mites and they covered distance did not differ significantly between the non-treated strings and the strings treated with dicofol, acrinathrin, flufenoxuron and tau-fluvalinate.

In the choice test, one female adult mite was put on a square of strings where one half was treated with acaricide and the half left untreated. Except for dicofol, there were no significant differences in the activity of the mites (distance covered, mean speed, and presence, immobility and mobility time) on the treated compared to the non-treated area (Table 3). With dicofol, the distance covered, time of presence and mobility period were significantly lower on the treated area compared to the non-treated area.

In the treated leaf disc test the mite state (dead or alive) was not independent of the leaf disc treatment. Compared with the control (4% mortality) all the tested chemicals were toxic to *T. urticae*, with mortalities as high as 90%. Except for dicofol (6%), the dead mites were mainly found on the cotton wool part around the treated leaves. Flufenoxuron resulted in 58%, acrinathrin 62%, propargite 70% and tau-fluvalinate 84% dead mites.

The rising mite numbers were also recorded by video tracking (Fig. 5). The wandering movement peak was between 1 p.m. and 3 p.m. (period with high temperature, strong light and low relative humidity) whatever the infestation level. It was observed that the higher the infestation level the longer the migration period and the higher the peak. The ascent and descent movements were regrouped into 1 h intervals.

using a thin hairbrush (50 leaf discs per chemical). After 24 h the location (leaf or cotton wool) of living or dead individuals was noted. A X^2 test was done with the software R version 2.12.0 following treatments, to see any location-dependent influence. If the variables were dependent, a linear generalized model (binomial model, logit link function) was used to determine the repellent effect. To check the migratory movement a black paper strip (1 × 20 cm) was attached to a 12 cm French dwarf bean with one leaf. Levels of infestation tested on the plant included 100, 300 or 500 mites with 3 replications. Under infrared light the Ethovision® software recorded the rise and fall of mite numbers.

2.6. Field test

The field test was carried out in a 1 ha rose (*Rosa* sp. hybrid) greenhouse of a production farm near Nairobi city in Kenya in July and August 2011. Using a rotation of treated string curtains on five days, a crop line was divided in five segments with a curtain protecting one in five segments (Fig. 2). Every day at 9.00 a.m. the curtain was moved to the following segment. The curtains were removed in the afternoon at 3 p.m. in case there was a chemical spray. In that experiment the efficiency of string curtains treated with LC_{90} of dicofol or acrinathrin on their own was compared to the farmer practice without curtain. There were 34 crop lines for each treated curtain and 68 for the control. The greenhouse was divided into four plots, including two for the control separating the two plots with treated curtains.

Actually, the treated string curtain protection was added to the usual IPM program of the producer *P. persimilis* predating mites released when the ratio of predator/pest was lower than 1/5 and a miticide sprayed when two-spotted spider mites were observed in the middle of the marketable rose stem (Fig. 3). During the experiment in the greenhouse (Day 0–21) the producer decided, after scouting, to spray chemicals on the bush or to release predators on the stem as follows: three spot treatments against *P. citri* (Risso) (D0: Cropgold®, D9: Silwet®, D16: Golan® + Cropgold®) infestations, five treatments against *S. pannosa* (Wallr.) infestation (D7 and D11: Mg SO_4, D14 and D20: Methatox®), three spot treatments against *Thrips* spp. infestations (D5: Synamec®, D9: Selva®; D12 and D19: *Neoseiulus cucumeris* [Oudemans]), one spot treatment against *D. fovealis* (Zeller) and *H. armigera* (Hubner) infestations (D11: Bipel®), five spot treatments against *T. urticae* infestations (D4, D12 and D15: Cropgold®; and D3, D12 and D19 *P. persimilis*).

The mite populations (*T. urticae* and *P. persimilis*) was evaluated twice a week in each segment of crop lines: 2 low- and 2 high-canopy leaves were observed at random and 4 other leaves were sampled on the last observed hotspots. Adults were counted and a score (0, 1, 2 or 3) assigned for the nymphs (all motile stages) and eggs: 0 for 0 individuals, 1 for 1–5 individuals, 2 for 6–10 individuals and 3 for more than 10 individuals. A Kruskal–Wallis test was done with the software R version 2.12.0 to determine if the median levels between treatments were different. If the test was significant a Siegel–Castellan test was used to compare the pairs of treatment effects. If this test did not show any difference a pairwise ranked Wilcoxon test adjusted by the Bonferroni method was done.

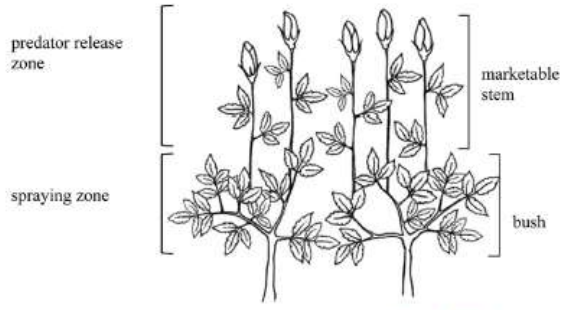


Fig. 3. Structure of a rose plant (adapted from Martin, 2000).

3. Results

3.1. Bioassays

Five of seven acaricides were pre-selected for their high toxicity to adults (>50% mortality at 12.5 g a.i./l). Their LC_{50} and LC_{90} value are presented in Fig. 4. Observing the confidence intervals of their LC_{50}, propargite, dicofol and acrinathrin were significantly more efficient on two-spotted spider mite adults than tau-fluvalinate or flufenoxuron (Table 1).

3.2. Behavioural study

In the choice and no choice tests, the mites explored the area then stopped moving or were trapped by the glue. The activity of adults on the treated and non-treated strings was recorded by video tracking simultaneously. Only the data obtained during the first 15–20 min were analysed because after that period there was more than 20% mortality in the control.

In the no choice test, the number of motile mites was significantly higher on the strings treated with propargite, acrinathrin or tau-fluvalinate compared with the non-treated strings (Table 2). In contrast, the number of motile mites was significantly lower on the strings treated with dicofol or flufenoxuron compared with the non-treated strings. The speed and distance covered by mites were significantly higher on the propargite treated strings compared to the control. But the mean speed of motile mites and they covered distance did not differ significantly between the non-treated strings and the strings treated with dicofol, acrinathrin, flufenoxuron and tau-fluvalinate.

In the choice test, one female adult mite was put on a square of strings where one half was treated with acaricide and the half left untreated. Except for dicofol, there were no significant differences in the activity of the mites (distance covered, mean speed, and presence, immobility and mobility time) on the treated compared to the non-treated area (Table 3). With dicofol, the distance covered, time of presence and mobility period were significantly lower on the treated area compared to the non-treated area.

In the treated leaf disc test the mite state (dead or alive) was not independent of the leaf disc treatment. Compared with the control (4% mortality) all the tested chemicals were toxic to *T. urticae*, with mortalities as high as 90%. Except for dicofol (6%), the dead mites were mainly found on the cotton wool part around the treated leaves. Flufenoxuron resulted in 58%, acrinathrin 62%, propargite 70% and tau-fluvalinate 84% dead mites.

The rising mite numbers were also recorded by video tracking (Fig. 5). The wandering movement peak was between 1 p.m. and 3 p.m. (period with high temperature, strong light and low relative humidity) whatever the infestation level. It was observed that the higher the infestation level the longer the migration period and the higher the peak. The ascent and descent movements were regrouped into 1 h intervals.

Table 1
Contact toxicity of 5 treated nets on *Tetranychus urticae* adult. Mortality was observed 48 h after 1 h exposure to the nets treated with dicofol, flufenoxuron, propargite, tau-fluvalinate and acrinathrin using bioassay method with modified Munger cell.

Acaricide	LC	Concentration	95% CI	Slope (±SE)	Chi^2	d.f.
Dicofol	LC_{90}	5.98	3.69–13.81			
	LC_{50}	1.22	0.80–1.78	1.85 ± 0.019	8.33	4
Flufenoxuron	LC_{90}	25.00	21.58–31.80			
	LC_{50}	14.89	13.24–16.63	5.7 ± 0.050	11.16	6
Propargite	LC_{90}	4.66	3.67–6.83			
	LC_{50}	1.99	1.56–2.43	3.46 ± 0.034	8.05	4
Tau-fluvalinate	LC_{90}	44.69	27.09–96.43			
	LC_{50}	6.59	2.62–11.29	1.54 ± 0.019	1.36	4
Acrinathrin	LC_{90}	30.48	18.04–70.99			
	LC_{50}	3.94	1.52–6.84	1.44 ± 0.017	6.28	4

et al. (1986) demonstrated the repellent effect of several pyrethroids on *T. urticae* that were concentration dependent. They also showed that the repellent concentration was lower than the toxic concentration, suggesting pyrethroid-based acaricides are not suitable for the control of mites. In our study, except for dicofol, the quantity of chemicals applied to the strings was insufficient to cause repellence after 20 min. This result could be due to the slow release of the chemical due to the binder. However, the binder did not prevent the toxic effect of chemicals after 1 h of contact. The immobility period was shorter in the choice test with 1 mite than in the no choice test with 16 *T. urticae* adult mites. This result points to group behaviour, i.e. when one mite moves the others do the same (Clotuche et al., 2011). This author also reported that *T. urticae* has an aggregation behaviour that hinders random dispersal.

The results from this study also revealed that propargite, acrinathrin and tau-fluvalinate had excitation effects. The number of actively moving mites was significantly higher with these three chemicals. Moreover, the mean speed of *T. urticae* was higher with propargite than with the control without treated string curtain. This result confirmed the observations of Penman et al. (1986) using pyrethroid based acaricides such as acrinathrin and tau-fluvalinate where they showed that the primary response of *T. urticae* was to escape from contact with the pyrethroid residues. This effect may explain the ineffectiveness of the string curtain treated with acrinathrin compared with the curtain treated with dicofol, which was not repellent at the concentration used.

The circadian upward and downward movement of *T. urticae* reported by Pralavorio et al. (1989) was confirmed during video tracking motile stage in our study. Peak migration was observed at the beginning of the afternoon (period with high temperature, strong light and low relative humidity). No mite activity was recorded during the night, justifying the use of treated string curtains only during the day. According to Pralavorio et al. (1989) and as confirmed by our field observations on roses, the migratory mites were the young sexually mature females, which is the stage most suited for colonization.

The field trials enabled us to test the design and efficiency of an acaricide-treated string curtain to protect roses. Field tests showed greater effectiveness of the dicofol-treated string curtain for the control of *T. urticae* on roses than the producer's IPM practice,

Table 2
No-choice behaviour of *Tetranychus urticae* on curtains treated with acaricides. Means (±standard error) of total covered distance by individuals, mobile individual numbers ($v > 0.1$ mm/s) and mean speed compared with a non-treated curtain as control.

		Dicofol	Flufenoxuron	Propargite	Acrinathrin	Tau-fluvalinate
Distance covered (mm)	Non-treated	44.61 ± 15.87	28. 59 ± 23.89	42.52 ± 8.24	51.47 ± 13.66	41.68 ± 9.49
	Treated	43.54 ± 13.94	28. 07 ± 19.41	61.40 ± 25.59	44.22 ± 10.88	40.39 ± 8.69
	P-value	0.636	0.598	**0.014**	0.081	0.656
Speed (mm/s)	Non treated	1.32 ± 0.65	0.81 ± 0.56	1.16 ± 0.42	1.35 ± 0.44	1.11 ± 0.33
	Treated	1.10 ± 0.39	0.57 ± 0.36	1.58 ± 0.68	1.27 ± 0.44	0.98 ± 0.27
	P-value	0.425	0.190	**0.022**	0.617	0.126
Mobile insects (s)	Non treated	7.00 ± 2.10	11.7 ± 1.56	8.90 ± 0.852	10.85 ± 2.46	11.90 ± 2.51
	Treated	4.05 ± 1.23	4.60 ± 1.42	12.7 ± 8.9	12.15 ± 3.51	15.35 ± 0.93
	P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

The data were recorded by video tracking the locomotory behaviour of 16 mites. The given *P*-value is the value of the Wilcoxon–Mann–Whitney test, the bold values are significative (p-value < 0.05).

Table 3
Choice behaviour of *Tetranychus urticae* adult on string curtain partly treated with acaricide and partly non-treated. Mean (±standard error) of covered distance, mean speed, presence on the net, and mobility and immobility time by individuals ($v > 0.1$ mm/s).

		Dicofol	Flufenoxuron	Propargite	Acrinathrin	Tau-fluvalinate
Covered distance (mm)	Non-treated	58.33 ± 27.39	43.27 ± 30.64	42.79 ± 18.02	38.34 ± 16.56	41.45 ± 27.67
	Treated	25.40 ± 18.93	36.53 ± 22.83	33.11 ± 21.77	30.63 ± 21.10	28. 12 ± 25.67
	P-value	**0.005**	0.643	0.245	0.368	0.277
Speed (mm/s)	Non-treated	0.20 ± 0.09	0.23 ± 0.11	0.26 ± 0.15	1.17 ± 0.51	0.19 ± 0.09
	Treated	0.18 ± 0.11	0.22 ± 0.11	0.31 ± 0.14	1.34 ± 0.43	0.20 ± 0.21
	P-value	0.541	0.540	0.083	0.246	0.812
Presence time (s)	Non-treated	619.66 ± 254.74	472.32 ± 225.94	518.44 ± 133.67	496.99 ± 229.57	537.89 ± 317.52
	Treated	280.16 ± 254.83	387.18 ± 225.69	336.62 ± 191.88	373.14 ± 198.30	361.69 ± 317.79
	P-value	**0.012**	0.463	0.107	0.177	0.261
Immobility time (s)	Non-treated	186.86 ± 233.40	121.30 ± 171.34	191.35 ± 169.68	3.81 ± 7.77	146.85 ± 155.26
	Treated	96.92 ± 172.42	116.90 ± 161.44	125.96 ± 162.91	1.52 ± 1.79	136.93 ± 183.08
	P-value	0.123	0.922	0.134	0.173	0.898
Mobility time (s)	Non treated	432.41 ± 257.94	350.65 ± 186.70	424.76 ± 96.56	493.10 ± 228.19	390.82 ± 253.30
	Treated	182.91 ± 155.09	270.03 ± 168.82	332. 87 ± 134.97	371.32 ± 197.41	224.47 ± 190.76
	P-value	**0.005**	0.540	0.0731	0.165	0.090

The data were recorded by video tracking of 20 replicates of the locomotory behaviour of 1 mite. The given *P*-value was the value of the Wilcoxon–Mann–Whitney test, the bold values are significative (p-value < 0.05).

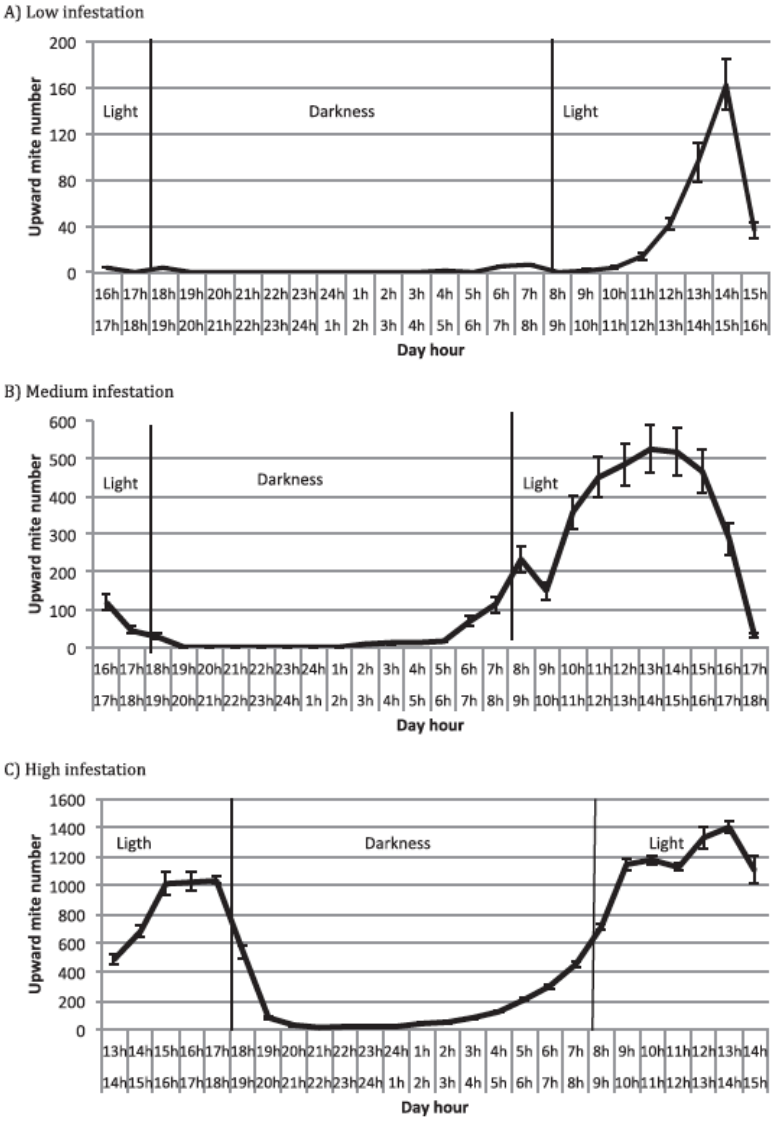


Fig. 5. Rising mite numbers calculated per hour on one day at different population pressure: 100 (A); 300 (B) and 600 (C) individuals on a French dwarf bean plant. The data were recorded by the Ethovision® software on a paper strip fixed to the plant with IR light.

despite dicofol's repellent effect, which was demonstrated in laboratory tests. On the contrary, the acrinathrin-treated string curtain was not effective. In addition, our control plot was not a source of infestation because *T. urticae* was controlled by the producer's usual IPM program (Giustina et al., 1983). However, as workers can disseminate the mites on their clothing, they should wear coats treated with repellent to prevent the mites from being carried on them. Depending on the concentration used, the two chemicals – dicofol and acrinathrin – are repellent and so the mites would not walk on the string curtains. We hypothesize that the contact surface between the string curtain and the rose plant is not large enough; although similar string curtains were used to harvest the predators on other plants. To improve the effectiveness of treated string curtains, an attractive olfactory (essential oil) or visual (colour) substance could be added to the curtain. The ineffectiveness of acrinathrin in the field tests could also be due to the resistance of the local mite population. Dr Philippe Auger (INRA–CBGP) identified the population in the greenhouse as *T. urticae* but no test was carried out in our study to check if this population was resistant to dicofol or acrinathrin as shown elsewhere (Grafton, 2005; Kim et al., 2006). Our method focused on the circadian migratory behaviour on the stems and leaves but during the field trials, a few mites were observed on flower buds. The climate was cold so the development of *T. urticae* was slow. The low rate of migration and hence the low efficacy of the method could be explained by the cold weather (Pralavorio et al., 1989). For that reason, the results of that field test concern only the control of a low population. The results of these trials led us to hypothesize that the

Table 4
Mean number of *Tetranychus urticae* on rose leaves. Mites were sampled on the stem or in the bush before and after setting the treated curtains. A rotation of 5 days (1 protected segment per day) was done with the dicofol (6 g a.i./l) and acrinathrin (15 g a.i./l) treated curtains.

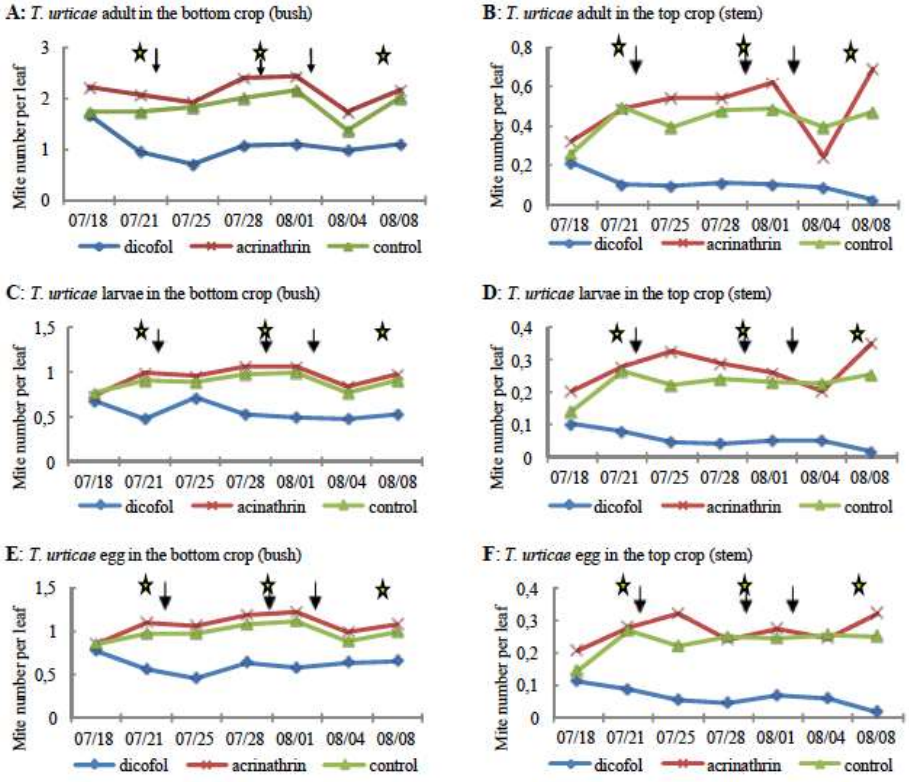
	Adults				Larvae				Eggs			
	Bush		Stem		Bush		Stem		Bush		Stem	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Dicofol	1.67 a[(a)]	0.98 a	0.22 a	0.09 a	0.68 a	0.49 a	0.11 a*	0.05 a	0.78 a	0.59 a	0.11 a*	0.06 a
Acrinathrin	1.59 a	2.11 b	0.32 a	0.52 b	0.74 a	0.98 b	0.21 b*	0.29 b	0.85 a	1.10 b	0.21 b*	0.28 b
Control	1.73 a	1.85 c	0.27 a	0.45 b	0.76 a	0.91 c	0.14 ab*	0.24 b	0.85 a	1.00 c	0.14 ab*	0.25 b
P-value[(1)]	0.95	**<0.001**	0.081	**<0.001**	0.629	**<0.001**	**0.026**	**<0.001**	0.650	**<0.001**	**0.042**	<0.001

[(1)] When the *P*-value from the Kruskal–Wallis test were inferior to 0.05, [(a)] the means were compared by pair and means with the same letter were not significantly different (*P*-value < 0.05 of the Siegel–Catellan or Wilcoxon test (*)) between two medians, the bold values are significative (*p*-value < 0.05).

Table 5
Mean number of *Phytoseiulus persimilis* on rose leaves sampled on the stem and in the bush before and after the setting of the treated curtains. A rotation of 5 days (1 protected segment per day) was done with the dicofol (6 g a.i./l) and acrinathrin (15 g a.i./l) treated curtains.

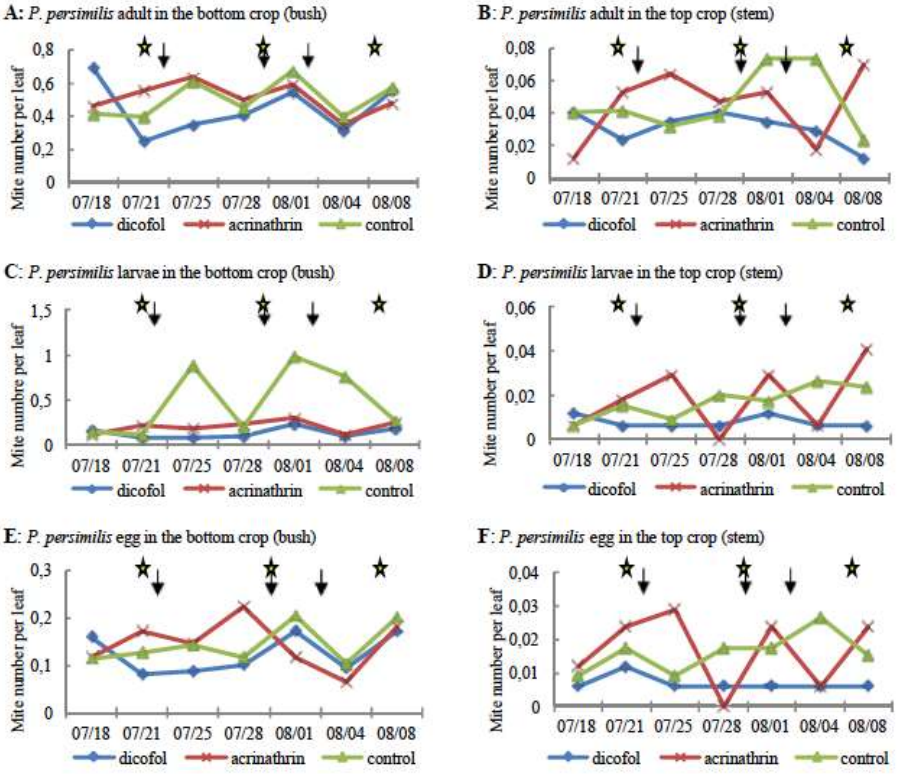
	Adults				Larvae				Eggs			
	Bush		Stem		Bush		Stem		Bush		Stem	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Dicofol	0.25 a[(a)]	0.41 a	0.04 a	0.03 a	0.17 a	0.13 a	0.01 a*	0.01 a	0.16 a	0.12 a*	0.01 a	0.01 a
Acrinathrin	0.55 b	0. 52 b	0.01 a	0.05 a	0.12 a	0.22 b	0.02 b*	0.01 a	0.12 a	0.15 b*	0.01 a	0.02 a
Control	0.40 ab	0.52 b	0.04 a	0.05 a	0.16 a	0.54 c	0.02 ab*	0.01 a	0.12 a	0.15 b*	0.01 a	0.02 a
P-value[(1)]	**<0.001**	**<0.001**	0.519	0.302	0.359	**<0.001**	**0.042**	1.000	0.341	**0.049**	0.845	0.062

[(1)] When the *P*-value from the Kruskal–Wallis test were inferior to 0.05, [(a)] the means were compared by pair and means with the same letter were not significantly different (*P*-value < 0.05 of the Siegel–Catellan or Wilcoxon test (*)) between two medians, the bold values are significative (*p*-value < 0.05).



[1]All the calculated standard erroroverlap for each observation.

Fig. 6. Evolution[1] of *Tetranycus urticae* population in a rose greenhouse during the dicofol and acrinathrin treated curtain test: a rotation on 5 days (1 protected segment per day) was done with the dicofol (6 g a.i./l) and acrinathrin (15 g a.i./l) treated curtains. +: chemical spray, ☆: *Phytoseiulus persimilis* release.



[1]All the calculated standard erroroverlap for each observation.

Fig. 7. Evolution[1] of *Phytoseiulus persimilis* population in a rose greenhouse during the dicofol and acrinathrin treated curtain test: a rotation on 5 days (1 protected segment per day) was done with the dicofol (6 g a.i./l) and acrinathrin (15 g a.i./l) treated curtains. ↓: chemical spray, ☆: *P. persimilis* release.

higher the mite infestation rate, the greater the reduction that could be achieved in the mite population with an acaricide-treated device (such as a string curtain). This hypothesis now needs to be tested on a crop other than roses, because for marketing reasons, the mechanical damage on the rose leaves is unacceptable. However, our choice of roses facilitated the implementation of the trials in the greenhouse where climatic conditions are controlled, and pest control is easier. The knowledge gained under greenhouse conditions will enable us to improve the design of an impregnated device for the control of mites on roses that will not damage the commercial stem. Our results also support the conclusion that the treated curtains have little effect on the predatory mite *P. persimilis*. For the impregnated curtain, the logical choice would be a vegetable crop such as tomato, where the economic threshold of mites is higher and the impact of mechanical damage caused by the string curtains will not influence sales.

Table 6
Percentage of chemical-treated segment of rose rows against *Tetranychus urticae*. A rotation on 5 days was done with treated curtain at 6 g a.i./l for dicofol and at 15 g a.i./l for acrinathrin, respectively.

	July17th	July 22nd	July 30th	August 2nd	*P*-value[(1)]
Dicofol	*2.35 a[(a)]	*5.29 a	*1.76 a	*1.76 a	0.477
Acrinathrin	**5.88 b	**23.52 c	**30.58 c	**24.70 c	**<0.001**
Control	**11.17 d	**21.17 d	**24.11 d	**22.35 d	0.096
P-value [(1)]	**0.001**	**<0.001**	**<0.001**	**<0.001**	

[(1)] When the *P*-value from the Kruskal–Wallis test were inferior to 0.05, the bold values are significative (*p*-value < 0.05) [(a)] the means were compared by pair and means with a same letter (comparison between dates) or sign (comparison between treatments) were not significantly different (*P*-value < 0.05 of the Siegel–Catellan) between two medians.

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RESEARCH ARTICLE OPEN ACCESS

The repellency of lemongrass oil against stable flies, tested using video tracking

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Abstract – Lemongrass oil (*Cymbopogon citratus*) is an effective repellent against mosquitoes (Diptera: Culicidae) and house flies (Diptera: Muscidae). In this study, its effectiveness was assessed on stable flies (Diptera: Muscidae) in laboratory conditions. First, we demonstrated that lemongrass oil is an active substance for antennal olfactory receptor cells of *Stomoxys calcitrans* as indicated by a significant increase in the electroantennogram responses to increasing doses of lemongrass oil. Feeding-choice tests in a flight cage with stable flies having access to two blood-soaked sanitary pads, one of which was treated with lemongrass oil, showed that stable flies (n = 24) spent significantly more time in the untreated zone (median value = 218.4 s) than in the treated zone (median value = 63.7 s). No stable flies fed on the treated pad, whereas nine fed on the untreated pad. These results suggest that lemongrass oil could be used as an effective repellent against stable flies. Additional studies to confirm its spatial repellent and feeding deterrent effects are warranted.

Key words: *Stomoxys calcitrans*, stable fly, repellent, lemongrass, *Cymbopogon citratus*, video tracking.

Résumé – Activité répulsive de l'huile essentielle de citronnelle contre les stomoxes, testée par tracking vidéo. L'huile essentielle de *Cymbopogon citratus* est un répulsif actif contre les moustiques (Diptera : Culicidae) et les mouches domestiques (Diptera : Muscidae). Dans cette étude, nous avons testé son efficacité contre les stomoxes (Diptera : Muscidae) en laboratoire. Nous avons tout d'abord démontré par électroantennographie (EAG) que l'huile essentielle de *C. citratus* était une substance active sur les récepteurs olfactifs des antennes de *Stomoxys calcitrans*, par la mise en évidence d'une augmentation significative des réponses EAG à des doses croissantes d'huile essentielle. Des tests de choix réalisés en cage de vol avec des stomoxes ayant à disposition deux supports imprégnés de sang, l'un ayant été traité avec de l'huile essentielle, montrent que les stomoxes (n = 24) ont passé significativement plus de temps dans la zone non traitée (valeur médiane = 218,4 s) que dans la zone traitée (valeur médiane = 63,7 s). Aucun stomoxe ne s'est nourri sur le support traité alors que neuf stomoxes se sont nourris sur le support non traité. Ces résultats suggèrent que l'huile essentielle de *C. citratus* pourrait être utilisée comme répulsif contre les stomoxes. Des études complémentaires sont nécessaires pour confirmer ses effets répulsifs et anti-gorgement.

Introduction

The stable fly *Stomoxys calcitrans* L. is among the most damaging arthropod pest of livestock worldwide [8, 15, 23], with a high economic impact on dairy and beef cattle production [3, 27, 39]. It is also a potential mechanical vector of animal pathogens such as equine infectious anemia virus, *Trypanosoma evansi*, and *Besnoitia besnoiti* [7, 9, 19]. Control of stable fly populations includes various methods, such as chemical control (pesticides and repellents), cultural control (sanitation), mechanical control (trapping devices), and biological control (parasitoids and entomopathogenic fungi) [9, 20]. The best approach is the simultaneous use of several methods in an integrated pest-management program [26]. Management of adult flies is accomplished mainly with topical insecticides, applied directly to animals. However, continued or repeated use of conventional insecticides often results in the development of resistance and fosters serious human health and environmental

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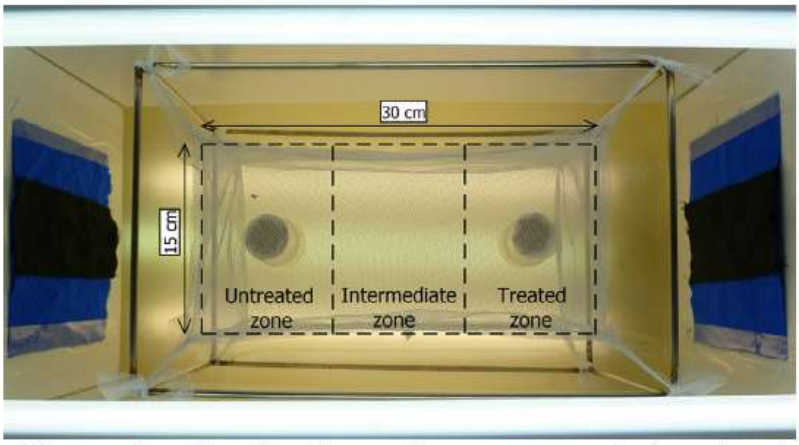

Figure 1. Aerial view of the system for spatial repellency bioassays. The screen cage was made of mosquito netting suspended on a metal frame and surrounded by white foam board with blue and black fabric on each side. Two blood-soaked sanitary pads were set under the cage: one, impregnated with lemongrass oil, was placed in the treated zone, and the other, impregnated with hexane, was placed in the untreated zone. For further details see Materials and Methods section.

concerns [13, 42]. Populations of *S. calcitrans* resistant to pyrethroids and/or organophosphates have already been described in North America and in Europe [4, 21, 31, 34]. As a result, there have been increased research efforts for natural and environmentally friendly repellents, particularly those based on essential oils [38]. Several plant-based repellents, such as citronella oil, eucalyptus oil, catnip oil, and zanthoxylum oil, have previously been tested against stable flies and have shown a reduction in attraction and in feeding [1, 13, 40, 43]. These repellents can be applied topically on animals or in livestock barns [13]. The first study demonstrating the potential application of a plant-based repellent was conducted by Zhu et al. [44], in which wax-based catnip pellets spread in the manure/soil areas of cattle feedlots resulted in over 99% repellency of stable flies.

Lemongrass oil is the essential oil obtained from the aerial parts of *Cymbopogon citratus* (DC.) Stapf., Poaceae [29]. Geranial (α-citral) and neral (β-citral) are the two main active components of lemongrass oil, but other compounds, such as geraniol and citronellol, which are known repellents, are also present in small amounts [2, 18, 38]. Lemongrass essential oil has previously shown a repellent effect, alone or in combination, against different species of disease-transmitting mosquitoes (Diptera: Culicidae) and the house fly *Musca domestica* L. (Diptera: Muscidae) [16, 25, 30, 37], and is already present in commercially available products [5, 32]. Therefore, our objectives were to verify the sensitivity of antennal receptor cells of *S. calcitrans* to lemongrass oil and to evaluate its repellency against stable flies using a video-tracking system.

Materials and methods

Insects

Stomoxys calcitrans pupae were obtained from the laboratory colony of the National Veterinary School of Toulouse (Toulouse, France) [35]. Newly emerged flies were not sexed. Males and females were enclosed together in a cotton mesh cage (40 cm W × 25 cm H × 25 cm D) at 24 ± 2 °C with 40–50% relative humidity. Flies were fed with 10% sugar water *ad libitum* and, once a day, with citrated bovine blood. Experiments were conducted with 2–4-day-old flies. Flies were not fed for 24 h prior to each test.

Electroantennogram recording

Following the method used in the study by Jeanbourquin and Guerin [14], electroantennogram (EAG) recordings from antennae of *S. calcitrans* were made with an EAG recording device (EAG combi probe internal gain ×10, CS-55 stimulus controller and IDAC-2 signal acquisition controller, Syntech, Hilversum, the Netherlands). Recordings were made using electrolyte-filled (0.1 M KCl) glass capillary electrodes (Ø 1.5 mm, 40 mm L), with Ag/AgCl wire (Ø 0.5 mm, 20 mm L) making contact with the recording apparatus. The antenna was maintained in a humidified charcoal-filtered air stream delivered at 14.6 mL/s through a metal tube. Aliquots of pure lemongrass oil (from *C. citratus* DC., citral ~75%, Sigma Aldrich Chemie GmbH, Buchs, Switzerland) were prepared using hexane (95%, Carlo Erba Reagenti, Arese, Italy) at 0.1, 0.01, 0.001, 0.0001 mg/µL. Tested solutions (10 µL) were deposited on a strip of filter paper (20 × 5 mm) placed in a glass Pasteur pipette. The solvent was allowed to evaporate for 15 min before first use. The tip of the pipette was connected to the metal tube, and the test stimulus was delivered to the antenna using an air pulse (20 mL/s for 0.6 s). Stimuli were released successively in random order at 90-s intervals to avoid receptor saturation. Octenol (1-octen-3-ol, 98%, Sigma Aldrich Chemie GmbH, Buchs, Switzerland) was used as a positive control and hexane was used as a negative control. Differences in EAG responses were evaluated using a Wilcoxon signed-rank test.

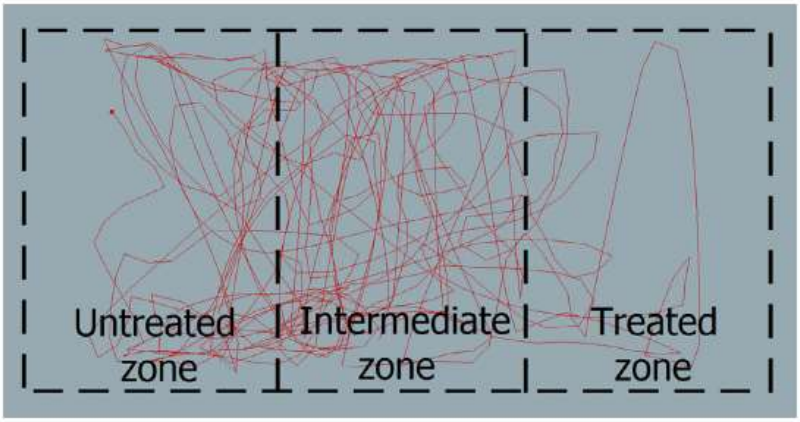

Figure 2. Track showing the 10-min recording of a stable fly in the bioassay cage divided into three zones: the untreated zone, the intermediate zone, and the treated zone.

Bioassays

To observe the flight behavior of stable flies, we used a screen cage (30 cm W × 15 cm H × 15 cm D) made of polyester mosquito netting suspended on a metal frame (Figure 1). A small hole in the middle of one side of the cage was sealed with a piece of cotton wool and was used to allow the release of one fly at a time into the cage. The cage was surrounded by a shield of white foam board to prevent optical stimulation of the flies. To stimulate the fly to move in the cage, pieces of blue and black fabric (SuperMaine 300 g cotton/polyester 65/35%; TDV industries, Laval, France), commonly used to attract biting flies, were hung on each side of the foam board [17, 24]. Illumination was provided by fluorescent tubes (frequency 50 Hz) placed below and above the screen cage. The light level in the middle of the cage was about 4600–5000 lux.

One fly was released into the cage 15 min before the test. Bioassays were conducted using male and female stable flies during the daytime at ambient laboratory temperatures of 22–26 °C and 40–50% relative humidity. The bioassays consisted of feeding-choice tests in which the fly had access to two blood sources, one of which was treated with lemongrass oil. Citrated bovine blood (1.5 mL), previously heated at 45 °C, was placed on two sanitary pads (Ø 4 cm) from which we removed the outer layer. The outer layer of one pad was impregnated with 100 µL of lemongrass oil solution at 0.1 mg/µL, and the other outer layer with 100 µL of hexane. When the solvent had evaporated, each outer layer was repositioned on top of one of the blood-soaked sanitary pads, which were placed just under the cage floor, 20 cm apart. Fly movement was recorded using a Digital Video Camera Recorder (DCR-SR21E; Sony, Japan) set 1 m above the center of the cage. The behavior of the fly was then recorded during a 10-min period. We tested 4–6 flies each day; the behavior of 24 flies was included in this study. The room was ventilated for at least 30 min between each test, and a new screen cage was used for successive flies. The positions of the pad treated with lemongrass oil and the untreated pad were inversed each time. The cages were cleaned every day by soaking them in a 2% solution of Decon 90 (Decon Laboratories Limited, Sussex, England) for 12 h.

Video analysis

The video records of fly movement were analyzed using EthoVison XT (v. 8.0; Noldus Information Technology, Wageningen, the Netherlands) [28]. The cage was defined as an arena (30 × 15 cm) divided into three zones (each 10 × 15 cm): untreated, intermediate, and treated (Figure 1). Movement was recorded at 25 video frames per second, and the fly was tracked by dynamic subtraction (Figure 2). In this method, the program compares each sampled image with a reference image that is updated regularly. Image processing algorithms are applied to detect the fly against the background and to extract relevant image features. During data acquisition, EthoVision displays the live video image, tracking statistics (elapsed time, number of samples), and the x, y co-ordinates of the fly [28]. Several parameters were calculated: the distance moved (in centimeters), the total time spent in each zone (in seconds), the time spent in movement (in seconds), and the mean velocity (centimeters per second). "Moving" and "not moving" were defined with thresholds at 1 and 0.9 cm/s. A comparison between males and females was made with the non-parametric Mann-Whitney test for independent samples. Comparisons of flight parameters between the treated zone and the untreated zone were made with the non-parametric Wilcoxon signed-rank test for two samples of univariate data. All analyses were performed using PAST version 2.12 [12].

Lemongrass oil volatiles

To estimate the diffusion of lemongrass oil volatiles in the bioassay cage, we compared the atmospheric concentrations of

Table 1. Comparison of the flight activity of male and female stable flies (Mann-Whitney *U* test), and comparison of the behavior of flies (both sexes) between the zone treated with lemongrass oil and the untreated zone (Wilcoxon *W* signed-rank test).

	N	Median value	Percentiles	Test
Time spent in movement (s)				
Males	11	**95.7**	**38.1–148.8**	***U* = 34**
Females	13	**144.8**	**110.8–177**	***p* = 0.030**
Velocity (cm/s)				
Males	11	**6.9**	**5.7–7.7**	***U* = 10**
Females	13	**15**	**10.4–16.9**	***p* = 0.0001**
Total time (s)				
Treated zone	24	**63.7**	**41–163.7**	***W* = 233**
Untreated zone	24	**218.4**	**94.2–434.2**	***p* = 0.016**
Time spent in movement (s)				
Treated zone	24	22.3	11.3–35.8	*W* = 200
Untreated zone	24	30.6	14–54.3	*p* = 0.160
Velocity (cm/s)				
Treated zone	24	9.8	7.2–14	*W* = 182
Untreated zone	24	9.1	6.7–12.2	*p* = 0.371

Data that show significant differences are indicated in bold.

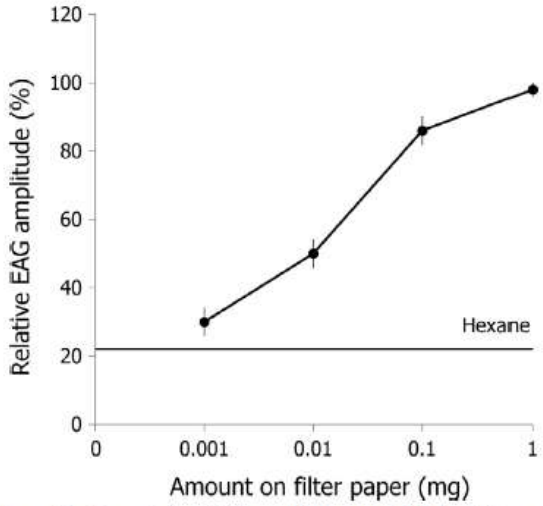

Figure 3. Mean relative EAG amplitudes recorded from *Stomoxys calcitrans* antennae ($n = 7$) stimulated with lemongrass essential oil at doses of 0.001 mg, 0.01 mg, 0.1 mg, and 1 mg. Hexane was used as negative control. EAG amplitudes are relative to the value of 100% for octenol at 1 mg in the stimulus syringe. Differences in EAG amplitudes were evaluated using the Wilcoxon signed-rank test. Significant differences are indicated by different letters ($p \leq 0.05$).

neral and geranial, its most abundant constituents. To accomplish this, three 65 µm Polydimethylsiloxane-Divinylbenzene (PDMS-DVB) fibers (Supelco, Sigma-Aldrich, Bellefonte, PA, USA) were conditioned in the inlet of a gas chromatograph (GC) held at 250 °C for 5 min before sampling. The SPME holders were exposed in the cage for 10 min at three positions. One SPME fiber was positioned 10 cm above each of the two blood-soaked sanitary pads, and another was positioned in the middle of the cage. Relative concentrations of volatile samples were analyzed in a GC-mass spectrometry (MS; Shimadzu QP2010plus, Shimadzu Scientific Instruments, Kyoto, Japan), using helium as the carrier gas (1 mL/min). Samples were injected in splitless mode. The temperature program for GC analyses was 40 °C for 5 min, 5 °C/min to 220 °C, and 10 °C/min to 250 °C.

Results and discussion

Our investigation showed that *S. calcitrans* EAG amplitudes increased significantly in a dose-dependent fashion with increasing doses of lemongrass oil in the stimulus pipette. The mean EAG amplitude elicited by each dose (0.001 mg: 2.06 ± 0.37 mV; 0.01 mg: 3.37 ± 0.47 mV; 0.1 mg: 5.80 ± 0.67 mV; 1 mg: 6.50 ± 0.57 mV) was significantly greater than that elicited by hexane (1.46 ± 0.29 mV) (Figure 3) and there was no significant difference between lemongrass oil and the octenol at 1 mg on filter paper (6.64 ± 0.55 mV). Octenol is a very strong chemostimulant for *S. calcitrans* antennae [36, 41] and a good attractant in the field [11]. The study by Zhu *et al.* [44] was the first to report that stable fly antennae are also capable of detecting repellents such as catnip oil. In our study, EAG responses to lemongrass oil at 10 µg (~3350 µV) were nearly five times higher than the EAG responses to the same amount of catnip oil (~700 µV recorded by Zhu *et al.* [44]). These results indicate that lemongrass oil is a strong stimulant for the olfactory receptor cells of *S. calcitrans* and thus a suitable candidate for behavioral tests.

In the bioassays, the amount of lemongrass oil on the treated pad used in all tests was 10 mg. Relative concentrations of

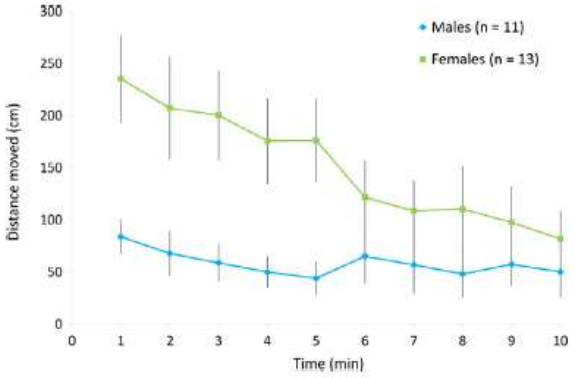

Figure 4. Time-course (mean ± *SE*) of the distance moved by stable fly males and females for 10 min following release into a bioassay cage. Curves represent the distance moved for each successive 1-min period during 10-min recordings.

neral and geranial in the arena were assessed by the height of their peaks in mass chromatograms to reveal a 12-fold decrease in the atmospheric concentration of lemongrass oil between the treated and untreated pads. It should be noted, however, that this measurement was taken in the absence of a fly. The air flow induced by the flight activity of a fly in the cage might partially disturb this ratio during a test.

We tested 11 males and 13 females in the bioassay cage (Table 1). First, we compared the flight activity between the two sexes. The distance moved is considered to be the main indicator of the activity level of a fly [22]. At the beginning, females were more active than males (in terms of time spent in movement and velocity) (Figure 4). Over the duration of the 10-min recordings, the distance moved by females gradually decreased to reach a level similar to males. This decrease in movement might have been due to exposure to lemongrass oil, or simply to acclimation to the bioassay cage. This is an open question as no tests were conducted without a treated pad. However, locomotor activity was sexually distinct, as has been observed in fruit flies, *Drosophila melanogaster* [10].

Comparing the behavior of stable flies in the zone treated with lemongrass oil with their behavior in the untreated zone did not reveal any significant differences between the two zones in terms of the time spent in movement or in the mean velocity of movement (Table 1). However, stable flies spent significantly more time flying in the untreated zone than in the treated zone during the tests. Moreover, we observed nine stable flies feeding on the untreated pad, whereas none fed on the treated pad. The attractiveness of the untreated blood-soaked pad versus the treated pad explains the difference in the total time spent between the two zones. These findings suggest that lemongrass oil could be used as a repellent against stable flies. However, further investigations on spatial repellency and feeding deterrence are necessary to demonstrate that lemongrass oil is as effective as catnip oil against stable flies in the field [44]. Video tracking appears to be a useful tool to study insect behavior in response to repellent volatiles [6, 33], especially for flies, which are otherwise difficult to track.

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De la répulsion chez les insectes
- cas d'étude du moustique *Anopheles gambiae* et de la mouche blanche *Bemisia tabaci* -

Tous les insectes recherchent un hôte, animal ou végétal, pour la nutrition, un site de refuge, d'accouplement, ou de ponte. La recherche et la reconnaissance de l'hôte se font à distance en utilisant les signaux olfactifs et visuels, et la sélection et l'acceptabilité de l'hôte se font à l'aide des indices tactiles, olfactifs et gustatifs au contact de celui-ci. Grâce à nos connaissances sur les mécanismes d'accouplement ou de recherche de l'hôte, des pièges attractifs ont été mis au point par les scientifiques pour surveiller et réduire certaines populations d'insectes. Pourquoi ne pas utiliser ce que nous savons sur la recherche de l'hôte pour repousser les insectes nuisibles ?

Un composé répulsif est un composé qui empêche un insecte de chercher, suivre, localiser, sélectionner, reconnaître ou accepter son hôte. Cinq phénomènes de répulsion ont été identifiés : la répulsion-expulsion, la répulsion-masquante, la répulsion-irritation, la répulsion-anti-appétante et la répulsion visuelle. Les composés répulsifs agissent sur le système olfactif et sur le système gustatif mais les modes d'actions restent inconnus. A partir des connaissances actuelles, des hypothèses ont été émises sur les modes d'actions des différents phénomènes de répulsion. Chaque répulsion a été étudiée sur un ou sur nos deux insectes modèles afin de discuter des modes d'actions potentielles et des applications possibles. Chez le moustique *Anopheles gambiae*, le lemongrass et la cannelle se sont montrés répulsif-expulsif. L'effet de la cannelle est du au cinnamaldéhyde. Le coleus et le thym se sont montrés répulsif-irritants. L'effet du thym est du au carvacrol. Chez la mouche blanche *Bemisia tabaci*, l'aframomum et la citronnelle se sont montrés répulsif-expulsif. L'effet de la citronnelle est du au géraniol. Aucun produit n'a été irritant. Le géranium, en particulier, son composé volatil majoritaire : le myrcène, a démontré un effet masquant pour les mouches blanches en présence de tomates. L'utilisation de filet, comme répulsion visuelle, s'est souvent révélé très efficace. L'effet anti-appétant pas toujours facile à évaluer chez les insectes piqueurs n'a pas été étudié chez nos deux insectes modèles. Avec une meilleure connaissance des mécanismes d'actions, de nouvelles définitions des phénomènes de répulsion pourraient être proposées prenant en compte non seulement le comportement de l'insecte mais aussi le mode d'action du composé. La découverte de nouvelles molécules pourrait en être facilitée.
Mots clés : huile essentielle, olfaction, gustation, toxicité, comportement, IPM.

Repellency in insects
- study on the *Anopheles gambiae* mosquito and the *Bemisia tabaci* whitefly -

All insects are looking for a host, animal or vegetable, for nutrition, refuge, mating or oviposition. Research and recognition of the host are at distance by using olfactory and visual signals, and the selection and acceptability of the host are by using touch, smell and taste cues in contact with it. With our knowledge of mechanisms of mating or host seeking, attractive traps were developed by scientists to monitor and control insect populations. Why not using our knowledge about the host seeking to repel insects?

A repellent compound is a compound that prevents an insect to search, track, locate, select, recognize or accept its host. Five repulsive phenomena are identified: expellency-repellency, masking-repellency, irritant-repellency, antifeedant-repellency and visual-repellency. Repellent compounds act on the olfactory system and the gustatory system but the modes of action remain unknown. Based on current knowledge, hypothesis were given about the modes of action of the various phenomena of repellency. Each repulsion was studied here on one or two insect models to discuss about the potential modes of actions and possible field application. With the mosquito *Anopheles gambiae*, lemongrass and cinnamon were shown expellent-repellent. The effect of cinnamon was due to cinnamaldehyde. Coleus and thyme showed irritant-repellent effect. The effect of thyme was due to carvacrol. With the whitefly *Bemisia tabaci*, the aframomum and lemongrass showed expellent-repellent effect. The effect of lemongrass was due to geraniol. No products were irritating. Geranium, in particular its major volatil compound myrcene, showed a masking effect in the presence of tomatoes. The use of nets, as visual repellent, appeared most of the time as a very effective barrier against whiteflies. The anti-feeding effect, not always easy to measure on sucking insect, was not studied here. With a better understanding of action mechanisms, new definitions of repellency phenomena could be proposed taking into account the mode of action of the compound and the behavior of insect. The discovery of new molecules could be facilitated.

Key words: essential oil, olfaction, gustatory, toxicity, behavior, IPM.