



Responses of stemborer *Chilo partellus* to volatiles emitted by maize landraces exposed to signal grass (*Brachiaria brizantha*)

Henlay J.O. Magara, Daniel M. Mutyambai, Midega A.O. Charles, Syprine A. Otieno, Teresia M. Nyaga, Saliou Niassy & Zeyaur R. Khan

To cite this article: Henlay J.O. Magara, Daniel M. Mutyambai, Midega A.O. Charles, Syprine A. Otieno, Teresia M. Nyaga, Saliou Niassy & Zeyaur R. Khan (2020) Responses of stemborer *Chilo partellus* to volatiles emitted by maize landraces exposed to signal grass (*Brachiaria brizantha*), Journal of Plant Interactions, 15:1, 345-357, DOI: [10.1080/17429145.2020.1827056](https://doi.org/10.1080/17429145.2020.1827056)

To link to this article: <https://doi.org/10.1080/17429145.2020.1827056>



© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



Published online: 06 Oct 2020.



Submit your article to this journal [↗](#)



Article views: 445



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 1 View citing articles [↗](#)

Responses of stemborer *Chilo partellus* to volatiles emitted by maize landraces exposed to signal grass (*Brachiaria brizantha*)

Henlay J.O. Magara^{a,b,c}, Daniel M. Mutyambai^{a,d}, Midega A.O. Charles^a, Syprine A. Otieno^b, Teresia M. Nyaga^e, Saliou Niassy^a and Zeyaur R. Khan^{id}^a

^aInternational Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya; ^bSchool of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya; ^cSchool of Agricultural and food Sciences, Jaramogi Oginga Odinga University Science and Technology (JOUST), Bondo, Kenya; ^dDepartment of Biology, South Eastern Kenya University, Kitui, Kenya; ^eDepartment of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

ABSTRACT

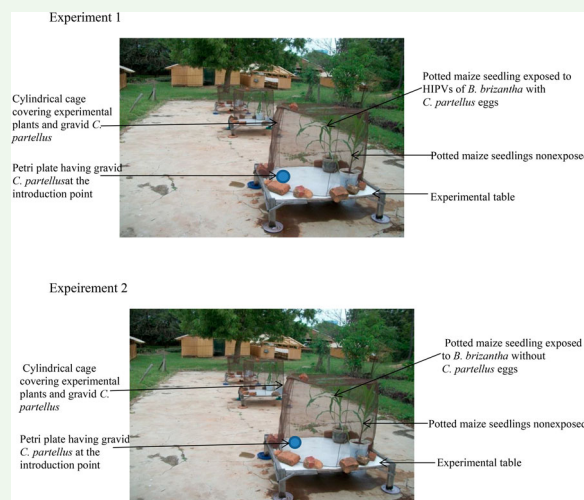
This study sought to evaluate the oviposition responses of *Chilo partellus* (Lepidoptera: Crambidae) on maize plants exposed to *Brachiaria brizantha* Stapf following oviposition by *C. partellus* and nonexposed maize. Two Kenyan maize landraces (Jowi and Nyamula), Latin America landrace (Cuba 91) and WH505 (hybrid variety) (control) were studied. The result demonstrated that *C. partellus* deposited a significantly lower number of eggs and egg batches on Nyamula, Jowi and Cuba 91 maize plants exposed to *B. brizantha* with *C. partellus* eggs compared to nonexposed ones and the exposed hybrid variety. This was because they emitted electrophysiologically active compounds such as (*E*)-4, 8-dimethyl-1, 3, 7-nonatriene, decanal, (*E*)-caryophyllene, linalool, linalool (plus nananal), *E*- β -fernesene, methyl salicylate and (3*E*, 7*E*)-4, 8, 12-trimethyl-1, 3, 7, 11-tri-decatetraene that deterred *C. partellus* from ovipositing more eggs on these plants. Therefore, herbivore-induced plant volatiles (HIPVs) of *B. brizantha* can be employed to protect the maize crop against *C. partellus*.

ARTICLE HISTORY

Received 7 May 2020
Accepted 16 September 2020

KEYWORDS

Brachiaria brizantha; *Chilo partellus*; herbivore induced plant volatiles; inducing defense; insect oviposition; *Zea mays*





1. Introduction

Maize (*Zea mays* L.), is a popular food crop in many households in Africa (Khan et al. 2010). It is a rich source of carbohydrates, oils and caloric energy. Maize also serves as a source of raw materials such as flour, animal feed and cooking oil for various industries (Tajamul et al. 2016). The farming of this crop in sub-Saharan Africa (SSA) is however constrained by environmental factors such as climate change and living factors such as pests. One of the economic pests damaging maize is the lepidopteran *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) (Seshu Reddy 1998; Khan et al. 2007; Tamiru et al. 2011). This pest is widely distributed in the tropical, warm temperate zones of Asia and the Mediterranean

region (Yonow et al. 2017). It is native to Asia (Harris 1990; Kfir 2000) and is presently well established in Kenya (Guofa et al. 2001; Sylvain et al. 2015). The larva of *C. partellus* is the damaging stage which makes tunnels into maize leaves, tassels and stems causing yield loss of up to 80% and therefore threatens food security (Kfir et al. 2002; Khan et al. 2007, 2010). *Chilo partellus* has also been reported to cause damage on other crops such as sorghum, sugar cane, forage crops and wild grasses (Harris 1990; Khan et al. 1997; Mutyambai et al. 2015a).

Due to the economic importance and widely known losses to maize crop, spraying and dusting of synthetic insecticides are considered the best method to manage *C. partellus* (Pest

CONTACT Henlay J.O. Magara  mhenlay@gmail.com or hmagara@icipe.org  International Centre for Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya; School of Pure and Applied Sciences, Kenyatta University, P.O. Box 43844, Nairobi, Kenya; School of Agricultural Sciences and Food, Jaramogi Oginga Odinga University Science and Technology (JOUST), P.O. BOX 210, Bondo, Kenya

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Control Products Board 2019). However, repeated use of these insecticides has resulted in the development of resistance in *C. partellus* (Bruce et al. 2010). Moreover, the use of insecticides to control *C. partellus* has proved to be too expensive and unsustainable to smallholder farmers who are resource constrained (Pest Control Products Board 2019). This has prompted a need for the search of alternative strategies which are sustainable and eco-friendly to manage this pest. One of the strategies is by exploiting herbivore-induced plant volatiles (HIPVs). The HIPVs are natural protective chemical compounds produced by damaged plants following oviposition or feeding by a pest which then primes neighboring undamaged plants, leading to warding off of the damaging insect (Bruce et al. 2010; Pickett et al. 2014; Magara et al. 2015; Khan et al. 2016; Tolosa et al. 2019). This strategy has been applied successfully in push-pull technology to manage stemborers in maize (Khan et al. 2001; Cook et al. 2007; Heil 2008).

Plant to plant communication is known to be a mechanism of pest control through herbivore-induced plant volatiles (HIPVs) (Khan et al. 2001; Cook et al. 2007; Heil 2008; Tamiru et al. 2012; Magara et al. 2015; Mutyambai et al. 2016). Studies have shown that *B. brizantha* is preferred to maize for oviposition by gravid *C. partellus* (Midega et al. 2011; Cheruiyot et al. 2018). This is as a result of *B. Brizantha* leaves emitting more green-leaf volatiles signals ((Z)-3-hexenyl acetate) which are the responsible cues for attracting gravid females of *C. partellus* to the grass to deposit eggs compared to maize (Bruce et al. 2010; Magara et al. 2015; Tolosa et al. 2019). Additionally, the *B. brizantha* supports minimal feeding and survival of *C. partellus* larvae (Midega et al. 2011). Moreover, this grass has a complex reaction to *C. partellus* herbivory that comprise HIPVs-mediated multi-trophic interactions with predators and parasitoids (Bruce et al. 2010). *Brachiaria brizantha* grass achieves this by inducing or priming neighboring plants that are undamaged to emit defensive signals against *C. partellus*, hence its common name signal grass. The emitted HIPVs also repel and inhibit further colonization of the plant by *C. partellus* female moths from depositing their eggs on the signal plants (Bruce et al. 2010; Kessler and Kalske 2018). Furthermore, some HIPVs are taken up by neighboring undamaged *B. brizantha* plants hence tailoring them as a part of their defence against attack from stemborers (Dicke et al. 2009; Bruce et al. 2010; Khan et al. 2016; Kessler and Kalske 2018).

Several studies on plant to plant signaling indicated that exposure to HIPVs can induce or prime undamaged plants to attain faster defence responses upon subsequent pest attack (Heil and Silva Bueno 2007; Himanen et al. 2010). For instance, research has shown that undamaged maize landraces can be induced to emit volatile that recruits natural enemies of *C. partellus* when exposed to HIPVs from damaged maize landraces or grass such signal grass *Brachiaria brizantha* (A. Rich.) Stapf and Molasses grass *Melinis minutiflora* (P. Beauv) (Bruce et al. 2010; Magara et al. 2015; Mutyambai et al. 2015a; Tolosa et al. 2019). Moreover, studies have demonstrated less oviposition preference by *C. partellus* on maize landraces exposed to HIPVs of other maize landraces with *C. partellus* eggs compared to nonexposed maize landraces (Tamiru et al. 2012; Mutyambai et al. 2016). Intra-specific genetic variation in HIPVs composition and HIPVs release rates does, however, exist in these maize

varieties (Tamiru et al. 2012; Mutyambai et al. 2015b). These variations in HIPVs blend impacts host choice by *C. partellus* within maize plant species. However, no research has been carried out to evaluate the response of gravid *C. partellus* to volatiles emitted by local maize varieties exposed to *B. brizantha* with *C. partellus* eggs. Because of this, our study aimed at determining the responses of *C. partellus* to volatiles emitted by maize landraces exposed to HIPVs of neighboring *B. Brizantha* grass following oviposition by *C. partellus* as a strategy to defend itself against *C. partellus* damage. This paper provides data on the inducement of African maize landraces and a South American landrace by HIPVs of *B. brizantha* following oviposition and the possible role of this grass in *C. partellus* management. This information will contribute to the understanding and exploitation of *B. brizantha* as a trap crop in the innovative push-pull system.

2. Materials and methods

2.1. Plants

Two African maize landraces: Jowi and Nyamula were obtained from local farmers in Mbita sub-county in Kenya while Cuba 91 (Latin America maize landrace) was procured from the International Maize and Wheat Improvement Centre (CIMMYT in Nairobi office, Kenya). Jowi, Nyamula, and Cuba 91 maize varieties and *B. brizantha* grass were selected because they have traits of being induced and primed by HIPVs from damaged neighboring plants. WH505 (a hybrid maize variety) (a control) was acquired from Western Seed Company Ltd (a licensed commercial seed supplier in Kenya). *Brachiaria brizantha* splits were obtained from International Centre of Insect Physiology and Ecology Thomas Odhiambo campus (ITOC). Maize seeds and *B. brizantha* splits were planted individually and allowed to grow in plastic containers filled with fertilized soil in an insect-proof screen house at ITOC, Mbita Point research station (0°25'S, 34° 12'E; 1200 m above sea level), in Homabay western Kenya. Plants for the experiment were all grown under environmental conditions such as 25 °C, 65% RH and 12L: 12D. The maize and *B. brizantha* plants were utilized for the experiments when they were 28 days old.

2.2. Insects

Chilo partellus trapped from the field were raised on a semi-synthetic diet made from sorghum leaf powder (Ochieng et al. 1985). These moths were maintained at the insectary at ITOC (24 ± 3 °C, 70 ± 5% RH and 12L: 12D). After every three months, the mass-reared *C. partellus* at *icipe* was mixed with field trapped insects to avoid genetic depletion and hence maintaining the initial behavioral traits of the *C. partellus* species. To obtain gravid *C. partellus* females, the modified protocol of Calatayud et al. (2007) was used. One-day-old *C. partellus* females were released into a mosquito-net cage (40×40×63 cm) at onset of the scotophase. The first females started to call 6 h later. One-day-old *C. partellus* males were introduced 1 h thereafter. During the ensuing 1-hour period, mating pairs were taken out of the cage and placed individually in plastic cylinders (8 cm high × 5 cm in diameter). After copulation, the male *C. partellus* were separated from female *C. partellus* and a

group of seven naïve gravid females were used the following night in all experiments. After each experiment, females were dissected to check for the presence of spermatophores in the bursa copulatrix, which in Lepidoptera indicates successful mating (Lum 1979). Only females bearing spermatophores were considered in the results.

2.3. Entrainment of volatiles

Volatile organic compounds from experimental plants that included potted *B. brizantha* with *C. partellus* eggs, intact potted maize seedlings exposed to *B. brizantha* with *C. partellus* eggs, *B. brizantha* without *C. partellus* eggs and nonexposed maize for use in subsequent electrophysiological and chemical analyses were collected by headspace sampling (Agelopoulos et al. 1999; Birkett et al. 2003; Magara et al. 2015; Tolosa et al. 2019). Precaution was taken not to damage the plants while setting up the grass and the maize plants for entrainment to avoid possible release of volatiles in response to mechanical damage of the plant. Before volatile collection, 28 days old *B. brizantha* were put in the oviposition cages measuring 80cm high × 40cm in length × 40 cm in width into which 20 newly mated female *C. partellus* moths were introduced and left throughout the night to deposit eggs on these plants. Each cage was standing in lids containing clean water to prevent predator arthropods such as brown ants and spiders from killing the *C. partellus* moths and also from attacking the eggs deposited by these moths on the grass. At the same time, control *B. brizantha* was put inside similar cages, but without *C. partellus* moths. The following day, *B. brizantha* grasses with oviposited eggs were removed from the cages; some were spared for entrainment while others were taken back into the screen house whereby they were arranged into two rows of 1 m apart. Each row comprised of five potted *B. brizantha* plants hence making a total of 10 plants in the two rows. One row of maize comprising of five potted maize plants was exposed to two rows of signal grass for three days (Figure 1a). The above set up was repeated for another 5 maize plants but with nonexposed *B. brizantha* to gravid *C. partellus* moths for three days (Figure 1b), lastly another set up comprised of nonexposed (clean) maize (Figure 1c).

Volatile compounds from the maize plants were entrained after the third day of exposure, starting at the last two hour of photo- phase, for 48 h. Leaves of maize seedlings (45 grams) exposed to *B. brizantha* with or without eggs and nonexposed (clean) ones were enclosed in polyethyleneterephthalate (PET) bags (volume 3.2 L, ~12.5 mm thickness) sterilized in an oven at 150 °C before use and fitted with Swagelock

inlet and outlet ports. Charcoal-filtered air was pumped at a flow rate of 600 mL per minute through the inlet port. Volatiles were trapped on Porapak Q (0.05 g, 60/80 mesh; Supelco Inc. Bellefonte, PA, USA) filters inserted in the outlet port through which air was sucked at a rate of 450 mL per minute. Pumping rates were controlled using flow meters on entrainment kits to make sure more purified air is pumped in than drawn out to avoid the influx of unfiltered air from outside. After collection, volatiles was eluted from the traps using 0.5 mL dichloromethane and used immediately in bioassays or stored at −20°C for later use. Each experiment was replicated three times. The above procedure was repeated for *B. brizantha* with eggs.

2.4. Coupled GC-mass spectrometry (GC-MS) analysis

Aliquots of attractive headspace samples were analysed using a Hewlett-Packard 5890GC machine (Agilent Technologies) on a capillary Gas Chromatography HP-1 column (50, 0.32 mm internal diameter, 0.52 µm film thickness) directly coupled to a mass spectrometer (VG Autospec; Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionization was performed by electron impact (70 eV at 250 °C). Four µL of headspace sample was injected into the injector port of the GC instrument. The oven temperature was maintained at 30°C for 5 min, and then programmed at 5 °C per minute to 250 °C. The carrier gas was hydrogen. Tentative identifications were made by comparison of spectra with mass spectral databases (Nist 2005). Tentative identifications of the compounds were confirmed through co-injection with authentic standards.

2.5. Oviposition bioassay

Oviposition responses of *C. partellus* to maize seedlings of Jowi, Nyamula, Cuba 91 and WH505 exposed to *B. brizantha* bearing *C. partellus* eggs versus non-exposed ones and maize seedlings exposed to *B. brizantha* without *C. partellus* eggs versus non-exposed ones were observed in a cage oviposition bioassay. Two sets of experiments were carried out. The first experimental set comprised of maize seedlings exposed to *B. brizantha* with *C. partellus* eggs versus non-exposed ones. During this experiment, two 28 d old potted maize plants of each variety at a time, one nonexposed (no prior exposure to HIPVs of *B. brizantha*) and one exposed to HIPVs of *B. brizantha* with *C. partellus* eggs were removed from the screen house. Then these plants were taken to an open oviposition bioassay field where they were placed side by side (at a distance of 40 cm away from

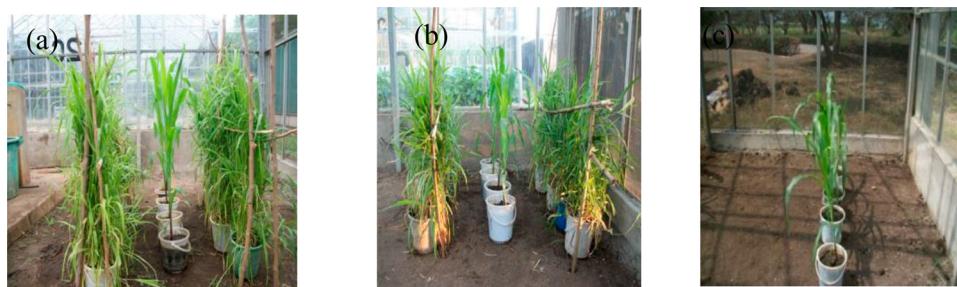


Figure 1. Experimental design (a) Potted maize plants exposed to potted *B. brizantha* with eggs (b) Potted maize plants exposed to potted *B. brizantha* without eggs (c) Potted nonexposed maize plants

each other) on a clean oviposition table measuring 1.2 m×1.2 m×0.3 m without touching each other. This was to prevent any communication through contact. A pad of cotton wool was moistened with clean water in a Petri plate (10 cm diameter) and was placed on a centrally marked point on the table in between the two potted maize for the moths to drink. Seven gravid females of *C. partellus* in a Petri plate (20 cm diameter) were introduced at a marked circular point on the table. Large cylindrical oviposition cages (Diameter = 100 cm and height = 100 cm) made of fine wire mesh (400 p~m) netting were then carefully introduced to cover the two maize seedlings to prevent the gravid *C. partellus* from escaping from the cage. The precaution was taken to prevent breaking of the maize leaves which could lead to the test plants producing volatiles due to mechanical damage. To free the moths from the Petri plate, the cage was raised up slightly at the point where the Petri plate containing the moths was. The lid of the Petri plate was removed and then the cage was dropped back immediately to prevent the moths from escaping. Then the cage was fastened and supported by debris onto the table to prevent it from being blown away by the strong winds that normally blow late in the evening at ITOC. Each experimental set up was replicated three times in each trial and trials were repeated six times ($n = 18$). The tables for the experiment were placed at a distance of 4 m from each other and 2m from the edge to prevent edge effect (Figure 2). The above set up was repeated for the second experiment that comprised of maize seedlings exposed to *B. brizantha* without *C. partellus* eggs versus nonexposed maize seedlings. Like in the first experiment, each experimental set up was replicated three times in each trial and trials were repeated six times ($n = 18$). At the same time tables for the experiment

were placed at a distance 4 m from each other and 2m from the edge to prevent edge effect.

The experiments were carried out during the last two hours of photophase when there was more emission of volatiles by the test plants (Bruce et al. 2010; Tamiru et al. 2012; Magara et al. 2015). The gravid female moths were allowed to make a choice on which plant in the cage to oviposit overnight. At 7.00 am the following day, plants were removed and the egg batches cut from both plants and placed into Petri dishes labeled with the variety, treatment (exposed or nonexposed), the date of harvesting the eggs and the replication. The egg batches were counted and recorded per maize in each replicate. Then they were taken into the laboratory where they were kept in the locker for 3–5 days depending on prevailing weather (12L: 12D photoperiod, 24 ± 2 °C, $70 \pm 5\%$ RH). The aim for this was to allow the heads of the larvae to turn black for easy counting. The number of eggs on each plant was counted under a light microscope at $Mg \times 6.5$ and recorded.

2.6. The Y-tube olfactometer bioassay (Two-Choice test)

Responses of *C. partellus* to plant produced volatiles and authentic standards were tested in a two-arm glass olfactometer as described in Calatayud et al. (2014). The Y-tube olfactometer had 2.4 cm internal diameter, 3.2 cm external diameter, 20 cm long arm length and 17 cm short arms' length, it had a 75° angle at the Y-junction. Air was drawn through the two arms towards the long arm at 300 ml per minute. The experiment was conducted in a dark room at 25 ± 1 °C, $60 \pm 5\%$ RH, and under four 16- Watt cool white

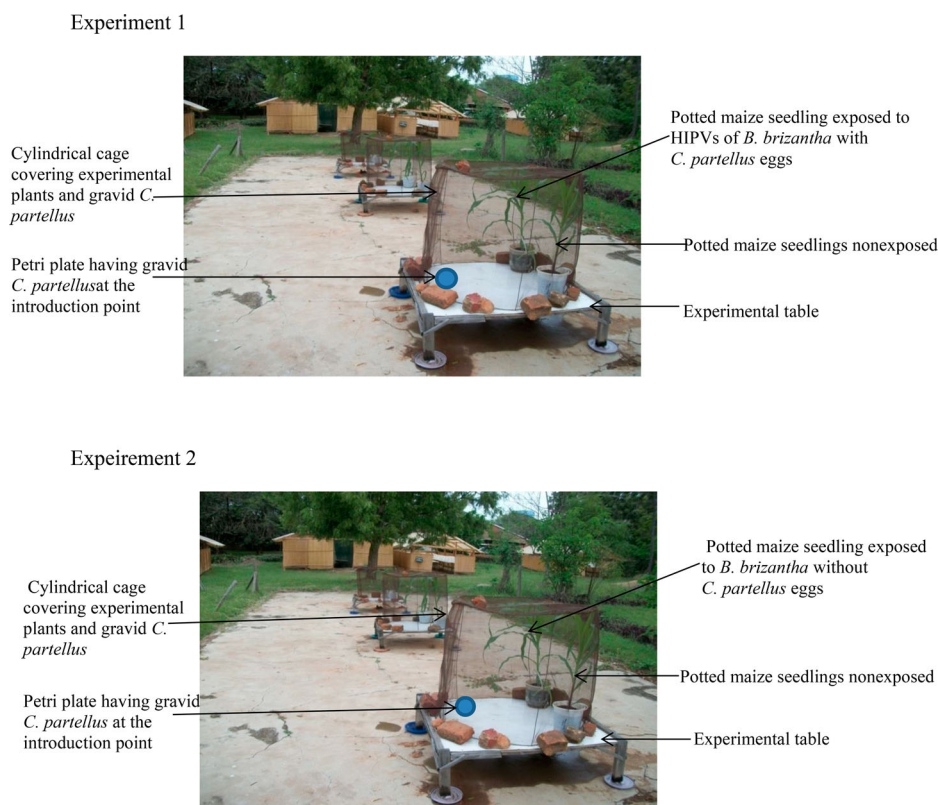


Figure 2. Schematic diagram of the experimental design for oviposition bioassay. Experiment (1) comprised of maize seedlings exposed to *B. brizantha* with *C. partellus* eggs versus non-exposed ones. Experiment (2) comprised of maize seedlings exposed to *B. brizantha* without *C. partellus* eggs versus non-exposed ones. Each experimental set was replicated three times.

Table 1. Responses of *Chilo partellus* to volatiles from maize exposed to *B. brizantha* ($n = 18$) with *C. partellus* eggs and nonexposed maize in an oviposition cage bioassay. Mean percentage of eggs (\pm SE) and egg batches (\pm SE) oviposited by gravid *C. partellus* on Jowi, Nyamula (African maize landraces), Cuba 91 (Latin America maize landrace), and WH505 (Commercial Hybrid).

Maize variety	Mean number of Eggs (\pm SE)		Mean number of egg batches (\pm SE)	
	Exposed to <i>B. brizantha</i> with eggs	Nonexposed	Exposed to <i>B. brizantha</i> with eggs	Nonexposed
Jowi	47.55 (\pm 17.3) a	122.7 (\pm 21.03) b	1.5 (\pm 0.34) a	2.9 (\pm 0.42) b
Cuba 91	52.7 (\pm 17.19) a	93.0 (\pm 17.39) b	1.8 (\pm 0.38) a	3.2 (\pm 0.61) b
Nyamula	54.6 (\pm 15.85) a	172.9 (\pm 40.26) b	1.6 (\pm 0.40) a	4.7 (\pm 0.91) b
WH505	96.4 (\pm 25.35) a	104.9 (\pm 25.20) a	2.7 (\pm 0.61) a	2.7 (\pm 0.54) a

Means followed by dissimilar letter, within a row, are significantly different from each other ($P < 0.05$). For each maize variety, $n = 18$.

lights at the top to ensure even distribution of light. The end tubes of the Y-junction of the olfactometer were connected to two Wheaton Micro Kit® adapters made of glass, having, attached 4 ml glass-vials, each containing 4×25 mm filter paper piece. A volume of 10 μ l of the volatile component from the maize exposed to *B. brizantha* with eggs, diluted in Dichloromethane (DCM) or nonexposed maize volatile diluted in DCM was applied at the control side, using a micropipette (Drummond ‘microcap’, Drummond Scientific Company, Broomal, PA, USA). This was then applied to the filter paper pieces 30 min before the first gravid stemborer was released, in order to allow the odor to reach a constant release rate. The airflow was initially purified by the passage through wash bottles filled with charcoal pellets and was then led into the vials containing volatiles of maize exposed to *B. brizantha* with eggs loaded in DCM and nonexposed maize volatile loaded in DCM as control. The test solutions were replaced after each bioassay (Magara et al. 2015). Twelve gravid *C. partellus* were tested in each trial and each trial was repeated three times ($n = 36$). Each *C. partellus* was allowed two minutes to acclimatize to the new environment and then given 10 min to respond and walk towards either the known attractant (Nonexposed maize volatiles) or repellent (exposed maize volatile). The same procedure was repeated using volatile compounds from maize exposed to *B. brizantha* without eggs as treatment and nonexposed maize volatiles as control. Lastly, the above procedure was repeated using specific volatile compounds to show how *C. partellus* responded to them *vis a vis* clean air as control. The following choices were tested: (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) vs Clean air, methyl salicylate vs clean air, (*E*)-caryophyllene vs clean air, decanal vs Clean air, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) vs Clean air, (*E*)- β -farnesene vs clean air, linalool+nananal vs clean air and linalool vs clean air.

A choice was recorded when an insect passed the intersection into either of the two arms and remained motionless there for 20 s. Those that made no choice were also recorded. Odor sources connections to the chambers were reversed after every five insects to minimize any position bias. Chambers were cleaned and rinsed thoroughly in water after each observation. In each case, the number of the gravid *C. partellus* that made a distinct choice was calculated.

2.7. Statistical analyses

Two-sample (unpaired) Student’s *t*-tests were used to analyse differences between exposed and non-exposed maize plants of the different varieties with regard to the number of eggs laid on each plant and number of egg batches after the data were $\log(x + 1)$ transformed. The results of Y-tube olfactory

bioassays were analyzed with an X^2 test to test the null hypothesis that there was equal preference of gravid *C. partellus* to volatiles of maize plants that are either exposed or nonexposed and the identified physiologically bioactive compounds from the maize landraces. The 95% confidence intervals were estimated using Agrest and Coull intervals. Individuals that did not make a choice were left out from the statistical analysis. Statistical analyses were done using R (version 4.00) (Development Core Team. 2020).

3. Results

3.1. Oviposition responses of *Chilo partellus* to headspace samples of volatiles from maize exposed to *Brachiaria brizantha* with and without eggs

Cage oviposition preference bioassay showed that the African landrace maize varieties (Jowi and Nyamula) and the Latin America landrace maize plant (Cuba 91) were significantly less preferred for oviposition following exposure to *B. brizantha* with *C. partellus* eggs compared to maize plants exposed to *B. brizantha* without eggs (Jowi, d.f. = 34, $t = -3.98$, $P = 0.0003$; Cuba 91, d.f. = 34, $t = -3.16$, $P = 0.0033$; Nyamula, d.f. = 34, $t = -3.42$, $P = 0.0017$) (Table 1). Similarly, the number of egg batches deposited by gravid female *C. partellus* on the plants exposed to *B. brizantha* with *C. partellus* eggs were significantly lower compared to those deposited on nonexposed plants (Jowi, d.f. = 34, $t = -2.92$, $P = 0.0061$; Cuba 91, d.f. = 34, $t = -2.45$, $P = 0.0195$; Nyamula, d.f. = 34, $t = -3.72$, $P = 0.0007$) (Table 1). Conversely, the bioassays showed no significant difference in mean number of eggs oviposited on plants exposed to *B. brizantha* without *C. partellus* eggs and those oviposited on nonexposed plants (Jowi, d.f. = 34; $t = -1.70$; $P = 0.0975$; Cuba 91, d.f. = 34; $t = 1.11$; $P = 0.2733$; Nyamula, d.f. = 34; $t = 0.34$; $P = 0.7374$) (Table 2). The same oviposition response was seen in the number of egg batches deposited by gravid female *C. partellus* on plants exposed to *B. brizantha* without *C. partellus* eggs (Jowi, d.f. = 34, $t = -1.03$, $P = 0.3081$; Cuba 91, d.f. = 34, $t = -0.02$, $P = 0.9834$; Nyamula, d.f. = 34, $t = -0.31$, $P = 0.7556$) compared to nonexposed plants (Table 2).

A contrast was seen when a standard commercial hybrid variety (WH505) was used. Cage oviposition preference bioassay revealed that for this variety, plants exposed to *B. brizantha* with *C. partellus* eggs showed no significant difference in the number of eggs oviposited compared to the nonexposed plants (WH505, d.f. = 34, $t = -0.73$, $P = 0.4694$) (Table 1). Notably, the same behavioral response was exhibited in the number of egg batches oviposited by the gravid female *C. partellus* on WH505 seedlings exposed to *B. brizantha* with *C. partellus* eggs relative to those oviposited on the non-exposed plants (WH505, d.f. = 34; $t = -0.46$;

Table 2. Responses of *Chilo partellus* to volatiles from maize exposed to *B. brizantha* ($n = 18$) without *C. partellus* eggs and nonexposed maize in an oviposition cage bioassay. Mean percentage of eggs (\pm SE) and egg batches (\pm SE) oviposited by *C. partellus* gravid female on Jowi, Nyamula (African maize landraces), Cuba 91 (Latin America maize landrace), and WH505 (Commercial Hybrid).

Maize variety	Mean (\pm SE) percentage of eggs		Mean number of egg batches (\pm SE)	
	Exposed to <i>B. brizantha</i> without eggs	Nonexposed	Exposed to <i>B. brizantha</i> without eggs	Nonexposed
Jowi	91.6 (\pm 22.96) a	98.6 (\pm 11.24) a	2.2 (\pm 0.40) a	2.4 (\pm 0.22) a
Cuba 91	89.2 (\pm 12.36) a	92.6 (\pm 22.87) a	2.4 (\pm 0.23) a	2.7 (\pm 0.44) a
Nyamula	111.8 (\pm 12.77) a	116.8 (\pm 21.60) a	2.7 (\pm 0.29) a	2.9 (\pm 0.42) a
WH505	101.3 (\pm 20.92) a	93.9 (\pm 18.28) a	2.8 (\pm 0.43) a	3.0 (\pm 0.51) a

Means followed by similar letter, within a row, are not significantly different from each other ($P < 0.05$). For each maize variety, $n = 18$.

$P = 0.6496$) (Table 1). Moreover, there were no significant differences between the number of eggs oviposited on maize plants exposed to *B. brizantha* without *C. partellus* eggs and those ones oviposited on the non-exposed plants (WH505, d.f. = 34, $t = -0.28$, $P = 0.7797$) (Table 2). A similar oviposition response was observed in the number of egg batches deposited by the gravid female *C. partellus* on WH505 exposed to *B. brizantha* without *C. partellus* eggs and those oviposited on nonexposed plants (WH505 d.f. = 34, $t = -0.20$, $P = 0.8435$) (Table 2).

3.2. Behavioral responses of gravid *Chilo partellus* to headspace samples of volatiles from maize exposed to *Brachiaria brizantha* with and without eggs in Y-tube olfactometer

In Y-tube olfactometer bioassays, the gravid *C. partellus* moths were significantly less attracted to the headspace of sample volatiles trapped from *B. brizantha* HIPVs-exposed maize landraces, Jowi, Cuba 91 and Nyamula, compared to

nonexposed maize landraces (Jowi, d.f. = 1, $X^2 = 14.69$, $P = 0.0001$; Cuba 91, d.f. = 1, $X^2 = 10.03$, $P = 0.0015$; Nyamula, d.f. = 1, $X^2 = 4.69$, $P = 0.0303$) (Figure 2). In contrast, there was no significant difference in the number of *C. partellus* that chose the arm with volatiles collected from a hybrid variety, WH505 exposed to *B. brizantha* with *C. partellus* eggs and nonexposed (WH505, d.f. = 1, $X^2 = 0.03$, $P = 0.8676$; Figure 3).

Besides, there were no significant differences between the number of gravid females that chose the Y-tube olfactometer arm with volatiles from maize plants exposed to *B. brizantha* without *C. partellus* eggs and the arm with the nonexposed plants' volatiles (Jowi, d.f. = 1, $X^2 = 3.36$, $P = 0.0668$; Cuba 91, d.f. = 1, $X^2 = 2.25$, $P = 0.1336$; Nyamula, d.f. = 1, $X^2 = 0.69$, $P = 0.4047$) (Figure 2). A similar behavioral response of no significant differences was also observed between the number of the gravid *C. partellus* on maize plants exposed to *B. brizantha* without *C. partellus* eggs and the one on the nonexposed plants (WH505, d.f. = 1, $X^2 = 0.25$, $P = 0.6171$) (Figure 4).

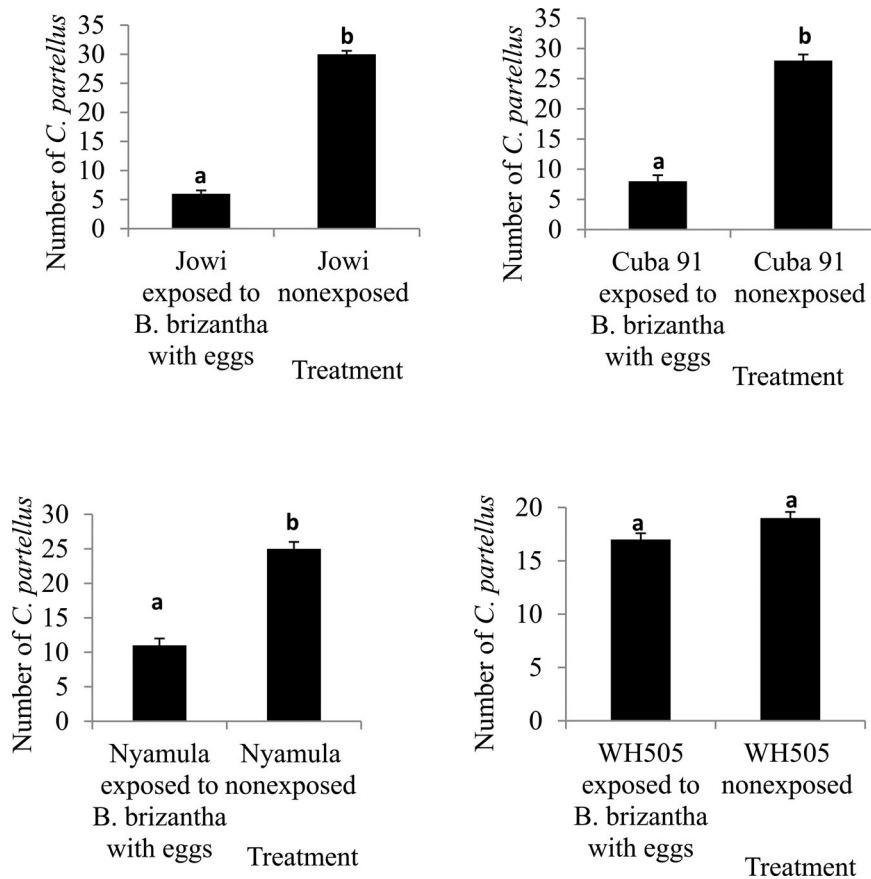


Figure 3. Y-tube olfactometer results of number of *Chilo partellus* choosing to walk towards headspace samples of volatiles from maize plants exposed to *Brachiaria brizantha* with eggs against nonexposed maize ($n = 36$). Number of *C. partellus* marked by different letters within a graph is significantly different ($P < 0.05$).

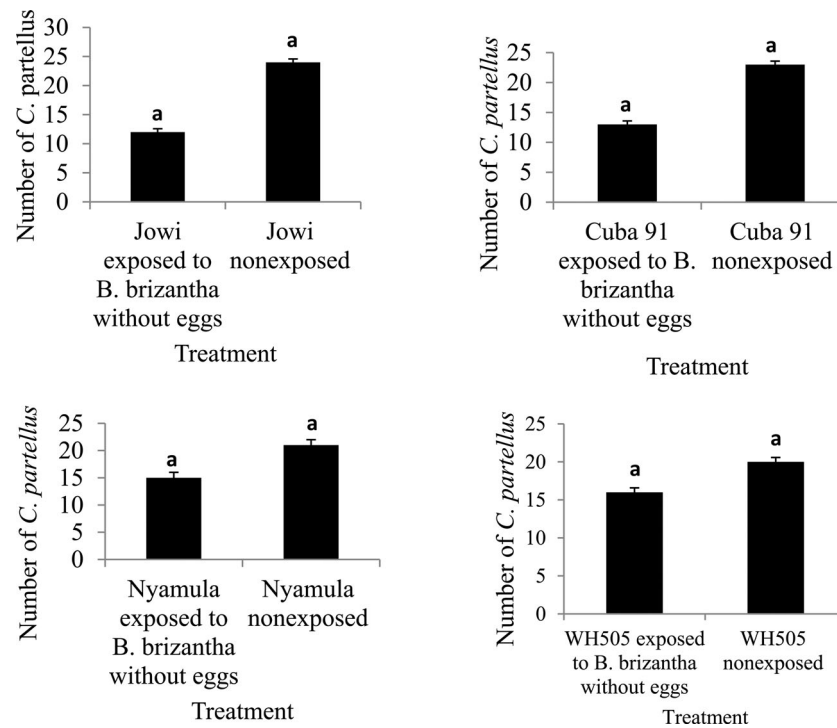


Figure 4. Y-tube olfactometer results of number of *Chilo partellus* choosing to walk towards headspace samples of volatiles from maize exposed to *Brachiaria brizantha* without eggs against nonexposed maize ($n = 36$). Numbers of *C. partellus* marked by same letters within a graph are not significantly different ($P < 0.05$).

3.3. Identification of physiological active herbivore induced plant volatiles (HIPVs)

The Gas chromatography (GC) analysis of the volatile samples collected from *B. brizantha* HIPVs-exposed and nonexposed and *B. brizantha* without eggs-exposed and nonexposed African maize landraces (Jowi and Nyamula) and landrace from Latin America (Cuba 91) lines revealed differences in volatile profile (Figures 5–7). There was strong induction of physiologically active volatile compounds that included (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), linalool+nananal, methyl salicylate, decanal, (*E*)-caryophyllene, (*E*)- β -farnesene and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and linalool in *B. brizantha* HIPVs-induced maize landraces (Figures 5–7). In contrast, *B. brizantha* HIPVs-exposed and nonexposed and *B. brizantha* without eggs-exposed and nonexposed WH505 (hybrid maize) were alike (Figure 8).

The result obtained from behavioral responses of gravid *C. partellus* to volatile compounds from maize exposed to *B. brizantha* with eggs using Y-tube olfactometer is presented in Table 3. The result show that *C. partellus* was less attracted to the arm containing individual volatile chemical compounds compared to the arm containing clean air ((*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), d.f. = 1, $X^2 = 17.63$, $P = 0.0001$; decanal, d.f. = 1, $X^2 = 13.89$, $P = 0.0002$; methyl salicylate, d.f. = 1, $X^2 = 12.03$, $P = 0.0005$; (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), d.f. = 1, $X^2 = 10.32$, $P = 0.0013$; (*E*)-caryophyllene, d.f. = 1, $X^2 = 9.63$, $P = 0.0019$; (*E*)- β -farnesene, d.f. = 1, $X^2 = 9.63$, $P = 0.0019$; linalool + nananal, d.f. = 1, $X^2 = 0.30$, $P = 0.5839$). The only exception was linalool (d.f. = 1, $X^2 = 0.14$, $P > 0.0140$) whereby more gravid stemborers were attracted to the arm containing linalool (plant volatile) compared to clean air. Based on the repellence of the respective chemical compounds against *C. partellus* stemborer, the order of repellence can be arranged as follows (*E*)-4,8-dimethyl-1,3,7-nonatriene

(DMNT) > decanal > methyl salicylate > (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) > (*E*)-caryophyllene > (*E*)- β -farnesene > linalool+nananal > linalool, Gas chromatography linked mass spectrometry analysis of volatiles from *B. brizantha* with eggs showed different bioactive HIPVs that provide direct and indirect defences consisting of 6-methyl-5-hepten-2-one, Z3HA, (*E*)-ocimene, linalool + nonanal, linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), decanal, methyl salicylate, (*E*)-caryophyllene, (*E*)- β -farnesene and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (Figure 9). Egg deposition by *C. partellus* on the *B. brizantha* studied was thus found to prime maize landraces (Jowi, Nyamula and Cuba 91) to emit volatile compounds which repelled the pest.

4. Discussion

The present study revealed that when African maize landraces Jowi and Nyamula and a Latin America landrace (Cuba 91) were exposed to *B. brizantha* with *C. partellus* eggs, the HIPVs made the maize plants to elicit enhanced direct and indirect defence responses against *C. partellus* pest through the plant to plant communication. Since the *B. brizantha* with the eggs and the maize landrace were potted, there was no direct link between them in the soil. Therefore the HIPVs from the grass rather than the microbiota and root exudates are involved in the plant to plant communication noted here. In oviposition bioassays, there was a lower number of *C. partellus* eggs oviposited on these exposed maize plants compared to nonexposed Jowi, Nyamula and Cuba 91. Moreover, the numbers of egg batches deposited on these maize varieties exposed to this grass with *C. Partellus* eggs were also significantly lower. The decreased oviposition choice for the exposed maize landraces could be associated with the observed changes in volatile emission profiles of the maize plants exposed to

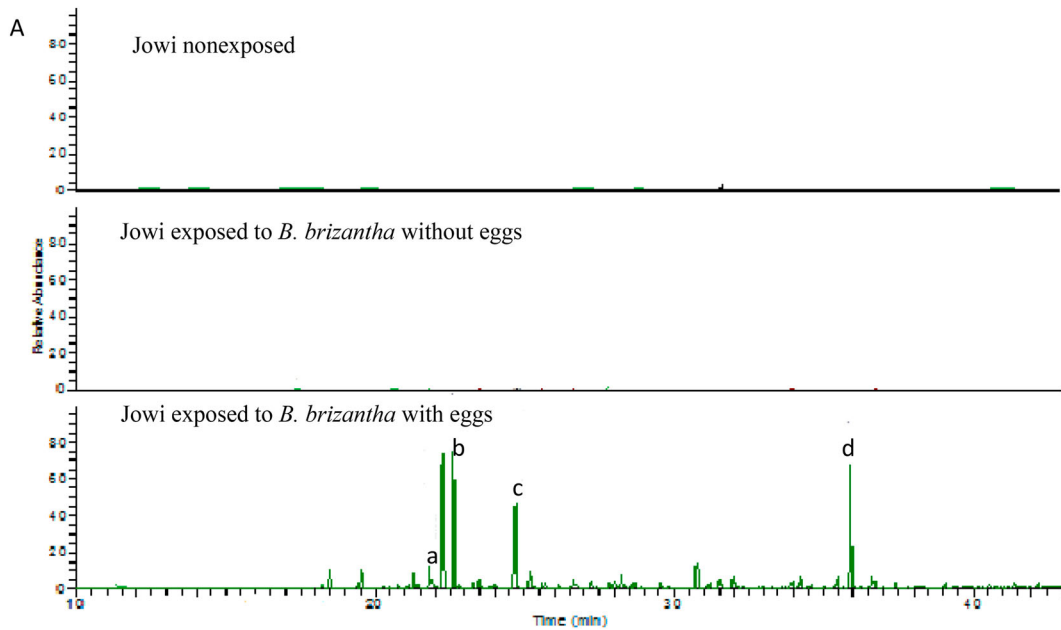


Figure 5. GC profiles of headspace volatiles from representative Jowi maize landrace line exposed to *B. brizantha* with and without *C. partellus* eggs and nonexposed maize: The identities of GC-MS active compounds are as follows: (a) linalool + nonanal, (b) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT) (c) methyl salicylate, (d) (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

B. brizantha with eggs. The choice of maize plants by ovipositing *C. partellus* is guided by intrinsic characteristics of the plants, especially plant volatile chemistry and central processing of olfactory signals by the *C. partellus* (Konstantopoulou et al. 2002; Bruce et al. 2005; Tolosa et al. 2019). These findings suggest that for *B. brizantha* to be able to induce and/or prime the maize landraces; the *C. partellus* must deposit its eggs on this grass. Then the eggs will trigger the *B. brizantha* to emit HIPVs that prime these landraces maize which in return deter the gravid *C. partellus* from colonizing and laying more eggs and egg batches on them.

In contrast, the number of eggs and egg batches deposited on the W505, a hybrid maize variety exposed to *B. brizantha* with *C. Partellus* and the ones deposited on nonexposed

W505 hybrid maize variety did not differ. This can be attributed to the effects of breeding on maize defense responses. In most cases, breeding is done for traits that are considered to be important such as high yield, rapid growth and adaptability to environmental conditions at the expense of other traits such as defense responses. Therefore breeding can be thought to be downregulating HIPVs-defense inducibility in hybrid maize variety. This trait, however, can be boosted by incorporating it into the hybrid maize varieties during breeding to enable the hybrid maize variety to protect itself against *C. partellus*. This observation is consistent with the previous studies (Tamiru et al. 2007; Bruce et al. 2010; Mutyambai et al. 2016). On the other hand, there was no significant difference in the number of eggs oviposited and the number of egg batches deposited on African maize landraces and a landrace

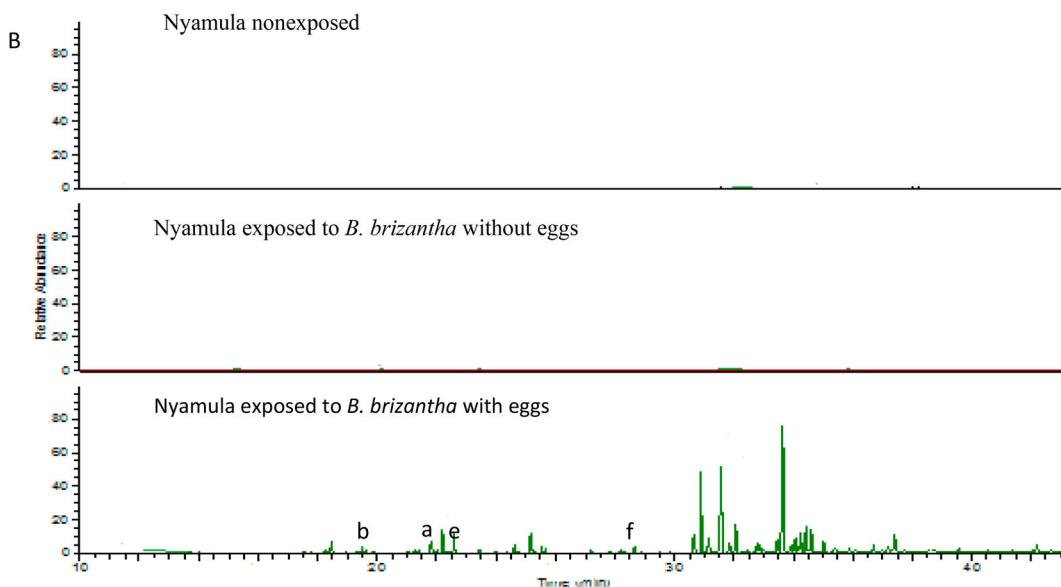


Figure 6. GC profiles of headspace volatiles from representative Nyamula maize landrace (Kenya) exposed to *B. brizantha* with and without *C. partellus* eggs and nonexposed maize. The identities of GC-MS active compounds are as follows: (a) linalool + nonanal, (b) (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT), (e) decanal, (f) (*E*)-caryophyllene

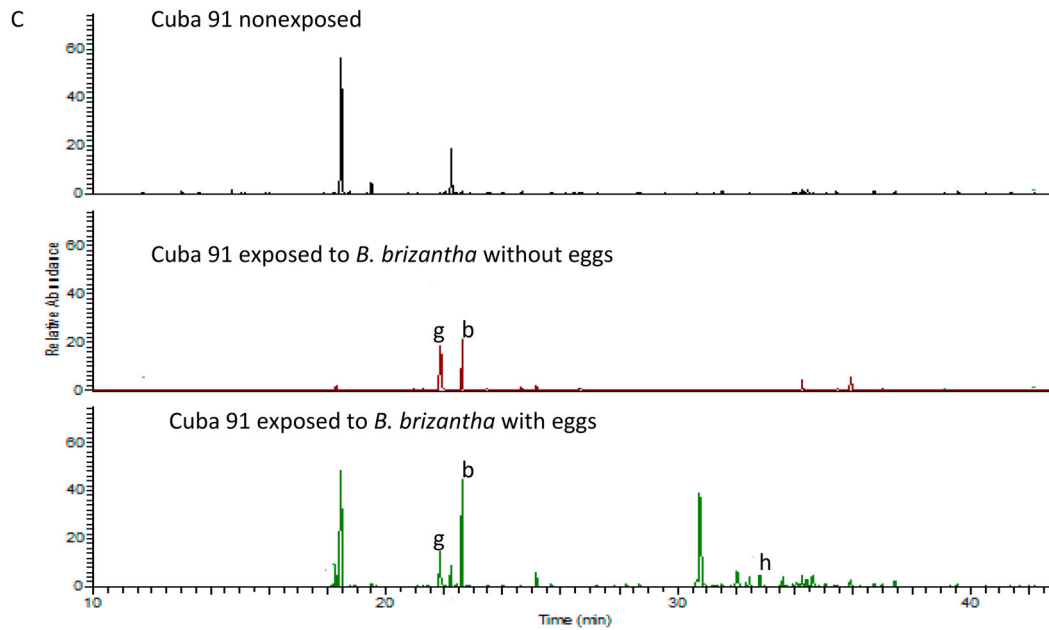


Figure 7. GC profiles of headspace volatiles from representative (Cuba 91) maize landrace (Latin America) exposed to *B. brizantha* with and without *C. partellus* eggs and nonexposed maize. The identities of GC-MS active compounds are as follows: (b) (*E*)-4, 8-dimethyl-1, 3, 7, nonatriene (DMNT), (g) linalool, (h) (*E*)- β -farnesene

from Latin America plants exposed to *B. brizantha* without eggs and the non-exposed ones. A similar trend was observed of egg oviposition and egg batches deposition on a commercial hybrid variety (WH505) exposed to *B. Brizantha* without eggs and the nonexposed maize plants of this variety. Comparison of gas chromatography-mass spectrometry (GC-MS) analysis of volatiles from Jowi and Nyamula and Cuba 91 maize plants revealed qualitative and quantitative differences in the volatile profiles. Eight bioactive chemical compounds including linalool+nananal, linalool, methyl salicylate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), decanal, (*E*)- β -farnesene and (*E*)-caryophyllene were produced in higher quantities following *C. partellus* egg laying on *B. brizantha* that in turn induced Jowi and Nyamula and Cuba 91 maize plants (Magara et al. 2015). The average abundance (ng kg per fresh weight per day) of most GC-MS active compounds was significantly higher on Jowi, Nyamula, and Cuba 91 maize plants exposed to *B. brizantha* following oviposition by *C. partellus* compared to Jowi, Nyamula and Cuba 91 maize plants exposed to *B. brizantha* without *C. partellus* eggs and nonexposed Jowi and Nyamula and Cuba 91 maize plants volatile controls. However, there were no changes in the volatile profiles in the WH 505 hybrid maize variety following *C. partellus* oviposition on *B. brizantha* (Magara et al. 2015). Differences between our results and others maybe because of variations in the abundance of plant volatile compounds among plant varieties and plant growth stages or be a product of the quality and quantity of volatiles (Bruce et al. 2005; Ghassemi-Dehkordi et al. 2015; Yang et al. 2019). Moreover, these differences may be due to difficulty in detecting compounds below the detection limit of the employed GC-MS system (Brattoli et al. 2013) since volatile compounds can be present at very low concentrations in a complex background of volatiles.

Our study also revealed that HIPVs from *B. brizantha* with *C. partellus* eggs induces changes in volatile profiles that have a function in direct defence responses of neighboring maize landraces. In the Y-tube olfactometer bioassays, the gravid

C. partellus moths were less significantly attracted to volatiles from maize landraces exposed *B. brizantha* with *C. partellus* eggs compared to nonexposed control maize plants. This implies that the HIPV-mediated communication between the infested grass and the maize landraces could deter *C. partellus* from depositing eggs and thus reducing their ecological fitness on *B. brizantha*-maize intercropped farms infested with *C. partellus*. This is as a result of infested *B. Brizantha* leaves emitting more green-leaf volatiles signal ((*Z*)-3-hexenyl acetate) and linalool which are responsible cues for attracting gravid females of *C. partellus* to the grass to deposit eggs hence diverting it away from maize (Bruce et al. 2010). Additionally, these volatile compounds have been implicated in recruiting egg parasitoids (*Trichogramma bournieri*) and larvae parasitoids (*Cotesia sesamiae*) that attack the *C. partellus* eggs before they hatch as well as the larvae hence reducing the damage on the maize crop (Magara et al. 2015; Khan et al. 1997, 2000, p. 2002; Mutyambai et al. 2015a, 2015b).

When behavioral responses of gravid *C. partellus* to 10 μ L of synthetic linalool+nananal, methyl salicylate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), decanal, (*E*)- β -farnesene and (*E*)-caryophyllene were tested in a Y-tube olfactometer, *C. partellus* showed less preference to seven of the compounds except linalool. This is consistent to previous studies that have shown volatile compounds such as DMNT, TMTT, methyl salicylate, (*E*)-caryophyllene, (*E*)- β -farnesene to be repellent to adult gravid stemborer moths (Khan et al. 2000, p. 2002; Bruce et al. 2010; Hassanali et al. 2007; Pickett et al. 2006; Mutyambai et al. 2015a, 2015b; Pickett and Khan 2016; Tolosa et al. 2019). Although there was attraction of *C. partellus* by synthetic compound linalool when used alone in the bioassay, the attraction of this compound seems to have been masked off when this compound is produced in a blend of volatiles with the other *C. partellus* repellent compounds produced by the maize landraces exposed to oviposited *B. Brizantha*. Even then, linalool like the compounds induced by exposure of maize landraces to oviposited

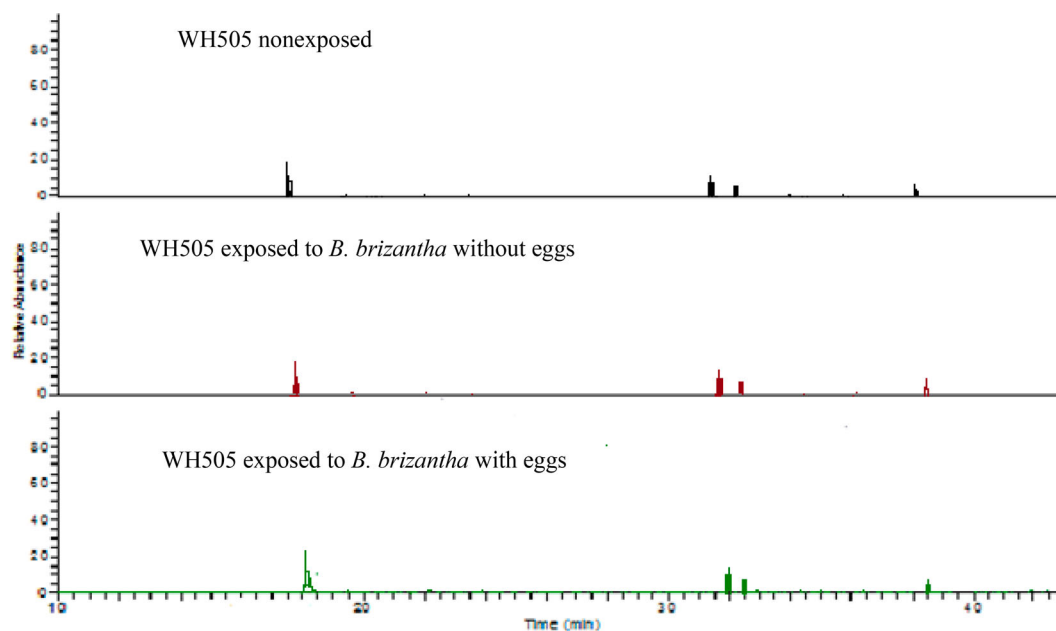


Figure 8. GC profiles of headspace volatiles from a representative maize hybrid (WH505) exposed to *B. brizantha* with and without *C. partellus* eggs and nonexposed maize. No profiles were formed hence no physiological active compounds formed.

Table 3. Responses of *Chilo partellus* to specific volatile compounds from maize exposed to *Bricharia brizantha* with eggs and clean air in Y-tube Olfactometer.

Treatment	Towards specific volatile compound	Towards clean air
(<i>E</i>)-4,8-dimethyl-1, 3,7-nonatriene (DMNT) vs clean air	3	27*
decanal vs clean air	3	23*
(<i>E</i>)-caryophyllene vs clean air	6	24*
(<i>E</i>)- β -farnesene vs clean air	5	21*
linalool vs clean air	21	7
linalool+ nonanal vs clean air	13	17
methyl Salicylate vs clean air	5	25*
(3 <i>E</i> ,7 <i>E</i>)-4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMTT) vs clean air	5	23*

B. brizantha has in previous studies been implicated in recruitment of natural enemies of the *C. partellus* which offer indirect protection to the maize crop (Magara et al. 2015). Therefore, we suggest that the production of these compounds by the landraces maize after being induced by the grass could have determined the oviposition choice of the gravid *C. partellus*. Also, the release of constitutive compounds such as linalool, which are produced by undamaged

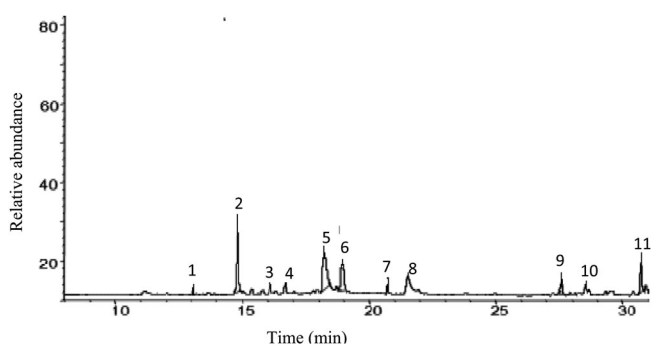


Figure 9. GC profile of head space volatiles from intact *B. brizantha* with *C. partellus* eggs. The identity of represented bioactive compounds are: (1) 6-methyl-5-hepten-2-one; (2) Z3HA; (3) (*E*)-ocimene; (4) linalool + nonanal, (5) linalool; (6) (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT); (7) decanal; (8) methyl salicylate; (9) (*E*)-caryophyllene; (10) (*E*)- β -farnesene; (11) (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

B. brizantha and maize plants (Zangerl 1999; D'Alessandro et al. 2006; Mutyambai et al. 2015a) and which are attractive to *C. partellus* moths (D'Alessandro et al. 2006; Bruce et al. 2010), are suppressed upon egg deposition on *B. brizantha* which in turn is less produced by the exposed maize plants. These HIPV compounds were therefore acting as signals determining *C. partellus* colonization, which incoming gravid females need to avoid egg deposition on these plants to enhance survival of their eggs and the emerging young larvae (Hassanali et al. 2007).

Our study is the first one in which the HIPVs emission from *B. brizantha* effect is shown to have a potential effect in neighboring maize, an economically important crop plant and staple food in SSA. However, the effect only occurred in tested African maize landraces and the Latin American landrace maize plants exposed to *B. brizantha* with *C. partellus* eggs. This oviposition deterrence was only observed in landrace maize exposed to *B. brizantha* with eggs but was lacking in the hybrid exposed to *B. brizantha* with eggs. Previous studies have shown landrace maize induction by egg deposition of *C. partellus* producing repellent HIPVs (Tamiru et al. 2011; 2012; Mutyambai et al. 2015b). However, infestation in other non-inducible maize have shown even further attraction of the moths to lay more eggs on the oviposited maize plant (Ntiri et al. 2018), the same scenario we observed with hybrid maize WH505 where there was no significant reduction in egg deposition in HIPVs exposed maize.

These induced defensive responses were not shown by the commercial hybrid maize varieties tested; this implies that the ability to be induced by HIPVs from *B. brizantha* with oviposition at this early stage of herbivory may have been lost during the breeding process. Previous reports have indicated a possible loss of direct defense (Sotelo 1997; Degen et al. 2004) and below ground indirect defenses (Köllner et al. 2008; Tamiru et al. 2011) during breeding and domestication processes. As far as our research is concerned, this is the first demonstration of grass-maize induction of indirect defense characteristic, caused by insect eggs, that is present in African

maize landraces and Latin America landrace, but absent in commercial hybrid maize varieties. We advocate the resource-poor farmers to incorporate the findings of our study into the field practically to protect their maize crop from phytophagous stemborers and in return improve maize yields.

5. Conclusion

This study shows that egg deposition by *C. partellus* on *B. brizantha* induces African maize landraces and Latin America landrace maize to release volatiles that repel *C. partellus*. The important finding of this study demonstrates that the oviposition associated HIPVs can prime neighboring maize plants for their protection from pest damage. This gives the neighboring maize crop an added advantage as it increases the total amount of induced volatiles by the maize plant neighboring *B. brizantha* with oviposition and hence the strength of the defense signals (Turlings and Tumlinson 1992; Zangerl 1999; Dicke and van Loon 2000; De Moraes et al. 2001; Van den Berg 2006; Hare 2011). The findings of this study will contribute to the development of management strategies that rely on the utilization of plant to plant communication through HIPVs to manipulate oviposition behavior of the *C. partellus*. Inclusion of these traits into commercial hybrids may open the gate for the development of novel and ecologically friendly strategies for *C. partellus* management.

Acknowledgements

We appreciate the local farmers in Mbita sub-county, Kenya for providing local maize seeds and CIMMYT-Nairobi office for providing Cuba 91 maize seeds. We thank Amos Gadi and Silas Ouko for technical assistance, insect rearing and screen house operations. Financial support was from European Union funded ADOPT Project DCI-FOOD.2010/230224 () to Prof. Zeyaur R. Khan. We also gratefully acknowledge the *icipe* core funding provided by UK Aid from the Government of the United Kingdom; Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); Federal Ministry for Economic Cooperation and Development (BMZ), Germany, Ethiopian Government and the Kenyan Government. *The views expressed herein do not necessarily reflect the official opinion of the donors.*

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by European Union funded ADOPT Project: [Grant Number B2238A-52005-004, Budget Code B2238A-52005-004].

Notes on contributors

Henlay J.O. Magara is interested in chemical ecology, insect science, tritrophic interactions to combat stemborers and fall armyworms in maize farms. He also works on insect for food and feed to mitigate food insecurity and malnutrition.

Zeyaur R. Khan is a Principal Scientist and Leader of Habitat Management in *icipe* Push-Pull program. He does research on chemical ecology, insect science, tritrophic interactions to combat stemborers and striga in cereal crops fields.

Charles A.O. Midega is a research scientist interested in insect science, chemical ecology, tritrophic interactions to combat stemborers and striga in cereal crops fields.

Syprine A. Otieno is a senior lecturer in the Department of Zoological Sciences, Kenyatta University. She is interested in animal physiology, insect science, tritrophic interactions.

Daniel M. Mutyambai is a chemical ecology scientist within the plant health department in *icipe* and department of life sciences in South Eastern Kenya University. He does research on chemical ecology. He is also involved in tritrophic interactions to combat stemborers and fall armyworms in cereal and horticulture crop fields.

Saliou Niassy is a research scientist interested in technology transfer, insect science, tritrophic interactions and insect for food and feed.

Teresia M. Nyaga is a research fellow interested in Bioinformatics, insect science and tritrophic interactions.

ORCID

Zeyaur R. Khan  <http://orcid.org/0000-0002-3548-7563>

References

- Agelopoulos NG, Hooper AM, Maniar SP, Pickett JA, Wadhams LJ. 1999. A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *J Chem Ecol.* 25:1411–1425.
- Birkett MA, Chamberlain K, Guerrieri ES, Pickett JA, Wadhams LJ, Yasuda T. 2003. Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid *Encarsia Formosa*. *Chem Ecol.* 29:1589–1600.
- Brattoli M, Cisternino E, Dambrosio PR, Gennaro G, Giungato P, Mazzone A, Palmisani J, Tutino M. 2013. Gas chromatography analysis with olfactometric detection (GC-O) as a useful methodology of chemical characterization of odorous compounds. *Sensors.* 13:16759–16800.
- Bruce TJA, Midega CAO, Birkett MA, Pickett JA, Khan ZR. 2010. Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. *Biol Lett.* 6:314–317.
- Bruce TJA, Wadhams LJ, Woodcock CM. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* 10(6):269–274.
- Calatayud P-A, Ahuya P, Le Ru B. 2014. Importance of the experimental setup in research on attractiveness of odours in moths: an example with *Busseola fusca*. *Entomol Exp Appl.* 152:72–76. DOI: 10.1111/eea.12201.
- Calatayud PA, Guénégo H, Le Rü B, Silvain JF, Frérot B. 2007. Temporal patterns of emergence, calling behaviour and oviposition period of the maize stem borer, *Busseola fusca* (Fuller 1901) (Lepidoptera: Noctuidae). *Annales de la Société Entomologique de France.* 43:63–68.
- Cheruiyot D, Midega CAO, Van den Berg J, Pickett JA, Khan ZR. 2018. Suitability of Brachiaria grass as a trap crop for management of *Chilo partellus*. *Entomol Exp Appl.* 166:139–148. DOI: 10.1111/eea.12651.
- Cook SM, Khan ZR, Pickett JA. 2007. The use of push-pull strategies in integrated pest management. *Annu Rev Entomol.* 52:375–400.
- D'Alessandro M, Held M, Triponez Y, Turlings TCJ. 2006. The role of indole and other shikimic acid derived maize volatiles in the attraction of two parasitic wasps. *J Chem Ecol.* 32:2733–2748.
- Degen T, Dillmann C, Marion-Poll F, Turlings TCJ. 2004. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiol.* 135:1928–1938.
- De Moraes CM, Mescher MC, Tumlinson JH. 2001. Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature.* 410:577–580.
- Dicke M, van Loon JJA. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol Exp Appl.* 97:237–249.
- Dicke M, van Loon JA, Soler R. 2009. Chemical complexity of volatiles from plants induced by multiple attack. *Nat Chem Biol.* 5:317–323.
- Ghassemi-Dehkordi N, Saghedi M, Kaviani M, Zolfaghari B. 2015. Analysis of *Helichrysum oligocephalum* DC. Essential oil Res J.Pharm. 2:47–52.

- Guofa Z, Overholt WA, Mochiah MB. 2001. Changes in the distribution of lepidopteran maize stem borers in Kenya from the 1950s to 1990s. *Int J Trop Insect Sci.* 21:395–402.
- Hare JD. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annu. Rev. Entomol.* 56:161–180.
- Harris KM. 1990. Bioecology of *Chilo species*. *Insect Sci Appl.* 11:467–477.
- Hassanali A, Herren H, Khan ZR, Pickett JA, Woodcock CM. 2007. Integrated pest management: the push–pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. *Phil Trans R Soc B.* 363:611–621. doi:10.1098/rstb.2007.2173.
- Heil M. 2008. Indirect defence via tritrophic interactions. *New Phytol.* 178:41–61.
- Heil M, Silva Bueno JC. 2007. Within–plant signalling by volatiles leads to induction and priming of an indirect plant defence in nature. *Proc Natl Acad Sci USA.* 104:5467–5472.
- Himanen SJ, Blande JD, Klemola T, et al. 2010. Birch (*Betula spp.*) leaves adsorb and re-release volatiles specific to neighbouring plants—A mechanism for associational herbivore resistance? *New Phytol.* 186(3):722–732.
- Kessler A, Kalske A. 2018. Plant secondary metabolite diversity and species interactions. *Annu Rev Ecol Evol Syst.* 49:1–24. doi:10.1146/annurev-ecolsys-110617-062406.
- Kfir R, Overholt WA, Khan ZR, Polaszek A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annu Rev Entomol.* 47:701–731.
- Khan ZR, AmpongNyarko K, Chiliswa P, Hassanali A, Kimani S, Lwande W, Overholt WA, Pickett JA, Smart LE, Woodcock CM. 1997. Intercropping increases parasitism of pests. *Nature.* 388:631–632.
- Khan ZR, Midega CAO, Bruce TJA, et al. 2010. Exploiting phytochemicals for developing a ‘push – pull’ crop protection strategy for cereal farmers in Africa. *J Exp Bot.* 61:4185–4196.
- Khan ZR, Midega CAO, Hooper A, Pickett J. 2016. Push-Pull: chemical ecology-based integrated pest management technology. *J Chem Ecol.* 42:689–697. doi:10.1007/s10886-016-0730-y.
- Khan ZR, Midega CAO, Wadhams LJ, Pickett JA, Mumuni A. 2007. Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stem borer (*Busseola fusca*) in a push–pull strategy. *Entomol Exp Appl.* 124:201–211. doi:10.1111/j.1570-7458.2007.00569.x.
- Khan ZR, Pickett JA, Van den Berg J, Wadhams LJ, Woodcock CM. 2000. Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Manag Sci.* 56:957–962.
- Khan ZR, Pickett JA, Wadhams LJ, Muyekho F. 2001. Habitat management strategies for the control of cereal stem borers and striga in maize in Kenya. *Insect Sci-Appl.* 21:375–380.
- Köllner TG, Held M, Lenk C, Hiltbold I, Turling TCJ, Gershenson J, Degenhardt J. 2008. A maize (E)- β -caryophyllene synthase implicated in indirect defense response against herbivores is not expressed in most American maize varieties. *Plant Cell.* 20:482–494.
- Konstantopoulou MA, Krokos FD, Mazomenos BE. 2002. Chemical stimuli from corn plants affect host selection and oviposition behaviour of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *J Econ Entomol.* 95:1289–1293.
- Lum PTM. 1979. Degeneration of ova in the bulla seminalis of Lepidoptera. *Insect Physiol.* 25:595–599.
- Magara JOH, Midega CAO, Otieno SA, Ogol CK, Bruce TJA, Pickett JA, Khan ZR. 2015. Signal grass (*Brachiaria brizantha*) oviposited by stem borer (*Chilo partellus*) emits herbivore-induced plant volatiles that induce neighbouring local maize (*Zea mays*) varieties to recruit cereal stem borer larval parasitoid *Cotesia sesamiae*. *Int J Sci.* 19(1):341–357.
- Midega CAO, Khan ZR, Pickett JA, Nylin S. 2011. Host plant selection behaviour of *Chilo partellus* and its implication for effectiveness of a trap crop. *Entomol Exp Appl.* 138:40–47.
- Mutyambai DM, Bruce TJ, Midega CAO., Woodcock CM, Cauliffield JC, Van den Berg J, Pickett J, Khan ZR. 2015b. Responses of parasitoids to volatiles induced by *Chilo partellus* oviposition on teosinte, a wild Ancestor of maize. *J Chem Ecol.* doi 10.1007/s10886-015-0570-1.
- Mutyambai DM, Bruce TJA, Van den Berg J, Midega CAO, Pickett JA, Khan ZR. 2016. An indirect defense trait mediated through egg-induced maize volatiles from neighbouring plants. *PLoS ONE.* 11:1–15.
- Mutyambai DM, Midega CAO, Bruce TJA., Van den Berg J, Pickett JA, Khan ZR. 2015a. Behaviour and biology of *Chilo partellus* on maize landraces. *Entomol Exp Appl.* 10:170–181. doi:10.1111/eea.12237.
- Nist. 2005. NIST mass spectral search for the NIST/EPA/NIH mass spectral library version 2.0. Office of the standard reference data base. Gaithersburg, Maryland: National Institute of Standards and Technology.
- Ntiri ES, Calatayud P, Musyoka B, Van den Berg J, Le Ru BP. 2018. Influence of feeding-damaged plants on the oviposition responses within a community of female moths. *Phytoparasitica.* 46:607–615. doi:10.1007/s12600-018-0695-1.
- Ochieng RS, Onyango FO, Bungu MDO. 1985. Improvement of techniques for mass-culture of *Chilo partellus* (Swinhoe). *Insect Sci Appl.* 6:425–428.
- Pest Control Products Board. 2019. Pest control products Registered for use in Kenya. Published by: Pest Control products Board, pp. 1–561.
- Pickett JA, Bruce TJA, Chamberlain K, Hassanali A, Khan ZR, Matthes MC, Napier JA, Smart LE, Wadhams LJ, Woodcock CM. 2006. Plant volatiles yielding new ways to exploit plant defence. In: Dicke M, Takken W, editors. *Chemical ecology from gene to ecosystem.* New York, The Netherlands: Springer; p. 161–173.
- Pickett JA, Khan ZR. 2016. Plant volatile-mediated signalling and its application in agriculture: success and challenges. *New Phytol.* 212(4):856–870.
- Pickett JA, Woodcock CM, Midega CAO, Khan ZR. 2014. Push-pull farming systems. *Curr Opin Biotechnol.* 26:125–132. doi:10.1016/j.copbio.2013.12.006.
- (R version 4.00) (R Development Core Team). 2020. R: A Language and environment for statistical computing. Vienna: R Foundation for statistical computing. Available online at: <http://www.R-project.org>.
- Seshu Reddy KV. 1998. Maize and sorghum in East Africa. In: Polaszek A, editor. *African cereal stem borers’ economic importance, Taxonomy, natural enemies and control.* Wallingford: CAB International; p. 25–27.
- Sotelo A. 1997. Constituents of wild food plants. In: Jones T, Romeo JJ, editors. *Functionality of food Phytochemicals.* New York: Plenum Press; p. 89–111.
- Sylvain NM, Manyangarirwa W, Tuarira M, Onesime M. 2015. Effect of lepidopterous stem borers, *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) on maize (*Zea mays* L.) yield: a review. *Int J Innov Res Dev.* 4:181–188.
- Tajamul RS, Kamlesh P, Pradyuman K. 2016. Maize-A potential source of human nutrition and health: a review. *Cogent Food & Agriculture.* 2(1):1166995. doi:10.1080/23311932.2016.1166995.
- Tamiru A, Bruce TJA, Midega CAO, Woodcock CM, Birkett MA, Pickett JA, Khan ZR. 2012. Oviposition induce volatile emissions from African small farmers’ maize varieties. *J Chem Ecol.* 38(3):231–234.
- Tamiru A, Bruce TJA, Woodcock CM., Cauliffield JC, Midega CAO, Ogol CKPO, Mayon P, Birkett MA, Pickett JA, Khan ZR. 2011. Maize landrace recruit egg and larval parasitoids in response to egg deposition by herbivore. *Ecol Lett.* doi:10.1111/J.141-0248.2011.01674.X.
- Tamiru A, Getu E, Jembere B. 2007. Role of some ecological factors for an altitudinal expansion of *Chilo partellus* (swinhoe) (Lepidoptera: Crambidae). *SINET: Ethio J Sci.* 30:71–76.
- Tolosa TA, Tamiru A, Midega CAO, et al. 2019. Molasses grass induces direct and indirect defense responses in neighbouring maize plants. *J Chem Ecol.* 45:982–992. doi:10.1007/s10886-019-01122-z.
- Turlings TCJ, Tumlinson JH. 1992. Systemic release of chemical signals by herbivore injured corn. *Proc Natl Acad Sci USA.* 89:8399–8402.
- Van den Berg J. 2006. Oviposition preference and larval survival of *Chilo partellus* (Lepidoptera: Pyralidae) on Napier grass (*Pennisetum purpureum*) trap crops. *Int Pest Manag.* 52:37–44.
- Yang L, Hu XP, Allan SA, Alborn HT, Bernier UR. 2019. Electrophysiological and behavioural responses of the Kudzu Bug, *Megacopta cribraria* (Hemiptera: Pltaspidae), to volatile

- compounds from Kudzu and Soybean plants. *J Agric Food Chem.* 67:4177–4183.
- Yonow T, Kriticos DJ, Ota N, Van den berg J, Hutchison WD. 2017. The potential global distribution of *Chilo partellus*, including consideration of irrigation and cropping patterns. *J Pest Sci.* 90:459–477. doi:10.1007/s10340-016-0801-4.
- Zangerl AR. 1999. Locally-induced responses in plants: the ecology and evolution of restrained defense. In: Agrawal ST, Bent E, editors. *Induced plant defenses against Herbivores and Pathogens: Biochemistry, ecology, and Agriculture.* St. Paul Minnesota: American Pathological Society Press; p. 231–232.