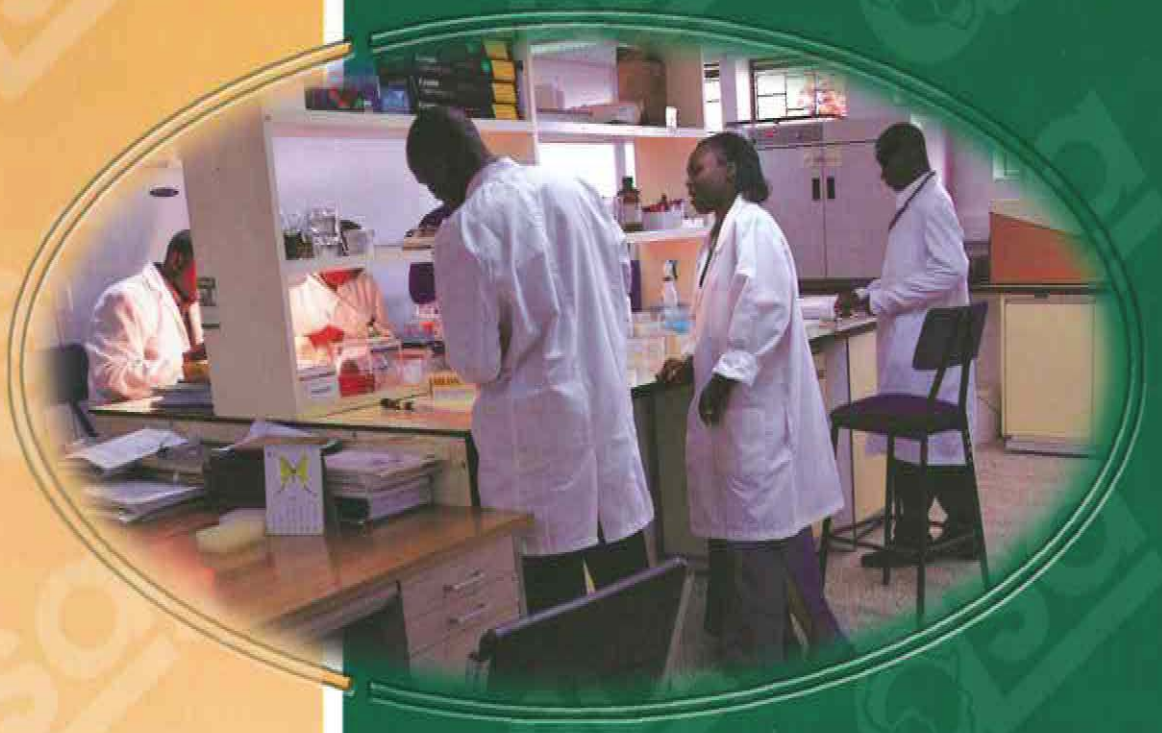

Africa-Wide Network of ARPPIS
Silver Jubilee
1983 – 2008

ASA
ARPPIS Scholars Association

icipe
African Insect Science for Food and Health

THE ROCKEFELLER FOUNDATION

Koninkrijk der Nederlanden
Netherlands Ministry of Foreign Affairs

DAAD

African Regional Postgraduate Programme in Insect Science (ARPPIS)

in collaboration with

ARPPIS Scholars' Association (ASA)

Proceedings of the

7th Biennial ASA Scientific Conference

Held at the *icipe* Headquarters, Kasarani, Nairobi, Kenya
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Acknowledgements

The ARPPIS Scholars Association (ASA) and the entire ARPPIS Alumni wish to sincerely acknowledge the Rockefeller Foundation, the Netherlands Ministry of Foreign Affairs and International Cooperation, and the German Academic Exchange Service (DAAD) for providing financial support that enabled the successful holding of the 7th Biennial ASA Scientific Conference at the *icipe* Headquarters, Kasarani, Nairobi, Kenya. At the same time this support also enabled successful hosting of the 25th ARPPIS Anniversary Celebrations and the publication of the conference proceedings, which we highly appreciate. The International Centre of Insect Physiology and Ecology (*icipe*), through its Director General Prof. Christian Borgemeister who is also the Chairperson, ARPPIS Academic Board provided generous and valuable financial and technical assistance and guidance for which we are very thankful. Special acknowledgements are due to Dr J. P. Ochieng'-Odero, the Head of *icipe*'s Capacity Building and Institutional Development Programme, Ms Susan Kariuki, Fundraising Coordinator, and Ms Lillian Igweta, Dr Henri Tonnang, Dr Paul Mireji and Mr Justo Otieno all who worked hard in raising funds and helping in organising the various events that ensured success. The role played by the workshop organising committee spearheaded by Prof. J.B. Okeyo-Owuor (ASA President), Prof. A. Deng (General Secretary) and Prof. R. Maranga (ASA Treasurer) ensured excellent performance during planning and holding the 7th Biennial ASA Scientific Conference and 25th ARPPIS Anniversary celebrations. Last but not least we wish to thank all the participants who attended and presented papers during the entire function.

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Contents

Foreword

The African Regional Postgraduate Programme in Insect Science (ARPPIS) was launched by the International Centre of Insect Physiology and Ecology (*icipe*) in 1983 to build capacity of talented individuals and institutions so as to contribute to improving food production and health through enhanced and sustained research for development in insect science in Africa. In partnership with 35 leading universities in Africa, ARPPIS has todate successfully trained 300 PhD- and 170 MSc-level young African scientists for leadership in insect science so as to meet the needs of the continent and the world at large. A further 250 postgraduate students from different African countries have been trained under the Dissertation Research Internship Programme (DRIP). These well-trained scientists continue to be leading researchers, capacity builders, policy makers and development agents in various countries in Africa and other parts of the world.

To keep the ARPPIS alumni alive and promote *icipe*'s noble mission and vision the ARPPIS alumni established the ARPPIS Scholars Association (ASA) with a vibrant membership all over the world. ASA holds scientific symposia every two years which amongst other activities contributes significantly to improved manpower needs, information exchange and national and continental development. This seventh (7th) ARPPIS Symposium held in Nairobi from 25–27th November 2008 coincided with the Silver Jubilee celebrations during which *icipe* and her partners observed 25 years of a successful ARPPIS story, examined lessons learned and reviewed the way forward for the ARPPIS programme. The event brought together ARPPIS alumni, representatives from the ARPPIS collaborating universities, previous programme coordinators and other stakeholders.

The conference provided an opportunity to listen to a keynote address, several world class papers from invited lead speakers and primary scientific research papers from ASA members presented and discussed from key thematic areas in 4 sessions. The session 2 focused on Human, Animal and Plant Health Care for Sustainable Natural Resources Utilisation. The session 3 focused on Arthropods Eco-technology Adoption and Utilisation for Sustainable Development in Africa. The scientific session 6 with 6 papers was on Climate Change and Management of Arthropod Pests and Vectors. Fifteen posters that addressed various research areas related to insect science and development in Africa were presented in this session.

The week-long conference and Silver Jubilee Celebrations provided a great opportunity for exchange and sharing of scientific research information, professional contacts and networking as well as enhancing the chances of developing national, regional and international research and development collaboration in insect related development challenges facing Africa. ASA's next biennial conference schedulded for November 2010 in Addis Ababa, Ethiopia, will greatly enhance the gains made so far as the Association, *icipe* and partners strive to improve food security, health and natural resources management in Africa and the entire globe. I believe the scientific information presented during

this conference and published in the current proceedings will provide readers with the desired insights to continue networking and supporting *icipe*, ARPPIS and ASA in the years to come.

On behalf of ASA, *icipe* Alumni and the organisers of this conference and ARPPIS Silver Jubilee Celebrations event, I thank the Rockefeller Foundation and the Netherlands Ministry of Foreign Affairs and International Cooperation and the German Academic Exchange Service (DAAD) as well as *icipe* for their support during this process and look forward to future partnership in achieving our joint mission and noble tasks. I also thank all participants to this conference for their valuable contributions and patience amidst the many challenges that normally face such functions.

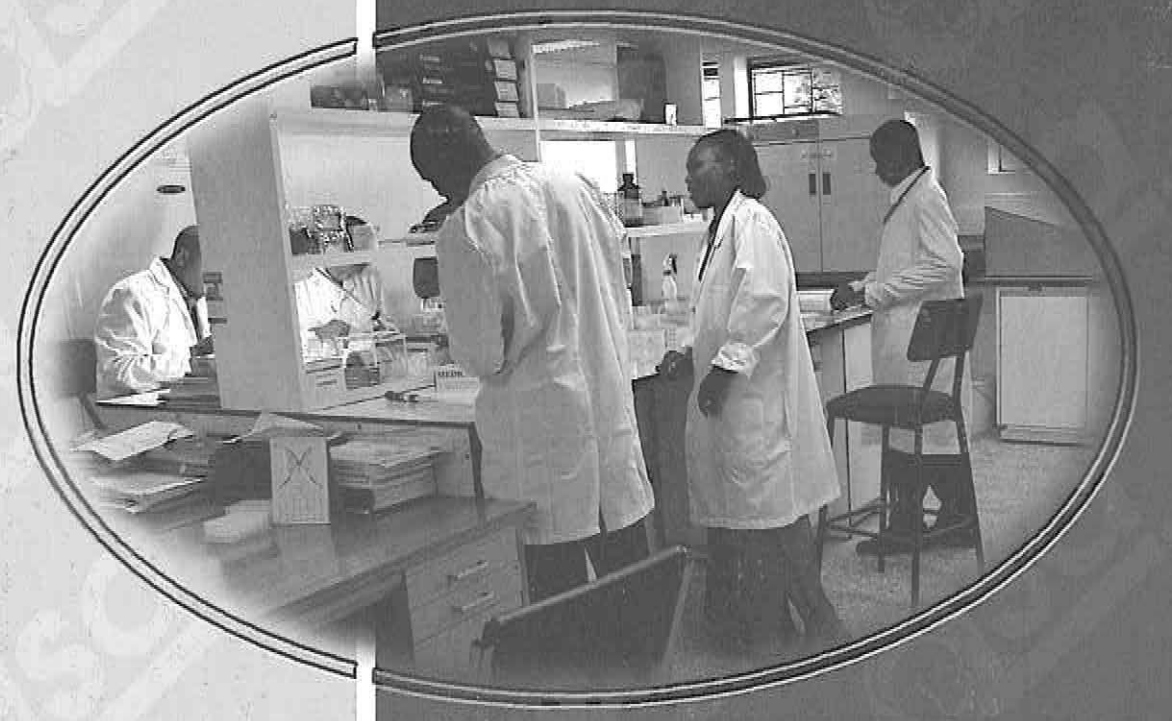
I am glad to present the proceedings of the 7th ASA Biennial Conference and hope that the information will be of benefit to all who are concerned about the role of insect science in development.

Professor J. B. Okeyo-Owour
ASA President

Acronyms and Abbreviations

AAS	African Academy of Sciences
AFFP	African Fruit Fly Programme
AMREF	African Medical and Research Foundation
APU	Arthropod Pathology Unit
ARI	agricultural research institute
ARPPIS	African Regional Postgraduate Programme in Insect Science
ASA	ARPPIS Scholars' Association
AVRDC	Asian Vegetable Research and Development Centre (World Vegetable Centre)
BMB	black maize beetle
BNF	biological nitrogen fixation
Bt	*Bacillus thuringiensis*
CABI	Centre for Agriculture and Biosciences International
CABI	Commonwealth Agricultural Bureaux International
CBD	Convention on Biological Diversity
CDC	Centres for Disease Control
CGIAR	Consultative Group on International Agricultural Research
CILSS	Permanent Interstates Committee for Drought Control in the Sahel
CIP	Commercial Insects Programme
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CORAF/WECARD	Conference of the Agronomic Research Organisations in West and Central Africa
DAAD	German Academic Exchange Service
DBM	diamondback moth
EHD	Environmental Health Division
EIAR	Ethiopian Institute of Agricultural Research
EU	European Union
GHG	greenhouse gases
HIV/AIDS	human immunodeficiency virus/acquired immune deficiency syndrome
IAEA	International Atomic Energy Agency
IBTPS	*icipe*'s Board of Training and Postgraduate Studies
icipe	International Centre of Insect Physiology and Ecology
ICRAF	World Agroforestry Centre
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IER	Institut d'Economie Rurale, Mali
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
INERA	Institut de l'Environnement et de Recherches Agricoles, Burkina Faso

IPCC	Intergovernmental Panel on Climate Change
IPM	integrated pest management
IRD	Institut de Recherche pour le Développement, France
ISRA	Institut Sénégalais de Recherche Agricole
IUCEA	The Inter-University Council for East Africa
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
KEMRI	Kenya Medical Research Institute
KU	Kenyatta University
KWS	Kenya Wildlife Service
LVB	Lake Victoria Basin
MDGs	millennium development goals
NAPA	National Adaptation Programme of Action
NAPs	National Action Plans to Combat Desertification
NARS	national agricultural research systems
NCAR	National Centre for Atmospheric Research
NCST	Kenya National Council for Science and Technology
NEAP	National Environmental Action Plans
NEPAD	New Partnership for Africa's Development
NGO	non-governmental organisation
NIH	National Institutes of Health
NMK	National Museums of Kenya
NOAA	National Oceanic and Atmospheric Administration
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PRECIS	Providing Regional Climates for Impact Studies model
PRSP	Poverty Reduction Strategy Papers
SEF	Sahelian Eco-Farm
SIDA/SAREC	Swedish International Development Cooperation Agency/ Swedish Agency for Research Cooperation with Developing Countries
SWC	soil and water conservation
UEA	University of Eastern Africa at Baraton
UNCCD	United Nations Convention to Combat Desertification
UNDP	United Nations Development Programme
UNFCCC	United Nations Framework Convention on Climate Change
USAID	United States Agency for International Development
VDSM	Visual Damage Scale Method
VIRED	Victoria Research on Environment and Development International
WARDA	West African Rice Development Association
WHO	World Health Organization
WNV	West Nile virus
ZSAES	Zimbabwe Sugar Association Experiment Station



Human, Animal and Plant Health Care for Sustainable Natural Resources Utilisation

Tsetse and Wild Hosts: Lessons From Waterbuck on How To Protect Cattle From Tsetse and Sleeping Sickness

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Abstract

Comparison of the behaviour of teneral *Glossina morsitans morsitans* on waterbuck *Kobus defassa* (a refractory host), and on two preferred hosts, buffalo *Syncerus caffer*, and ox *Bos indicus*, suggested the presence of allomones in the waterbuck odour. Examination of the volatile odours by coupled gas chromatography–electroantennographic detection showed that the antennal receptors of the flies detected constituents common to the three bovids (phenols and aldehydes), as well as a series of compounds specific to waterbuck, including C_8–C_{13} methyl ketones, δ-octalactone and phenols. Behavioural respones of teneral *G. m. morsitans* to different blends of these compounds were evaluated in a choice wind tunnel. The flies were attracted to known or putative attractant blends (the latter comprising EAG-active constituents common to all three animals and those common to buffalo and ox, excluding the known tseste attractants, 4-methylphenol and 3-*n*-propylphenol), and were aversive to putative repellent (the blend of EAG-active compounds specific to the waterbuck volatiles). Racemic δ-octalactone synthesised via an abbreviated route, was assayed against 3-day-old starved teneral female *G.m. morsitans* in the choice wind tunnel and found to be a potent tsetse repellent. The results lend support to indications for the existence of a tsetse repellent blend in waterbuck body odour that masks the attractive constituents in buffalo and ox body odours.

Key words: *Glossina morsitans morsitans, Kobus defassa, Syncerus caffer, Bos indicus,* sleeping sickness, semiochemicals, behaviour

Introduction

Surveys on feeding patterns of different species of tsetse (*Glossina* spp.) based on blood-meal analyses have shown varying degree of specialisation on different groups of vertebrate hosts (Weitz, 1963; Moloo, 1993; Clausen *et al.*, 1998). Although overlap or conjunction of habitats preferred by tsetse and their hosts may be a factor in promoting some specialisation (Mihok *et al.*, 1996; Clausen *et al.*, 1998), no relationship exists between the relative abundance of different vertebrates that are available in different habitats and the frequency with which they are fed on (Vale, 1974a; Turner, 1987; Snow *et al.*, 1988; Clausen *et al.*, 1998). For example, in a study carried out in Lambwe Valley in Kenya where *Glossina pallidipes* is the dominant species, over 80% of feeds were derived from bushbuck,

buffalo and bushpig, but none or hardly any from other numerically common game species such as oribi, impala, waterbuck, reedbuck and hartebeest (Turner, 1987). In the Luangwa Valley of Zambia, *G. m. morsitans* and *G. m. submorsitans* derived over 90% of their bloodmeals from warthog, hippopotamus, bushpig, cattle, bushbuck, elephant and buffalo. Other mammalian hosts in the valley such as black rhinoceros, zebra, giraffe, waterbuck, hartebeest and impala were rarely fed on (Clausen *et al.*, 1998).

Field experiments in which ox, buffalo, eland, oryx and waterbuck were observed in enclosures in a *G. pallidipes* habitat provided clear evidence that the fly has a gradation of host preferences (Grootenhuis, 1986; Grootenhuis and Olubayo, 1993). In this study, buffalo and cattle were equally attractive to tsetse (600–800 flies during the observation period), 20–25% of which engorged. On the other hand, very few flies (<10) appeared to be attracted to the waterbuck, none of which were seen to engorge. Eland and oryx were intermediate attracting a third and a sixth, respectively, of the number associated with cattle (or buffalo), of which even smaller proportions (10% and 3%, respectively) engorged. Vale (1974b) observed very few landings and no engorgement on the impala by *G. m. morsitans* compared to the ox, bushpig, dog and warthog on which many flies alighted and engorged.

No methodical studies have been carried out to elucidate the basis of these differences. Although host body size and mass are known to affect close-range attraction and landing behaviour of tsetse (Vale 1974 a,b; Hargrove, 1976), they cannot account for differences between animals of roughly the same sizes nor the extent of differences observed. A key question is whether preferences by tsetse are related to suitability of host blood, which may have influenced the adaptive behaviour of the fly, or whether they reflect the existence of defence mechanisms of varying effectiveness in different wild animals. Moloo *et al.* (1988) studied the survival and reproductive performance of *G. m. morsitans* when maintained *in vitro* on the blood of eight wild mammals comprising of both unpreferred (e.g. waterbuck, oryx) and preferred (e.g. buffalo, cattle, warthog) hosts. No significant differences were found, showing that tsetse host preferences are not based on host blood characteristics. A well-documented defensive reaction of hosts to tsetse involves their grooming behaviour such as tail flicking, skin twitching, kicking and stamping (Vale, 1977; Torr, 1994; Baylis, 1996; Torr and Hargrove, 1998). However, detailed observations todate have not revealed significant negative correlation between host grooming and feeding success by tsetse (Baylis, 1996; Torr and Hargrove, 1998).

Tsetse flies locate stationary hosts beyond their visual range upwind (60–120 m) through odour-mediated anemotaxis (Vale, 1977). Closer to the host (~10 m), visual cues supplement host odours (Vale, 1974b). Thermal, tactile and contact chemical stimuli on the host induce landing, probing and feeding, and contribute to host acceptability (Reinouts van Haga and Mitchell, 1975; Packer and Warner, 1991; Van der Goes van Naters and Rinkes, 1993; Saini *et al.*, 1993; Van der Goes van Naters and Den Otter, 1998; Van der Goes van Naters *et al.*, 1998). Some of the kairomones associated with host odours used by some tsetse species to locate their hosts have been identified and include breath constituents, such as carbon dioxide, acetone and 1-octen-3-ol (Hall *et al.*, 1984; Vale and Hall, 1985) and phenolic microbial breakdown products of host skin secretions and urine, particularly the blend of 4-cresol and 3-*n*-propylphenol (Hassanali *et al.*,

1986; Owaga *et al.*, 1988; Bursell *et al.*, 1988; Vale *et al.*, 1988; Warnes, 1990; Saini *et al.*, 1993). The propylphenol has so far been detected only in bovids including, interestingly, waterbuck urine (Madubunyi *et al.*, 1996). Combinations of these kairomones have been successfully used as baits to enhance the efficacy of traps or insecticide-treated targets for the control of *G. pallidipes* and *G.m. morsitans* in the field (Vale *et al.*, 1988; Brightwell *et al.*, 1991; Willemse, 1991).

It was speculated that the refractoriness of wild animals like waterbuck to tsetse could be due to the absence and/or presence of specific semiochemicals. That is, key kairomone components are absent, or present in sub-optimal amounts, rendering the flies relatively indifferent to the animals; or allomones may be present that repel the flies from a distance and, when close to the animal, deter them from feeding. This paper reports several studies that evaluated these possibilities.

Materials and Methods

Insects

Glossina m. morsitans and *G. pallidipes* flies were obtained from the International Centre of Insect Physiology and Ecology (*icipe*) insectary where they were maintained under 12:12 normal photoperiod and 25 ± 2 °C and 70 ± 5% RH and fed off rabbits' ears. *Glossina pallidipes* was also caught from the wild at Nguruman, South-West Kenya and maintained as above. Teneral females (2- or 3-day-old) were used for experiments. On the day of feeding experiments, the flies were individually placed in 18 x 7 x 5 cm^3 cages made of PVC frames covered with nylon mesh (Kaaya and Alemu, 1984).

Pupae of *G. m. morsitans* were also obtained from insectaries of the International Atomic Energy Agency (IAEA), Vienna, Austria and International Livestock Research Institute (ILRI), Nairobi, Kenya. After emergence, teneral females *G. m. morsitans* were placed in 20 x 15 x 15 cm perspex cages and kept under 25 ± 2 °C and 70 ± 5% RH until they were three days old. On the day of wind-tunnel experiments, flies were individually transferred into cylindrical PVC release cages (4 cm long and 3 cm in diameter) closed on one side with dark PVC gauze. The open end was plugged loosely with a small piece of cotton wool.

Host animals

Waterbucks, *Kobus defassa* Ruppel, were born and reared in captivity at the National Veterinary Research Centre, Kenya Agricultural Research Institute, Kabete, in an area free of tsetse flies. They were maintained on hay and concentrates and grazed freely within the pens. The oxen were kept within the *icipe* animal confinement pens and maintained on hay and concentrates.

Behavioural experiments

Feeding responses on live animals: A cage containing one fly was firmly held on a randomly selected part of the body of the animal. The fly was then observed and

scoring taken at 1-minute intervals for a maximum of 10 minutes. The following observations and measurements were made:

(a) landing on the animal, time taken to do so and its subsequent behaviour;
(b) probing and feeding;
(c) time spent in probing before initiation of feeding.

Probing was scored when the protruded haustellum was brought into contact with the animal skin. An observation cycle was terminated once a fly started to feed. Feeding was scored at the onset of swelling of the abdomen, confirmed by subsequent change of colour of the abdominal wall. For each treatment between 34 and 70 flies were observed.

Feeding responses on membranes: The top part of a silicone feeding membrane (Bauer and Wetzel, 1976) was marked out using a waterproof leukoplast adhesive, such that two equal zones of dimensions 6 x 6 cm were separated by a strip of 2 cm width. Waterbuck sebum of known weight was dissolved in 1 ml of ethanol and applied evenly on one zone of the membrane using a micropipette, to give one of the following concentrations of the sebum: 0.1, 0.7, 1.0 and 1.4 mg/cm^2 (the concentration of sebum on the waterbuck was estimated to be close to 1.0 mg/cm^2). For the control (blank) zone, the membrane was similarly treated with an equal volume of the solvent. The solvent was allowed to evaporate, and the membrane then placed on the aluminium plate containing heparinised ox blood such that the experimental arena was directly above the blood. The preparation was placed on a heating matt and the temperature on the membrane maintained at 34.5 ± 0.5 °C. A cage containing a single fly resting on its upper part was cautiously placed on the membrane such that the lower meshed side sat squarely on top of the marked out experimental arena. The observations made were similar to those on the animals except that a direct comparison of the behaviours of the flies landing on treated and untreated zones could be made. About 10 flies were individually observed in any one treatment, after which a fresh membrane was used.

Flight behaviour in the wind-tunnel: Tests were conducted in a cylindrical Plexiglass tunnel (180 cm long, 24 cm internal diameter) in a bioassay room maintained at 25 ± 2 °C and 70 ± 5% RH. A duct (20 cm diameter) in the middle of the tunnel was connected to a PVC pipe in the terminal end of which an air extracting fan was mounted. The duct in the middle thus divided the tunnel into two equal arms with a 20 cm wide middle zone where air from either arm mixed (Figure 1). When the fan was switched on, air flowed into the tunnel from both arms thereby making the middle of the tunnel downwind. The upwind ends of the tunnel were closed with white PVC gauze, while the downwind end was closed with a metallic wire mesh cover. The two upwind ends of the tunnel were connected to air filters made of PVC containing activated charcoal. The wind tunnel had three windows, one on each arm (15 cm x 10 cm) for introducing sample dispensers and one in the middle (4.7 cm in diameter) for introducing a release cage.

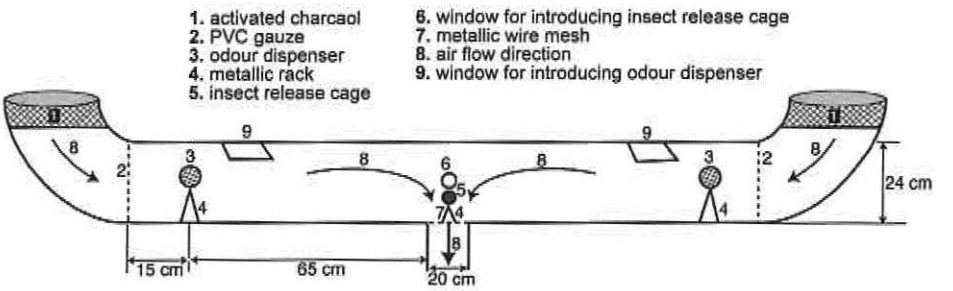


Figure 1. The wind tunnel

Light from fluorescent tubes and bulbs controlled through a dimmer switch was diffused through a frosted glass sheeting placed 35 cm above the tunnel giving about 1000 lux of the incident light. A white sheet of paper with black stripes marked about 2 cm apart was placed beneath the tunnel floor to provide contrast to a fly during anemotaxis. The wind speed in the tunnel was adjusted to 10 cm/sec.

Odour trapping

This was carried out with adsorbent sachets placed on the animals. Activated charcoal and octadecyl bonded silica, Soxhlet-cleaned with dichloromethane for 24 hr and then dried at 40 °C, were enclosed in sachets (4 x 4 cm) made of either filter paper (Whatman No.1) or stainless steel mesh-wire sheet (250 mesh, UK) folded from larger pieces (5 x 10 cm) and stapled securely together. Twenty such sachets were placed along mesh belts (8 x 41 or 126 cm) made from strips of the stainless steel mesh-wire sheet folded lengthwise in the middle (Figure 2). A strip of clean aluminium foil formed the outer part of the belt. Adsorbent sachets were arranged at intervals above the wire mesh along the inside of the belt, which was hemmed and stapled tightly on one side. The sachets were thus exposed to air on the inner side, and sealed by the aluminium foil on the other. The belts were transported wrapped lengthwise in aluminium foil and immediately tied around the neck, and belly near the front and hind legs of animals restrained in a metallic crush (Figure 3). The belted animals were then released into the pens to feed freely for the rest of the day. Control belts were exposed to the air around the crush for the duration taken to tie a

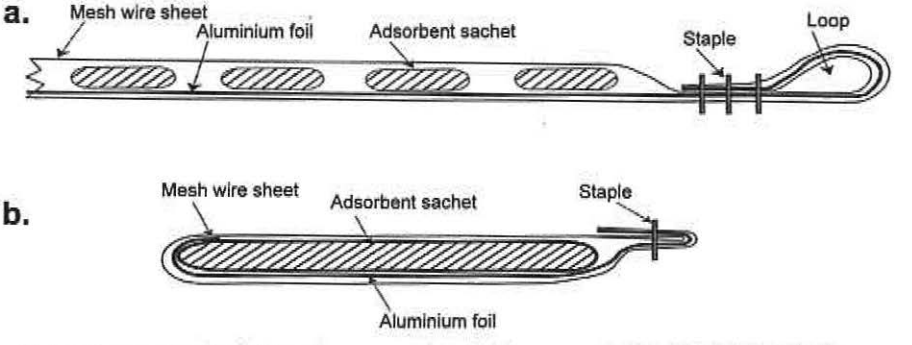


Figure 2. Longitudinal (a) and cross-sectional (b) representation of adsorbent belt

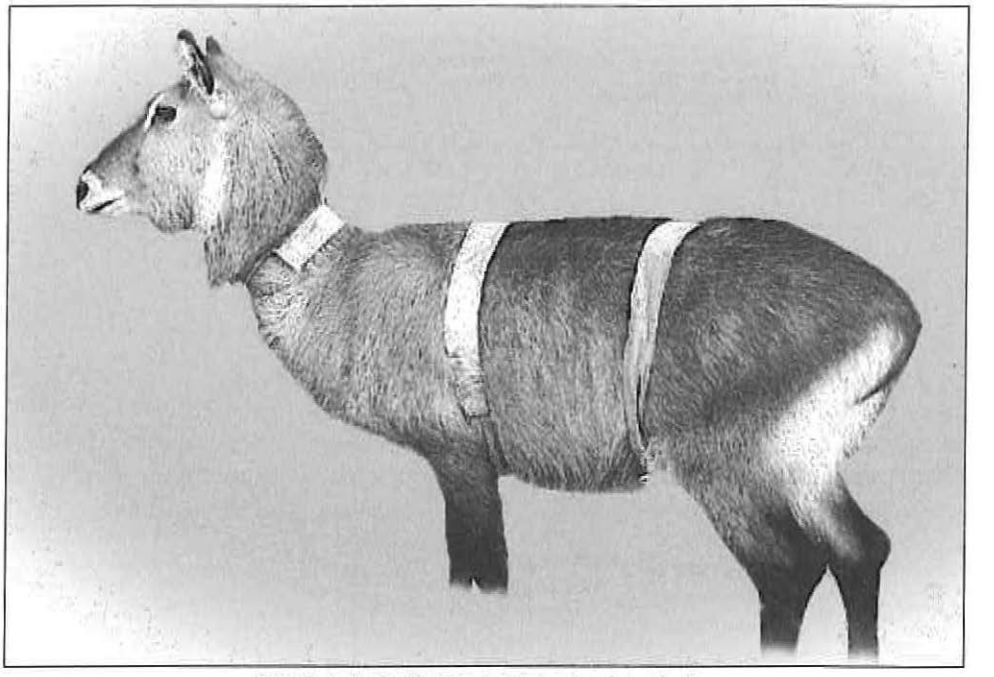


Figure 3. Waterbuck wearing adsorbent belts

single belt around an animal. After trapping, the animals were herded into the crush, the belts removed, wrapped in clean aluminium foil, placed in a cool box, transported to the laboratory and stored at -20 °C.

Chemical analyses

Gas chromatographic (GC) analyses were carried out on a Hewlett Packard (HP) model 5890 Series II gas chromatograph equipped with a splitless capillary injector system, a flame ionisation detector (FID) and an HP 3396 Series II integrator. The GC oven was fitted with a HP fused silica capillary column (50 m x 0.2 mm x 0.33 μm) and temperature programmed at 40 °C for 15 min, 8 °C/min to 280 °C and held for 15 min. GC-MS analyses were carried out on a VG Masslab 12-250 mass spectrometer (EI, 70 eV) coupled to a HP 5790 Series A gas chromatograph, which was operated under the same conditions as for GC analyses. The synthetic standard chemicals were purchased from Aldrich Chemicals (Dorset, UK).

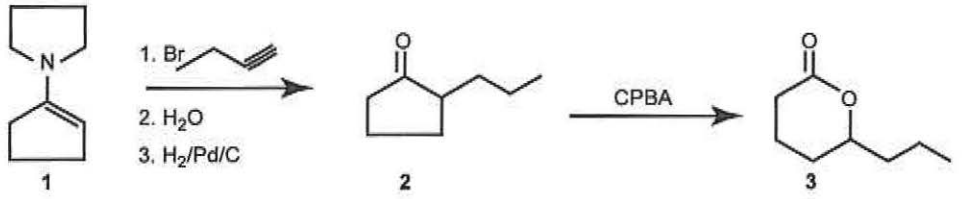
Electroantennographic analyses (GC-EAD)

Antennae were prepared from 3-day-old adult female laboratory-reared *G. m. morsitans*, and from laboratory-reared as well as field-trapped *G. pallidipes*. All flies had been fed 2 days earlier. GC-EAD was performed on an HP model 5890 Series II gas chromatograph equipped with an FID and an HP 3396 Series II integrator, and a splitter. The same conditions were employed as in the GC analyses. A make-up gas with a flow rate of 40 ml/min was added just before

the split point to accelerate the effluent through 50 cm deactivated fused silica columns. The effluents for antennal detection were driven through a transfer line maintained at 150 °C (Syntech THC-3) into a moistened air stream (25 °C, 90% RH, 4.0 ml/sec) and delivered over the antennal preparation via a stainless steel tube (5 mm i.d.). FID and EAD signals were monitored synchronously using a programme on a GC-EAD interface card (Syntech) installed in a PC. Recordings were obtained from at least 3 antennae for each stimulus. EAG-active compounds were initially identified from their mass spectra, and then confirmed by co-injections of authentic compounds and trapped odours into GC, GC-EAD runs on *G. m. morsitans* or *G. pallidipes*, and GC-MS comparisons.

Synthesis

δ-Octalactone (3), identified from waterbuck volatiles was prepared according to the scheme below, adopted from Fieser and Fieser (1967) and Furniss *et al.* (1991). 1-Pyrrolidino-1-cyclopentene (1) was allowed to react with propargylbromide in acetonitrile and the resulting 2-propylcyclopentanone (2) was converted to the lactone using *m*-chloroperbenzoic acid (CPBA). The structure of δ-octalactone (3) was confirmed by MS, ^{1}H and ^{13}C NMR spectra. The synthetic product (an enantiomeric blend) was EAG-active on both *G. m. morsitans* and *G. pallidipes* and co-eluted in GC with the target peak in the waterbuck odour.



Test odour in the wind-tunnel

Test odour: Test odour consisted of selected blends of compounds identified in body volatile odours of buffalo, ox and waterbuck, that elicited electroantennographic (EAG) responses on the antenna of *G. m. morsitans*. The following odour blends were tested:

- Blend A: All the EAG-active compounds in waterbuck body volatiles.
- Blend B: Compounds present in waterbuck but absent in both ox and buffalo volatiles.
- Blend C: Compounds common in waterbuck and ox or buffalo volatiles.
- Blend D: Compounds present in buffalo and ox volatiles minus known tsetse attractants, 4-methylphenol and 3-*n*-propylphenol.
- Blend E: Known tsetse fly attractants previously identified from ox breath, body and urine (acetone, 1-octen-3-ol, 4-methylphenol and 3-*n*-propylphenol) in the ratio 1000:10:4:1 (Pynter and Brady, 1993; Owaga *et al.*, 1988; Saini and Hassanali, 1992).
- Octalactone: The synthetic racemic δ-octalactone.

The flies responses to the above odour blends were compared in the following ways: (a) three or four doses (0.01, 0.1, 1.0 and 10.0 mg) in 200 μl paraffin oil of blends A, B, C and D set against a control (200 μl paraffin oil); (b) 1.0 mg of blend E in 200 μl paraffin oil against control; (c) 0.1, 0.5, 1.0 and 2.50 mg of octalactone in 200 μl paraffin oil and (d) a blank test involving equal volumes of paraffin oil (200 μl).

Odour dispensers: The odour dispenser consisted of a black cloth that was secured using Soxhlet-cleaned (dichloromethane) rubber bands placed on one end of an open-ended Plexiglass tube (5 cm long and 4.5 cm in diameter). The appropriate dose of the odour in paraffin oil was pipetted onto the centre of the cloth on one of the dispensers, while air was allowed to flow at 10 cm/sec. For the control, the same volume of paraffin oil was pipetted onto a similar dispenser placed in the other arm of the tunnel. All the windows were then closed and air was allowed to flow inside the tunnel from the two ends for 5 minutes before the first fly was introduced.

Behavioural experiments: A 3-day-old teneral female tsetse fly in a release cage was introduced cautiously through the window in the middle of the tunnel. The behaviour of the fly was observed and recorded for three minutes, after which it was aspirated out using a vacuum pump. In each replicate cycle, the responses of about 10 individual flies were recorded after which the tunnel, the metallic racks and the release cages were cleaned. Between five to eight replicates were carried out for each treatment.

Analysis of data

For the feeding experiments, the average number of probing sites changed was calculated and means compared by Student's *t* test (SAS, 1996). The percentage of flies that fed on waterbuck and ox, and on treated and control zones of the membrane, was plotted against observation time and regression curves computed using probits (SAS, 1996). The parameters and lack-of-fit of the regression were tested using chi-square. Similar plots were used to calculate the probing times for 50% of flies that had initiated feeding under different conditions.

The differences in proportions of flies that were engaged in specific behaviours (activation, departure from mid-section, flight preferences, upwind progression and avoidance) in the choice wind tunnel were analysed using Chi-square (SAS, 1996).

Results and Discussion

Feeding responses on live animals and on membranes treated with sebum

The behavioural responses of caged individual teneral *G. m. morsitans* on waterbuck and ox and on feeding membranes with and without smears of different doses of waterbuck sebum were different (Figures 4 and 5). However

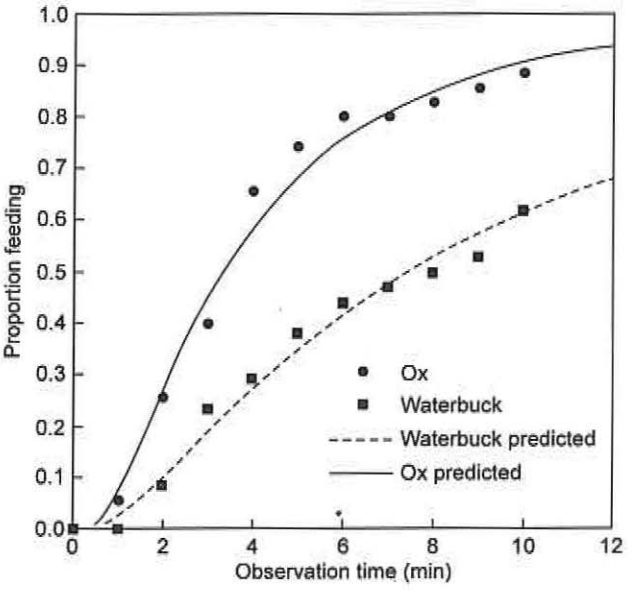


Figure 4. Proportion of 3-day teneral *Glossina m. morsitans* females feeding on waterbuck and ox (regression curves derived using probit analysis). Proportions feeding on waterbuck and ox differ significantly from the 2nd to 10th minute ($P < 0.05$–$P < 0.001$)

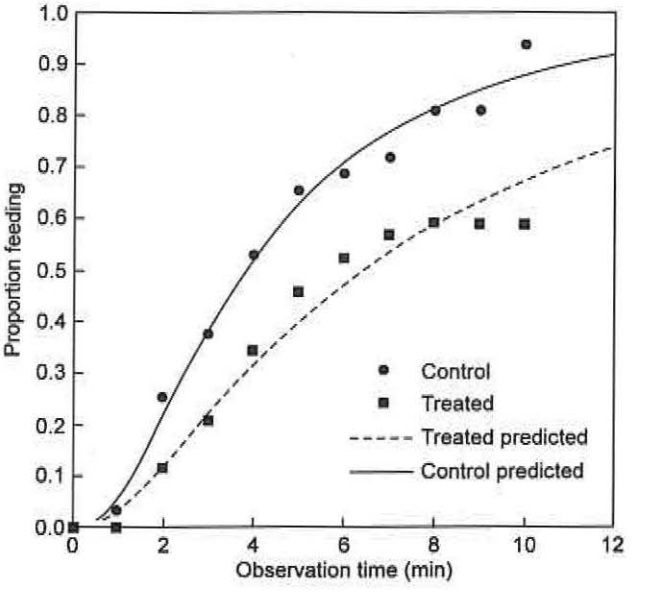


Figure 5. Proportion of 3-day teneral *Glossina m. morsitans* females feeding through a membrane partly treated with 1.0 mg/cm² of waterbuck sebum. Regression curves were derived using probit analysis. Proportions feeding on treated and control zones differ significantly from the 3rd to 10th minute ($P < 0.05$–$P < 0.001$)

no, significant difference was found in the initial landing behaviour on the two animals, nor on treated and control parts of the membrane. Whereas none of the flies that landed on the ox showed any escape behaviour, more than a third of those that initially landed on waterbuck escaped. Similar results were obtained on feeding membranes treated in part with 1.0 or 1.4 mg/cm² of waterbuck sebum. Moreover, flies that landed on waterbuck or its sebum changed probing sites more often and probed significantly longer. The proportions that initiated feeding during the 10 min. observation period were also significantly less. These results suggest presence of both volatile and non-volatile allomones on waterbuck, which would account for low numbers of flies found attracted to and feeding on waterbuck in the wild (Gikonyo *et al.*, 2000).

Electroantennogram-active compounds in odours

Electroantennogram-active compounds in odours from waterbuck were compared with those of buffalo and ox (Table 1). The GC-EAD profiles (with *G. m. morsitans* antennae) of the odours of the two preferred hosts were comparable, comprising medium-chain, saturated or unsaturated aldehydes and phenols, with buffalo emitting a few more EAG-active aldehydes (Figures 6 and 7). Waterbuck odour gave a richer profile with *G. m. morsitans* antennae, consisting of fewer aldehydes but more phenolic components, and a series of 2-ketones (C8–C13) and δ-octalactone (Figure 8). This bovid also emits moderate amounts of C5–C9 straight-chain fatty acids, some of which were detected in buffalo and ox only in trace amounts. However, these did not elicit significant GC-EAD responses. Waterbuck profiles from the antennae of *G. pallidipes* showed broad similarity with those from *G. m. morsitans*, although the composition of aldehydes and ketones was somewhat different, indicating

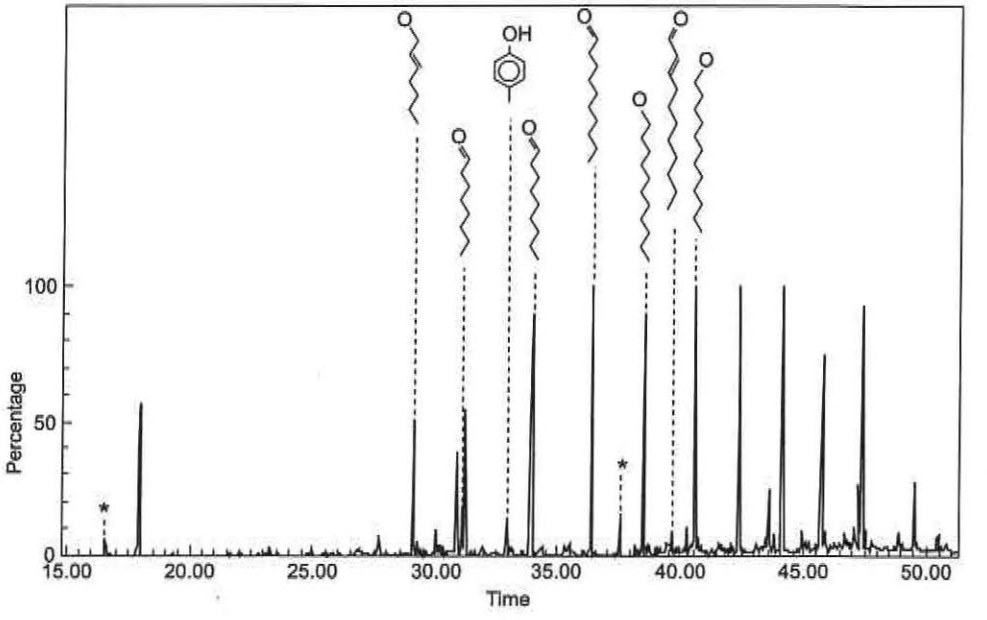


Figure 6. EAG-active compounds from the body volatiles of buffalo

Table 1 Compounds detected by GC and GC-EAD in the body odours of buffalo, ox and waterbuck: (+) detected; (-) undetected; * EAG-active on *G. m. morsitans;* † EAG-active on *G. pallidipes* (waterbuck only)

Peak number	Compound	Presence in body odours		
		Buffalo	Ox	Waterbuck
3	Hexanal	-	-	+
4	Heptanal	+	-	+
5	(E)-2-Heptenal	+ (*)	-	+ (* †)
6	Octanal	+ (*)	-	-
7	Nonanal	+ (*)	+ (*)	+ (* †)
8	(E)-2-Nonenal	-	-	+ (†)
9	Decanal	+ (*)	+ (*)	+ (* †)
10	Undecanal	+ (*)	+ (*)	+ (*)
11	(E)-2-Undecenal	+ (*)	+ (*)	-
12	Dodecanal	+ (*)	+ (*)	-
13	Tridecanal	+	+	-
14	Tetradecanal	+	+	-
15	Pentadecanal	+	+	-
16	Hexadecanal	+	+	-
17	6-Methyl-5-hepten-2-one	-	-	+
18	2-Octanone	-	-	+ (* †)
19	2-Nonanone	-	-	+ (* †)
20	2-Decanone	-	-	(*)
21	2-Undecanone	-	-	+
22	2-Dodecanone	-	-	+
23	(E)-6,10-Dimethyl-5,9-Undecadien-2-one	-	-	+ (* †)
24	δ-Octalactone	-	-	+ (* †)
25	Dimethyl sulfone	-	+	-
26	4-Methylphenol	+ (*)	+ (*)	+ (* †)
27	2-Methoxyphenol	+	+	+ (†)
28	3-*n*-Propylphenol	+ (*)	+ (*)	+ (* †)
29	3-Isopropyl-6 methylphenol	-	-	+ (*)
30	Pentanoic acid	-	-	+
31	Hexanoic acid	-	-	+
32	Heptanoic acid	-	-	+
33	Octanoic acid	-	-	+
34	Nonanoic acid	-	-	+
35	Decanoic acid	-	-	+
36	Toluene	-	-	+
37	1-Octene	-	-	+
38	(E)-4-Octene	-	-	+
39	3-Ethyl-2-methyl-1-heptene	-	+	-
40	Unidentified	+ (*)	-	-
41	Unidentified	+ (*)	-	-
42	Unidentified	-	+ (*)	-
43	Unidentified	-	+ (*)	-
44	Unidentified	-	+ (*)	-
45	Unidentified	-	-	+ (†)
46	Unidentified	-	-	+ (†)

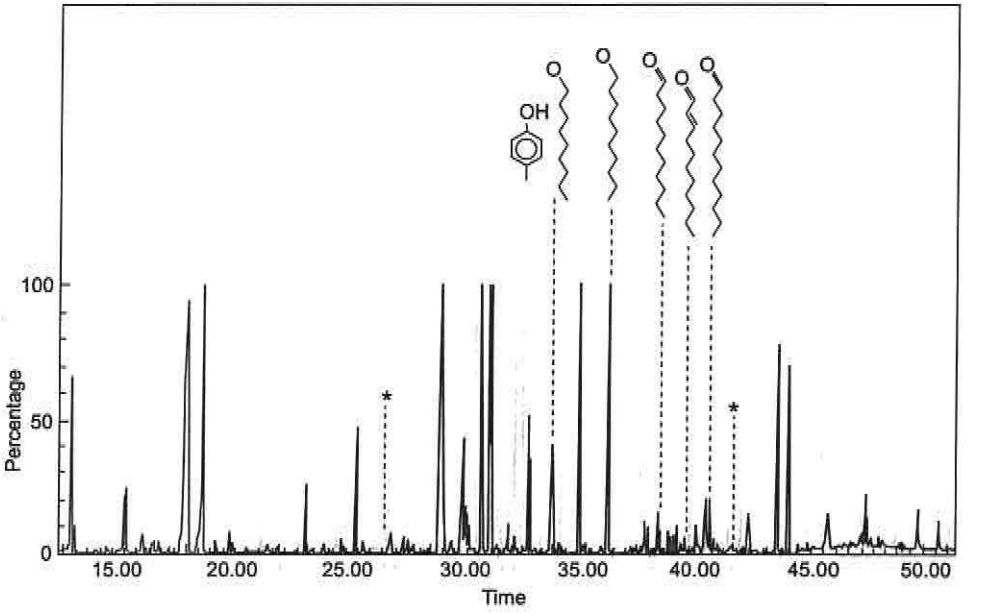


Figure 7. EAG-active compounds from the body volatiles of ox

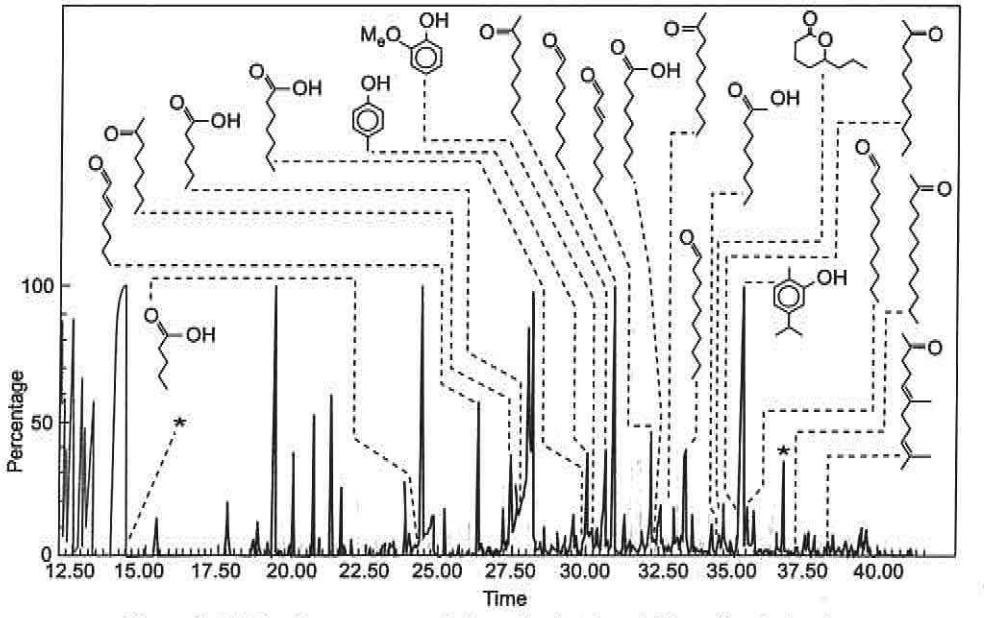


Figure 8. EAG-active compounds from the body volatiles of waterbuck

some species-specific differences in the detection of host odours (Gikonyo *et al.*, 2002). Certain waterbuck-specific EAG-active components, particularly the 2-ketones and lactone, were suspected to constitute a candidate allomonal blend in waterbuck odour.

Behavioural responses in the wind-tunnel

Behavioural responses of teneral *G. m. morsitans* to different blends of EAG-active compounds from the three bovids were evaluated in a choice wind tunnel. The flies' responses to known or putative attractant blends [the latter comprising electroantennogram (EAG)-active constituents common to all three animals and those common to buffalo and ox, excluding the known tsetse attractants, 4-methylphenol and 3-*n*-propylphenol], and that to putative repellent (the blend of EAG-active compounds specific to the waterbuck volatiles), were different. A major difference related to their initial and final behaviours. When a choice of attractant blends (known or putative) and clean air was presented, flies initially responded by flying upwind towards the odour source, but later moved downwind and rested on either side of the tunnel, with some evidence of preference for the side with the odour treatments. However, when presented with a choice of waterbuck-specific blend (putative repellent) and clean air, the flies' initial reaction appeared random, but they eventually settled down on the odourless side of the tunnel (Gikonyo et al., 2003). Racemic δ-octalactone was found to be repellent to teneral *G. m. morsitans* on its own (Mwangi et al., 2008).

The flies' responses to the blend of EAG-active constituents of waterbuck odour, comprising both attractive and aversive compounds is interesting. Upwind flight choices of the flies showed no discrimination between this odour blend and clean air. On the other hand, significantly more flies eventually settled on the odourless control arm of the tunnel compared to the odour arm at one of the doses (1.00 mg, $P<0.05$). The first response suggests neutralisation of the effects of the attractive constituents by the waterbuck-specific compounds through a mechanism that may involve jamming of the sensory information system (Davis, 1985; Ouden et al., 1996). The second involves orientation away from the total blend implying repellency in the sense defined by Dethier et al. (1960). The actual mechanism that operates in nature remains to be established through detailed observations in the field. However, preliminary results from field studies suggest an active repellent effect. Moreover, an additional feature in the volatile odour chemistry of waterbuck is the presence of moderate amounts of C_5–C_9 straight-chain fatty acids, which were not present in ox or buffalo odours (Gikonyo et al., 2002). Although none of these fatty acids elicited EAG responses, some are known to reduce tsetse fly trap catches in the field (Vale, 1980; Torr et al., 1996). It is suggested that C_5–C_9 acids may act additively or in concert at the attennal receptors that result in a repellent action, which may augment the blend of ketones, octalactone and phenols (Gikonyo et al., 2002). Preliminary field results confirm that the fatty acid mixture contributes to the overall repellency of waterbuck-specific constituents.

In conclusion, the following inferences can be made: (i) each host animal emits semiochemicals that act as a 'signature' recognised by tsetse and hence the observed gradation of bloodmeal source in the wild; (ii) the blend of EAG-active constituents specific to tsetse-refractory waterbuck is allomonal to the fly; and (iii) the aldehyde blend associated with tsetse hosts is attractive to the fly and may be a significant component of the kairomone system of the insect. Additionally, this study suggests a greater tactical diversity in the odour- and host-location behaviour of tsetse, which warrants further investigation.

Acknowledgements

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Predation on Termites of the Subfamily Macrotermitinae by the Ponerine Ant *Pachycondyla analis* (Latreille)

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Abstract

Predation on termites of the subfamily Macrotermitinae by the ant *Pachycondyla analis* (Latreille) was studied over a period of six months (April–September 2007) at Mpala, in a semiarid savanna in central Kenya. A total of 33 nests were studied and 330 raids were observed and recorded. There were 17.0 nests/ha, with most nests located under rocks. There were mainly two peaks of raiding activity one in the morning and the other in the evening. However, during the dry season, an additional minor raid occurred occasionally in the early hours of the night. The duration of different phases during the morning and evening raids did not differ significantly. Number of ants in a raid reflects the number of ants carrying termites as well as the number of termites carried. *Microtermes* spp. and *Odontotermes* spp. were the prey species with *Microtermes* spp. most frequently preyed upon in June and *Odontotermes* spp. in August. Overall raiding activity of *P. analis* was synchronised by the prey activity.

Key words: *Pachycondyla analis*, *Microtermes* spp., *Odontotermes* spp., termites, nests, trail pheromones, raids

Introduction

During their 100 million years of coexistence, ants and termites have engaged in a co-evolutionary arms race (Hölldobler and Wilson, 1990) with ants evolving several predatory strategies and termites responding with defensive ones. Group raiding strategies are considered to be more advanced than solitary raiding, because they involve cooperative behaviours among workers and enable the capture of larger prey and thus energy saving (Corbara and Dejean, 2000).

The African ponerine ant *Pachycondyla analis* (Latr.) (Hymenoptera: Formicidae), (formerly, *Megaponera foetens* Fabr.), is widespread and relatively common throughout most of sub-Saharan Africa. The number of workers in a colony varies with an average of 400 (Lévieux, 1966) to 1475 (Lepage, 1981). *Pachycondyla analis* feeds exclusively on termites, mainly of the subfamily Macrotermitinae. Raids occur when scouting ants detect a termite source, and lay pheromone trails back to their nests (Longhurst *et al.*, 1978). Once in the nest, a scout recruits between 22–840 nest mates and guides a column back to the prey by following the trail. Both major and minor workers of *P. analis* take part in the raids (Crewe *et al.*, 1984). On arrival at the termite source, major worker ants break open the termite galleries and the minor workers invade them. Those that capture termites, sting them, bring them out of the termite

galleries and place them near the gallery entrance, before returning to continue hunting. Piles of paralysed termites grow at the entrance. After about 13–20 minutes, the workers stop hunting and return to the pile of paralysed termites. Major workers grasp between 1–7 termites with their mandibles, while minors grasp 1–3 termites. Some do not carry any termites, but lead columns of ants loaded with prey back to their nest.

This study was carried out to establish the abundance and location of nests in the study area for the purpose of collecting experimental materials for chemical ecological work. The study also documents the raiding dynamics and prey preferences as measured by frequencies of raids on different termite species.

Materials and Methods

Study site

This study was conducted at Mpala Research Centre (MRC) in Laikipia district, Central Kenya, between the months of April to September 2007. Mpala Research Centre (0°17'N, 37°52'E, elevation: 1600 masl) occupies an area of 19,465 ha of semi-arid land. The dry season is normally from December to February; the rest of the year is usually characterised by long, continental and short rains.

Nest location, distribution and density

Nests of *P. analis* were located using three different approaches (Longhurst *et al.*, 1978: Bayliss and Fielding, 2002: Marcus Stüben; [personal communication]). These included following ants returning from raids, following scout ants and looking for pupal cases, termite body fragments, or dead ants near likely nest sites, which are usually ejected from nests. Nest density was calculated using standard methods (Bayliss and Fielding, 2002). Eight quadrants (50 x 50 m each) were set randomly within the study area, and the number of nests counted and expressed as nests per hectare.

Foraging parties

Nests were selected opportunistically from each of the eight quadrants set to observe morning and evening raids between 0700–1100 hr and between 1600–1930 hr respectively. Ant colonies were also monitored for late evening raids between 2200–2400 hr (times based on preliminary observations for 14 days on the raiding behaviour). Observation was performed continuously for the whole study period.

The number of ants in a raiding column, those carrying termites, and, number of termites carried per ant in a random sample of 10 ants from each raiding column were counted. The number of termites carried per ant was determined by carefully grabbing the ants by the thorax using entomological forceps. By attacking the forceps, this made the ant release the termites it was carrying onto a paper held underneath it. Termites were identified to the generic level by their caste information as outlined in Pearce *et al.* (1996). Duration of the different phases in a raid (outward, journey to termites source, time spent at termite source and time spent on return journeys back to nest) were determined for each raid.

Predation rates

Predation rate was calculated for each month based on number of termites taken per nest per day using the equation of Lepage (1981) as modified by Bayliss and Fielding (2002):

$$P = F \times T \times R \times N \times t$$

Where;

P = Termites predated per nest per day
F = Average number of ants carrying termites of that species per raid
T = Average number of termites per ant per calendar month
R = Average number of raids per day per nest per calendar month
N = Number of ant nests per hectare
t = Average duration of raid per calendar month.

Results and Discussion

Nest location, distribution and density

Of the 33 nests located, majority (46%, 15) were under rocks while 12% (4) were in an open field (Figure 1). Mean number of nests per quadrant was 4.25 ± 2.71 which is equivalent to ~17 nests per hectare. Nesting under rocks and in old termite mounds by *P. analis* at Mpala might have advantages in the regulation of the nest temperature and could offer protection against eroding effects of rains.

Relatively high nest density, (~17 nests/ha) in a savanna was reported in this study compared to 9.0 nests/ha, from a Nigerian Guinea savanna (Longhurst *et al.*, 1978) and 3.90 nests/ha from Kajiado a Kenyan savanna (Lepage, 1981). This high density can be attributed to the availability of suitable nesting sites (rocks) as well as the availability of prey at Mpala.

Foraging parties

During the study period 330 raids were observed with an average of 60 raids per month. Most raids occurred during the morning hours (0700–1100 hr) (Figure 2). In April, which was dry, nocturnal raids (2000–2400 hr) occurred; similar raids were observed in May and June with the onset of the rains (Figure 2). Nocturnal raiding behaviour and frequent morning raids could be attributed to the physiology and behaviour of ants as well as that of the termite prey. This is because *P. analis* uses trail pheromones, which are volatile under high temperatures and when these pheromones are lost

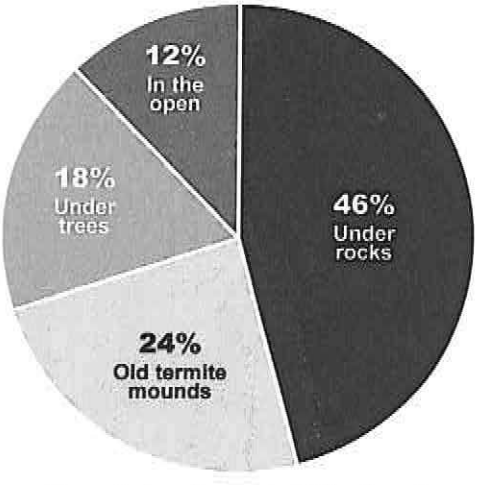

Figure 1. Location and distribution of *Pachycondyla analis* nests at Mpala

a raiding party will not be able to trace its way back to the nest. On the other hand termites are most active when the humidity is high as opposed to high temperatures.

Number of ants in raids increased steadily during the study period with an average of 170 ants in a raid. As expected the number of ants carrying termites also increased reflecting the size of the raiding party (Figure 3). On average each ant carried two termites, with the highest amount of termites carried in the month of July (Figure 3). Duration of the different phases in raids between morning and evening raids did not differ. Ants spent the same time (minutes) for outgoing and return journeys to termites' source. But they spent more time raiding than on outgoing and return journeys (Figure 4). On average raids at Mpala lasted for about 28 minutes. Duration of raids at Mpala was shorter than those reported by Longhurst *et al.*, (1978) and Lepage (1981). Shorter duration of raids could be linked to shorter distances to termite sources (6.6–13.4 m) (personal observation) which reflects the availability of food sources at Mpala.

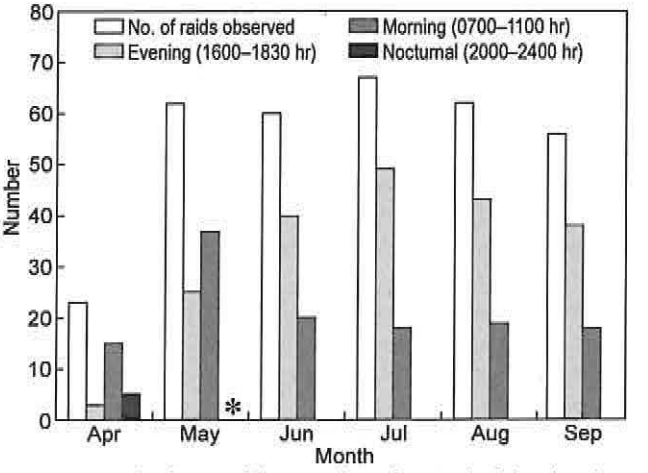

Figure 2. Raids observed by month and period of the day they occurred. Star represents presence of nocturnal raids

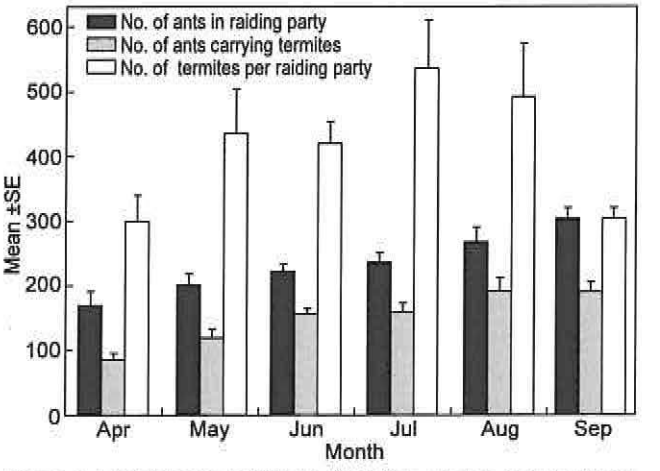

Figure 3. Monthly composition of raiding parties, ants carrying termites and number of termites carried by ants during raids at Mpala

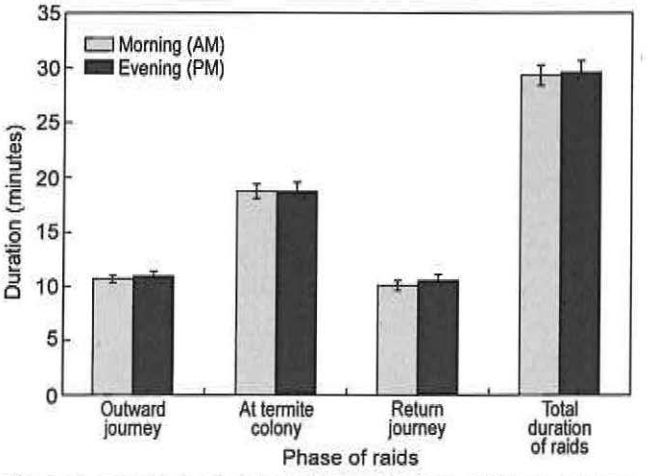

Figure 4. Duration of outward journey, time spent at termite colony, return journey and total time spent in raids for morning and evening raids

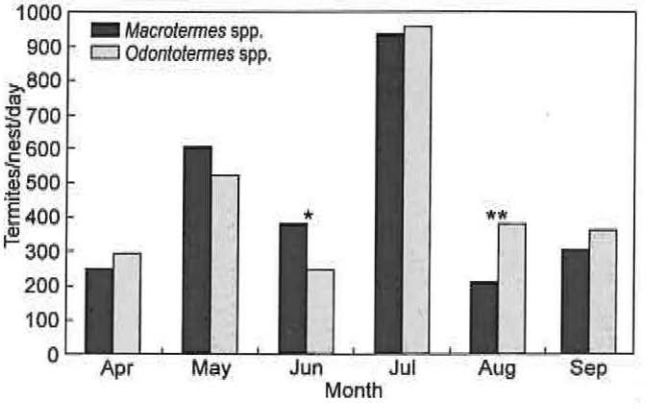

Figure 5. Monthly predation rate of *Pachycondyla analis* based on termite prey captures, * indicates significant differences at $P < 0.05$ and **$P < 0.01$

Predation rates

Pachycondyla analis was only observed to prey on two genera of termites at Mpala during the study period in varying proportions. Of the 330 raids, it was only possible to identify prey for 237 raids. *Microtermes* spp. was targeted on average in 66% of these raids and *Odontotermes* spp. in 34%. The latter was significantly more preyed on in April and May (75% and 59%, $\chi^2 = 28.2$, $P < 0.0001$), where *Microtermes* spp. was the main prey in subsequent months (69, 74, 75 and 70% respectively, $\chi^2 = 83$, $P < 0.0001$).

The average abundance of *Microtermes* spp. was higher, based on transects surveys in the field (81%) compared to the observed prey frequency by *P. analis* (70%) in September (n=21). However, when comparing the predation rate on the two species for each month, the ants preyed significantly more often on *Microtermes* in June ($\chi^2 = 4.9$, $P < 0.05$) and more on *Odontotermes* in August ($\chi^2 = 8.14$, $P < 0.01$; Figure 5).

Conclusions

Nests of *P. analis* at Mpala were mainly located beneath rocks and in old termite mounds, which may serve as a form of temperature regulation (nest homoeostasis) during the nights hence maintaining an optimum temperature throughout the day. High abundance of nests at Mpala can be attributed to high amount of food (termites) this is reflected by high frequencies of raids and predation rates. Overall, raiding activity of *P. analis* is synchronised by the behaviour of prey activity (foraging, abundance and defence).

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Potency of Endod (*Phytolacca dodecandra*) to Control Mosquito Vectors of Malaria along the Shores of Lake Victoria Basin, Kenya

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Abstract

Malaria still remains one of the leading killer diseases in the tropics as its control remains elusive with an estimated 300–500 million cases and 1.4–2.6 million deaths occurring annually, mainly among African children. In the Lake Victoria Basin (LVB) the disease vector *Anopheles gambiae* complex has increased its distribution range to highland areas where it was absent before making malaria to be a major killer for people of all age groups in this highly populated region. During the last two decades, malaria cases have continued to increase in the region leading to increased mortality, morbidity and declining productivity amongst the resident population. This may be due to increased mosquito populations and enhanced human–vector interactions arising from numerous occurrences of mosquito habitats in form of fresh water bodies such as many wetlands, natural and artificial ponds and paddy fields created by human activities. These, together with the warm tropical climate, favour breeding, survival and dispersal of mosquitoes, thereby enhancing risks of malaria transmission throughout the year. Thus control approaches of the vector have been a real threat to the government and local residents even as mortalities due to malaria keep rising especially among the poor. The green berries of *Phytolacca dodecandra* L'Herit, locally known as endod, an indigenous plant species, contain biochemicals which have been proven to be toxic to various aquatic life forms including bilharzia vector snails, zebra mussels, leeches and larvae of various aquatic insect species. This paper discusses work done in western Kenya on the efficacy of using endod (*Phytolacca dodecandra* L' Herit) commonly known as the African or Ethiopian soapberry plant for controlling immature mosquito stages in stagnant water pools. Powder from green berries of this plant was tested against immature stages of *An. gambiae* in the laboratory and under semi-field conditions along the basin. It was found that endod powder form is significantly effective against anopheles immature stages. The larvicidal activity varied with geographical sources of endod plant. For instance, plants from Nandi Hills had a higher larvicidal effect compared to those from Eldoret and Nyando. The crude powder was only more effective within 12–24 hr larval exposure and beyond this time larval mortality declined suggesting fast biodegradation of the extracts once in water. It is our belief that inclusion of endod in malaria control programmes at local community level in the LVB especially in the context of Integrated Mosquito Vector Control will help improve the health status of the vulnerable poor community in this region. More detailed studies are required to improve on the potency, packaging and application methods of endod products against mosquitoes in the malaria endemic areas of the region.

Key words: endod, *Anopheles gambiae*, Lake Victoria Basin, malaria control, larvicide

Introduction

Malaria is one of the major public health challenges eroding development in the developing world. It exerts its heaviest toll in Africa, with about 90% of the deaths from malaria worldwide occurring in sub-Saharan Africa (http://www.rbm.who.int/amd2003/ch 1.htm, accessed 21 October 2004). It is a major killer for people of all age groups in the highly populated Kenyan portion of Lake Victoria Basin (LVB). In the unborn children attack by the disease may result from placental transmission from their infected mothers (GoK, 1997). During the last two decades, malaria cases have continued to increase in the region leading to increased mortality, morbidity and declining productivity among the resident population. This may be due to increased mosquito populations and enhanced human–vector interactions arising from numerous occurrence of fresh water bodies such as many wetlands, natural and artificial ponds and paddy fields, which together with the warm tropical climate, favour breeding, survival and dispersal of mosquitoes thereby enhancing risks of malaria transmission throughout the year (Bayoh and Lindsay, 2003, 2004). Vector control is still one of the WHO approaches for Roll Back Malaria technical strategies (http://www.rbm.who.int/amd2003/ch 1.htm, accessed 21 October 2004). The principal objective of vector control is the reduction in morbidity and mortality due to malaria by reducing the level of transmission. Taking environmental and economic concerns into consideration, botanical larvicides should be examined. Interest in phytochemicals as larvicides has been fuelled by the rising costs of medicines and synthetic chemicals as well as resistance to drugs and to chemical pesticides. For maintenance of personal health and well being and sustainable control of malaria, larvicides, which are more environmentally friendly, readily available or capable of being locally produced, have become the interest of many scientists. Botanical pesticides would offer such tools, as they are biodegradable, locally available and readily acceptable by local communities. Their application does not necessarily require expensive equipment. Natural products in vector control have been proven to be effective and have little or no undesirable side effects on the environment and non-target aquatic animal species. For the control of mosquito larvae, extracts from the Meliaceae species, *Azadirachta indica* A. Juss (neem) and *Melia volkensii* (Gurke), have shown the greatest potential. Neem products serve primarily as larvicides against mosquitoes (Su and Mulla, 1998a; Mulla and Su, 1999), though technical AZ and experimental formulations of AZ have also shown ovicidal capability (Su and Mulla, 1998b), and crude preparations of neem seed or technical AZ exerted effects on reproductive events of adult mosquitoes (Ludlum and Sieber, 1988; Dhar *et al.*, 1996). A locally available plant species, endod (*Phytolacca dodecandra*), has proven to have various active compounds with molluscicidal properties against the bilharzias vector snails and has been used to control schistosome snails in Ethiopia (Gundersen and Esser, 1999) and zebra mussels in North American waters (Lee *et al.*, 1993) and as well proved toxic against 3rd instar larvae of *Aedes aegypti*, *Culex pipiens* and *Anopheles quadrimaculatus* in the USA (Spielman and Lemma, 1973). Endod has been proven to not only be effective under a wide range of environmental conditions (such as pH and temperature), but is easily degradable under a wide range of environmental conditions and

in a relatively short period of time with no residual accumulation. It is cost effective and is being economically produced, stored, transported, and applied with a low dose requirement. It is also non-toxic to mammals at molluscicidal concentrations.

Hypothesis

Crude endod material has larvicidal effects on *Anopheles gambiae*, hence potential for use in *Anopheles gambiae* control operations.

General objective

The general objective of this study was to investigate the potential of using *Phytolacca dodecandra* (endod) powder to control *Anopheles gambiae* in the LVB, Kenya.

Specific objectives

1. To establish the efficacy of endod on different immature stages of *An. gambiae*.
2. To evaluate the larvicidal effects of endod in relation to different plant's geographical origin.
3. To assess the efficacy of applying endod extracts against mosquito larvae in semi-field conditions and establish its degradability.

Materials and Methods

The plant materials, mature unripe berries, were collected from Nandi Hills, Eldoret and Nyando in Kenya. They were dried under shade in the laboratory, ground into fine powder and sieved with a sieve of 0.25 mm mesh size. Powder of different plant parts was used to prepare solutions. Mosquito test species was *An. gambiae*, uninfected laboratory strain reared in KEMRI-Kisumu insectary. Endod was subjected to laboratory bioassays and small-scale field-testing under simulated field conditions against the mosquito larvae in the LVB. In these trials guidelines for laboratory and field-testing of mosquito larvicides (WHO, 2005) were used.

Efficacy of endod from different regions on immature stages of An. gambiae

Ground powder from mature unripe endod berries from different regions (Eldoret, Nandi Hills and Nyando) was used to prepare experimental solutions by dissolving the appropriate mg of powder in distilled water to give the desired experimental concentrations of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 g/l in 1000 ml of solution, which were then bioassayed against 3rd instar larvae of *An. gambiae* according to WHO procedures (1996, 2005). In the laboratory, each of these five concentrations was replicated three times. The solutions were made up and dispensed into experimental vessels or units (plastic bowls of 1500 ml capacity, 20 x 13 x 8 cm) and incubated for 1 hr at laboratory temperature of 33 °C (Lee *et*

al., 1993). At the end of this incubation period some 100 healthy late 3rd instar larvae were then introduced into each experimental unit and monitored for 24 hours. For each assay a set of controls consisting of endod-free water with equal number and age group of mosquito larvae were set up simultaneously. Both the experimental containers/units and controls were held at 25–28 °C and a photoperiod of 12 hours light and 12 hours dark (12L: 12D). No larval food was provided to larvae during treatment time since the exposure period of 24 hours was short (WHO, 2005). Larval mortality was recorded after 12 hours and 24 hours exposure periods by counting dead larvae in both treated and control units. The larvae that neither responded to gentle prodding nor failed to rise to the solution surface even after gentle disturbance of water were considered and counted as dead. Each test was repeated 3 times on 3 different days, using fresh endod solutions (and new batches of larvae) each time. Mortality data were used in subsequent data analysis to determine lethal concentrations (LC). The lethal concentrations were defined as the concentrations at which 50% (LC_{50}) and 90% (LC_{90}) of the experimental larvae died during the exposure period.

The biopotency of powder from dried mature unripe berries of endod against 1st, 2nd, 3rd and 4th instar larvae and pupae of *An. gambiae* was tested from Nandi Hills following the above procedure.

Semi-field bioassays

Under semi-field conditions, pools were prepared by digging 15 holes of similar sizes 1 meter apart in the field outside KEMRI-Kisumu Station. Troughs of 20-litres capacity were placed in the holes and smeared with a thin layer of equal volume of soil obtained from the same field. These were filled with water and allowed to condition/acclimate for 1 week prior to treatment to simulate the natural breeding conditions of *Anopheles* mosquitoes. During this period the water level was sustained, troughs were covered with a netting material to prevent other mosquitoes or other insects from laying eggs and to protect the water from falling debris. The troughs were designed to simulate temporary pools and they provided an arrangement by which the mosquitoes could be treated and compared with untreated ones under identical conditions.

At experiment time the troughs were filled to a depth of 15 cm with 2 litres of water and left for 24 hours. A batch of 100 laboratory-reared 3rd instar larvae of *An. gambiae* was released into each trough and larval food (brewer's yeast) added due to the long exposure duration of 48 hours. After 2–3 hours of larval acclimatisation, the troughs were treated with selected endod extract dosages based on the findings of laboratory studies by broadcasting the appropriate mass of solid endod powder from mature unripe berries over the water as described by WHO (2005) to give the desired concentration of 0.15, 0.25, 0.35, 0.45 and 0.50 g/l. Higher dosages than those used in laboratory studies were required to cause substantial larval mortality under semi field conditions. Controls of water without endod powder were set up. The experiment was run in three replicates. The pools were then covered with netting material, the water level maintained and larval mortality recorded at 12, 24, 36 and 48 hr of larval exposure. The slow activity of material under field conditions necessitated extension of exposure time to 48 hours. The test was repeated 3 times on 3 different days. Mortality

data were used to determine mosquito larvicidal lethal concentrations of endod under semi-field conditions.

Biodegradability of endod

Biodegradability of endod was investigated by testing effectiveness of aged endod solutions in the laboratory using concentrations of 0.25 g/l. Solutions of 0.25 g/l concentrations were prepared and stored in experiment vessels for different periods (72, 60, 48, 36, 24, 12 and 0 hr). For each storage period the solutions were replicated 3 times. At the end of the incubation period these solutions were assayed against 3rd instar larvae of *An. gambiae*, 100 larvae for each experiment vessel. Larval mortality was recorded at 12, 24, 36 and 48 hr after exposure to endod. The exposure period was extended to 48 hours due to slow activity of the most aged (72-hr-old) solution. The data was used to determine longevity of endod preparations. A similar method was used by Lee *et al.* (1993) to determine biodegradability of endod by testing the molluscicidal effectiveness of aged endod solutions on zebra mussels.

Data analysis

The experiments involved dose response treatments consisting of binary variables of survival and mortality. Dose response curves were drawn using mean percentage larval mortalities after 12 and 24 hours of larval exposure to endod treatment and from these curves optimal larval efficacies, LC_{50} and LC_{90} values were determined.

To determine relationship between larval mortality and endod powder, mortality response of larvae was tested at each concentration by fitting logistic model to the data. The logit model

$$\text{logit}\left[\theta(x)\right] = \log\left[\frac{\theta(x)}{1-\theta(x)}\right] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i$$

is a general logistic model for testing mortality response at each concentration as described by

$$\log\left[\frac{\rho}{1-\rho}\right] = \beta_0 + \beta_1 C + \beta_2 C^2 + \beta_3 C^3$$

in dose response treatments (Agresti, 1990); where ρ denotes the probability of survival, β_0 is an intercept, β_1 is the coefficient of concentration C, and β2 is the coefficient of quadratic response in C while β_3 is the coefficient of cubic response in C. The model of efficacy ranges for the treatment dosages of endod powder was fitted using GENSTAT (GenStat Release 4.24DE). Model fit was based on residual likelihood ratio chi-square statistic. Data that fully described the model was ascertained using scaled deviance. Otherwise all the model fits that did not fully describe the data were based on residual likelihood ratio chi-square statistic based on the Wald statistics. The differences in mean percentage

larval mortalities, optimal larval efficacies, and LC_{50} and LC_{90} values were tested using Tukey's HSD multiple comparision tests (Michael and Douglas, 2004). Significance was accepted at $P \leq 0.05$.

Results and Discussion

Efficacy of endod plants from different geographical regions

Results of logistic regression test indicated that endod plants from all the three regions had significant larvicidal effects ($R^2 = 0.9665$) on all larvae exposed to them. Figures 1a and 1b show the results of observed percentage mortality of 3rd instar larvae of *An. gambiae* exposed to solutions of powder of mature unripe endod berries from different geographical regions. Table 1 describes the optimal efficacy, LC_{50} and LC_{90} values of endod plants from various regions in Kenya. Significant differences were also recorded in the minimum lethal concentrations causing 50% (LC_{50}) death ($F = 3.5443$; $P = 0.0009$) and ($F = 2.4551$; $P = 0.0000$)

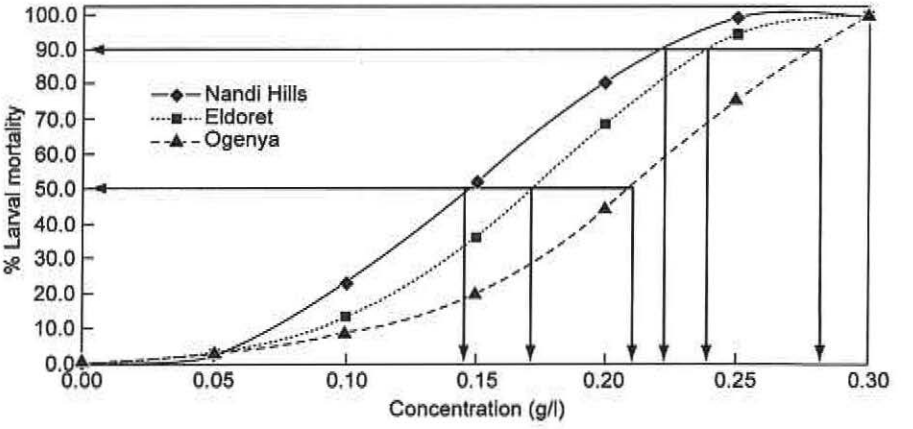

Figure 1a. Mortality of mosquito larvae after 12-hr exposure to endod suspensions of plants from different areas

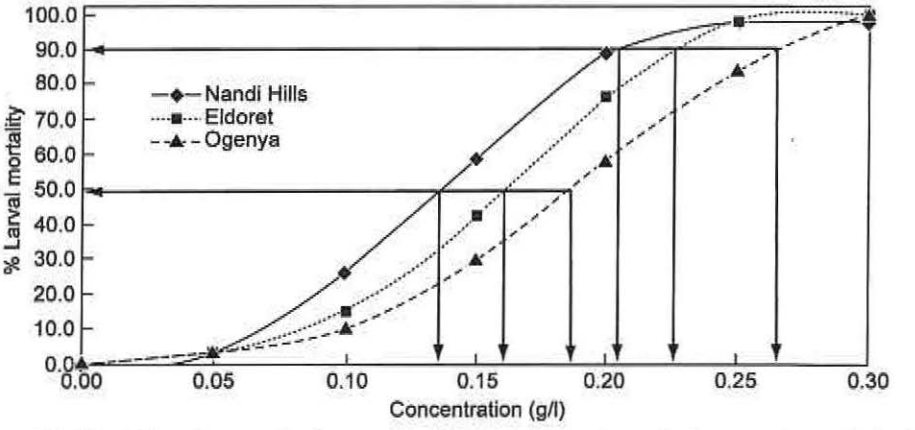

Figure 1b. Mortality of mosquito larvae after 24-hr exposure to endod suspensions of plants from different areas

at 12-hr and 24-hr exposure respectively and 90% (LC_{90}) death (F = 3.8771; P = 0.0011) and (F = 8.8776; P = 0.0001) in the larvae at 12-hr and 24-hr exposure respectively. Based on LC_{50} and LC_{90} values plants from Nandi Hills on overall had highest larvicidal activity both at 12- and 24-hr-exposure periods, while those from Nyando had lowest potency.

Table 1. Optimal efficacy, LC_{90} and LC_{50} of endod powder from various regions on *Anopheles gambiae* larvae after exposure for 12 and 24 hrs

Exposure time	Geographical origin of plant				F-value	P-value
		Nandi Hills	Eldoret	Nyando		
24 h	Optimal efficacy	98.8[a]	99.9[a]	99.9[a]	2.332	0.0727
	LC_{90}	0.21[a]	0.23[b]	0.26[c]	8.8776	0.0001
	LC_{50}	0.14[a]	0.16[b]	0.18[c]	2.4551	0.0000
12 h	Optimal efficacy	99.1[a]	99.1[a]	99.0[a]	2.4911	0.0612
	LC_{90}	0.22[a]	0.24[b]	0.28[c]	3.8771	0.0011
	LC_{50}	0.15[a]	0.17[b]	0.21[c]	3.5443	0.0009

Letters with the same superscript across the row are not significantly different (P > 0.05).

Susceptibility of different immature An. gambiae *stages to endod*

Results showing the observed mortalities of the various immature developmental stages of *An. gambiae* mosquito when treated with solutions of mature unripe endod berry powder are shown in Figures 2a and 2b at treatment times of 12 and 24 hours respectively. After a 12- and 24-hr treatment period, larval mortalities fully fitted the model equations ($R^2 > 0.9649$), and at 24 hr exposure the LC_{50} and LC_{90} values for the different stages were statistically different (F = 21.4242; P = 0.0001) and (F = 27.3325; P = 0.0001) respectively (Table 2). At 12 hrs, based on LC_{50} values susceptibility of 3rd and 4th instars was not significantly different but higher than 2nd. Pupae were least susceptible. At 24 hrs susceptibility of 2nd, 3rd and 4th instars was not significantly different but higher than pupae. 1st instars were least susceptible.

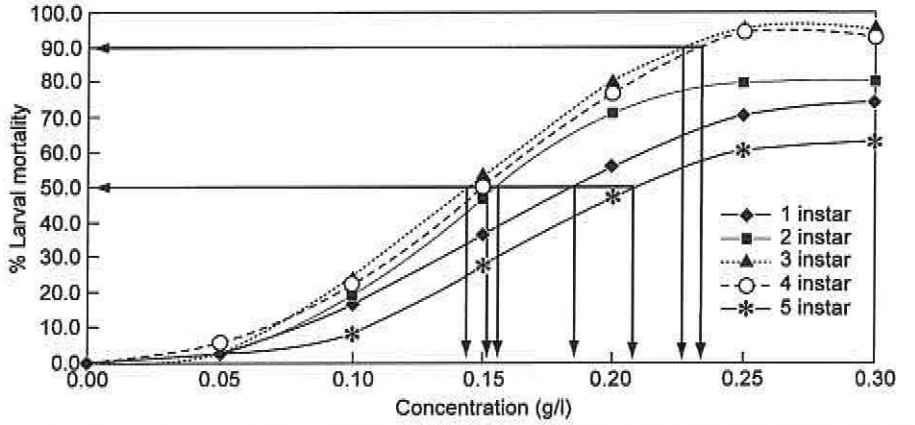

Figure 2a. Percentage mortality of immature developmental stages of *Anopheles gambiae* exposed for 12 h to endod solution

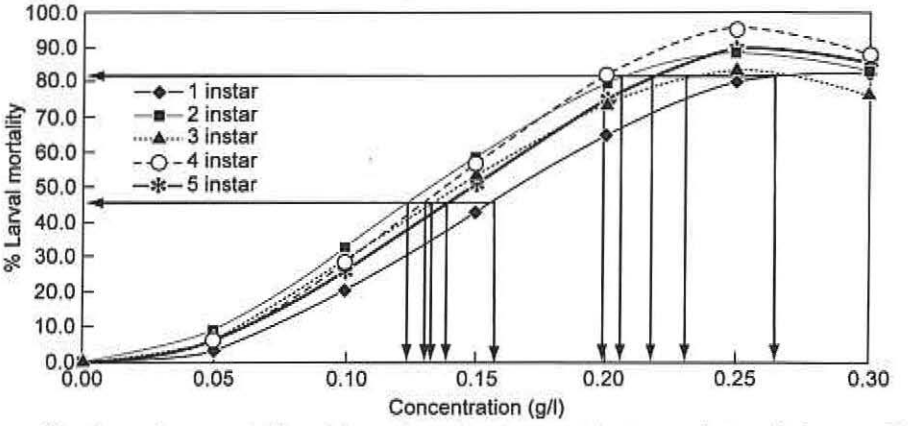

Figure 2b. Percentage mortality of immature developmental stages of *Anopheles gambiae* exposed for 24 h to endod solution

Table 2. Optimal efficacy, LC_{90} and LC_{50} of endod on immature developmental stages of *Anopheles gambiae* for 12- and 24-hr exposure periods

Exposure time		Mosquito developmental stage					F-value	P-value
		1st instar	2nd instar	3rd instar	4th instar	Pupae		
24 h	Optimal efficacy	90.2^{a}	97.1^{b}	97.2^{b}	100^{c}	98.1^{b}	24.4711	0.0000
	LC_{90}	0.26^{c}	0.21^{a}	0.24^{b}	0.20^{a}	0.22^{a}	27.3325	0.00001
	LC_{50}	0.16^{b}	0.12^{a}	0.13^{a}	0.13^{a}	$0.14^{a,b}$	21.4242	0.0001
12 h	Optimal efficacy	$74.6^{a,b}$	79.8^{b}	95.4^{c}	93.8^{c}	60.0^{a}	88.1737	0.0000
	LC_{90}	–	–	0.23^{a}	0.24^{a}	-	1.2334	0.0922
	LC_{50}	0.19^{c}	0.16^{b}	0.14^{a}	0.15^{a}	0.21^{a}	44.1587	0.0000

Letters with the same superscript in the same rows are significantly different ($P < 0.05$)

There is evidence from this study that susceptibility of *An. gambiae* to endod varies with development stage among its immature stages. The 3rd and 4th instar larvae were more susceptible than other immature stages. Among the larval stages, the 1st instar was least susceptible to endod. Death of pupae confirms larvicidal effect is not through ingestion of the powder but must be through chemical effects because pupal stage does not feed and may have died as a result of absorption of the active chemical ingredient present in endod powder into their bodies, causing toxicity which resulted in a physiological response, terminating in death. Their low susceptibility in comparison with other stages at 12 hr suggests low absorption rate of the chemical through their spiracles, the cuticle being an impediment in the absorption process. This difference in certain developmental stages of mosquitoes being more susceptible to phytochemicals has been observed in other studies, and is due to inherent physiological differences between different immature mosquito stages tested. A butanol extract of endod in USA was found toxic against the 3rd instar larvae of *Ae. aegypti*, *Cx. pipiens* and *An. quadrimaculatus* with LC_{50} values ranging from 0.3 to 0.4 mg/l (Spielman and Lemma, 1973), though direct comparision with the current study cannot be made because of the difference in solvents used.

Amorose (1995) testing the larvicidal efficacy of neem oil and defatted neem cake on *Cx. quinquefasciatus* found 3rd stage larvae more susceptible than 4th stage larvae. Pelha *et al.* (2002) reported that the larvicidal activity of commercial bark saponin extract from *Quillaja saponaria* was toxic to the 3rd and 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, but did not affect egg hatching ability in either species. Rongsriyam *et al.* (2006) testing the larvicidal activity of granular formulation of methanol extract of *Rhinacanthus nasutus* on *Ae. aegypti* and *Cx. quinquefasciatus* found 3rd stage larvae more sensitive than 4th stage larvae. Lapcharoen *et al.* (2005) on testing the potential larvicidal effect of Thai medicinal plants on *Ae. aegypti* and *Cx. quinquefasciatus*, discovered 3rd stage larvae to be more susceptible than 4th stage larvae to all plant species tested.

These results have shown that 0.3 g/l of mature endod berries is the minimum larvicidal concentration yielding 100% mortality in 12 hours. This is a low dosage and shows endod as a very effective larvicide. Regarding the difference in exposure times, larvicidal activity is seen to be higher at 24-hour exposure than at 12 hours in all the experiments, higher mortality being between 12 and 24 hours post addition than within the first 12 hrs. This can signify that the plant is more potent or effective after 24 hours exposure, suggesting that more of the active compound is dissolved and becomes available when powder stays in water for a longer time. When the powder is soaked hydrolysis occurs releasing the active ingredient into water. Mwangi and Rembold (1988) obtained a yield of 0.59% for the most active acetone extract of *Melia volkensii* fruits; the 24-hr LC_{50} of the active fraction was reported for 2nd instar *Ae. aegypti* larvae (78 mg/l) to be far higher than the 48-hr LC_{50} values. Lapcharoen *et al.* (2005) on testing the potential larvicidal effect of Thai medicinal plants on *Ae. aegypti* and *Cx. quinquefasciatus* found that all tested plants were more potent or effective after 48-hr exposures than after 24-hr exposures. These results strongly support the findings of this study.

There was a marked correlation between larvicidal activities of endod powder with place of origin of plant material. It is clear that plants from Nandi Hills were the most potent, while those from Nyando had the least potency against *An. gambiae* larvae. This suggests a variation in concentration of the larvicidal bioactive ingredient in the berries in relation to geographical factors or influence of these factors on its concentration. A marked difference has been reported in the yield of azadirachtin (mosquito bioactive compound in neem) from neem seeds from different geographical origins (Schmutterer and Zebitz, 1984; Charamamjaree *et al.*, 1997), or even in different seasons in the same geographical area (Sidhu and Behl, 1996). Plants from all the three regions are, however, effective against mosquito larvae. They yield over 98% mortality at as low concentrations as 0.28 g/l after 12 hours of application. Plants from Nandi Hills contain the highest concentration of the larvicidal compound, having the lowest 24-hr LC_{50} of 0.140 g/l. The edaphic and climatic factors of this region could be favourable for manufacture and concentration of the larvicidal components in endod berries. Plants from Nyando had lowest larvicidal potency probably due to influence of the floodplain environment where these plants were cultivated and transplantation from a different region with different environmental conditions could exert physiological stress resulting in lower concentration of larvicidal compounds in their berries. Nevertheless these

plants can be used by the local people to treat their water bodies in mosquito control programmes because the optimal efficacy after 12 hours is above 95% at a low concentration of 0.28 g/l.

Efficacy of endod to An. gambiae *under laboratory and semi-field conditions*

Treatment of *An. gambiae* larvae with endod under laboratory and field conditions indicated that similar concentrations of endod caused different larval mortalities under the two conditions at all exposure times (Figures 3a, 3b and 3c). The larvae were more susceptible to endod in the laboratory (12-hr LC_{50} = 0.22 g/l; 24-hr LC_{50} = 0.14 g/l; 36-hr LC_{50} = 0.11 g/l) than in the field (12-hr LC_{50} = 0.35 g/l; 24-hr LC_{50} = 0.23 g/l; 36-hr LC_{50} = 0.20 g/l) throughout the experimental period (Table 3). It was also clear that the material took a longer time to take effect in the field than in the laboratory (Figures 3a and 3b). Nevertheless the material is effective in field conditions considering the low

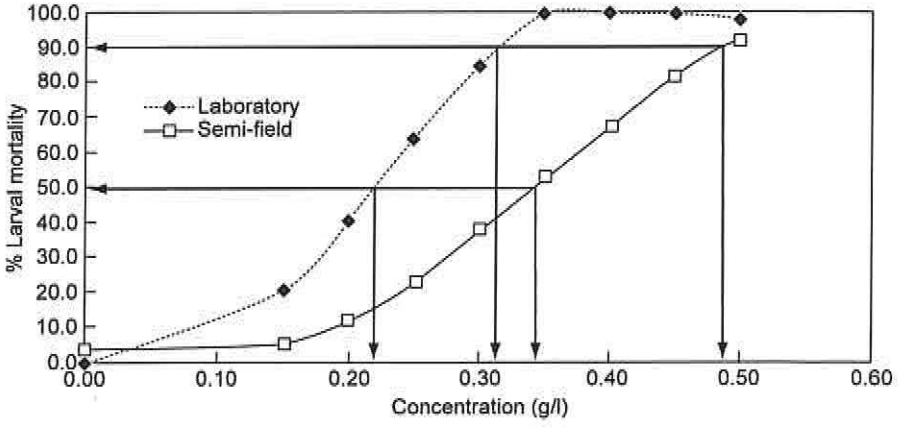

Figure 3a. Efficacy of endod to *Anopheles gambiae* larvae under laboratory and semi-field conditions

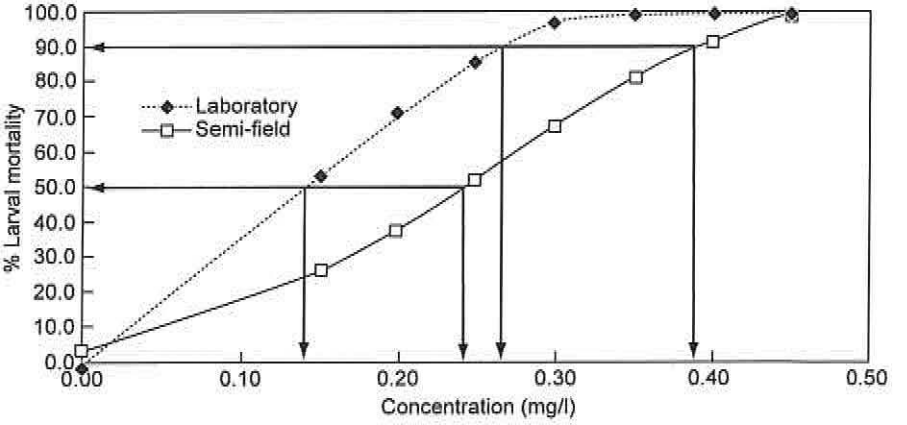

Figure 3b. Efficacy of endod to *Anopheles gambiae* larvae under laboratory and semi-field conditions after a 24-hour exposure period

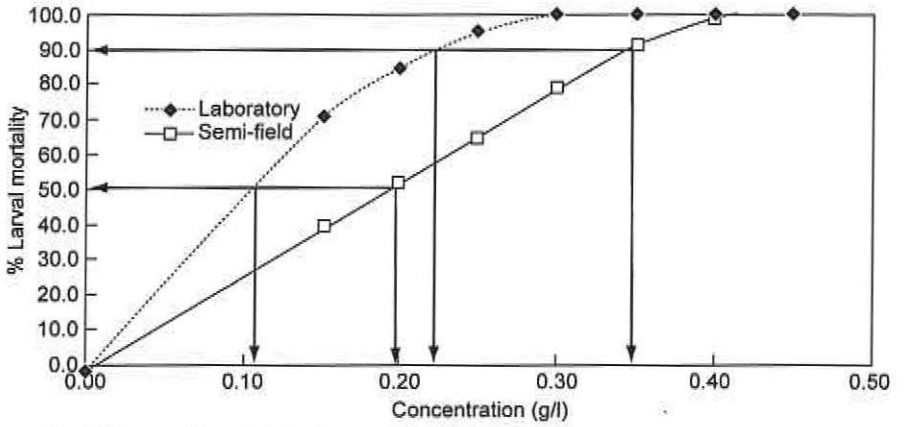

Figure 3c. Efficacy of endod to *Anopheles gambiae* larvae under laboratory and semi-field conditions after a 36-hour exposure period

effective dosage (0.55 g/l) required to cause 100% larval mortality even after 12 hours of application. Larval susceptibility under both conditions was also higher at 24-hr exposures than at 12-hr for all the concentrations used (Figures 3a and 3b).

The results obtained from acute bioassays conducted under semi-field conditions using endod berry powder have demonstrated that endod can be effective in the field as a larvicide against anopheles mosquitoes. These bioassays were useful in proving that toxicity of endod was altered by exposure under field conditions compared to laboratory acute toxicity tests at similar concentrations. They suggest that chemical constituents in endod would degrade faster in the field because UV light is stronger under natural conditions whereas the toxicity will be higher in the laboratory where the exposure period to the compounds is extended. Half-life of endod under sunlight has been shown to be 15 hours (A.I. Chihaka, personal communication). Probably the lower toxicity under semi-field conditions could be attributed to dissipation mechanisms for the active chemical constituent such as volatilisation, hydrolysis, oxidation and photolysis, thus leaving less of the active compound exposed to the larvae. There could also be more binding of material to the soil surface (adsorption to the natural substrate) and so less compound remains available in the water.

Table 3. LC_{50} and LC_{90} values under field and laboratory conditions

Exposure time		Laboratory conditions	Semi-field conditions
12hrs	LC_{50}mg/l	0.22	0.35
12hrs	LC_{90}mg/l	0.31	0.49
24hrs	LC_{50}mg/l	0.14	0.23
24hrs	LC_{90}mg/l	0.27	0.39
36hrs	LC_{50}mg/l	0.11	0.20
36hrs	LC_{90}mg/l	0.22	0.34

Biodegradability of endod

An age-dependent decrease in larvicidal activity was discovered when 3rd instar larvae of *An. gambiae* were exposed to endod solutions soaked for different lengths of time prior to treatment under laboratory conditions (Figures 4a and 4b). Soaking time had a significant effect on mortality (P= 0.0000). Results show that larval mortality generally decreased with an increase in age of solution at all exposure hours (Figure 4a). Exposure time also had a significant effect on mortality (P < 0.0000). Larvicidal activity was higher at 48 hours exposure period than at 12 hours for all the endod preparations used.

The study on degradability or modification of larvicidal activity shows there is a loss of larvicidal activities of endod (degradability is achieved) by merely storing it at room temperature for 24 hours or longer. Lee *et al.* (1993) demonstrated loss of molluscicidal activities of endod in laboratory bioassays on zebra mussels by merely storing the endod aqueous solution for 24 hours or longer. This degradation phenomenon has been attributed to the breaking of the glycosidic bonds in saponins by enzymatic procedures since glycosidase is a common cellular enzyme (Lee *et al.*, 1993). Reduced larvicidal potency with longer soaking period is presumably due to biodegradation by microbial activity (Lemma, 1970; Lemma and Yau, 1974). Microbial degradation of endod in water was confirmed in studies using filtered and unfiltered 25 mg/l endod solutions (Lemma *et al.*, 1972). When microbes were filtered out from the solution, endod molluscicidal potency was retained even after 72-hour storage at room temperature, while unfiltered preparations showed a reduced effect after storage for only 24 hours. Other studies have also shown that molluscicidal activity of endod disappeared in 1 or 2 days in field trials in Africa (Lemma and Yau, 1974) and is biodegradable in North American waters (Lee *et al.*, 1993). Chihaka (personal communication) demonstrated that molluscicidal components in aqueous endod extracts were stable during the first two days, and then rapidly declined during the 3rd and 4th days, with only traces of saponin mixture detected later than 5 days after application of the molluscicide and also indicated no molluscicidal activity of endod after 5 days. He also showed the compound is biodegraded after a lag phase of 30.7 hours with a half life of 15.8

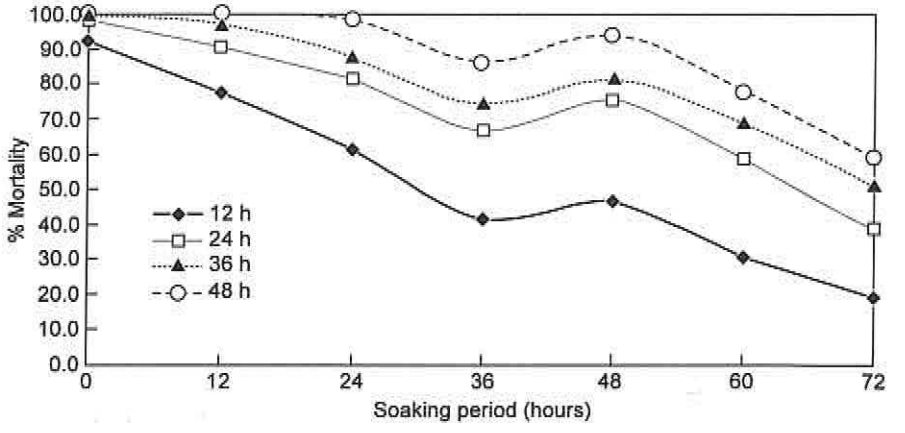

Figure 4a. Efficacy of 0.25 g/l endod solutions of different ages on *Anopheles gambiae* larvae under laboratory conditions

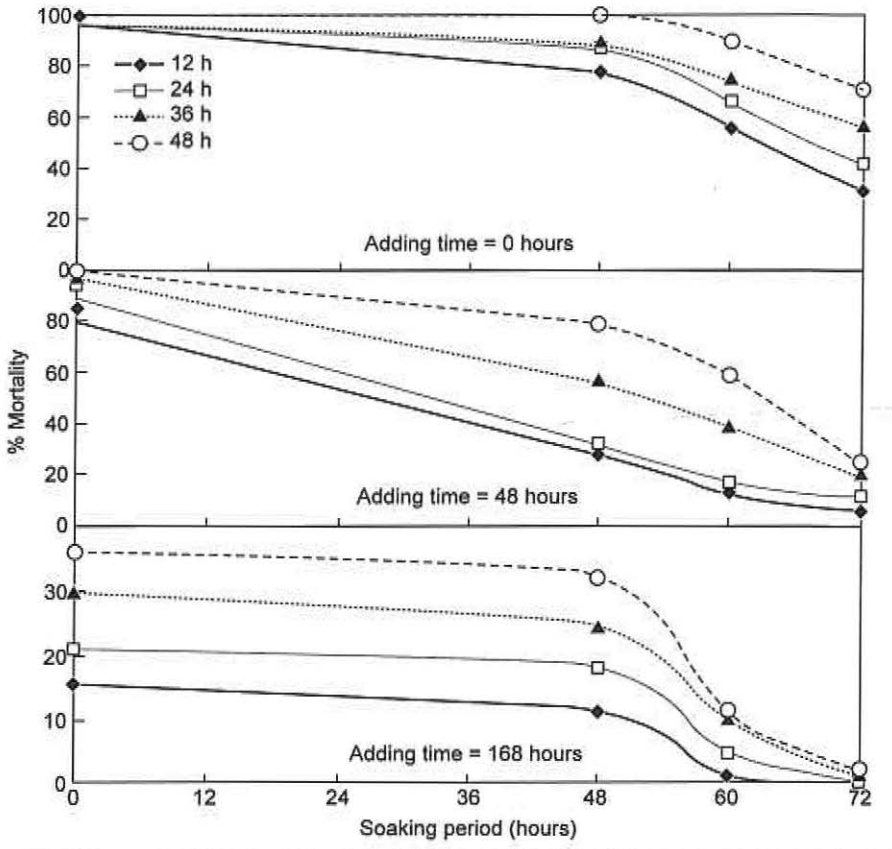

Figure 4b. Efficacy of 0.45 g/l endod solutions soaked for different time periods on *Anopheles gambiae* larvae in the laboratory

hours and biodegradation occurred within a 10-day window. The extract was considered to be readily biodegradable in most environments under aerobic conditions. The nontoxicity on mammals (Lambert *et al.*, 1991) has also been speculated to be due to enzymatic degradation of endod in animal systems. All these support the results from this study by indicating the ability of endod to biodegrade and probably the larvicidal components biodegrade in the same way molluscicidal constituents in endod do. The loss or decrease in biopotency or biological activities of endod due to biodegradation implies that endod toxicity to nontarget organisms would elicit a minimal risk, an advantage over the use of nonbiodegradable chemical compounds. Unlike other larvicides, which accumulate (gradually increase in concentration), endod active components will disappear after they exert their lethal effects to mosquito larvae and the insoluble material (pulps of plant materials) decays. The higher activity at 48 hours than at 36 hours shows that toxicity of endod to larvae may not be totally related to the active ingredient concentration, but rather is correlated with degradation/breakdown products which also play a part in the toxicity of the powder, suggesting breakdown products are larvicidal. These products could be having a dissipation rate different from that of active ingredient.

Conclusion

Endod berry powder is lethal to all immature developmental stages (larval stages as well as pupal stage) of *An. gambiae* and thus can be used to control these stages in their aquatic breeding habitats by applying endod powder to these habitats.

Larvicidal effects of endod on *An. gambiae* are greatly influenced by the geographical origin of the plant material.

Larvicidal activity of endod is higher at 24-hr exposure period than at 12-hr exposure time.

Powder from endod berries is effective under semi-field conditions as a larvicide against *An. gambiae*. Endod takes a longer time to take effect and its larvicidal efficacy is lower under semi-field conditions than under laboratory conditions.

Larvicidal components of endod in crude powder from dried ground endod berries degrade rapidly under laboratory conditions.

Thus endod berry powder has promising larvicidal activity against *An. gambiae*, having potency as a larvicide against immature stages of mosquitoes and high potential for inclusion in integrated vector and malaria management with reasonable safety. The plant products as powder have an economic effectiveness, as these can be purified to get more active ingredients. Endod may present an appropriate and sustainable solution to the control of malaria in the LVB. Through development and use of simple, appropriate agronomic techniques and extraction and application procedures, people could easily grow, process locally and use endod products to control malaria on a community self-help basis. It is a sustainable technology.

Recommendations

Based on the findings of this study, the following recommendations can be made:

1. Endod should be introduced to the rural local community as a larvicide to control mosquito larvae and pupae through its application to mosquito larval breeding aquatic habitats in an integrated malaria control programme in the LVB.
2. There is need for the Ministry of Health and other stakeholders to conduct education and awareness programmes to the rural local communities in the LVB on how to grow, process and use endod berries in controlling mosquito larvae in their aquatic breeding habitats.
3. For further research, focus can be on the following areas:
   - investigating the mode of mosquito larvicidal action of endod and efficacy of endod on a wider range of mosquito and nontarget species;
   - carrying out structural characterisation of larvicidal components of endod to determine the chemical structure of the active compound with larvicidal effects.
   - investigating the residual effects of treating the larvae with sublethal dosages of endod on their development and on blood feeding, fecundity and survivorship of adult mosquitoes.

Acknowledgement

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Arthropods Eco-Technology Adoption and Utilisation for Sustainable Development in Africa

West Nile Virus: What Africa Can Learn From a Re-emerging Global Health Threat

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Abstract

In 1999, West Nile virus (WNV) was identified for the first time in the Western Hemisphere in New York City (NYC). This event was unprecedented, as WNV had been considered local to the Middle East, Africa and Asia. This paper discusses the West Nile virus epidemic in the US and lessons Africa can learn from the management of this disease.

In the US, the first reported human cases were identified on 2 August 1999 with additional cases through 22 September 1999. By the end of the 1999 WNV season, 62 human cases were identified. Fifty-five (55) of these patients were hospitalised with encephalitis and 6 of them died. Further investigation revealed that a WNV epidemic among birds had preceded the human and equine epidemics by nearly a month. The virus was found to be capable of overwintering in local vector mosquitoes, and thus had gained permanent ecological establishment in the US.

In Washington, DC, WNV was detected within the context of one of the most intensive coordinated local biosurveillance efforts in the country. To prevent WN virus infection in humans, a comprehensive arboviral surveillance programme that includes avian, mosquito, human, remotely sensed and ground meteorological surveillance, and extensive early season larval control is recommended. Research is needed to define surveillance markers that identify unusual biological events and the efficacy and cost-effectiveness of various prevention measures in Africa.

Key words: West Nile virus, District of Columbia, mosquitoes, *Aedes albopictus*, *Culex pipiens*, early warning systems

Introduction

WNV was first isolated in Uganda in 1937 and since its isolation it has been associated with several outbreaks in humans and horses in both the Old and New World. The pathogen that causes the disease belongs to a group of disease-causing viruses known as flaviviruses that are usually spread by ticks or mosquitoes. Other well-known diseases caused by flaviviruses are yellow fever, Japanese encephalitis and dengue. West Nile virus consists of two lineages and it has been determined that lineage 1 is responsible for clinical human disease that has been isolated from Africa, Europe, Asia and North America (Berthet *et al.*, 1997; Scherret *et al.*, 2001). This paper describes West Nile virus activity in the US with particular reference to the management of the epidemic in the District of Columbia, USA, and lessons Africa can learn from this re-emerging vector-borne disease.

West Nile Virus Activity in the US

WNV is the leading cause of arboviral encephalitis in the United States. It has caused seasonal epidemics of febrile illness and neurologic disease in the United States since it was first identified in New York in 1999. This event was unprecedented, as WNV had been considered local to the Middle East, Africa and Asia. The first reported human cases were identified on 2 August 1999 with additional cases through 22 September 1999 (Nash *et al.*, 2001). The Centers for Disease Control (CDC) reported 62 WNV cases by the end of the 1999 WNV season (CDC, 2004). Fifty-five (55) of these patients were hospitalised with encephalitis and 6 of them died. Further investigation revealed that a WNV epidemic among birds had preceded the human and equine epidemics by nearly a month. The virus was found to be capable of overwintering in local vector mosquitoes and was well established in the country.

The spread of WNV across the US in 2002 (Figure 1) became the largest vector-borne encephalitis epidemic recorded in the history of the Western Hemisphere and the largest WNV-related meningo-encephalitis epidemic recorded in world history (CDC, 2002). By the end of the 2002 season, 4156 human cases of WNV had been identified in the US, with 284 deaths (CDC, 2004). By the end of 2002, the disease had spread widely in the country with only 6 states (Arizona, Utah, Nevada, Oregon, Alaska and Hawaii) that did not report WNV activity. By the end of 2003, only 4 states had not reported WNV activity (Figure 2).

The US continues to wage a steady but difficult war against the disease and recent figures show that the disease is still spreading. By 2007, WNV transmission to humans or animals expanded into 19 counties that had not reported transmission previously and recurred in 1148 counties where transmission had

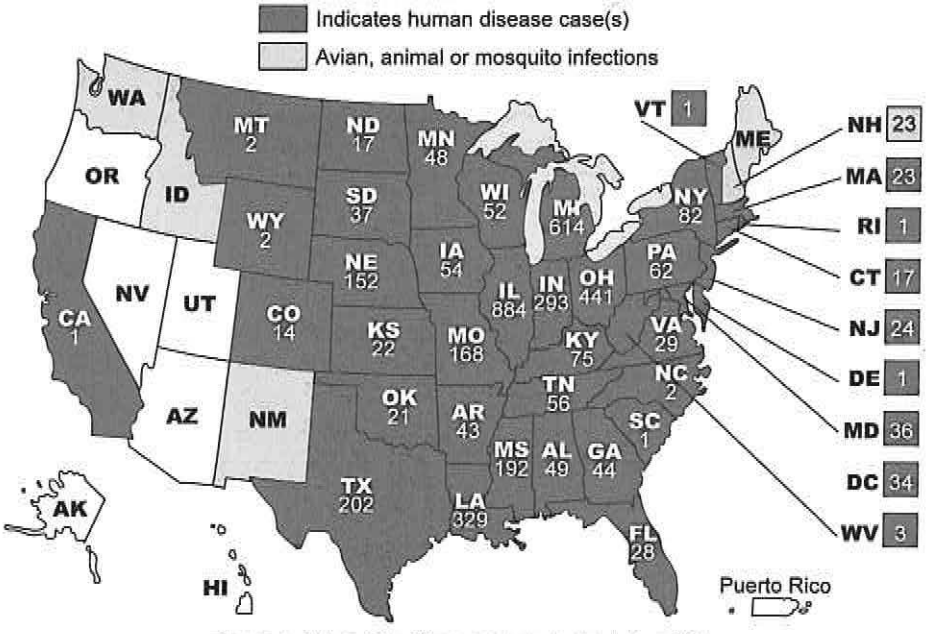

Figure 1. West Nile Virus activity in the US, 2002
(Source: Centers for Disease Control, USA)

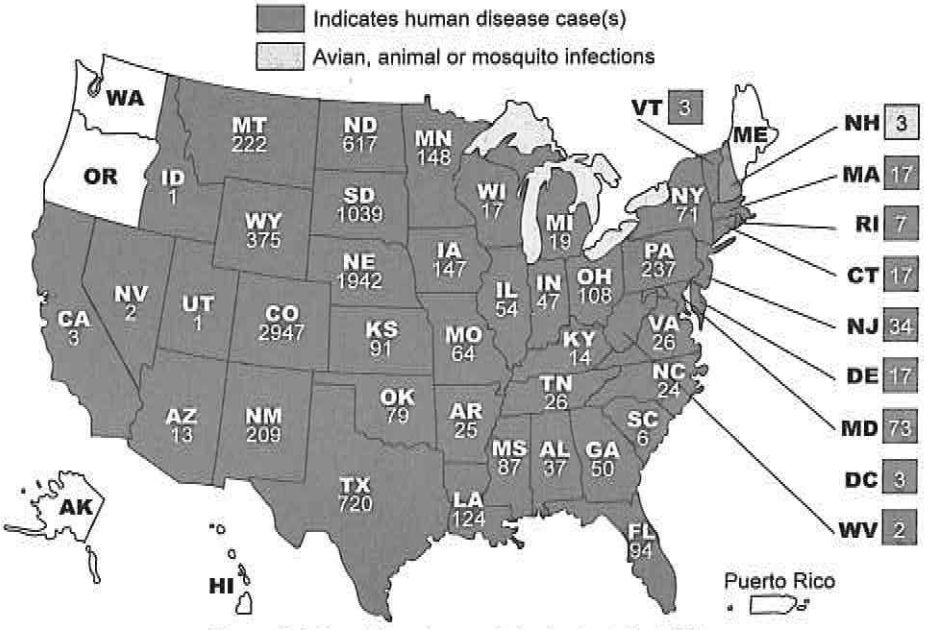

Figure 2. West Nile virus activity in the US, 2003
(Source: Centers for Disease Control, USA)

been reported in previous years. A total of 1227 cases of WNV neuroinvasive disease (WNND) and 117 deaths were reported (CDC, 2007). The reported incidence of WNND in the United States in 2007 was 0.4 per 100,000 population. This incidence is similar to that reported in 2004 (0.4), 2005 (0.4) and 2006 (0.5), but substantially lower than the reported incidence for 2002 (1.0) and 2003 (1.0). CDC therefore continues to stress the need for ongoing surveillance, mosquito control, promotion of personal protection from mosquito bites, and research into additional prevention strategies, including a WNV human vaccine.

West Nile Virus Activity in the District of Columbia (Washington, DC), 2001–2007

Between 1999 and 2000, there were no positive WNV cases reported to the District of Columbia Department of Health (DOH). The District of Columbia recorded its first case in 2002 and by the end of the year a total of 31 cases had been reported (Figure 3). Control measures intensified during that period and the number of positive human cases dropped markedly in 2003 with only 3 human cases recorded at that time. The number of positive cases continued to drop in subsequent years, and by 2007, not a single positive human case was recorded. The District of Columbia Department of Health stopped collecting and testing dead birds in 2002 because WNV was considered endemic in the District. Furthermore, positive results of dead bird testing did not provide any relevant information. Sightings of dead birds are still received and compiled by DOH. In 2001, 360 (81%) birds tested positive for WNV and in 2002, 175 (85%) were positive. The main mosquito WNV carriers in the District of Columbia are *Aedes albopictus* and *Culex pipiens*. In 2001, 0.36% of female mosquitoes were positive for WNV (Table 1). This was lower than in 2002 which had a positive

rate of (6.39%). The rate continued to decline in recent years with the lowest rate reported in 2007 (1.18%).

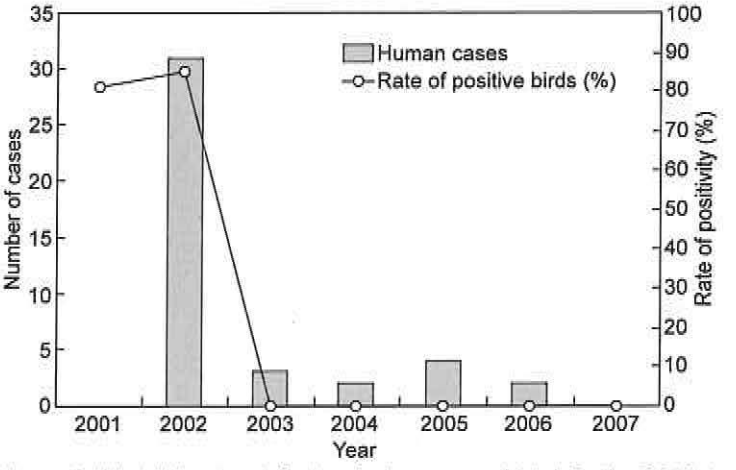

Figure 3. West Nile virus infection in humans and birds in the District of Columbia, 2001–2007

Table 1. Mosquito surveillance in the District of Columbia, 2001–2007

YEAR	2001	2002	2003	2004	2005	2006	2007
Pools tested	841	1315	2114	1671	1399	1917	1014
Positive pools	3	84	49	42	55	56	12
Rate of positivity for female mosquitoes tested (%)	0.36	6.39	2.31	2.51	3.93	2.92	1.18

In 2001, The Metropolitan Council of Governments (MCOG) in collaboration with health officers in the District of Columbia, Maryland and Virginia, developed a plan to prevent the spread of WNV in the region (MCOG, 2001). The plan emphasised a graduated risk level approach to planning and outlined activities and resources suitable for implementing a response to the threat of a West Nile virus outbreak in a locality as determined by the risk level.

By 2003, WNV had become endemic in the US and therefore a new plan was developed that was not based on risk levels but on intervention measures to manage the disease (MCOG, 2003). The goal of the plan was to reduce the threat of mosquito species that carry WNV to human populations. The control measures adopted in the District of Columbia were focused on human surveillance, mosquito control, personal protection and public education. For human surveillance, public health personnel distributed WNV physician alerts by blast fax to healthcare providers and hospitals detailing the West Nile virus reporting and specimen submission criteria. Public health personnel also contacted hospital infectious disease practitioners weekly to determine if any patients met the testing and reporting criteria.

The most effective mosquito and arbovirus control programmes utilises a system of integrated pest management (IPM). IPM utilises knowledge of

the current mosquito problem to consider from among all available control strategies, and the best ones to use. Intensive public education on mosquito and avoidance techniques was conducted throughout the District of Columbia. Although adulticiding was carried out in other jurisdictions like Maryland and Virginia, no adulticiding was carried out in the District of Columbia. This was mainly due to concern for the vulnerable population, like those with various chronic respiratory diseases like asthma, which is highly prevalent in the population.

Larviciding was the main method of mosquito control adopted. DOH staff applied larvicides in response to positive birds and mosquitoes and community concerns until dead bird collection was terminated and the focus switched to mainly positive mosquito identification and control. The larvicide, a biological product that kills mosquitoes in the larval stage, is placed in catch basins and in areas of standing or stagnant water. Killing mosquito larvae and eliminating mosquito breeding sites appeared to be the most effective measures in reducing the numbers of mosquitoes. It has been shown that larviciding is far more effective in reducing mosquitoes than spraying over time (Walker and Lynch, 2007; Fillinger et al., 2008). The control of WNV in Washington, DC is highly dependent on mainly federal and some local funding. It is this combined infusion of resources, including the use of a relatively cheap method of control, and aggressive surveillance that has resulted in a successful control programme.

Implications for Africa

Ecological changes have caused some vector-borne diseases to appear for the first time and resurgence of others that were quiescent or under control (Katz, 1999). Some of these diseases have become serious public health concerns in Africa and other parts of the world. For example, there has been a resurgence of several well-known vector-borne diseases like malaria and dengue, and in the last decade, the incidence of malaria increased in some endemic countries as a result of ecological changes (Katz, 1999).

West Nile virus in North America is a re-emerging vector-borne disease that presents entomologists and other public health practitioners in Africa with an excellent opportunity to learn and prepare for similar epidemics. Lessons learned from this disease will help in the design of effective control programmes for other vector-borne diseases.

After the initial isolation of West Nile virus in Uganda, sporadic cases and outbreaks of febrile disease were recorded in humans in other parts of Africa, the Near East and Asia. The largest outbreak occurred in South Africa in 1974 (McIntosh *et al.*, 1976). Although WNV causes few problems for most livestock farmers, it has been suggested resource-poor farmers, especially those living near lakes on bird migratory routes, have cause to fear the debilitating effects of severe illness (The New Agriculturist http://www.new-ag.info/03-2/focuson/focuson4.html).

The frequency and spread of West Nile virus neurologic infections, the increase in human cases and deaths including outbreaks in horses and birds in the Northern Hemisphere have raised the question of whether a recent emergence of WNV strains with increased pathogenicities occurred or whether

the virulence of the virus had previously been underestimated (Katz, 1999). It has also made health authorities aware of the potential of previously forgotten viruses to become a threat to public health. To address this threat, in the US, health officials worked hard to limit the spread of the disease. Local health departments in the US focused on intensive control measures in partnership with other jurisdictions. This was more so in the Washington, DC Metro Area where people commute extensively from one jurisdiction to the other daily. Local authorities in Maryland, Virginia and the District of Columbia embarked on human and animal surveillance, larviciding, and encouraged personal protection strategies (MCOG, 2003).

While these strategies are essential for effective vector control, the WNV epidemic in the US clearly showed that enormous resources are required to control the disease. In Louisiana, the estimated cost of the epidemic was found to be $20.1 million from June 2002 to February 2003, including a $10.9 million cost of illness ($4.4 million medical and $6.5 million nonmedical costs) and a $9.2 million cost of public health response (Zohrabian *et al.*, 2004). Since infectious disease control programmes in local public were seriously underfunded, the federal government allocated massive resources to build the public health infrastructure for effective management of the epidemic. Therefore, to achieve success in Africa, the public health infrastructure in Africa would have to be rebuilt and policy changes made to support public health approaches to disease prevention.

Cost is always an issue in Africa for extensive disease control programmes. In this regard, practical methods that have been found to be effective and cheap in the US and other parts of the world could be employed in vector-borne disease management. For example, larviciding of vector breeding sites and eliminating breeding habitats by flushing streams through the seasonal release of water from upstream reservoirs have been found to be cheaper than other preventive measures in controlling mosquito-borne diseases (Konradsen *et al.*, 1999; Suaya *et al.*, 2007). Larviciding was extensively employed in the District of Columbia to control WNV.

Research is also needed to understand the dynamics of emerging and re-emerging vector-borne diseases. Since mosquitoes and mosquito-borne disease transmission are sensitive to hydrologic variability, it is necessary to monitor hydrologic conditions or model population dynamics of vector mosquitoes so that early warning systems can be developed. Effective government involvement, financial support and political will to address emerging and re-emerging diseases will be necessary for achieving success in Africa.

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Climate Change and Management of Arthropod Pests and Vectors

Climate Change, Agriculture and Food Security in the Sahel Region

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Abstract

The Sahel is highly vulnerable to climate change due its geographic location at the southern edge of the Sahara desert and the strong dependence of its population on climate sensitive activities such as agriculture, livestock and forestry. The primary sector employs more than 60 percent of the active population and contributes 40 percent of the GDP. Rainfall variability and desertification are common processes. This, coupled with the low use of modern technologies such as improved germplasm, fertilisers, mechanisation and irrigation, makes the Sahel one of the most food insecure regions in the world. Although many climate models predict an increase in precipitation in the region during the 21st century, positive impacts may be neutralised by the coupled effect of higher temperatures and more frequent climatic extremes.

The objective of this paper is to highlight some of the coping mechanisms the Sahelian communities have used, and the contribution of scientific research and technological innovations in addressing the major constraints of drought and land degradation. The use of climate information, coupled with an improvement in crop management, including the use of techniques such as rainwater harvesting, soil conservation and agroforestry can enhance productivity among smallholder farming systems and buffer them against the uncertainties related to climate change.

Key words: agriculture, climate change, climate variability, drought, Sahel

Definitions of the Sahel

The Sahel represents the southern edge of the Sahara desert, extending roughly 4500 km from Cape Verde through Senegal, Mauritania, Mali, Burkina Faso, Niger and Chad. It is limited to the south by the less arid Sudan-Sahel belt, and constitutes a transitional zone between the arid Sahara and the tropical forest that borders the maritime coast. The climate is generally warm and characterised by irregular summer rains that vary between 200 mm and 600 mm falling in a period of 3 to 4 months. The succession of dry years and wet years is a typical feature of the climate.

The term 'Sahel' also refers to a geopolitical entity. In 1973, nine countries (Table 1) formed the Permanent Interstates Committee for Drought Control in the Sahel (CILSS). The CILSS space covers approximately 5.7 million km^2 and is home to about 60 million people. Livelihood strategies are based on agriculture, livestock, fishing, short and long-distance trading, and a variety of urban occupations. Dryland crops such as millet, sorghum and cowpea provide the staple food for the populations while groundnut and cotton are the major cash

crops. Rain-fed agriculture dominates the production systems although some irrigation activities are undertaken around the river banks. Livestock provides the major source of income in many areas.

Why is the Sahel Vulnerable to Climate Change?

Sahelian countries are among the poorest in the world (Table 1), where climate variability poses a major obstacle to the achievement of the MDGs. Droughts with varying degrees of severity occur in two out of every five years. In the 20th century, the Sahel region experienced three major drought periods: 1910–1916, 1941–1945 and the long period of sustained rainfall decline that spanned the 1970s and most of the 1980s, and continued with some interruptions into the 1990s. The annual rainfall values of 1983 and 1984 were among the lowest ever recorded in the history of the Sahel, but severe droughts also occurred in 1972, 1973 and 1977. In 1984, drought severely affected all countries from Mauritania to Ethiopia, including several bordering countries on the southern edge of the Sahel. In contrast, the 1973 drought was more localised and affected mostly Mali, Niger and Chad.

Soils are inherently fragile, with low carbon and nutrients contents. This, coupled with poor management, has often produced disastrous socioeconomic and environmental outcomes. In the Sahel, the use of fertilisers, improved seeds and other technologies is very limited due to a number of reasons including unreliable rainfall and poor infrastructure that does not facilitate access to input and output markets.

Aggregate food production has increased over the last few decades, but this has not kept pace with the rapid growth of the population, resulting in a decline of per capita food availability. Furthermore, this increase has been mainly based on the expansion of the cultivated area since cereal yields per unit area have largely stagnated (Figure 1). The consequence is the mining of nutrients and the deterioration of the land resources especially when marginal lands are brought under crop production. In many areas, the disappearance of fallows and the reduction of grazing areas have led to overgrazing, which is a recognised cause of desertification.

Table 1. CILSS member countries (source: www.hdr.undp.org/en/countries)

	Population (millions)	Per capita GDP (PPP)	HDI rank/177
Burkina Faso	13.9	1,084	173
Cape Verde	0.5	2,833	118
Chad	10.1	1,470	170
Gambia	1.6	1,152	160
Guinea Bissau	1.6	467	171
Mali	11.6	1,058	168
Mauritania	3.0	1,890	140
Niger	13.3	612	174
Senegal	11.8	1,592	153

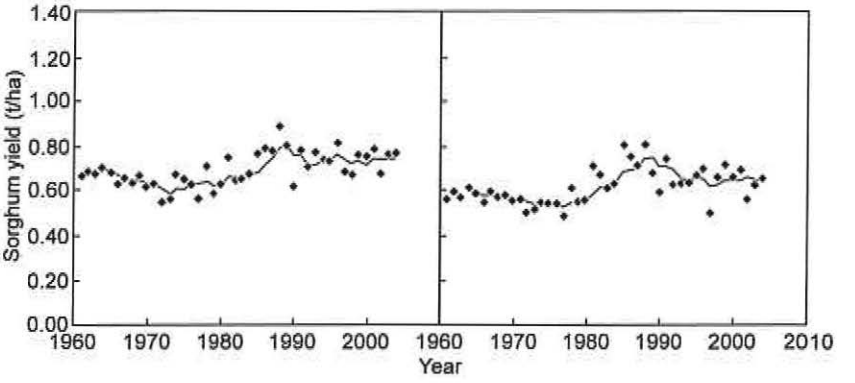

Figure 1. Averaged sorghum and millet yield for the nine CILSS member countries since 1961 (data from FAOSTAT)

Although rainfall and poor soil conditions are key factors, they are only part of a complex combination of processes that makes the Sahel a highly vulnerable entity. Over the last half-century, the combined effects of population growth, land degradation, reduced and erratic rainfall, lack of coherent environmental policies and misplaced development priorities, have contributed to transform a large proportion of the Sahel into barren land. The desertification process, which has prevailed in the Sahel over the last few decades, is nothing more than the embodiment of a degenerative process that started several decades back. The droughts of the 1970s, 1980s and 1990s were not necessarily the cause, but certainly the culmination, of this environmental crisis. Even if rainfall has come back to near normal in recent years, the Sahel remains an environmentally sensitive region and climate change will only exacerbate the vulnerability of its ecological and socio-economic systems.

Is the Climate Changing in the Sahel?

Climatic trends in the 20th century

There is now scientific consensus that the global climate is changing. Global mean temperature increased by 0.6 °C in the last century, with the hottest years ever in record occurring after 1990. This warming of the world climate has been linked to a higher concentration of greenhouse gases (GHGs) in the atmosphere, and one consequence of this is the increased frequency of climate extremes such as floods, droughts and cyclones. The Sahel region has had its fair share of changes. While rainfall variability is a major characteristic of its climate, the second half of the 20th century witnessed a dramatic reduction in mean annual rainfall throughout the region (Figure 2). According to the IPCC (Intergovernmental Panel on Climate Change), a 29–49% rainfall decrease was observed within the Sahel region between 1968 and 1997 compared to the 1931–1960 baseline period. This long-term decline of rainfall ended in 1988, which marked the reappearance of 'normal' rainfall years.

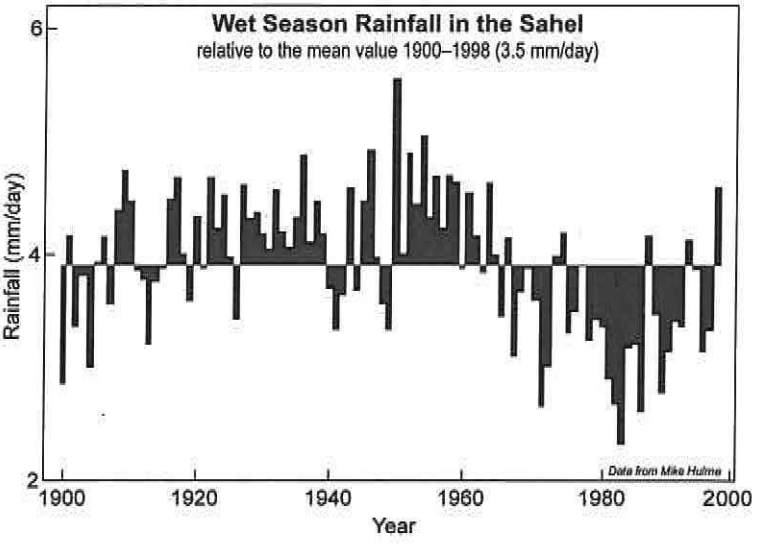

Figure 2. Wet season daily rainfall in the Sahel relative to the 1900–1998 mean value (3.5 mm per day) (Source: Taylor, 2001)

Future changes

While much is known about the climatic past and present of the Sahel, predicting its future climate appears to be a more complicated task. Models have often shown conflicting outcomes. In the third assessment report of the IPCC, general circulation model (GCM) simulations suggest a future warming of 0.2 °C per decade (low warming scenario) to more than 0.5 °C per decade (high warming scenario) across the African region, with warming expected to be greatest in the interior of the Sahel and in central southern Africa. Rainfall increases may also be expected in the Sahel. In a recent study, scientists from the National Centre for Atmospheric Research (NCAR) and the National Oceanic and Atmospheric Administration (NOAA) showed that the Sahel would be 20 to 30 percent wetter by 2050 compared to the 1950–1999 average.

Implications of Climate Change for the Sahel

The socio-economic impacts of the drying

The drought that hit the Sahel from the late 1960s to the 1980s was a wake up call to the international community (Batterbury, 2001; Mortimore and Adams, 2001; Raynaut, 2001). There were an estimated 100,000 deaths. Other consequences include hunger, malnutrition and widespread misery. Many people migrated in search of relief. Squatter settlements and urban overcrowding increased, accompanied by rising unemployment. Additional burdens were placed on limited social services, and political instability intensified in many countries.

Chad was one of the worst affected. It is estimated that more than 900,000 people severely suffered from the drought (DMC, 1995). In 1973, the GDP of Chad dipped by 9 percent; by 1975, its per capita GNP dropped to US$ 120, ranking Chad as one of the poorest countries in the world. The 1984 drought

had similar impacts on Mali and Niger, who saw their GDP shrink by 9 and 18 percent respectively (World Conference on Disaster Reduction, 1994).

Climate change and food security

Despite the lack of consensus over the direction future changes in precipitations will take, climate change is likely to have negative consequences on agricultural production and food security in the Sahel region. Arid conditions may be exacerbated because of a higher evapotranspiration regime. Extremes in the form of droughts and floods will be more frequent, putting additional pressure on already stressed systems. While global food supplies may not be affected by future shifts in climate due to gains in arable land in boreal and temperate areas, many projections show food production declining in Africa, and the Sahel will not be spared by the deficit (Table 2).

Table 2. Anticipated impact of climate change on maize production (Jones and Thornton, 2003)

	Simulated yield in 2000	Simulated yield in 2055	Production change (t)	Production change (%)
Burkina Faso	966	809	-43,960	-16
Chad	1127	914	-20,987	-19
Gambia	1160	940	-3,300	-19
Guinea Bissau	1306	1126	-4,860	-14
Mali	1053	740	-43,820	-30
Mauritania	591	583	-104	-1
Niger	649	539	-660	-17
Senegal	1013	849	-16,400	-16
Total	**983**	**813**	**-134,091**	**-17**

Analysis of Adaptation Efforts in the Sahel

The negative effects of the 1970s droughts, which led to losses of human lives and visited hunger and poverty on millions of people, were exacerbated by the fact that the coping capacity of the rural populations was stretched to its limits (Hulme, 2001). Climatologically speaking, the 1984 drought was more severe than that of 1973. Yet, the former made relatively less damage as the economies and societies of the Sahelian countries had, by the mid-1980s, developed more appropriate coping mechanisms to deal with such extreme situations (Batterbury, 2001).

Local responses to climate variability

Owing to rainfall variability, farmers in the Sahel have to show enough wits and flexibility every year in the timing of the various farming operations and in the management of household labour. They use local climatic indicators

including tree fruit and flower production, duration and intensity of cold and hot periods, bird and insect behaviour, and movement of stars and the moon to predict precipitations (Roncoli *et al.*, 2002) (although the reliability of these traditional approaches may need to be investigated further). For example, in a good rainfall year, farm households, which are limited by labour, may decide to reduce the cultivated area, use more manure and focus on weeding to maximise yield while households that have more labour can expand the cultivated area to make maximum use of good rainfall conditions.

Maintaining a high level of plant diversity in the farms and agricultural landscapes has also been a recognised strategy to reduce food insecurity. Simultaneous growing of different types of crops or cultivars of the same crops is not uncommon in the Sahel. Despite the dry conditions, inventories have shown a surprisingly high number of non-domesticated species (trees, shrubs, herbs) in the Sahelian landscapes, each of them playing a useful role. The unrelenting conversion of natural systems to croplands is no doubt reducing plant populations, but diversity is not as significantly affected as one would expect since many indigenous tree species are well conserved through a system of selective clearing (the parkland system). People's reliance on indigenous trees becomes more important during droughts (Joet *et al.*, 1998; Ong and Leakey, 1999; Mortimore and Adams, 2001). Farmers also spread risks by simultaneously using various types of soil that respond differently to droughts and floods (Reenberg *et al.*, 1998; Mazzucato and Niemeijer, 2000; Reenberg, 2001; Warren, 2002).

These few illustrations represent a small portion of the myriad of adaptive strategies of the Sahelian farmers. Yet, they give an idea of the rural communities' flexibility vis-à-vis the management of their resources. These strategies have their own strengths and have helped rural dwellers withstand some of the pressure posed by climate variability. They also have their limits, especially when extremes become more persistent leading to so-called entitlement failure (inability to command sufficient food to prevent starvation) (Sen, 1981). Moreover, adaptation to changes including climate variability is a dynamic process and should, as a survival mechanism, display enough flexibility to accommodate new components. The need for alternative sources of income outside agriculture is real and calls for a diversification of livelihood strategies, which starts usually with livestock ownership (Figure 3).

Keeping animals has become an important aspect of the farmers' livelihoods in the Sahel. Recent surveys show that the number of animals kept by sedentary farmers is becoming more important than the number held by specialised nomadic pastoralists. A progressive shift from cattle to small ruminants has also been observed, which can be viewed as a strategic move since small stock reproduce much faster than cattle and are more hardy, less costly and easier to feed (Mortimore and Adams, 2001).

As part of a wider economy, farmers are aware of other income-generating opportunities existing around them that can help supplement their farm-derived income. These vary from small trade and business activities within the confines of the village territory, to seasonal migration or travel to distant places (Batterbury, 2001; Mortimore and Adams, 2001). Thanks to rapid urbanisation and improved mobility in West Africa, farmers are now able to export their

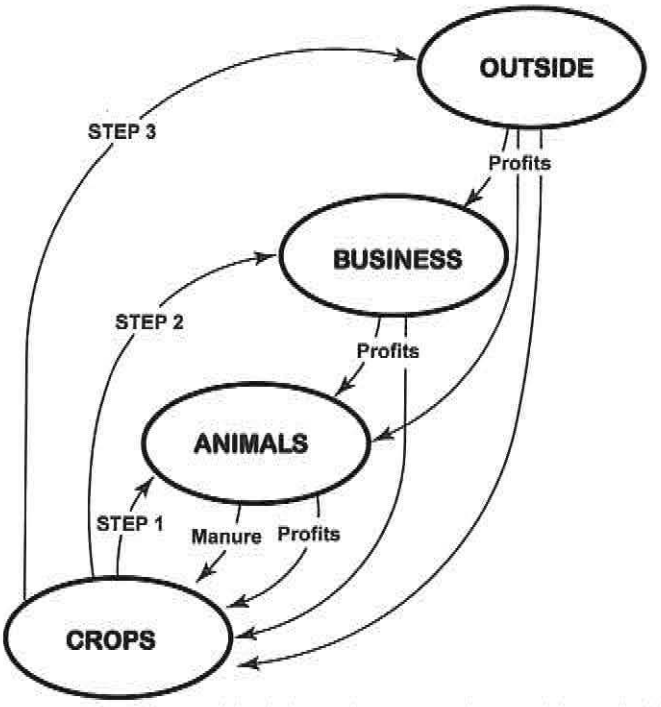

Figure 3. A simple model of diversification in the rural household economy (adapted from Mortimore and Adams, 1999)

labour force towards the cities, neighbouring coastal countries or even to other continents (Raynaut, 2001). In some cases, seasonal migration offers farmers an opportunity to improve farm productivity, not only by investing part of their remittances in the farm, but also by applying technologies learnt from elsewhere.

Regional and national efforts

The Sahelian states recognised drought as a major roadblock to overcome years before climate change became a subject of international debate. This materialised with the creation of CILSS in 1973. Its mandate: "to invest into the research for food security and in the fight against the effects of drought and desertification in order to achieve a new ecological equilibrium" in the Sahel (www.cilss.bf) bears testimony to the commitment of the Sahelian governments to address the pressing challenge posed by climate variability in the region.

This commitment was reiterated with the ratification by the various states of the three major international conventions, namely the Convention on Biological Diversity (CBD), the United Nations Convention to Combat Desertification (UNCCD) and the UNFCCC. A growing number of countries in the region is also ratifying the Kyoto Protocol. In an effort to implement the Climate Convention, the Sahelian countries continue to produce and submit national communications to the UNFCCC. They are also finalising their NAPAs (National Adaptation Programme of Action) through which the most urgent adaptation needs are identified and addressed.

The Sahelian countries place agriculture at the core of their socio-economic development and poverty reduction strategies. The primary sector plays many important roles including: (1) improvement of food security; (2) job creation and income for the rural population; (3) supply of raw material to agro-industries; (4) the absorption of a part of the industrial and semi-industrial sector's outputs (fertilisers, pesticides, machinery, etc); and (5) the generation of foreign exchange.

A whole range of development plans, programmes, policies and projects have been developed by the Sahelian nations and most of them contain provisions for improving agricultural productivity. Policy documents such as the National Environmental Action Plans (NEAPs), the National Action Plans to Combat Desertification (NAPs), the Poverty Reduction Strategy Papers (PRSPs) and many other sectoral plans contain strategies and interventions aimed at accelerating agricultural growth as an engine of economic development and poverty reduction. Although the issue of climate change is not often addressed directly in these policies and programmes, climate variability (especially drought) is often recognised as the major problem to which responses need to be found if food insecurity and poverty are to be significantly reduced in the Sahelian countries. Implementing these strategies can contribute meaningfully to reducing the vulnerability of the rural populations in the face of climate change.

The Role of Research in Climate Change Adaptation

Potential of seasonal weather forecasting for local populations

Seasonal weather forecasting can help farmers and other land users in their decision making processes. A study by Ingram et al. (2002) in Burkina Faso showed that farmers had a strong interest in receiving forecast information since their traditional methods of predicting rainfall have become unreliable due to climate change. Seasonal forecasts could help them adjust their planting dates and management strategies, choose suitable crops or crop varieties and make a wide range of agricultural and non-agricultural decisions, based on timely and reliable information (Table 3). Awareness over an impending drought can help livestock farmers sell animals at good prices and avoid competition for insufficient pasture resources and unnecessary death of animals. Climate forecasts can also influence decisions on alternative livelihood strategies such as seasonal migration and other income generating activities.

There exist, however, a number of constraints which need to be addressed before the potential of climate forecasts can be fully exploited for the local communities. These include the downscaling of climate models to fit local conditions. In that respect, the PRECIS (Providing Regional Climates for Impact Studies) model should be tested more widely; the provision of relevant and timely information (for instance, the occurrence of a dry spell may be more important than seasonal rainfall); and a good communication strategy given the probabilistic nature of weather forecasts.

Table 3. Potential response strategies that farmers may implement in response to receipt of rainfall forecast of high probability for higher or lower than normal seasonal rainfall and lead-time needed to implement strategies in Burkina Faso (Ingram *et al.*, 2002)

Above normal	Below normal	Month required
1. Agricultural responses		
Clear upland areas for planting	Implement soil and water conservation	January
Order less insecticide (cotton)	Order less herbicide (cotton)	January
	Sell livestock or go on transhumance	February
Orient furrows along slope	Orient furrows across slope	May
Plant longer duration crops/ varieties	Plant shorter duration crops/varieties	May
Plant flood tolerant crops	Plant drought tolerant crops/varieties	May
Decide planting sequence based on location and toposequence position		May
		May
Increase area planted in uplands	Plant more cereal crops	May
Plant more cash crops		June
Decrease total area planted		
Apply more fertiliser or manure	Apply less fertiliser or manure	June
Sell grain stocks during rainy season	Store grain stocks	July
2. Non-agricultural responses		
Acquire capital to purchase inputs	Ration food	January
	Increase income-generating enterprises	January
	Migrate	March
	Purchase or borrow food grain	April
	Send younger men abroad to work	June

Improved cultivars

Shift in annual rainfall, increased frequency of dry spells during the growing season, or reduced length of the growing season in the Sahel, have prompted the need to introduce or develop new crop varieties to help stabilise food production. Varieties that were productive until the late 1960s have become maladaptive in recent years with the reduction of both annual rainfall and length of the growing season (Hall *et al.*, 2003). In the last two decades, several programmes and projects have been implemented to address these problems. Breeding programmes and technological development, based on partnerships between international research organisations, advanced research institutes and African national agricultural research systems, have permitted the creation and release of new improved varieties of the major food crops in the region.

A partnership triangle involving the Institut Sénégalais de Recherche Agricole (ISRA), the University of California-Riverside (UC-R) and IITA, has enabled the development, release and adoption of new cowpea varieties adapted to the novel climatic conditions, thus contributing to improving food security and income in Senegal (Hall *et al.*, 2003). An economic impact study estimates the present net benefit of these cowpea varieties at about US$ 19 million. Similar

results were observed in Sudan. The popularity of these cultivars among farmers was due to a number of features including drought adaptation, resistance to insects and diseases, high yield and earliness. Following this success, the NGO World Vision International has been actively involved in the field-testing and promotion of these varieties in many other countries such as Niger, Ghana, Mali and Chad (Hall *et al.*, 2003).

A large number of new millet and sorghum cultivars has also been released in Africa over the last 20 years. By 2000, ICRISAT had released 96 improved varieties and hybrids (63 for sorghum and 33 for pearl millet). Most of the cultivars that have been promoted in the Sahel are early maturing and are capable of producing more than local landraces in low rainfall years. In 1984 (one of the driest years in the Sahel's recent history), the drought-escaping sorghum variety 'S-35', selected from ICRISAT material, produced twice as much as the local landraces in Northern Cameroon (Ahmed et al., 2000), which facilitated its rapid adoption by farmers.

With feeding habits changing due to urbanisation, rice is fast becoming a staple for a growing number of West Africans. To respond to this demand and to reduce over-reliance on rice imports, the West African Rice Development Association (WARDA) has been implementing breeding programmes aimed at developing varieties adapted to the Sahelian conditions. Nerica 2 was developed in 1994 and is one such variety; it is adapted to rain-fed conditions and said to be significantly earlier (by 30–50 days) and more productive (+50 percent) than the traditional varieties. The tolerance of Nerica 2's drought tolerance has also been well documented. The Sahel Institute of the CILSS has a rich catalogue of crop varieties, many of them having drought tolerance features (Table 4).

Table 4. List of drought resistant/tolerant varieties on the Sahel Institute (INSAH) seed catalogue

	Origin	Adaptation level	Potential yield (t/ha)
Millet			
GB 8735	ICRISAT/Sahel	Tolerant	1.9–2
SOSSAT C 88	IER (Mali), ICRISAT	Tolerant	2.5
Cowpea			
TN 88-63	Niger	Good	1–2
TN 5-78	Burkina Faso	Tolerant	0.8–1
KVX 30-309-6G	Burkina Faso	Good	1.5
Maize			
MAKA	Mauritania	Good	3–6
JEKA	Gambia	Tolerant	2–4
Sorghum			
IRAT 204	Senegal	Tolerant	2.4

These new cultivars are appreciated by farmers because they have a double advantage compared to the local landraces. In a good rainfall year, they can

produce food during the hunger period before other crops/varieties reach maturity. These early harvests not only guarantee food to hunger-prone rural families during this most sensitive period, but significantly bolster household income as well, since the market prices are double those received at the end of the season. In addition, early-maturing or drought tolerant varieties can assure minimal production in the occurrence of a drought whereas the use of traditional varieties would lead to certain crop failure. This is a very important aspect from a climate change perspective.

Despite the high number of new varieties released in Africa over the last few decades, studies on adoption are not common. However, there are some few success stories that are worth mentioning. A good example is the ICRISAT-bred sorghum variety S-35, which, officially released in Cameroon in 1986, now occupies about 33 percent of the total rain-fed sorghum area of the country. The same variety was released in Chad in 1989 and now covers 27 percent of the sorghum growing area in the country. Compared to the farmers' best traditional varieties across all study sites in Cameroon and Chad, S-35 yields 27 percent more output (grain) and reduces unit production costs by 20 percent. These impacts are larger in Chad, where yield gain is 51 percent higher and cost reduction 33 percent higher. The net present value of benefits from S-35 research spillover was estimated to be US$ 15 million and US$ 11 million in Chad and in Cameroon, respectively. In Nigeria, the sorghum varieties ICSV 400 and ICSV 111 are grown in 30 percent of the total sorghum area in the Jigwa region. In the Sahelian zone of Sudan, where the cowpea variety Ein El Gazal was promoted, the first on-station yield trials started in 1983. By 2001, 500,000 farmers were growing it and the demand from the Arabian Sudanese Seed Company has kept growing (Elawad and Hall, 2002; Hall *et al.*, 2003).

Efforts to diffuse improved crop varieties have not always been successful. A common problem is that researchers and farmers may have conflicting objectives: yield improvement in average rainfall years for the former and avoiding total failure in low rainfall years for the latter. The lack of understanding of the manifestation of drought has resulted in farmers not using new varieties, for instance when early maturing varieties are promoted in places where mid-season drought is the problem (de Rouw, 2004). Failing to consider other aspects that are of interest such as taste, nutritional value or resistance to pests and diseases has also led to the rejection of some otherwise adapted varieties. In many cases, however, farmers cite inability to access seeds as the major obstacle to the adoption of new varieties.

The significant achievements of research partnerships in developing drought-escaping and drought-tolerant cultivars for the major food crops of the Sahel (millet, sorghum and cowpea) needs support by establishing the necessary policy mechanisms that will foster diffusion and adoption by farmers. One critical factor is how to make seeds available to farmers. Given the general failure of the public sector to supply seeds and the lack of interest from private seed companies in open pollinated varieties, new actors such as NGOs have been strongly involved in seed production and delivery.

These NGOs typically distribute seed to selected individual farmers or farmers' cooperatives for wide scale seed production. While this type of seed

delivery scheme has been successful in reaching a large number of farmers, it may not be sustainable nonetheless. Various schemes, involving the public seed sector, NGOs and charity organisations are being experimented in various countries. Valuable lessons can be learned from this kind of experience (see www.icrisat.org). It is also important that aspects other than drought tolerance and productivity be considered when developing new varieties. These include taste, nutritional value, ease of processing and resistance to storage pests. Genetic engineering is emerging as a new technology and the opportunities it offers should be better explored to breed more robust cultivars, which could combine drought resistance, productivity and various other desired features that will enhance the acceptability of new varieties among farmers.

Soil and water conservation (SWC)

In the Sahel, 20 to 40 percent of annual rainfall is lost as runoff. This often results in agricultural drought and soil erosion in some sites (hillsides, plateau edges) and flooding in others (low lying land, valley bottoms). Agricultural drought is, to a significant extent, responsible for shortfalls in food production in semi-arid areas. Yet, agricultural drought cannot always be linked to low rainfall (meteorological drought). In the Sahel, the loss of rainwater through runoff, soil evaporation and drainage below the rooting zone is often considered as the major cause of moisture stress (Zougmoré *et al.*, 2004). Thus, it appears that capturing the little rainfall that falls and making it available to crops could provide an effective and sensible way of improving farm productivity and reducing farmers' vulnerability to drought. At the same time, improving water infiltration in uplands and hillsides can reduce flooding in the lower end of the toposequence, i.e., in valley bottoms. This constitutes the very rationale of SWC strategies in the Sahel. The International Network for Research on Drought Resistance (R3S), launched jointly by the Conference of the Agronomic Research Organisations in West and Central Africa (CORAF/WECARD) and CILSS, has disseminated several SWC techniques in West Africa in collaboration with NARS and ARIs such as the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) (Ruelle *et al.*, 1990 a, b, c).

The Central Plateau of Burkina Faso and the Koutiala region of Mali have been important testing areas for SWC. In these regions and many others in the Sahel, soil crusting has rendered vast areas of land uncultivable. Yet, for those involved in SWC research and development, these hardened soils represent some 'open laboratories' (Batterbury, 2001) that provide the ideal conditions for testing a suite of techniques (conservation tillage, stone lines, earth bunds, dikes, half-moons, tied ridges, etc.), aimed at checking soil erosion, enhancing the capacity of soils to retain and store water and increasing food or fodder availability in the Sahel (Mando, 1997; Zougmoré *et al.*, 2000; Zougmoré *et al.*, 2003).

The 'zaï' technique is a popular farming system in the Sahel. It consists in several 20-to-80 cm-diameter pits scattered over the field and partly filled with organic residues at the end of the dry season. During the rainy season, the pits trap rain water and termite activities facilitate water infiltration and release of nutrients to crops. Half-moons have also been widely tested throughout the

Sahel as a means to harvest runoff water and to facilitate plant establishment. In an experiment conducted by Zougmoré *et al.* (2003), half-moon practice, associated with different fertilisation treatments, has enabled the production of between 500 kg per hectare and 1600 kg per hectare of sorghum grain (Table 5). The relatively low yield with the recommended mineral fertiliser was due to increased soil acidity. Half-moon without fertiliser or amendment produced 41 kg/ha while sorghum in the control plot died because the soil was too hard for the roots to establish. This study also showed much greater soil water content in the rooting zone of the half-moon plots compared to the control. However, the low yield in the 'half-moon alone' treatment shows that beside water, low soil fertility and acidity are other constraints that need to be addressed.

Table 5. Effect of half-moon practice on sorghum performance in Pougyango village, Burkina Faso (modified from Zougmoré *et al.*, 2003)

Treatment	Grain yield (kg/ha)	
	1998	1999
Control (no digging, no amendment)	0	0
Half-moon without amendment	41	42
Half-moon + animal manure	1614	1104
Half-moon + compost	1000	875
Half-moon + compost + rock phosphate (BP)	927	1104
Half-moon + BP + NPK fertiliser + urea	500	521

Stone lines and grass strips have shown similar results, although grass strips have the disadvantage of needing time for regrowth after the long dry season and hence may not be effective during the first and most erosive storms (Zougmoré *et al.*, 2004). These technologies have proved to be very practical in erosion prone soils, and more so in erratic rainy seasons. However, their effects are enhanced with the use of organic amendments. It has been demonstrated that the establishment of stone lines in a degraded soil in Burkina Faso allows the maintenance of sorghum yield at a level twice as high as that of control plots (Zougmoré *et al.*, 2004). Runoff control, water infiltration and sorghum yield increases with the number of stone lines on the plot. Modelling carried out in Mali showed that increasing rainfall infiltration from its current 40% level to 60% (combined with small amounts of chemical fertilisers) could help farmers improve food grain production by 60 to 90%, depending on rainfall. This, in financial terms, would translate into a two- to fourfold increase in disposable income. With 80% infiltration, income could even be increased another 50% (Day and Aillery, 1988).

Hengsdijk and van Keulen (2002) also demonstrated that the use of tied ridges in the semi-arid zone of Mali could increase average millet yield by 40 to 230% depending on soil type while reducing inter-annual variability in yield. Other options such as supplemental irrigation using harvested rainwater in low-cost manually dug farm ponds have also been explored and have proved to be helpful in bridging dry spells. A study by Fox and Rockström (2003) showed the

effect that 60 to 90 mm of supplemental irrigation applied at actual occurrence of dry spells had on sorghum yield. Supplemental irrigation alone (712 kg per hectare) and fertiliser alone (975 kg per hectare) significantly increased yields compared to control plots, which received neither fertiliser nor irrigation (455 kg per hectare). When supplemental irrigation and fertiliser were combined, 1403 kg of sorghum was produced per hectare, i.e. more than three times what the control plot yielded.

Given the role of land degradation in the exacerbation of climatic problems, investing in SWC is an almost unavoidable step in the quest for solutions to climatic hazards and future climate change in the Sahel. Losses of agricultural soils and rain water needs to be checked to reduce drought impacts, flooding and inter-annual variability in food production, which exacerbates vulnerability among rural communities. In the deep sandy soils where water loss is mostly by drainage, the use of conservation tillage, organic amendments, combined with low levels of fertilisers and dense planting, has proven to be effective in reducing agricultural drought with the improvement of the water use efficiency of crops. There is no shortage of manure in the Sahel given the abundance of livestock and the tendency of sedentary farmers to keep animals. Corralling animals directly in the fields can provide solution where the application of manure can be constrained by transport and labour. The use of other SWC techniques such as tied ridges and stone or vegetation lines should also be encouraged to improve water infiltration and storage in the soil.

One major advantage of pursuing SWC is that it can constitute an entry point for agricultural intensification. One factor that discourages smallholder growers in semi-arid areas to adopt improved technologies is moisture stress. Pit planting techniques significantly contribute to solving this problem since runoff water is gathered and directly made available to crops. Moreover, they can facilitate the use of supplemental irrigation. Given the acidity of these soils, the use of organic amendment (manure), supplemented with small doses of inorganic fertilisers, is the best option to improve food security and income, hence the adaptive capacities of farmers. SWC can therefore be a powerful tool not only to restore degraded land and control desertification but also could contribute meaningfully to sustaining crop production and mitigating the negative effects of climate change.

The labour-intensive nature of most SWC techniques constitutes a major constraint that can discourage farmers. Thus, the implementation of these techniques warrants a strong support from governmental institutions, NGOs and other agencies involved in rural development. Indeed, because of the strong environmental implications at the watershed and regional levels, the recovery of degraded land should be considered as top priority by the Sahelian governments. The work carried out in areas such as the Central Plateau of Burkina Faso should be scaled up/out to other areas of the Sahel having similar constraints. The local people can supply labour in the form of in-kind contribution. Schemes such as 'food for work' or the distribution of agricultural inputs to farmers have proven to be practical in mobilising local labour.

Agroforestry

Agroforestry has been practised in the Sahel since time immemorial, the traditional parkland system being a trademark practice throughout the region. However, agroforestry research and development gained momentum only recently with the creation of the ICRAF (World Agroforestry Centre) Sahel Programme in the 1990s. This Programme, which covers Burkina Faso, Mali, Niger and Senegal, was primarily launched as a strategy for ICRAF to contribute to the implementation of the Desertification Convention in the Sahel region.

The effects of different agroforestry techniques in enhancing the resilience of agricultural systems against adverse impacts of rainfall variability, shifting weather patterns, reduced water availability, soil erosion as well as pests, diseases and weeds are well documented. A successful and well-managed integration of trees on farms and in agricultural landscapes results in diversified and sustainable crop production, in addition to providing a wide range of environmental benefits.

In the African drylands, where climate variability is commonplace and adverse impacts of climate change are expected, farmers appreciate the role of trees in buffering against production risk (Ong and Leakey, 1999). The parkland farming system, a farming practice whereby a few selected useful trees are encouraged to grow in a scattered distribution on agricultural land, is one interesting example. One of the most valued in the Sahel is *Faidherbia albida*. Thanks to its reversed phenology (the tree sheds its leaves during the rainy season), *F. albida* significantly contributes to maintaining crop yield through biological nitrogen fixation (BNF) and favourable micro-climate while minimising tree–crop competition. A study on a *Faidherbia albida*–millet parkland system in Niger demonstrated that shade-induced reduction of soil temperatures, particularly at the time of crop establishment, is critical for good millet growth (Vandenbeldt and Williams, 1992). Furthermore, the protein-rich leaves, twigs and pods of *F. albida* constitute a precious source of animal feed for livestock during the long dry seasons in the Sahel.

The benefits of live fence technology are also well appreciated by farmers in Mali, Burkina Faso and Niger. Since there are no rules for animal browsing during the dry season, live fencing is emerging as an efficient method to keep off wandering animals, which are a major impediment to off-season agricultural activities. The development of dry season gardening for fruits and vegetables provides many Sahelian farmers with a useful fallback to compensate for the loss of crop production due to low rainfall and degraded soils. This is also a sub-sector many governments are supporting in their poverty reduction strategies to diversify crop production and increase exports. While the primary goal of fencing with trees is naturally to protect crops against animal encroachment, products such as fruits, fodder and firewood can also be of great contribution in improving nutrition, farmer income and easing pressure on natural resources.

Beside the biophysical resilience, which allows the various components of the agroforestry systems to withstand shocks related to climate variability, the presence of trees in agricultural croplands can provide farmers with alternative or additional sources of income strengthening the socio-economic resilience of rural populations. Tree products (timber, fodder, resins and fruits) are normally

of higher value compared to cereals and can buffer against income risks. Studies in the Sahel have shown that products from *Parkia biglobosa* can earn a family a yearly income of up to US$ 270, which in fact is double what crops normally produce. Other species such as *Vitellaria paradoxa, Adansonia digitata* (baobab) and *Tamarindus indica* (tamarind) are of similar importance (Bonkoungou *et al.*, 2002).

The dense planting of trees for the provision of useful products such as fruits and fodder is also becoming more popular in the region. Improved varieties of *Ziziphus mauritiana* are now available throughout the region and have potential to improve nutrition and income. Other tree species such as *Gliricidia sepium* and *Pterocarpus erinaceus* are planted for fodder production (Bonkoungou *et al.*, 2002). The lucrative fodder market that exists in the vicinity of Bamako has inspired ICRAF and its partners to launch a special programme on fodder trees. Preliminary results showed that *P. erinaceus* can be densely cultivated and yield enough leaf biomass to solve fodder problems during the dry periods in this part of the Sahel. From a financial standpoint, it is estimated that one hectare of densely planted *P. erinaceus* can earn its producer US$ 630 annually on fodder alone. This is a significant contribution in a country where yearly income averages US$ 270.

Recently, ICRISAT researchers developed an interesting model farm for the Sahel: the Sahelian Eco-Farm (SEF). The SEF is an integrated land use system that incorporates high value multipurpose trees/shrubs with soil and water conservation structures (Pasternak *et al.*, 2005). The premise is to provide a wide range of products and services: food, firewood, biomass for mulch and forage, cash, plant nutrients, increased infiltration of rainfall, improved soil organic matter, and protection from water and wind erosion. The economics of the SEF makes it a very attractive system: income from a one-hectare farm is estimated at US$ 600, a figure that represents 12 times the value of a typical millet crop (Table 6), whereas the establishment costs are relatively low: about US$ 60 per ha for the plant material and US$ 10 for the one time application of fertiliser. Labour for land preparation and tree planting was not considered since it is normally provided by the farming household. The strength of the Sahel Eco-Farm lies in the fact that it promotes crop diversification and system resilience by combining

Table 6. Value of Sahelian Eco-Farm (SEF) products from SEF-ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Sadore station during 2002 (adapted from Pasternak *et al.*, 2005)

Species	Quantity per unit area	Yield per unit	Unit value (US$)	Total revenue (US$)/ha
Acacia colei	320 trees/ha	2 kg seeds/tree	0.14/kg	90
Zizyphus mauritania	63 trees/ha	30 kg fresh fruit/tree	0.12/kg	225
Andropogon gayanus	567 metres/ha	1 bundle/10 m	0.8/bundle	45
Millet	1/3 ha	1500 kg/ha	0.1/kg	50
Cowpea	1/3 ha	1260 kg/ha	0.2/kg	84
Roselle	1/3 ha	400 kg/ha	0.8/kg	106
Total				**600**

various species of trees or shrubs (*Acacia colei* and *Zizyphus mauritiana*), grass (*Andropogon gayanus*) and annual crops such as roselle (*Hibiscus sabdariffa*), a relatively high value crop compared with millet or sorghum.

Establishment costs, labour and seed availability are elements that have a strong influence on the adoption rate of agroforestry technologies. It is generally believed that farmers will be more inclined to adopt agroforestry technologies if these can produce immediate benefits. Therefore, even the agroforestry techniques that are designed to serve long-term environmental purposes may need to include short-term benefits to stand a chance of being adopted at a large scale. In addition, since these agroforestry technologies are new to the farmers, training will be needed on areas such as nursery establishment, planting methods (timing, tree spacing, management, etc.).

Many agroforestry trees establish well when seedlings are used because they can withstand the biotic and abiotic pressure better than directly seeded plants in the initial stages. As shown in Zambia (Scherr and Franzel, 2002), seedling production can be assured with the establishment of small-scale village nurseries, which are easier to manage than centralised nurseries. Furthermore, they require less labour and can reduce transportation costs and damage related to handling. In the Sahel, village nurseries make more sense than individual nurseries because of water constraints. Given the short growing season, seedlings need to be prepared during the dry period of the year to have any chance of being planted at the beginning of the rains.

Seed availability is another barrier to overcome in the promotion of agroforestry. The main reason why tree seeds are problematic is that, without any insurance, neither the private sector nor individual producers are ready to invest in such a highly uncertain domain. Trees may take years before producing seeds and therefore the delay in investment return may be a deterrent to many who would consider venturing into tree seed production. In western Kenya, ICRAF has been involving individual farmers and farmer groups in tree seed production by volunteering germplasm and information to them and agreeing to purchase seed in the first years of production (Scherr and Franzel, 2002). The new seeds are then distributed to other farmers to diffuse the technology in other areas. The same strategy could be extended to the Sahel.

Conclusions

The Sahel region is highly vulnerable to climate change due to a combination of factors including geographic location, structural problems, inadequate infrastructure and weak institutions. One major problem is the overdependence of the region's population and economies on climate sensitive sectors such as agriculture, livestock and fisheries. The low use of modern technologies such as improved germplasm, fertilisers, mechanisation and irrigation makes the agricultural sector in the Sahel particularly vulnerable to climate change. The unreliable rainfall does not encourage investments in soil conservation, explaining the conservative attitude of many land users that does nothing but exacerbate nutrient mining and land degradation.

Per capita food production has been declining over the last three decades, making the Sahel one of the most food insecure regions of the planet. The recurrent droughts of the 1980s and 1990s have dealt a major blow to the

development objectives of many countries and deeply strained their social services. Food imports have drastically increased over the last few years, which constitute a diversion of the limited financial resources of these countries from development goals.

Efforts are ongoing at regional, national and local level to cope with the changing environmental conditions, including climate change. Many policy measures and interventions undertaken in the framework of the UNCCD and the poverty reduction strategy programmes are likely to have spillover effects for addressing climate change. However, in its third assessment report the IPCC concluded that, like in the rest of the world, a change in climate would occur in the Sahel leading to warmer conditions in many areas. An increased frequency of extremes such as droughts and floods is also expected, with serious food security, socio-economic and environmental consequences. That is why, within the framework of the UNFCCC all countries have undertaken impact studies on the agricultural sector and devised adaptation strategies and policies to deal with future climate change.

Agricultural research undertaken through strategic partnerships between international research organisations under the CGIAR and national programmes plays an important role in overcoming the constraints facing smallholder farmers in Africa. The development of new varieties that are tolerant to drought, mature early and are adapted to various other stresses such as nitrogen deficiency, pests and diseases, are already making an impact and are likely to play a major role in addressing the issue of climate change. The use of climate information, coupled with an improvement in crop management, including the use of techniques such as rainwater harvesting, soil conservation and agroforestry can enhance productivity among smallholder farming systems and buffer them against the uncertainties related to climate change.

Agroforestry, by improving soil conditions, producing firewood and enhancing cereal yields or producing fruits for home consumption or for sale at the local markets (domestication of indigenous trees), can provide an effective means to buffer vulnerable farmers against climatic uncertainties. Policies should be developed and implemented to enable the widespread utilisation of these various technologies and strategies to make the agricultural sector more resilient in the face of climate change.

The greatest challenge posed by climate change is that nobody knows exactly the magnitude (and sometimes direction) of the changes in climate variables, especially precipitation. Climate models are based on scenarios and can, at best, give a range of possible (sometimes conflicting) outcomes. Given these uncertainties, it is difficult to know whether the technologies that are used to cope with current climate variability will be effective to respond to future climate change. It is imperative, therefore, to develop a mix of no-regret technology options and policies geared at promoting the emergence of productive, sustainable and flexible agricultural systems that show enough resilience regardless of the direction and magnitude of climate change. Some of the options discussed in this paper should provide a foundation for such a process since they are useful even without climate change. There are clear linkages between poverty and vulnerability to climate change. Therefore, using agricultural development as an engine for development in general has the potential to help both local communities and African governments to be better

prepared for the future.

Climate change is going to be one of the biggest challenges for the African continent and developing resilient systems to face these changes will require more important investments by governments and their development partners. There is widespread consensus that at least 10% of national budgets need to be dedicated to agriculture within the next 5 years to address problems related to food insecurity and poverty in Africa. In the framework of the NEPAD process, African heads of state and government committed themselves to that, as expressed in the Maputo Declaration of 2003. These commitments need to be translated into practice.

There is now a strong commitment from the international community to address climate change. The various initiatives and funding opportunities that exist should be utilised judiciously to carry out vulnerability and adaptation studies and develop priority projects for funding in the agricultural sector. The NAPA process constitutes an important framework for the least developed countries. The adaptation funds, which are going to be available through the UNFCCC, the Kyoto protocol and other bilateral sources should be utilised in a more effective way to strengthen agriculture in Africa. Debt cancellation initiatives are also affording additional resources to a growing number of countries. Some of these resources should be used to make the agricultural sector more resilient to climate change.

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Biological Control and Integrated Management of Vegetable Pests in Ethiopia

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Abstract

This paper reviews progress on biological control and integrated pest management (IPM) of vegetable pests in Ethiopia. Experiments on the components of IPM including cultural control, host plant resistance and botanical control have been conducted against one or more vegetable pests by different crop protection researchers in Ethiopia over the years. However, limited efforts have been made to integrate the available IPM components with the objective of avoiding dependence on pesticidal control. There has been a dearth of information on the natural enemy complex associated with vegetable insect pests like in many other crops. In recent years, national and regional interest for strengthening biological control resulted in the documentation of the indigenous natural enemy complex of some vegetable pests and implementation of biological control. Classical biocontrol of diamondback moth using the larval parasitoid, *Diadegma semiclausum* (Hellen) in highland brassica production areas of Ethiopia is currently underway.

Key words: biological control, IPM, vegetable pests, diamondback moth, *Diadegma semiclausum*, Ethiopia

Introduction

Most of the research effort in vegetable pest management in Ethiopia in the past concentrated in the identification of effective insecticides and as a result control measures of vegetable pests in Ethiopia, as in several countries of Africa, rely almost exclusively on the application of insecticides. These are often ineffective because of resistance of the pest to the insecticides and the decimation of the natural enemies associated with the pest, which can play an important role in regulating the pest population. Arthropod pests that were regarded as minor or secondary have now attained pest status because of reliance on insecticides to combat vegetable pest problems. For example, whiteflies (*Bemisia tabaci*) and spider mites (*Tetranychus* spp.) were regarded as minor pests on tomato. Currently, they probably stand first as arthropod pests of vegetables in Ethiopia. *Thrips tabaci* on onion and *Plutella xylostella* on cabbage were effectively controlled by pyrethroid insecticides such as cyhalothrin in the past. Currently, such insecticides do not provide any level of control against these pests, which could be due to the development of pyrethroid resistant population of both insect pests (Ayalew *et al.*, 2008). Losses higher than 90% have been recorded due to some of the major vegetable pests (Ayalew and Abate, 1994; Ayalew, 2006). In a desperate attempt to achieve a desirable level

of control, vegetable growers use insecticide cocktails that in turn aggravate the problem of insect pests in vegetable production. This has necessitated a search for other nonchemical options including host plant resistance, cultural control, botanical and biological control. Crop protection researchers in the Ethiopian Institute of Agricultural Research have carried out IPM-related experiments in recent years and identified components that form integral components in vegetable IPM although efforts in implementation are minimal. In recent years, national and regional interest for strengthening biological control resulted in the documentation of the indigenous natural enemy complex of some vegetable pests and implementation of biological control. This paper reviews ecological studies including survey on insect pests and their natural enemies, components of IPM developed (including biological control) against major pests of common vegetable crops and attempts made to implement it and suggests future research direction to promote development and use of IPM in vegetable production in Ethiopia.

Arthropod Pests of Vegetables

Abate (1988a) documented insect pest species on vegetable crops cultivated in Ethiopia, which include cabbages, tomatoes, cucurbits, capsicum, eggplant, okra, onions, lettuce, green beans and others. Although a large number of insect pests is recorded on each crop, only a few of them are economically important. These include thrips (*Thrips tabaci*) on onion, diamondback moth (*Plutella xylostella*) and the mealy cabbage aphid (*Brevicoryne brassicae*) on cabbage, fruit worms (potato tuber moth, *Phthorimaea operculella* and African bollworm, *Helicoverpa armigera*) on tomato and termites and African bollworm on pepper (*Capsicum* spp.). Recently, spider mites (*Tetranychus* spp.) and whiteflies (*Bemisia tabaci*) have become increasingly important in irrigated tomato production. Ayalew *et al.* (2008) lists the insect species and their status on common vegetable crops.

Ecological Studies

Studies on the biology and ecology of some of the major insect pests have been made and information is documented. These include studies on the biology and ecology of diamondback moth attacking brassicas (Ayalew *et al.*, 2006a, b, c); the potato tuber moth (*Phthorimaea operculella*) attacking tomato (Mulatu *et al.*, 2004; Mulatu and Tadesse, 2004; Mulatu *et al.*, 2006a, b) and thrips attacking onion (Abate, 1986; Merene, 2005). These studies have generated useful baseline information for studies on management options and their implementation.

Biological Control

Biological control study in vegetable pest management has not been given the attention it deserves as in many other crops in Ethiopia and several countries of Africa. Most of the efforts are limited to documentation of indigenous natural enemies. Assessing the indigenous natural enemy complexes and the level of natural control exerted by them lays the foundation for a classical biological

control using proven natural enemies from anywhere in the world (Ayalew and Ogol, 2006).

Entomophagous arthropods (predators and parasitoids)

Abate (1991) lists natural enemies of insect pests attacking various groups of crops including horticultural crops, cereals, legumes and stimulants. Specific surveys targeted to determine natural enemy complex were reported only for a limited number of insect pests of a few crops. Due to this, the list does not have much information on natural enemies of major pests of vegetable crops. The available information with regard to this is on parasitoids of the whitefly (*Bemisia tabaci*) attacking tomato, the cabbage aphid *Brevicoryne brassicae* attacking cabbage and predators of thrips (*Thrips tabaci*) attacking onion, which are shown in Table 1. Ayalew and Ogol (2006) give an account on the diversity of the parasitoids species associated with diamondback moth and Negri (2004) on the diversity of egg parasitoids associated with African bollworm on tomato.

Classical biocontrol of diamondback moth

Extensive studies were made on the ecology of diamondback moth and associated parasitoids with the objective of gathering baseline information to implement biocontrol based IPM in Ethiopia between 2000 and 2002 (Ayalew, 2003). These studies documented the geographic distribution of DBM and parasitoid guilds including level of natural control (Ayalew and Ogol, 2006). It has been reported that the level of natural control by the indigenous parasitoids particularly in the major brassica production areas of Ethiopia which include the central Rift Valley areas and Arsi highland was low suggesting the need to import proven effective parasitoids from elsewhere in a classical biocontrol programme. Accordingly, *Diadegma semiclausum* (Hellen), the most effective larval parasitoid of diamondback moth in highland brassica production was imported from the International Centre of Insect Physiology and Ecology (*icipe*) in June 2008, which was originally introduced from the Asian Vegetable Research and Development Centre (AVRDC). Releases were made in 12 cabbage fields of Kofele area in the western Arsi Zone of Oromia region. The parasitoid was recovered three months after releases were made. Currently, monitoring on its establishment and expansion is being made through periodic surveys.

Use of entomopathogens

Studies on efficacy of entomopathogens have been made against diamondback moth on cabbage, fruit worms (potato tuber moth and African bollworm) on tomato and thrips on onion. The microbial tested against diamondback moth and fruit worms was the bacterial preparation *Bacillus thuringiensis* (Bt) (Abate and Ayalew, 1994; Ayalew, 2006; Lidet *et al.*, 2008) and on thrips the fungi *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces fumosoroseus* (Mendesil *et al.*, 2006). Although the effect of Bt was not satisfactory against fruit worms (Abate and Ayalew, 1994), it was found very effective against diamondback moth. Yield of cabbage in Bt-treated plot was more than double

Table 1. Parasitoids and predators associated with major insect pests of common vegetables cultivated in Ethiopia

Crop	Pest species	Parasitoid/predator species	Reference
Cabbage	*Plutella xylostella*	*Oomyzus sokolowskii* (Hymenoptera: Eulophidae) *Diadegma* sp. (Hymenoptera: Ichneumonidae) *Apanteles* sp. (Hymenoptera: Braconidae) *Brachymeria* sp. (Hymenoptera: Chalcididae) *Mesopolobus* sp. (Hymenoptera: Pteromalidae) *Pediobius* sp. (Hymenoptera: Eulophidae) *Itoplectis* sp. (Hymenoptera: Ichneumonidae) *Meloboris* sp. (Hymenoptera: Ichneumonidae)	Ayalew and Ogol (2006)
	Brevicoryne brassicae	*Aphidius* sp. (Hymenoptera: Aphidiidae) *Diaeretiella rapae* (Hymenoptera: Aphidiidae) *Pachyneuron* sp. (Hymenoptera: Pteromalidae)	Abate (1991)
Tomato	*Bemisia tabaci*	*Encarsia formosa* (Hymenoptera: Aphelinidae) *Eretmocerus mundus* (Hymenoptera: Aphelinidae)	Abate (1991)
	Helicoverpa armigera	*Telenomus* spp. (Hymenoptera: Scelionidae) *Trichogrammatoidea* sp. nr *lutea* (Hymenoptera: Trichogrammatidae) *Trichogrammatoidea* sp. nr *armigera* (Hymenoptera: Trichogrammatidae) *Trichogramma* sp. nr *mwanzai* (Hymenoptera: Trichogrammatidae) *Trichogramma* sp. nr *bournieri* (Hymenoptera: Trichogrammatidae)	Negeri (2004)
Onion	*Thrips tabaci*	*Adonia variegata* (Coleoptera: Coccinellidae) *Orius* sp. (Heteroptera: Anthocoridae) *Chrysopa* spp. (Neuroptera: Chrysopidae) *Baccha* spp. (Diptera: Syrphidae)	Abate (1991) Dejene (2006)

over the untreated plot and the synthetic insecticide, cyhalothrin-treated plots in some cases (Ayalew, 2006; Lidet *et al.*, 2008). All the three fungi tested against thrips reduced the pest density significantly ($P < 0.01$) compared to the control (Mendesil *et al.*, 2006).

IPM Components (Cultural, Varietal and Botanical Controls)

Pests of brassicas (cabbage)

Diamondback moth and the mealy cabbage aphid (*Brevicoryne brassicae*) are the only major insect pests on brassicas currently. Neem (*Azadirachta indica*) seed was found effective in reducing both diamondback moth and cabbage aphid infestation and minimising yield loss. From an experiment conducted at Wonji

in the central Rift Valley region, Lidet *et al.* (2008) reported that DBM number in neem seed preparation treated plot had significantly lower DBM numbers than the untreated as well as synthetic insecticide, cyhalothrin-treated plot with a yield advantage of more than 60% over both treatments. Similarly aphid infestation was significantly lower in neem seed treated plot than the untreated plot with a yield advantage of more than 50% (Lidet et al., unpublished).

Fruit worms on tomato

The potato tuber moth (*Phthorimaea operculella*) and African bollworm (*Helicoverpa armigera*) have been the major pests on tomato. Resistant varieties have been identified and released for production (Abate and Ayalew, 1997). These include 'Serio', 'Pusa Early Dwarf', 'Pussa Ruby' and 'Seedathing'. Strip cropping of tomatoes with maize is reported to serve as a trap for attracting the egg laying adult female moth (Abate, 1988b).

Thrips on onion

Thrips tabaci is the only major insect pest of thrips. Currently another thrips species namely *Frankliniella occidentalis* has been recorded from Melkassa Research Centre (Dejene, 2006).

Studies on varietal, cultural and botanical control have been made against thrips on onion (Ayalew *et al.*, 2008). From preliminary work on screening of onion germplasms against the pest, existence of variability in susceptibility to thrips was observed (MARC, 2004). Botanical control studies documented the potential of different parts of several plant species in reducing thrips infestation (Ayalew *et al.*, 2008). These include ethanol extracts of ground seeds of neem (*Azadiractha indica*) and pepper tree (*Schinus molli*), and leaves of bersema (*Bersema abyssinica*) (Ayalew, 2005); neem leaf, garlic, ginger and chilli and their mixtures (Merene, 2005). Mulching onion plot with white plastic significantly ($P < 0.05$) suppressed thrips population and consequently improved yield compared to mulching with black plastic and organic materials including tef straw and sawdust (Dejene, 2006).

Pests of pepper (Capsicum spp.)

African bollworm (*Helicoverpa armigera*) and termites are the major insect pests on pepper (*Capsicum* spp.) in Ethiopia. Mulching pepper field with maize stover, haricot bean residues and grass is reported to minimise termite damage (BARC, 2000). Strip cropping of hot pepper with lupine (*Lupinus* sp.) reduces ABW infestation on hot pepper by attracting the egg laying adult female moth (Abate, 1988b).

Demonstration of IPM components on farmers' field

A collaborative project on vegetable IPM was carried out between the Ethiopian Institute of Agricultural Research (EIAR) and the International Centre of Insect Physiology and Ecology (*icipe*) between 1999 and 2001 with the objective of

developing IPM options for sustainable vegetable cultivation by small-scale vegetable growers through:

- Improving farmers' perception of the pest problem, knowledge of pest identification and damage caused through group learning.
- Building awareness on the safe and proper use of pesticides; and
- Identification of suitable options.

Methodologies followed and results obtained are reported (Yesuf *et al.*, 2006). The following major lessons were learned:

- Farmers' group could be motivated and actively participate if the project is developed based on their need;
- Farmers' group members could identify major insect pests of vegetable crops they are growing;
- Farmers are aware about the range of insecticide groups for insect pest control and their rational use in vegetable pest management;
- Farmers are aware about the existence of natural controlling factors such as parasitoids and the care they need to take to use them as a component of IPM;
- Farmers are aware about the availability of non-chemical options of pest control and the benefit that can be obtained from options demonstrated on-farm. These include:
  - Availability of resistant variety for tomato fruit worms management.
  - Use of botanicals and biological products for DBM management on cabbage.
- The approach employed was found to be time and resource demanding as it required frequent visit to farmers' group.
- Unavailability of registered pesticides in local market was observed to be the major reason for use of banned or restricted pesticides by most vegetable farmers.

Conclusion and Recommendations

Integrated pest management related efforts in Ethiopia have resulted in identifying the major insect pests, their biology, ecology, natural enemy complex and management options in vegetables and many other crops in Ethiopia (Abate, 2007; Ayalew *et al.*, 2008). However, efforts to integrate and utilise the available IPM components are minimal. The only example of targeted effort to implement vegetable IPM in Ethiopia is work reported by Yesuf *et al.* (2006). Most of the IPM components developed by crop protection researchers are cultural, botanical and varietal controls. Although one or a combination of two or more renders effective management in some cases, practical feasibility in most cases is questionable. For example, efforts in host plant resistance against fruit worms on tomato resulted in identifying resistant fruit worms as well as high yielding varieties (Abate and Ayalew, 1997), which are under production. On the other hand socioeconomic factors hinder the adoption and utilisation of some technologies. For example lupine (*Lupinus* sp.) has been reported as effective trap crop against African bollworm on pepper (*Capsicum* spp.) (Abate,

1988b) but because of poor research extension effort as well as lack of interest by growers to incorporate a plant species in their farming system nullifies research efforts. The implication is that researchers need to consider socioeconomic factors in project development to make their research effort successful. Classical biological control is an important integral component of IPM that can be easily implemented if effective natural enemies are available. However, less emphasis has been given to biological control in vegetable pest management as it is in many other crops. For example, until recently no single attempt was made to implement biological control on any of the insect pests of vegetables. Similarly, effort to determine the natural enemy complex through a concerted survey was minimal. National and regional interest to promote biological control based IPM in recent years resulted in understanding the indigenous natural enemy complexes and their ecology (Ayalew *et al.*, 2008). Recent initiatives on bio-control of diamondback moth on cabbage in highland production areas stimulate similar efforts on other vegetable pests. Future work on IPM of vegetables should focus on biological control together with varietal, cultural and botanical controls. Identification of insecticides effective against vegetable pests and safe to their natural enemies should also be considered considering the commercial nature of vegetable production. The dedication of national professionals, policy support from the government and material support from the donor community will play a crucial role in IPM of vegetables and other crops in Ethiopia as stated by Abate (2007).

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Arthropods Knowledge Integration and Dissemination for Community Livelihood in Africa

Inventory of Indigenous Pest Management Practices in the Lake Victoria Basin, Kenya

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Abstract

A farm survey was conducted to document farmers' indigenous knowledge and pest management practices in Kenya's Lake Victoria basin districts of Bondo, Busia and Teso. Specifically, the survey inventoried farmers' traits, landholding, major pre- and post-harvest crop pests and pest control methods including the level of use of synthetic insecticides and associated problems. Results showed that farming in the LVB was predominantly at subsistence level. Stemborers, aphids, pod feeders, beanfly, cutworms and termites were the key pests in the field while grain weevils (*Sitophilus* spp.), bruchid beetles (*Acanthoscelides obtectus* Say and *Callosobruchus* spp.), grain moth (*Sitotroga cerealella* Olivier), rodents, larger grain borer (*Prostephanus truncatus* Horn) and red-flour beetle (*Tribolium castaneum* Herbst) were the leading storage problems. Farmers use a variety of pest control methods, with preference on botanicals such as wild sunflower (*Tithonia* sp.), neem (*Azadirachta indica* A. Juss), pepper (*Capsicum annum* L.), Mexican marigold (*Tagetes minuta* L.), sodom apple (*Solanum incanum* L.) among others, against field insect pests while general plant ash, cow-dung ash and bean husks ash were used in storage. Although synthetic pesticides were available and accessible, their high cost, toxicity concerns and inconsistency in efficacy were cited as reasons for lack of adoption. The study has shown that small-scale farmers in the LVB resort to an age-old wealth of anti-pest knowledge and practices to protect their produce, which demands scientific improvement and value addition.

Key words: survey, crop pests, control, botanicals, synthetic pesticides

Introduction

Food security has remained the main agenda in regional food policies. Food grain production and post-harvest handling in the LVB are greatly affected by pests with insects accounting for 25 and 15% pre- and post-harvest grain losses, respectively (Saxena *et al.*, 1990; Ngatia, 1997; Ogendo *et al.*, 2004, 2006).

Although the use of synthetic pesticides has been promoted in the region for the past 3–4 decades, subsistence farmers are yet to fully integrate them into their insect pest management systems owing to the high costs and associated toxicity risks (Mihale and Kishimba, 2004; Ogendo *et al.*, 2004). Thus more than 70% of subsistence farmers in the LVB never apply any pest control measure against

field insect pests (Ogendo *et al.*, 2004), but rely on local practices to protect their farm produce (Kamatenesi-Mugisha, 2004), which are most relevant to the rural poor and marginalised population.

Revelations that subsistence farmers in the tropics use traditional methods to manage field and storage insect pests and the realisation that a farmer's indigenous knowledge ('putting the last first') holds the key to the success of any pest management endeavour at farm level has shifted the research focus and approach. Recent local studies in the region identified several indigenous plant-based pest management options used for the control of field and storage insect pests and reconfirmed the usefulness of traditional claims (Ogendo *et al.*, 2006).

Although locally accessible and culturally relevant, the traditional insecticidal herbal products have not been accurately and extensively documented, scientifically improved and validated. Hence, this study was aimed at documenting the indigenous pest management practices in the Kenya's LVB districts of Bondo, Busia and Teso.

Materials and Methods

Sampling procedure

A stratified random sampling method was used in the three study districts of Bondo, Busia and Teso between 19 August–3 September 2007 according to Ogendo *et al.* (2004; 2006). Each study district was considered a homogeneous sampling block. Administrative divisions, locations, sub-locations and villages within each study district were appropriately represented during the sampling. A total of 65 farmers were randomly selected and interviewed using a structured questionnaire. Additional pre- and post-harvest observations were made on pests and pest management practices.

Farmers traits and land holding

Information on farmers' residential address (village, sub-location, location and division) within a district, age, educational level and land acreage were gathered. These variables were considered to have influence on the decision-making and crop pest management at the farm family level.

Field and storage pests

The major pest species and their infestation status, pre- and post-harvest, were inventoried. Identification of field and storage insect and non-insect pests was carried out by the researchers on the basis of expertise and available literature materials during the survey (Bohln, 1973; Singh, 1990). Identification also relied on farmers' description and ability to recognise the said pest from among other species in pictorial aids (NRI poster, 1999) availed by the survey team.

Level of use of synthetic insecticides and associated problems

The level of synthetic insecticides use, problems, technical advice, sellers, availability and accessibility were investigated.

Botanicals used in field and storage pest control

The botanicals / products locally used by small-scale farmers in the management of field and storage pests were screened. Samples of plants reported to be used by the farmers were collected, preserved and identified with the help of expertise, pictorial aids and literature materials (Kokwaro and Johns, 1998). Where the available expertise and literature proved inadequate, preserved specimens were forwarded to a plant taxonomist in the Department of Biological Sciences, Egerton University for authentic identification and verification.

Statistical analysis

Data collected on farmers' traits, landholding, crop pests and control methods were subjected to descriptive statistics using SPSS computer package, version 11.5.

Results

Farmers' traits and landholding

The sampled farmers in the three districts had an average age of 55.74 ± 7.7 years and primary educational level and below (2.06 ± 0.46). Results revealed that farming in LVB districts is mainly subsistence with a mean cultivated per capita land holding under different cropping systems of 1.19 acres (0.48 ha). Cereals and legumes occupied much of the cultivated land with a mean of 1.26 (0.51 ha) and 1.12 acres (0.45 ha), respectively.

Field and storage pests

Although farmers reported an array of pests across the three districts, it emerged that most crop types were infested by similar major pests. The major cereal insect pests were stemborers, aphids, cutworms and termites. Stemborers and aphids were reported as major insect pests by 12 and 10% of the farmers in the study area, respectively. Rodents and birds were the major non-insect pests recorded in the three districts with 9 and 3% occurrence, respectively (Table 1).

With respect to storage pests, grain weevils (*Sitophilus* spp.) dominated among cereals whereas bruchid beetles topped in the stored legumes. On average, 17% of farmers reported grain weevils compared to 11% for bean bruchid beetle in their stored grains (Table 1). Other storage pests reported were grain moths (*Sitotroga cerealella* Olivier), LGB (*P. truncatus*), rodents and red-flour beetle (*Tribolium castaneum*, Table 1).

Table 1. Major field and storage pests in the Lake Victoria Basin, Kenya

Major field pests	Percentage	Major storage pests	Percentage
Stemborers	12	Grain weevil	17
Aphids	10	Bean beetle	11
Rodents	9	Grain moth and grain weevil	9
Cut-worms	5		
Beanfly	5	Larger grain borer	6
Pod-feeders	5	Rodents	4
Termites	3	Pulse beetle	4
Birds	3	Flour beetle	3

Level of use of synthetic insecticides and associated problems

The survey showed that synthetic pesticides were supplied by the stockists and were available in abundance and easily accessible by the farmers within < 5–10 km. Moreover, farmers receive technical advice on the use from the agricultural extension officers (Figure 1 a–e). However, farmers cited high prices, toxicity concerns, inconsistent efficacies or the combined effects of these as the main factors hampering the use of insecticides for protection of produce in the field or storage (Figure 1 f).

Botanicals used in pest management practices

The inventory of botanicals / products used in traditional pest control has shown that majority of farmers never use botanicals to control insect pests in the field (78%) and storage (82%) (Table 2). In the field, wild sunflower (*Tithonia* spp.) as an aqueous concoction is the most commonly used (9% of farmers) for insect pest control. Pepper (*Capsicum annum* L.), neem (*Azadirachta indica*), 'Emusi' (*Urera hypseldendron*), 'Mululusia' (*Vernonia amygdalina*), *Acacia sial*, Mexican marigold (*Tagetes minuta*), *Eucalyptus* spp. and Sodom apple (*Solanum incanum* L.) solutions were also used by the farmers as sources of insecticides (Table 2). Indigenous options involving general and cow-dung ashes were the dominant methods of grains protection in storage used by 15 and 3% of farmers, respectively (Table 2). Other specific plant ashes were also used in the protection of stored produce (grains) for all the crop types and across the districts (Table 2).

Discussion

The majority of farmers are old, with primary level of education, and hence are unable to conceptualise technical farming skills, including handling and safe use of synthetic insecticides. These results showed that the younger generation, likely to be more educated are not practising agriculture because of its low income.

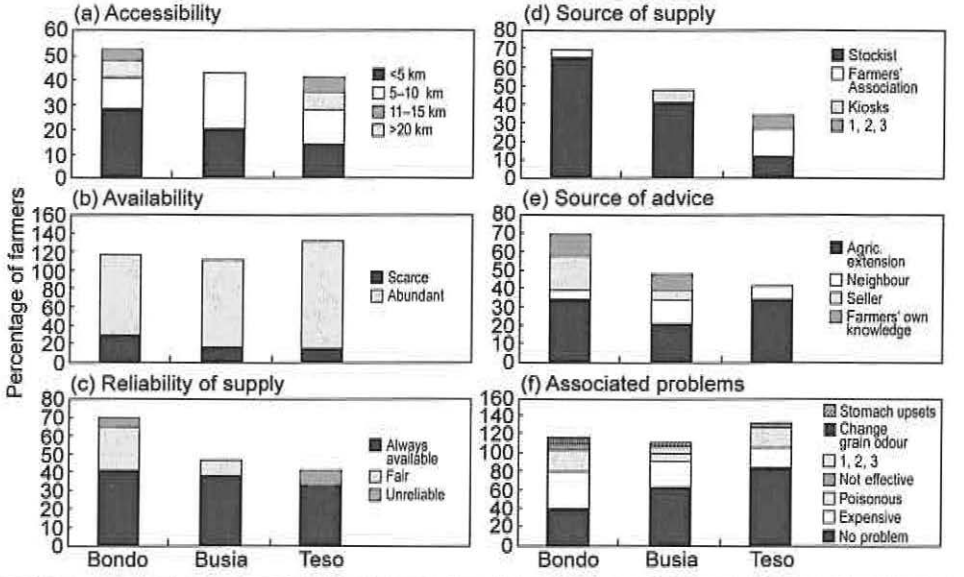

Figure 1. Level of use of synthetic insecticides and associated problems in the Lake Victoria Basin, Kenya

Table 2. Use of botanicals to control field and storage insect pests in the LVB, Kenya

Botanicals used to control field insect pests	Percentage	Botanicals used to control storage insect pests	Percentage
Tithonia (*Tithonia diversifolia*)	9.0	General ash	15
Pepper (*Capsicum annum*)	3.0	Cow-dung ash	03
Neem leaves (*Azadirachta indica*)	3.0	Bean husk ash	0.01
Emusi (*Urera hypseldendron*)	2.0	Sisal ash	0.01
Mululusia (*Vernonia amygdalina*)	1.8	Mululusia (*Vernonia amygdalina*) ash	0.01
Acacia (*Acacia sial*)	1.6	Neem (*Azadirachta indica*) leaves	0.01
Tagetes (*Tagetes minuta*)	1.4	Maize husk ash	0.002
Eucalyptus (*Eucalyptus* spp.)	1.0	–	–
Sodom apple (*Solanum incanum*)	1.0	–	–

Accordingly, this tends to promote continued subsistence farming among the majority of farmers, which impedes modernisation and commercialisation of agriculture in the LVB. In general, therefore, crop yield losses could largely be attributed to stemborers, aphids, pod feeder and beanfly in the field, and grain weevils, grain moth, larger grain borer, rodents and bruchid beetles in storage among other pests, incorporating earlier independent findings (Teetes *et al.*, 1983; Allen *et al.*, 1996 and Ogendo *et al.*, 2006).

Majority of farmers do not apply pest control methods both in the field and storage unless provoked to do so after appearance of pests and damage symptoms. A similar observation was made by Tefera (2004) in Ethiopia where

majority of subsistence farmers never applied any chemical insecticide against any field insect pest. Under this scenario farmers invest mostly in no or less costly control options such as botanicals. However, few farmers use synthetic insecticides in the field and storage in LVB.

Although promoted by the governments in the region for the last decade, subsistence farmers have not adopted synthetic insecticides. The results showed that synthetic pesticides were available in abundance, easily accessible but health risks, high cost and inefficacy were cited as reasons for their lack of adoption. The results agree with those of Saxena *et al.* (1990) who attributed non-adoption of synthetic insecticides to poverty, which has rendered them uneconomic and incompatible with farmers' existing resource base. In the present scenario, these farmers have resorted to an age long knowledge of the botanical products which are readily available, cheap, relatively safe and effective. These attributes might have contributed towards the popularity of botanicals among the subsistence farmers in the study area. More so, this preference is strongly associated with farmers' age and level of education, which prevent them from obtaining information on the synthetic insecticides though available. Therefore, the botanical control techniques should be improved, packaged and incorporated into modern integrated pest management (IPM) policy.

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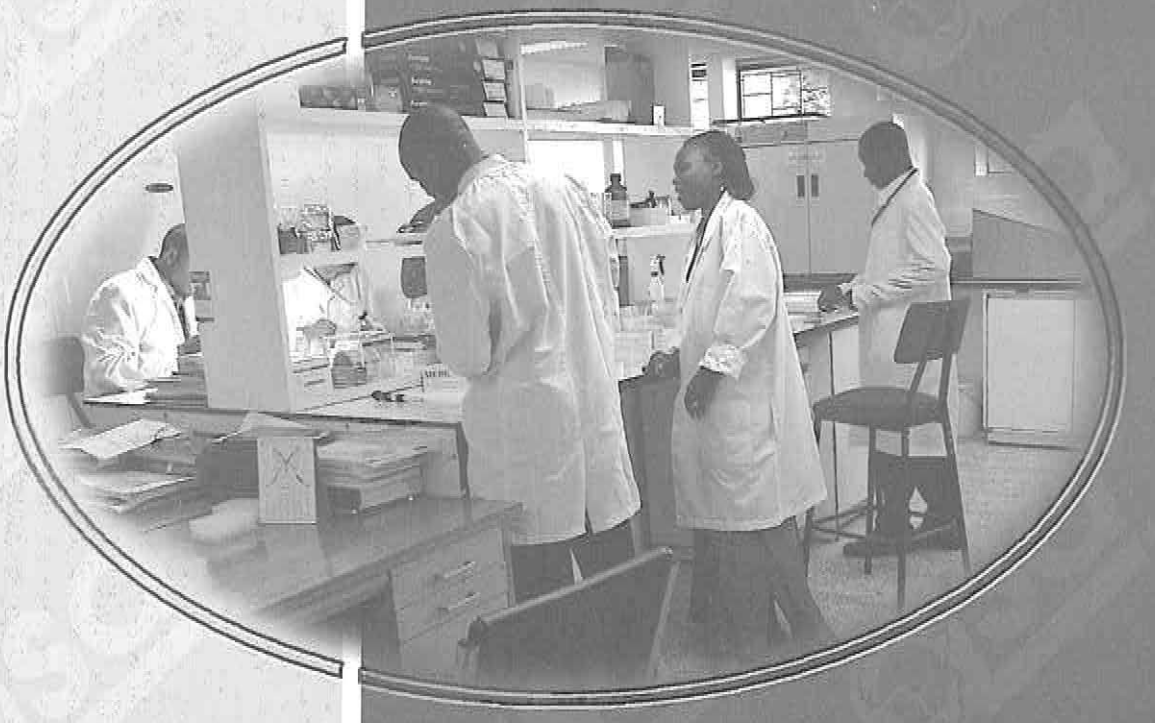

Poster Session Papers

Effects of Processing, Storage Time and Infestation of Dried Yam Chips by *Araeacerus fasciculatus* (Coleoptera: Anthribidae) on the Biochemical Components of the Yam Product in Ghana

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Abstract

Effects of processing, storage time and infestation of yam chips by *Araeacerus fasciculatus* on the biochemical components of the yam product were carried out in the laboratory in Ghana. The species of yam used were *Dioscorea rotundata* (Poir.) and *Dioscorea alata* (L.). Three varieties of the yams,'Asaana' and 'Pona' (*D. rotundata*) and 'Afasie' (*D. alata*) were used. The biochemical components of the yam that were analysed were moisture content, crude proteins, carbohydrates, crude fibre, fats, ash, reducing and non-reducing sugars. These were determined on yam that had undergone four different forms of processing (treatments): parboiled oven dried yam chips (PO), non-parboiled oven dried yam chips (NO), parboiled sun-dried yam chips (PS) and non-parboiled sun-dried yam chips (NS). These biochemical components were analysed before and after infestation by the insects. The biochemical analyses were carried out over three storage durations; 1 month, 2 months and 3 months. No significant differences were recorded for crude fibre, fat, ash and crude proteins but significant differences were observed in all other biochemical components in the different treatments. *Araeacerus fasciculatus* could not survive and establish on PO and NO yam chips; therefore, no damage was recorded but it survived on the other processed forms (PS and NS). Contaminants of various kinds arising from insect damage were also recorded on the dried yam chips that were infested by the beetle. Remnants of the damaged dried yam chips also produced offensive odour.

Key words: Asaana, Pona, Afasie, biochemical components, *D. rotundata, D. alata,* parboiled oven dried yam chips (PO), non-parboiled oven dried yam chips (NO), parboiled sun-dried yam chips (PS), non-parboiled sun-dried yam chips (NS), *A. fasciculatus*

Introduction

In West Africa, especially Nigeria, Benin Republic, Togo and Ghana, dried yam chips constitute an important food material. This is a stabilised product with low moisture content of about 12–14%. Dry chips can be kept for up to a year when stored under insect-proof conditions (CIRAD-IITA, 1998).

The major cause of post-harvest losses during chip storage is infestation by insects. A wide range of species that feed directly on the dried chips have been reported as causes of weight and quality losses in the stored produce. Pests which cause the greatest damage to the chips belong to the family Bostrichidae,

whose members are characterised by the presence of powerful mandibles with which they can cut directly into wood and other vegetable material (Adesuyi, 1975). Some of the important species of insect pests are *Rhyzopertha dominica* (Fabricius), *Araecerus fasciculatus* (Degeer), *Ahasverus advena* (Waltl), *Stegobium paniceum* (L.), *Tribolium castaneum* (Herbst), *Dinoderus minutus* (Fabricius) and *Prostephanus truncatus* (Horn.) (Pingale *et al.*, 1954; Ingram and Humphries, 1972; Parker and Booth, 1979; Hodges *et al.*, 1985; Balagopolan *et al.*, 1988; Katere and Giga, 1990).

Insect infestation of dried yam chips leads to damage, weight loss and changes in biochemical composition of the chips. Some farmers do not dispose of infested chips and the resultant powdery residues, which they sell at a lower price or consume within the household without ascertaining the safety of such products. The presence of insects on yam chips leads to contamination, including their by-products such as uric acid, fungal and bacterial inoculates, faecal matter and cast-off skins in and on the chips, foul odour, etc. The potential harmful effects of fungal and bacterial metabolites on consumers have so far not been sufficiently appreciated in developing countries (Majunder, 1982).

In spite of this, no work has been carried out to ascertain the biochemical changes in chips of *D. rotundata* and *D. alata* yams and their contrasting varieties commonly found in Ghana after infestation by insect pests. This work was carried out to investigate and determine the changes that could occur in the biochemical components of dried yam chips before and after infestation by insect pests. These were carried out on chips that had undergone different forms of processing and storage times. Contaminants that could result from insect infestation on dried yam chips were also determined.

Materials and Methods

The experiments were conducted at the Ministry of Food and Agriculture (MOFA) Pokuase, Ghana, the Department of Nutrition and Food Science, the Department of Biochemistry and the Ecological Laboratory, University of Ghana, Legon. Yam varieties were selected in such a way as to combine a number of basic contrasting characteristics of commonly grown yams found in Ghana, so that specific comparisons could be made. Three varieties of yams: 'Asaana', 'Pona' (*D. rotundata* Poir) and 'Afasie' (*D. alata* L.) were used for the study. Each variety was processed into dried chips (FSA-UNB, 1998). Yam tubers were sliced into chips of approximately 1 cm thickness. The yam chips were dried in the oven for 48 hr at 50 °C while chips dried in the sun were exposed over a period of 2 weeks (the rate and time of drying with the sun depended on the weather). Dried chips were placed separately, depending on the treatment and variety, in large transparent plastic bags. To kill any insects or developmental stages that might result from field infestation on sun dried chips, all dried chips were stored in a cold room for a period of four weeks, after which they were exposed for a period of one week for conditioning to the environmental conditions of the laboratory where the culture was set-up. Insect cultures were set up on the chips for three distinct periods as follows: 1 month, 2 months and 3 months respectively. Jars filled with chips without insects served as controls.

After the specified exposure period, dried yam chips were collected and ground into fine particles. Samples of about 100 g/kg were collected from each

treated dried yam chip; these were tightly packed in polythene bags and kept in the cold room. These processes were carried out on both uninfested and infested dried yam chips. The biochemical components analysed before and after infestation by *A. fasciculatus* were: moisture, crude proteins, carbohydrates, crude fibre, fats, ash, reducing and non-reducing sugars.

The hot air oven method was used for moisture determination; the dry ashing method was used for ash determination and the Micro Kjeldahl method was used for crude protein determination (Osborne and Voogt, 1978). The Manual Clegg Anthrone method was used for carbohydrate determination (Osborne and Voogt, 1978). The method used for fat determination was as described by Radin (1981). The spectrophotometric method was used for reducing sugar and non-reducing sugar analysis (AOAC, 1990). Crude fibre was determined based on the International Starch Institute Methods (1999).

Effects of processing on the damage caused by *A. fasciculatus*, its survival and establishment on dried yam chips and infestation for each storage period (1 month, 2 months and 3 months respectively) were determined by using the Visual Damage Scale Method (VDSM) to determine the damage recorded on different processed yam chips (treatments) for the different storage periods as described by Compton (1991). Contaminants were determined by pouring the contents of each culture on serially arranged sieves of mesh sizes 2.00 mm, 1.00 mm, 500 μm, 250 μm and 125 μm to allow for the separation of insects and contaminants comprising of feeding residues, frass, fragments of insect parts, insect excrement and damaged yam chips.

Results and Discussion

Initial biochemical components of dried yam chips before infestation by insects

Mean and ranges of values obtained for biochemical components of the varieties of *D. rotundata* and *D. alata* determined on g/100 kg dry weight basis are summarised in Table 1.

On the basis of moisture content *D. alata* (Afasie) had slightly higher moisture content than *D. rotundata* (Asaana and Pona) while no difference was observed between the *D. rotundata* varieties. The levels of total carbohydrates obtained for all the varieties were similar, but the mean value for Asaana was significantly higher than the values for Pona and Afasie while no difference was observed between Asaana and Afasie (Table 1). No significant differences were observed in the mean values of the following components: fats, reducing sugar, non-reducing sugar, ash, fibre and crude protein (Table 1).

Biochemical components of yam varieties subjected to different processing treatments

Based on the treatments applied to the varieties of yam species studied, slight variations in their compositions were observed. The variations observed were significant in some cases and insignificant in others (Tables 2, 3 and 4).

Table 1. Biochemical composition of chips of varieties of *Dioscorea rotundata* and *D. alata* yam prior to infestation

Biochemical component	Biochemical component ± SE		
	D. rotundata		*D. alata*
	Asaana	Pona	Afasie
Moisture	8.88 ± 0.27[a]	9.05 ± 0.32[a]	12.09 ± 2.00[b]
Fats	0.31 ± 0.21[a]	0.23 ± 0.14[a]	0.29 ± 0.08[a]
Reducing sugar	7.53 ± 1.05[a]	8.09 ± 1.56[a]	7.31 ± 0.90[a]
Non-reducing sugar	7.10 ± 1.82[a]	9.60 ± 0.81[a]	9.04 ± 1.29[a]
Carbohydrate	67.46 ± 1.17[b]	60.59 ± 0.72[a]	64.37 ± 2.51[ab]
Ash	2.92 ± 0.21[a]	2.63 ± 0.13[a]	2.78 ± 0.09[a]
Fibre	1.62 ± 0.06[a]	1.50 ± 0.05[a]	3.61 ± 0.07[a]
Crude protein	3.74 ± 0.09[a]	3.59 ± 0.15[a]	3.61 ± 0.07[a]

Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other at 5% significance level.

Asaana (D. rotundata)

A comparison of the biochemical composition of yam varieties subjected to different processing procedures revealed that the moisture contents for PO and NO dried yam chips of the Asaana variety of *D. rotundata* were significantly higher ($P < 0.05$) than the moisture contents of PS and NS treatments of the same variety. The values for fat content were similar for all the treatments. A similar trend was observed for crude protein and crude fibre. The mean values for reducing sugar observed for PO and NO were also significantly different ($P < 0.05$) from the mean values of PS and NS treated dried yam chips. Non-reducing sugar showed the same trend as was observed for reducing sugars. Mean values for carbohydrate for PO dried yam chips were significantly lower ($P < 0.05$) than the other treatments. The same trend was observed for ash contents (Table 2).

Pona (D. rotundata)

The moisture content for Pona was significantly higher for both PO and NO dried yam chips. NO dried yam chips were significantly higher in reducing sugar. Significantly higher values of non-reducing sugar were observed for NO dried and PS. The following, ash, fat, carbohydrate, fibre and protein showed no significant differences in the values observed for all treatments (Table 3).

Afasie (D. alata)

For the Afasie yam variety, NS treated chips had significantly more moisture than the other treated chips. A significantly higher value was also observed for reducing sugars in PO and NO dried yam chips and PO dried yam chips also had significantly higher values for non-reducing sugars. The carbohydrates

Table 2. Biochemical components of Asaana variety of yam (*Dioscorea rotundata*) dried chips

Biochemical component	Biochemical component ± SE+			
	PO	NO	PS	NS
Moisture	9.79 ± 0.28^{b}	8.69 ± 0.28^{b}	8.43 ± 0.15^{a}	8.60 ± 0.03^{a}
Fats	0.32 ± 0.05^{a}	0.30 ± 0.07^{a}	0.31 ± 0.06^{a}	0.33 ± 0.13^{a}
Reducing sugar	8.26 ± 1.69^{b}	9.63 ± 0.35^{b}	5.77 ± 0.48^{a}	5.45 ± 0.27^{a}
Non-reducing sugar	8.56 ± 0.53^{b}	8.85 ± 0.36^{b}	5.14 ± 0.55^{a}	5.83 ± 0.21^{a}
Carbohydrate	63.56 ± 1.45^{a}	69.18 ± 2.00^{b}	67.74 ± 2.21^{b}	69.36 ± 0.76^{b}
Ash	2.22 ± 0.05^{a}	3.01 ± 0.07^{b}	3.07 ± 0.05^{b}	3.17 ± 0.06^{b}
Fibre	1.42 ± 0.07^{a}	1.63 ± 0.10^{a}	1.66 ± 0.09^{a}	1.75 ± 0.09^{a}
Crude protein	3.90 ± 0.28^{a}	3.43 ± 0.14^{a}	3.83 ± 0.11^{a}	3.81 ± 0.10^{a}

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other.

Table 3. Biochemical components of Pona variety of yam (*Dioscorea rotundata*) dried chips

Biochemical component	Biochemical component ± SE+			
	PO	NO	PS	NS
Moisture	9.39 ± 0.15^{b}	9.82 ± 0.42^{b}	8.49 ± 0.34^{a}	8.41 ± 0.09^{a}
Fats	0.22 ± 0.02^{a}	0.27 ± 0.15^{a}	0.20 ± 0.02^{a}	0.25 ± 0.12^{a}
Reducing sugar	10.06 ± 0.42^{b}	12.14 ± 0.12^{c}	4.65 ± 0.87^{a}	5.49 ± 0.92^{a}
Non-reducing sugar	9.09 ± 0.59^{b}	11.24 ± 0.26^{b}	10.90 ± 1.50^{b}	7.16 ± 1.13^{a}
Carbohydrate	60.90 ± 1.70^{a}	58.60 ± 1.56^{a}	62.63 ± 3.69^{a}	60.22 ± 0.41^{a}
Ash	2.35 ± 0.10^{a}	2.83 ± 0.45^{a}	2.94 ± 0.51^{a}	2.39 ± 0.11^{a}
Fibre	1.47 ± 0.13^{a}	1.66 ± 0.09^{a}	1.40 ± 0.21^{a}	1.45 ± 0.28^{a}
Crude protein	3.34 ± 0.12^{a}	3.31 ± 0.13^{a}	3.40 ± 0.24^{a}	3.55 ± 0.12^{a}

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other.

content of PS and NS dried yam chips was also significantly higher than in the remaining treatments. No significant differences were observed among the following components, fat, ash, fibre and protein for all the treatments (Table 4).

Table 4. Biochemical components of Afasie variety of yam (*Dioscorea alata*) chips

Biochemical component	Biochemical component ± SE+			
	PO	NO	PS	NS
Moisture	9.25 ± 0.05[a]	11.80 ± 1.15[b]	12.37 ± 0.36[b]	14.94 ± 1.59[c]
Fats	0.29 ± 0.01[a]	0.26 ± 0.06[a]	0.29 ± 0.02[a]	0.31 ± 0.07[a]
Reducing sugar	8.91 ± 0.65[b]	9.01 ± 0.48[b]	4.68 ± 0.75[a]	6.63 ± 1.31[a]
Non-reducing sugar	13.01 ± 1.56[c]	9.86 ± 0.33[b]	6.32 ± 0.85[a]	7.28 ± 1.82[a]
Carbohydrate	59.17 ± 1.70[a]	59.57 ± 2.34[a]	69.81 ± 3.51[b]	68.94 ± 2.29[b]
Ash	2.69 ± 0.29[a]	2.54 ± 0.26[a]	2.93 ± 0.11[a]	2.96 ± 0.12[a]
Fibre	1.57 ± 0.12[a]	1.60 ± 0.10[a]	1.65 ± 0.09[a]	1.72 ± 0.07[a]
Crude protein	3.69 ± 0.14[a]	3.57 ± 0.23[a]	3.38 ± 0.16[a]	3.78 ± 0.26[a]

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other.

Biochemical changes observed in dried yam chips after infestation by insects

Infestation of yam chips by *A. fasciculatus* resulted in quality loss and product deterioration. Such deterioration also resulted in inadvertent deleterious biochemical reactions and changes in environmental factors. To ascertain the levels of quality loss, investigations were carried out into the biochemical changes associated with the dried yam chips after infestation. Analysed samples were taken from damaged chips and powdery residues and ground into flour.

Moisture content of dried yam chips after infestation by A. fasciculatus

Infestation by *A. fasciculatus* led to increases in the moisture content of the chips. It was also observed that significant ($P < 0.05$) differences were observed among the moisture content values of the various treatments. The percentage moisture content also increased significantly with time and level of infestation with value ranging between 12.73 and 20.00% (Table 5). The lowest moisture contents were recorded in the first month while the highest were recorded in the third month. Dried yam chips absorb moisture rapidly from the humid environment and from insect excreta to reach equilibrium moisture levels. At room temperature and relative humidity (30 ± 3 °C and 90 ± 2% RH), chips had moisture contents that ranged from 12.73–20% as compared to the initial moisture content with a range of 8–12% before the culture was set-up. This is more than the 14% acceptable upper limit for safe storage for dried root and tuber chips (Anon, 1952; Anon, 1965; CIRAD-IITA, 1998). Therefore, the high moisture contents observed in the chips after infestation enhanced the insect activities and the growth of micro-organisms such as fungi and bacteria which led to the deterioration of the chips (Stumpf, 1998).

Table 5. Percentage moisture content of dried yam chips with different treatments following infestation by *Araeacerus fasciculatus* over a period of three months

Variety	Percentage means ± SE+			
	Treatment	Month 1	Month 2	Month 3
Asaana	PO	14.67 ± 0.29^{ab}	14.97 ± 0.06^{ab}	16.50 ± 1.41^{bc}
	NO	14.70 ± 0.36^{ab}	14.07 ± 0.78^{ab}	15.00 ± 0.03^{ab}
	PS	17.37 ± 2.51^{bc}	15.00 ± 0.07^{ab}	19.40 ± 0.53^{c}
	NS	15.06 ± 0.12^{ab}	18.10 ± 0.46^{bc}	20.00 ± 0.02^{c}
Pona	PO	14.23 ± 1.08^{ab}	15.13 ± 0.91^{ab}	14.60 ± 0.46^{ab}
	NO	13.90 ± 0.69^{ab}	12.73 ± 0.28^{a}	14.63 ± 0.55^{ab}
	PS	15.87 ± 2.80^{ab}	17.33 ± 1.56^{bc}	18.17 ± 2.75^{bc}
	NS	18.60 ± 0.69^{bc}	18.33 ± 2.77^{bc}	18.00 ± 1.80^{bc}
Afasie	PO	12.57 ± 0.91^{a}	12.60 ± 1.51^{a}	14.07 ± 0.57^{ab}
	NO	12.30 ± 0.44^{a}	13.73 ± 0.91^{ab}	14.77 ± 0.40^{ab}
	PS	15.33 ± 0.76^{ab}	17.47 ± 2.21^{c}	20.00 ± 0.23^{c}
	NS	15.43 ± 0.70^{ab}	15.93 ± 1.48^{ab}	20.02 ± 0.08^{c}

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other.

Fat content of dried yam chips after infestation by A. fasciculatus

The fat content of the yam chips increased slightly following infestation by *A. fasciculatus* though not significant. The fat content of the chips initially ranged from 0.23–0.31%, but this range increased after infestation to 0.42–0.91%. Yam is known to have very low fat content with values similar to other root and tuber crops (Rickard and Coursey, 1981; Bradbury and Halloway, 1988; Agbor-Egbe and Rickard, 1990). This increase may therefore be due to the presence of the insect parts in the analysed samples, especially as it was observed to change with time and levels of infestation. This increase may also be attributed to the fact that fats in produce are likely to be broken down by lipases into free fatty acids and glycerol during storage, particularly when temperature and moisture contents are high (Christensen, 1974). This type of change is greatly accelerated by mould. It is possible that the insects may have carried some storage moulds and introduced them onto the yam chips. This may support the finding of Christensen (1974), that, at least some of the common stored produce insects regularly carry into the products they infest a large load of inoculums of storage fungi.

Reducing and non-reducing sugar content of dried yam chips after infestation by A. fasciculatus

The reducing and non-reducing sugar levels were observed to have increased for chips that were not damaged by *A. fasciculatus* (PO and NO), while those infested by these insects were drastically reduced. The reduction was from the first month to the third month of storage (Table 6a and b). This finding is similar to the report of Afoakwa (1999), who observed that different treatments given to yam tuber prior to storage, such as cooking and chopping into pieces, produce varied changes in sugar levels with storage time and temperature. Marked increases in sugars were noted in tubers chopped into pieces before storage than in whole tubers. This increase in the level of sugars may be due to the break down of starch molecules during storage under high temperatures (Afoakwa, 1999). The reduction in percentage sugars observed due to insects' infestation may be because the insects used up the sugars for their biological activities.

Carbohydrates content of dried yam chips after infestation by A. fasciculatus

Slight reduction in percentage carbohydrate was recorded after infestation by insects. Infestation of dried yam chips by insects led to significantly lower carbohydrate content when compared with the values observed for uninfested chips (Table 7). Carbohydrate content of the chips decreased in all treatments used for the processing of chips. This decrease is largely due to infestation by *A. fasciculatus*, and to the breakdown of carbohydrate into sugars. This result confirms the finding of Wright *et al.* (1993) that *P. truncatus* infestation reduced

Table 6a. Percentage reducing sugar content of dried yam chips with different treatments following infestation by *Araeacerus fasciculatus* over a period of three months

Variety	Percentage means ± SE*			
	Treatment	Month 1	Month 2	Month 3
Asaana	PO	10.74 ± 2.25^{e}	10.83 ± 2.06^{e}	9.06 ± 1.64^{e}
	NO	9.50 ± 1.93^{e}	8.93 ± 1.80^{e}	10.13 ± 1.10^{e}
	PS	5.37 ± 0.82^{c}	5.06 ± 0.99^{c}	4.64 ± 0.70^{c}
	NS	4.63 ± 1.91^{c}	4.53 ± 1.59^{c}	2.82 ± 0.23^{b}
Pona	PO	9.20 ± 1.03^{e}	10.24 ± 1.94^{e}	8.25 ± 1.83^{e}
	NO	10.43 ± 1.23^{e}	9.07 ± 2.61^{e}	10.50 ± 0.51^{e}
	PS	4.15 ± 0.86^{c}	4.72 ± 0.25^{c}	3.61 ± 1.05^{c}
	NS	4.84 ± 1.35^{c}	3.50 ± 1.33^{c}	3.14 ± 1.09^{c}
Afasie	PO	8.39 ± 1.40^{e}	10.49 ± 1.58^{e}	9.08 ± 1.40^{e}
	NO	8.01 ± 1.93^{e}	8.63 ± 1.22^{e}	7.39 ± 1.59^{de}
	PS	4.11 ± 1.36^{c}	2.84 ± 0.73^{b}	0.89 ± 0.07^{a}
	NS	6.06 ± 1.15^{d}	3.83 ± 1.14^{c}	0.52 ± 0.37^{a}

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.

*Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.

Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other.

Table 6b. Percentage non-reducing sugar content of dried yam chips with different treatments following infestation by *Araeacerus fasciculatus* over a period of three months

Variety	Percentage means ± SE+			
	Treatment	Month 1	Month 2	Month 3
Asaana	PO	8.79 ± 0.88^{d}	7.81 ± 1.99^{cd}	8.24 ± 1.60^{d}
	NO	8.42 ± 1.13^{d}	8.12 ± 1.45^{d}	8.83 ± 1.38^{d}
	PS	4.50 ± 1.76^{b}	4.11 ± 0.86^{b}	4.14 ± 1.15^{b}
	NS	4.95 ± 1.52^{b}	4.70 ± 1.17^{b}	4.13 ± 1.14^{b}
Pona	PO	8.97 ± 1.09^{d}	9.01 ± 1.30^{d}	8.74 ± 1.92^{d}
	NO	11.13 ± 0.49^{d}	10.66 ± 1.32^{d}	10.79 ± 0.75^{d}
	PS	9.73 ± 1.68^{d}	6.33 ± 1.50^{c}	4.68 ± 1.18^{b}
	NS	6.31 ± 0.87^{c}	4.61 ± 0.51^{b}	3.34 ± 1.03^{b}
Afasie	PO	12.53 ± 2.27^{d}	12.58 ± 2.46^{d}	11.22 ± 0.99^{d}
	NO	8.46 ± 1.13^{d}	9.03 ± 0.96^{d}	9.60 ± 0.98^{d}
	PS	5.55 ± 1.69^{c}	3.96 ± 1.02^{b}	0.99 ± 0.15^{a}
	NS	6.66 ± 1.11^{c}	3.18 ± 0.93^{b}	0.72 ± 0.11^{a}

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in the rows are significantly different ($P < 0.05$) from each other.

Table 7. Percentage carbohydrate content of dried yam chips with different treatments following infestation by *Araeacerus fasciculatus* over a period of three months

Variety	Percentage means ± SE+			
	Treatment	Month 1	Month 2	Month 3
Asaana	PO	58.33 ± 1.99^{b}	57.27 ± 1.16^{b}	56.97 ± 1.83^{b}
	NO	63.07 ± 1.11^{c}	65.40 ± 2.25^{c}	62.43 ± 1.21^{c}
	PS	61.40 ± 2.72^{bc}	62.47 ± 1.86^{c}	64.10 ± 1.56^{c}
	NS	63.07 ± 2.57^{c}	63.17 ± 2.31^{c}	63.13 ± 2.12^{c}
Pona	PO	58.52 ± 1.60^{b}	58.70 ± 1.28^{b}	57.47 ± 2.81^{b}
	NO	57.53 ± 0.78^{b}	53.03 ± 2.15^{a}	52.63 ± 2.72^{a}
	PS	58.07 ± 2.64^{b}	58.77 ± 1.21^{b}	59.17 ± 1.26^{b}
	NS	58.87 ± 1.27^{b}	56.23 ± 1.50^{ab}	58.03 ± 1.71^{b}
Afasie	PO	55.97 ± 2.22^{a}	56.33 ± 2.70^{ab}	54.90 ± 1.16^{a}
	NO	56.40 ± 1.65^{ab}	54.13 ± 1.23^{a}	55.17 ± 1.89^{a}
	PS	60.10 ± 1.96^{bc}	64.43 ± 0.78^{c}	63.83 ± 1.00^{c}
	NS	58.73 ± 1.83^{b}	63.40 ± 1.90^{c}	64.43 ± 1.86^{c}

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Mean values (g/100 g dry matter basis) from triplicate analysis ± standard error.
Means followed by different letters in both columns and rows are significantly different ($P < 0.05$) from each other.

starch levels by about 4% in station trials in Togo. Kumar and Okworonkwo (1991) found that in trials under control conditions, about 7% reduction of the starch level was measured on plain dried chips. In contrast, losses measured during field studies in Ghana, gave only a slight decrease of 0.3% of starch levels of local cassava varieties (Stumpf, 1998).

Ash content of dried yam chips after infestation by A. fasciculatus

Infestation by *A. fasciculatus* led to slight increases in the values of the ash in most treatments though this increase was not significant. The increase was high in the percentage ash observed for PS and NS yam chips infested by insects in the third month. Coursey (1967) reported that the ash content of yam makes it rich in minerals and even though the amount varies from species to species and from cultivar to cultivar, it is always considerable. In this study, it was found that the ash content increased slightly for the chips infested by *A. fasciculatus* as compared to the uninfested chips. Specifically, chips damaged by the insect recorded some high levels of ash contents and this may be due to the presence of insect parts and other products in the chips.

Crude fibre content of dried yam chips after infestation by A. fasciculatus

The percentage crude fibre recorded for this study ranged from 1.12–2.55%. Crude fibre content of the dried yam chips increased slightly during the duration of the study probably attributable to processing treatments and infestation by insects though the differences were not significant. This confirms the finding of Afoakwa (1999) that the fibre content of tubers chopped into pieces prior to storage was increased rapidly. It is already known that sample treatment, cultivars, storage condition and storage time had significant effects on the fibre content of *D. dumetorum* during storage (Treche and Delpeuch, 1982; Brillouet *et al.*, 1985; Sealy *et al.*, 1985; Treche and Agbor-agbe, 1996).

Protein content of dried yam chips after infestation by A. fasciculatus

The protein levels found in this study ranged from 2.99–5.90%. These protein levels were also observed to increase with time, probably due to treatments, infestation by insects and presence of insect parts in the yam powder analysed.

Effect of processing on percentage damage caused by *A. fasciculatus* to different yam chips

No damage was recorded on yam chips processed into PO and NO for all the yam varieties investigated in this study. The level of damage caused by *A. fasciculatus* to PS and NS treated yam chips during the three months of storage was significantly ($P < 0.05$) high (Table 8). Damage increased with time; with the lowest damage recorded on the dried yam chips stored for a month and the highest damage recorded on yam chips stored for three months. Percentage damage ranged from 23.06 to 92.00% from the first month to the third month of storage resulting in 12.51 and 42.56% weight loss respectively.

Table 8. Percentage damage caused by *Araeacerus fasciculatus* to differently processed yam chips

Variety	Percentage means ± SE+			
	Treatment	Month 1	Month 2	Month 3
Asaana	PO	0.0[a]	0.0[a]	0.0[a]
	NO	0.0[a]	0.0[a]	0.0[a]
	PS	23.06 ± 2.14[b]	48.38 ± 8.19[c]	61.26 ± 7.82[d]
	NS	31.18 ± 2.43[b]	85.78 ± 3.15[e]	92.00 ± 4.90[e]
Pona	PO	0.0[a]	0.0[a]	0.0[a]
	NO	0.0[a]	0.0[a]	0.0[a]
	PS	31.14 ± 1.90[b]	52.34 ± 4.43[c]	77.38 ± 5.04[d]
	NS	28.22 ± 2.64[b]	67.70 ± 6.91[d]	73.64 ± 3.25[d]
Afasie	PO	0.0[a]	0.0[a]	0.0[a]
	NO	0.0[a]	0.0[a]	0.0[a]
	PS	26.50 ± 2.40[b]	62.54 ± 1.35[d]	84.55 ± 3.00[e]
	NS	52.50 ± 2.26[c]	70.56 ± 6.51[d]	90.12 ± 10.09[e]

PO, Parboiled oven-dried, NO, non-parboiled oven-dried, PS, parboiled sun-dried and NS, non-parboiled sun-dried.
+Values are means of five replicates.
Means followed by different letters in both column and rows are significantly ($P < 0.05$) different from each other at 5% significance level.

Araeacerus fasciculatus has been recorded on dried yam chips as a major pest causing considerable damage and weight losses (Coursey, 1967). The factor of high moisture has been found to be the cause of high damage by this pest to cassava chips (Parker and Booth, 1979; Haines, 1991; Stumpf, 1998). The inability of *A. fasciculatus* to survive on the oven-dried yam chips may be due to the very low moisture content and method of drying the yam chips. Meanwhile *A. fasciculatus* is known to survive on produce with moisture contents of 8.5% and above (Haines, 1991). However, in contrast to Haines, William (1999) working on cocoa showed that *A. fasciculatus* could not establish on cocoa beans at 7.5% moisture content. Therefore, based on the quality standards which companies are already introducing for cassava chips, such as 12–14% moisture content, minimum 70% starch, and composition free of pests, extraneous materials and aflatoxin (Stumpf, 1994; Laryea, 1995), the acceptability and marketability of yam chips infested by pests is already threatened. This study shows that if *A. fasciculatus* is left uncontrolled on dried yam chips for more than one month, the chips will be considered sub-standard and attract low market value.

Contaminants produced by A. fasciculatus *on dried yam chips*

Based on the results obtained from the study, it was observed that the weight and complexity of contaminants produced by *A. fasciculatus* increased with time. This may be because increased insect numbers led to increased biological activities. The major contaminants recorded during the study period were powdery residues, dead insects, frass and insect fragments. These categories of

contaminants tended to vary according to the various treatments and length of infestation.

No contaminants were recorded on both PO and NO dried yam chips because the insect was unable to survive and establish on these chips. The contaminants were significantly high for PS and NS dried yam chips. The level of contaminants also increased with time. In the second and the third months, the level of contaminants increased and this was accompanied by a pungent odour and contaminants were clumped. The pungent odour could be due to the formation of ammonia products through heavy infestation and insect activities such as excretion, reproduction and even congestion (Mullins and Cochran, 1972).

Conclusion

Processing, storage period and infestation by *A. fasciculatus* lead to changes in the biochemical properties of dried yam chips. Processing and storage period also determine the level of damage caused by *A. fasciculatus* to dried yam chips. From the results of this study, it is advisable therefore, that farmers ensure that their chips are parboiled and dried properly using the oven dried method before storage to eliminate infestation by *A. fasciculatus*. Furthermore, any sign of infestation by *A. fasciculatus* should be controlled thoroughly to avoid extensive damage over a long period.

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Effect of Acute Heavy Metal Selection on Horizontal Life Table Characteristics of *Anopheles gambiae* (Diptera: Culicidae) Malaria Vector

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Abstract

Anopheles mosquito malaria vector appears to tolerate heavy metal presence, in excess of natural loads, in aquatic habitats. Relatively little is known about how this tolerance impacts on their survivorship. We selected the mosquito for tolerance to heavy metals by exposure of third instar *An. gambiae s.s.* larvae to sub-lethal LC_{30} cadmium, copper and lead concentration, through five successive generations. Horizontal life characteristics of the strain were the sixth generation, in the absence of the metal to assess potential impact of heavy metal pollution on mosquito biology. The metal-selected strains had a significantly lower magnitude of egg viability, pupation and adult emergence, larval survivorship and fecundity, and net reproductive rate than the control strain. The duration of larval stages and population doubling times were significantly longer, and the instantaneous birth rates lower in most metal-selected strains relative to the control strain. Although *An. gambiae s.s.* display the potential to develop tolerance to a wide range of heavy metal concentrations, this occurs at a significant biological cost to the insect, which can adversely affect its ecological fitness.

Key words: *Anopheles gambiae*, cadmium, copper, heavy metals, lead, life table

Introduction

Anopheles gambiae sensu stricto mosquito malaria vector has demonstrated adept ability to adapt to environmental changes (Coluzzi *et al.*, 1979; Coluzzi, 1994; Toure *et al.*, 1998), including to organically polluted habitats, such as drains containing domestic effluents (Coene, 1993) and other human-made aquatic habitats (Chinery, 1984, 1995), some with heavy metals in excess of natural loads (Mireji *et al.*, 2008). Similar niche expansions have been exhibited by other insects, including *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Bolnick, 2001) and *Culex pipiens quinquefasciatus* Say (Subra, 1981). The adaptations to the heavy metal pollutants in their environment can impact on

ecology of the mosquito (Reed *et al.*, 2003), which may in turn be closely tied to the effect on biology of the mosquito (Orr, 1998). Such effect would be reflected on adverse changes in life table parameters, such as survivorship and fecundity, of the mosquito, culminating in a decline in metal-tolerant individuals in environments devoid of heavy metals, wherein they would be displaced by naïve populations with greater reproductive and growth rates (Agnew *et al.*, 2004). Our aim in this study was to define salient effects of tolerance to heavy metals in the horizontal life table parameters in *An. gambiae s.s.* following acute generational selection by cadmium, copper or lead.

Materials and Methods

Heavy metals

Cadmium, copper and lead were used in the forms; cadmium chloride ($CdCl_2$) 99.99% pure, copper II nitrate hydrate ($Cu(NO_3)_2 \cdot 2.5H_2O$) >99 % pure and lead II nitrate ($Pb(NO_3)_2$) 99.5% pure analytical salts, sourced from Fisher Scientific, Fair Lawn, NJ, USA, Sigma-Aldrich, Laborchemikalien, GMBH, Germany and Prolabo, Fontenay, France, respectively.

Test insects

Anopheles gambiae s.s. mosquitoes were obtained from a colony kept by the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. This colony was originally collected from Mbita field station (00025'S, 34013'E), South Nyanza province, Kenya in December, 2000, where *An. gambiae s.s.* is abundant. At the time of this work, the colony was in the 35th filial generation post-field sampling and had not been exposed knowingly to heavy metals.

Mosquito rearing

Standard procedures for rearing *Anopheles* mosquitoes were followed (Ford and Green, 1972). All life stages were reared in an insectary under controlled environmental conditions (29 ± 2°C, 57–72% RH and LD 12: 12 h photoperiod) in the Animal Rearing and Quarantine Unit (ARQU) of *icipe*, Nairobi, Kenya. From the day of emergence, adult mosquitoes were provided with cotton wool soaked in a 10% sugar solution. Female mosquitoes were blood-fed on anaesthetized mice. Larvae were fed pulverised Tetramin fish food (Tetra GmbH, Melle, Germany).

Selection for heavy metal tolerance

Anopheles gambiae s.s. third-instar larvae were selected for heavy metal tolerance in the F_1–F_5 generations by exposing them to lethal concentrations (LC_{30}) of cadmium, copper, or lead resulting in 30% mortality. The usual practice of selecting for 50% mortality (LC_{50}) resulted in adult survival rates that were too low for analysis. In each of 3 replicates for each metal treatment, 300 larvae were

selected from each generation and exposed to the test treatment for 24 h in 1500 ml of water in polypropylene cylindrical pans (radius 10.5 cm and height 24.1 cm). The larvae were not fed during the exposure period The susceptibility of *An. gambiae* s.s. to each metal in successive generations was monitored by determining the LC_{50} values, as indicated below. A control colony not exposed to metals was reared simultaneously in a separate room and handled in the same manner through all manipulations.

Acute toxicity tests

Twenty-four hour toxicity range tests of cadmium, copper, or lead were conducted on each generation using third-instar *An. gambiae s.s.* larvae. Three replicates (n = 25 larvae per replicate) were exposed to five lead, cadmium, or copper concentrations within established toxicity response ranges (Finney, 1971), in 400 ml of distilled water in the polypropylene cylindrical pans. The concentrations were validated by direct quantitative determination of cadmium, copper or lead separately in each exposure concentration and replicate using a Buck Scientific 210VGP flame atomic absorption spectrophotometer (Buck Scientific, East Norwalk, Connecticut, USA). Quality control was achieved using certified reference sediment material for cadmium, copper, and lead (IAEA 433) from the International Atomic Energy Agency (Wyse *et al.*, 2004). Larval mortalities were evaluated 24 h post exposure; lethal concentrations and linear regressions were conducted using probit analysis (Finney, 1971). Changes in slope between generations indicated development of tolerance (Brown and Pal, 1971). The LC_{30} was used for selection, while LC_{50} was used to assess changes in tolerance.

Effect of heavy metal selections on juvenile An. gambiae s.s. *survivorship*

Samples of eggs (n = 300) were collected separately from the fifth generation of selection for each of the strains. Each sample of eggs was placed in 1500 ml chlorine-free distilled water and the proportions of eggs that hatched were counted under a dissecting microscope (Leica WILD M3Z) 48 h post-exposure. Sample of larvae was equivalent to those used in the selection (n = 300) from each of the fifth generation strains. The larvae were reared in separate 1500 ml containers, as described above. The number of larvae successfully pupating each day was noted and the pupae were placed into jars within emergence cages, separated by day and treatment. The numbers and sexes of adults emerging from each jar were recorded daily until the last adult emerged.

Effect of heavy metal selections on An. gambiae s.s. *adult survivorship*

Subsequent emergent adults from each replicate were sampled for adult life studies by the methods of Reisen and Mahmood (1980). After males and females had been together in a cage long enough for mating to have occurred, samples of 30 males and 30 females (< 12 h old) from the control and each of the metal selected strains were placed in separate 4 L plastic containers in three replicates.

Each sample of mosquitoes was supplied with balls of cotton wool soaked in 10% sucrose, and anaesthetised mice were provided daily as blood-meal source. For oviposition, water in a plastic cup lined with filter paper (9-cm radius) was provided. Egg-cups and sucrose cotton wool were changed daily. The eggs collected represented contribution by 30 females. Males were provided with the 10% sucrose solution only. Mortality of both sexes was recorded daily until the last mosquito died. The three replicates of each metal and control treatment were reared separately throughout post-selection processes and the replicates had separate growing pans/cages for each line in all assessments.

Data analysis

Mortality data for each metal selection was corrected by Abbott's formula (Busvine, 1971) and then transformed to Probits (Finney, 1971) for linear regression analyses and the determination of 30% (LC_{30}) and 50% (LC_{50}) lethal concentrations. Data sets with more than 10% control mortality were not considered for analysis (Finney, 1971). Horizontal life table analytical methods were applied to data for both juvenile and adult stages of various selection categories, since the cohorts were from distinct lines and were followed consistently through time. For adult life table characteristics, the calculation procedures, formulae and rationale employed were essentially those of the methods outlined by Reisen and Mahmood (1980) and Elkinton (1993).

Age-specific survivorship of adults (l_x) was determined as

$$l_x = y_x / y_o \quad (1)$$

where y_x = the number of males and females alive on each day x and y_o was the original number of the sample counted.

The age-specific life expectancy (e_x) was computed as

$$e_x = T_x / l_x \quad (2)$$

where

$$T_x = \sum_{x}^{w} L_x \quad (3)$$

$$L_x = (l_x + l_{x+1})/2 \quad (4)$$

and w = the day the last individual died; i.e. e_1 = the adult life expectancy at emergence in days.

To transmit *Plasmodium falciparum*, *P. malariae* or *P. vivax*, the anopheline vector must survive for ~8, 14 and 7 days, respectively, at temperatures and humidities similar to those applied in this study (Siddons, 1944). Assuming the infective meal is taken during the mosquito's second and third nights of adult life, the potential infective proportion of the population would consist of females not less than 10 days of age. Mean life expectancy at 10 days (e_{10}), was therefore computed for the control and metal-selected strains. The net reproductive rate per cohort, or the total number of living females produced per female (R_o), was established as

$$R_o = a\sum_{x=1}^{w} l_x m_x \quad (5)$$

where **a** = the mean proportion of females that survived from egg through adult emergence, and

$$m_x = E_x p \quad (6)$$

where E_x is the mean number of larvae (i.e. hatched eggs) produced per female per age interval **x**, and **p** is the proportion of the offspring that were female.

In this study, the mean value of '**a**' (proportions of females surviving to adult) for the control, cadmium-, copper- and lead-selected populations was 0.340, 0.096, 0.015 and 0.110, respectively, and '**p**' (proportion of offspring that were female) was 0.56, 0.52, 0.50, and 0.51, respectively. The **p** values were based on the observed sex ratio of the emerging adults from the non-selected control strain and metal-selected strains.

The age of mean cohort reproduction in days (T_o) was established as

$$T_o = a\sum_{x}^{w} l_x m_x x / R_o \quad (7)$$

starting at **x** =1, the day of adult emergence.

The instantaneous rate of increase in females per female (r_m), was calculated using the Dobzhansky *et al.* (1964) modification of the original Euler-Lotka equation by the Newton Raphason iteration method where

$$1 = a\sum_{x=1}^{w} l_x m_x e^{-r_m(x+D)} \quad (8)$$

e is the base of natural logarithm and **D** is the duration in days from oviposition in the present generation to first oviposition in the offspring generation. **D** was considered to be the observed mean median emergence time for females plus the duration of the nulliparous period for that cohort. For non-selected control, cadmium-, copper- and lead-selected strains, **D** (in days) ranges were 16.5–17.50, 17.0 –19.0, 16.5 – 19.0 and 14.0 – 14.0 days, respectively.

The mean generation time in days (**G**) was computed as

$$G = \ln R_o / r_m \quad (9)$$

Since this value included **D** in the calculation, **G** was a realistic estimate of the time from mean oviposition in the present generation to mean oviposition of the offspring generation.

The instantaneous birth rate (**b**) was calculated as

$$b = \ln(1 + \beta) \quad (10)$$

and the instantaneous death rate (d) as

$$d = (b - r_m) \quad (11)$$

where

$$1/\beta = \sum_{x=1}^{w} L_x e^{-r_m(x+1)}$$ (Birch, 1948) **(12)**

Population doubling time in days (T_d) was calculated as

$$T_d = \frac{\ln(2)}{r}$$ (Elkinton, 1993) **(13)**

The effects of the metal selection on egg viability/hatchability, larval and pupal mortalities, and male and female emergence were evaluated by one-way ANOVA on the three replicate data sets with the control and each of the metal treatments as factors. Means that were significantly different were separated by Tukey HSD post-hoc analysis. Similarly, the effects of heavy metal selection on the wing-length measurements, sex ratios, fecundities, male and female mean life expectancies from emergence (e_1), net reproductive rates, mean life expectancies at 10 days (e_{10}), (R_o) ages at mean cohort reproductions (T_o), instantaneous rates of increases (r_m), mean generation times (G), instantaneous birth rates (**b**), death rates (**d**), population doubling times (T_d), r_m/b and **b/d** among the treatments were also evaluated by one-way ANOVA on respective triplicate data sets with the control, and each of the metal treatments as factors, using 95% confidence limits. Means that were significantly different were separated by Tukey HSD post-hoc analysis. ANOVA and Tukey HSD post-hoc analyses were conducted using SPSS statistical software (SPSS Corporation, Chicago, Illinois Statistical Package version 11.5).

Results

Effects of metal-selection on An. gambiae s.s. *juvenile survivorship, adult emergence and fecundity*

There were significant changes in tolerance among metal and between generations, discussed elsewhere (Mireji *et al.*, 2009) in more details. Mean number of eggs that hatched was significantly higher ($F_{(3,11)} = 272.34$, $P < 0.001$) in the controls than in metal-selected strains. The mean egg eclosion from control, cadmium, copper and lead selected strains was 299 ± 0.58, 261 ± 1.76, 256 ± 2.33, 266 ± 2.31 respectively. Similarly, the mean number of larvae that pupated was significantly higher in control than in any of the metals selected strains $F_{(3, 11)} = 101.04$, $P < 0.001$). The mean larval pupation was 212.33 ± 3.28, 92.67 ± 2.73, 110.33 ± 2.91, and 101.00 ± 9.87 in control, cadmium, copper and lead tolerant strains respectively, representing about a 2-fold difference. Significantly more males ($F_{(3,11)} = 65.81$, $P < 0.001$) or females ($F_{(3,11)} = 48.37$, $P < 0.001$) also emerged from control than from any of the metal selected strains. There were 84.33 ± 1.76, 35.00 ± 2.65, 46.67 ± 3.28 and 35.00 ± 3.51 males and 107.00 ± 2.52, 34.67 ± 4.81, 48.33 ± 4.91 and 38.67 ± 6.36 females emergence from the control, cadmium, copper and lead tolerant strains. However, the male: female sex ratios were similar ($F_{(3, 11)} = 0.69$, $P > 0.05$) among the strains. Overall, there was significant similarity between the pattern of pupation and adult emergence ($\chi^2_{df=3} = 1.849$, P

> 0.05), absent between larvae exposed and pupation among the strains ($\chi^2_{df=3}$ = 45.85, P < 0.001). Fecundity (i.e. the mean number of eggs per female per group) was significantly higher ($F_{(3,11)}$ = 48.77, P < 0.001) in control than metal-selected strains by 2.4-, 1.9- and 2.1-fold in cadmium-, copper- and lead-selected strains, respectively. The fecundities were 78.71 ± 1.17, 33.22 ± 3.09, 37.80 ± 3.62 and 41.97 ± 3.40 eggs per female per group.

Effects of metal selection on *An. gambiae s.s.* pupation and adult emergence are summarized in Table 1. Median duration to pupation was shorter in lead than in cadmium or copper selected populations. Median pupae formation time for the control strain could not be precisely determined. Median durations to male emergence were also longer in cadmium or copper than in lead selected or control strains. Similar pattern was observed in the median durations to female emergence. Overall rates of male or female emergences (indicated by the slopes of regression lines) were higher in metal selected than in control strains.

Table 1. Mean (± SE) development attributes of metal-selected and non-selected control *Anopheles gambiae s.s.* strains

Aspect	Attribute	Control	Cadmium	Copper	Lead
Egg	Viability	1477.00 ± 8.50^a	997.33 ± 34.72^b	998.67 ± 18.98^b	957.00 ± 32.75^b
Larvae	Mortality	149.67 ± 2.67^a	687.00 ± 19.47^b	687.67 ± 20.67^b	756.00 ± 42.58^b
	Survivorship	1327.33 ± 6.06^a	310.33 ± 27.42^b	311.00 ± 29.72^b	201.00 ± 15.70^c
Pupae	Mortality	33.33 ± 2.03^a	61.33 ± 9.17^{ab}	46.00 ± 8.50^a	28.33 ± 2.33^{ac}
	Survivorship	1293.33 ± 4.33^a	248.67 ± 18.22^b	264.33 ± 21.94^b	172.33 ± 14.26^c
Adult male	Emergence	658.33 ± 11.46^a	126.33 ± 8.29^b	128.67 ± 12.47^b	87.33 ± 7.62^b
Adut female	Emergence	635.00 ± 11.14^a	121.67 ± 10.04^b	135.00 ± 9.54^b	84.33 ± 6.69^b
	Fecundity	129.48 ± 8.11^a	54.88 ± 0.76^b	47.18 ± 6.27^{bc}	61.69 ± 2.09^{bd}
Sex ratio	(Male/Total)	0.51 ± 0.01^a	0.51 ± 0.00^a	0.49 ± 0.01^a	0.51 ± 0.00^a

Different letters (superscripts) in the same row (treatments) denote mean differences that are significant at the 0.05 level of P by Tukey HSD multiple comparisons. Viability, no. of eggs hatching from 1500 eggs exposed; mortality, no. of larvae or pupae that died in larval or pupae stage respectively; survivorship, no. of larvae or pupae that developed into the next stage of the life cycle; emergence, no. of male or female adult mosquitoes that emerged from the 1500 eggs exposed; fecundity, mean number of eggs laid per female per group.

Effect of metal selection on An. gambiae s.s. *adult survivorship and fitness*

Adult survivorship was similar among treatments (Figure 1). The effects of metal tolerance on the biological fitness of *An. gambiae s.s.* adults are highlighted in Table 2. The copper-selected strain had higher mean life expectancy (e1) than cadmium or lead-selected strains. Female life expectancies at 10 days (e10) were similar ($F_{(3,11)}$ = 1.173, P > 0.05) between all treatments. Net reproductive rates (R_0) were significantly higher ($F_{(3,11)}$ = 62.943, P < 0.001) in controls by 12-, 18- and 10-fold than in the cadmium-, copper- and lead-selected strains, respectively. Metal-selected strains had significantly lower natural rates of increase (r_m) ($F_{(3,11)}$ = 16.281, P < 0.001) but longer population doubling times (T_d) ($F_{(3,11)}$ = 10.370,

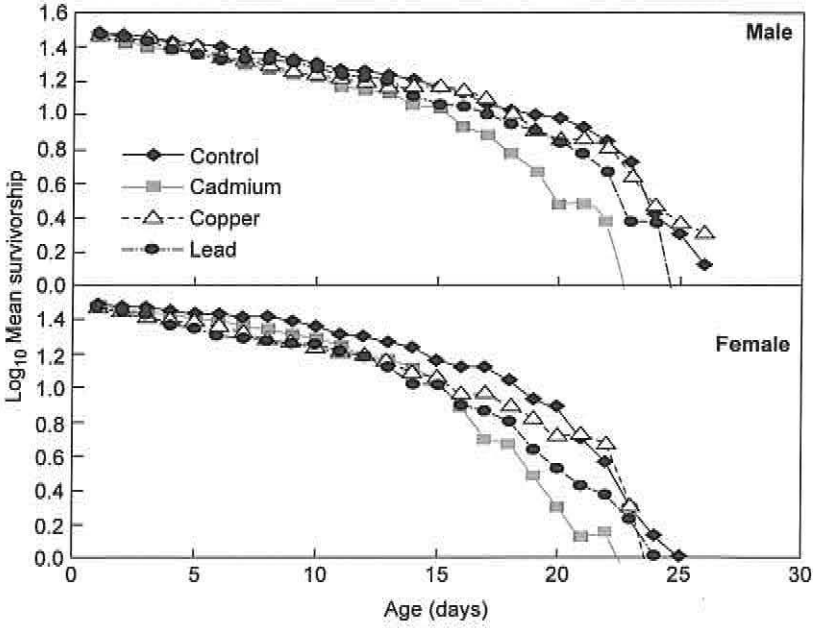

Figure 1. Survivorship (log_{10} mean) of male and female heavy metal tolerant *Anopheles gambiae* adults, and their respective controls. The mosquitoes were subjected to five successive generational selections with separate exposures to cadmium, copper and lead of which each selection exposure lasted 24 h. The survivorships were measured in independent triplicate; the means of the survivorships are presented

Table 2. Adult life table characteristics of control and heavy metal selected *Anopheles gambiae s.s.* strains

Attribute	Control	Cadmium	Copper	Lead
e_1 Male	8.05 ± 0.98^a	5.84 ± 0.53^a	5.95 ± 0.94^a	6.15 ± 0.79^a
e_1 Female	6.99 ± 0.89^a	5.45 ± 0.35^a	4.66 ± 0.27^b	5.02 ± 0.48^a
e_{10}	5.43 ± 0.60^a	3.42 ± 0.27^a	3.20 ± 0.91^a	3.61 ± 0.51^a
R_0	26.75 ± 8.01^a	4.62 ± 3.07^b	1.36 ± 0.23^b	1.29 ± 0.06^b
T_0	7.72 ± 0.46^a	8.00 ± 0.49^a	6.51 ± 0.39^a	6.99 ± 0.27^a
r_m	0.19 ± 0.06^a	0.05 ± 0.03^b	0.04 ± 0.02^b	0.01 ± 0.00^b
G	21.58 ± 0.29^a	23.79 ± 1.24^a	18.53 ± 5.42^a	23.10 ± 0.43^a
b	0.24 ± 0.01^a	0.16 ± 0.04^a	0.15 ± 0.01^b	0.12 ± 0.01^b
d	0.10 ± 0.02^a	0.11 ± 0.01^a	0.13 ± 0.01^a	0.11 ± 0.01^a
T_d	4.90 ± 0.66^a	28.83 ± 11.61^b	36.80 ± 6.53^b	67.52 ± 12.81^b
r_m/b	0.60 ± 0.08^a	0.25 ± 0.10^b	0.09 ± 0.04^b	0.09 ± 0.02^b
b/d	2.68 ± 0.47^a	1.40 ± 0.22^b	1.10 ± 0.05^b	1.10 ± 0.02^b

Different letters (superscripts) denote mean differences that are significant at the 0.05 level by Dunnett t-tests comparison of all other groups against control. e_1, mean life expectancy from emergence in days; e_{10}, mean life expectancy in days at 10 days post emergence; R_o, net reproductive rate in living female progeny per female per generation; T_o, age in days at mean cohort reproduction; r_m, instantaneous rate of increase in living female per female; G, mean generation time in days; b, instantaneous birth; d, death rate, assuming stable age distribution and T_d, population doubling time in days.

$P < 0.01$) than the respective control strains. Additionally, instantaneous birth rates (b) were significantly lower ($F_{(3,11)} = 6.392$, $P < 0.05$) in cadmium- or copper selected strains than in the respective control strains. However, R_m/b ($F_{(3, 11)} = 1.348$, $P > 0.05$) and b/d ratios ($F_{(3,11)} = 3.326$, $P > 0.05$) were similar among the strains.

Discussion

The present study indicates that tolerance in *Anopheles gambiae* to heavy metals following selection by acute concentrations of the cadmium, copper and lead metal significantly distort horizontal life table parameters of the species, reflected in changes in survivorship and fecundity, as previously suggested (Orr, 1998; Reed *et al.*, 2003). Ecological effects of such changes in the biology of the mosquito may impact on the vectorial capacity and fitness of the mosquito. Relatively high net reproductive rate (>1) of the metal-selected strains, for instance, indicates prowess of this species to successfully colonise metal polluted habitats (Elkinton, 1993). This would occur at lower numbers than that of their naïve counterparts in the absence of the metals, due to the adverse effect of the metals on viability of the eggs and fecundity. Similarly, distortion of the generation times by the metals would also increase the period to maturity in the metal tolerant populations, suggesting that such population would be more susceptible to control measures targeting juvenile stages.

The comparable patterns of tolerance induced by the metals suggest similar underlying biological regulatory processes. However, significant variation between the effects of the metals on survivorship and fecundity indicates major differences in their effective toxicities to mosquitoes (Hare, 1992; Beyersmann and Hechtenberg, 1997). This may be due to delayed toxicity resulting from different levels of residual metals present in the larvae 24 h post-exposure.

In conclusion, *An. gambiae s.s.* tolerance to heavy metals significantly distorts the horizontal life table characteristics of *An. gambiae* mosquito, with possible ecological implications on the biology of the mosquito.

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Survey of Cockroach Pests in Homes, with the Emergence of the German Cockroach *Blatella germanica* (L.) as One of the Most Serious Urban Pests in Cameroon

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Abstract

Some cockroaches are serious cosmopolitan household pests worldwide. Despite the abundant literature available on their destructive action, little information is available on their status in sub-Saharan Africa.

Recent complaints from populations in Cameroon, prompted us to design a study consisting of a survey and trapping activities in homes to assess their pest status as well as the awareness of people on their presence in Cameroon, with reference to the major cities of Douala, Bafoussam and Buea.

Of the 1750 homes inspected, we observed a household infestation of 86.5% that appeared homogeneous throughout the cities under study. The German cockroach *Blatella germanica* was the most prevalent (57.3%) and abundant species, followed by the American cockroach *Periplaneta americana* (46.6%), the Oriental cockroach *Blatta orientalis* (05.6%) and the Madeira cockroach *Leucophaea maderae* (05.0%). The public seems to be well aware of the pest status of the cockroaches including their potential implication in induction of allergies and dissemination of pathogens, food spoilage, and destruction of valuable fabrics, but poverty and low level of education make the fight against them very weak.

The lack of previous data on the cockroach fauna of households in Cameroon or even in neighbouring countries makes it impossible to investigate at the moment the alleged competitive displacement of *P. americana* by *B. germanica*.

Key words: *Blatella germanica, Periplaneta americana, Blatta orientalis, Leucophaea maderae,* household infestations, Cameroon

Introduction

Some cockroach species (Insecta: Blattodea) have a long-standing history as serious cosmopolitan household pests (Roth and Willis, 1960; Tarshis, 1962; Nojima *et al.*, 2005). Their movements between human and animal waste and food materials allow them not only to spoil the latter, but also to acquire, transport and disseminate pathogens (Ash and Greenburg, 1980; Miller and Peters, 2004; Chaichanawongsaroj *et al.*, 2004; Pai *et al.*, 2005). The foul smells they leave in and around places they haunt have made them most unwelcome in many places. Cockroaches have also been incriminated in many allergic diseases and asthma, as a result of exposure of people, especially children, to allergens from their body (Eggleston *et al.*, 1999; Gergen *et al.*, 1999; Arruda *et al.*, 2001; Litonjua *et al.*, 2001; Miller and Peters, 2004; Oishi *et al.*, 2004).

Despite this notoriety established worldwide over the years (Brenner, 1995), and the very important wealth of information gathered on their nuisance and especially their implication in food contamination/spoilage and induction of allergies, very little is known on the pest status of house-dwelling roaches in Africa. The only accounts of cockroaches on the African continent are those of Kumar (1975), and a few recent online publications on the allergens of cockroaches in South Africa (South African Allergy Working Group, 1996). The absence of valuable data on their biology, ability to serve as vector of various micro-organisms including amoebas and salmonellas, and implication in autoimmune diseases may have allowed grounds for their silent, but efficient destructive action.

Following recent complaints of heavy home and public transportation vehicles (buses, taxis and private cars) infestation with some "tiny cockroaches" in many cities in Cameroon, we designed a study consisting of a survey and the sampling of houses, to assess the reality of, and the awareness of populations to the cockroach problem. In this paper, we report on the cockroach problem in Cameroon, with reference to the situation in 3 major cities.

Materials and Methods

Study sites

The present study was conducted in Douala, Bafoussam and Buea, provincial capitals of 3 of the 10 provinces, and major cities/towns of Cameroon (Figure 1). Douala is the largest and most populated city of Cameroon, with a population projected to be about 3.8 millions, while Bafoussam is the 3rd largest city with a population of about 600,000 (Anon, 1999). Buea is a small town of about 45,000 inhabitants and plays host to the University of Buea, where this study was designed. Bafoussam is located in the Western Highlands of Cameroon (altitude of 1600–1700 m), while Douala and Buea are located on the coast. The most prominent feature of the relief is Mt Cameroon, the highest mountain in West Africa, which culminates at 4900 m.

Study design

A questionnaire, coupled with insect trapping, was designed to assess the effectiveness of the cockroach problem, and the awareness of populations about it in Cameroon. This survey involved volunteers or groups of volunteers, each (individual or group) belonging to a different household. Hence, a total of 1750 consenting persons or groups of persons were interviewed, either in French, English or Pidgin (Creole), for their knowledge/awareness of the cockroach problem, and their homes searched for the presence of cockroaches. One thousand questionnaires/searches were administered in Douala, 500 in Bafoussam and 250 in Buea. Young children (6–10 years old), knew the hiding places for the roaches and assisted us in our investigations in many houses. Cockroaches were either hand-picked or collected using sticky traps (Lee *et al.*, 2003; Miller and Meek, 2004) made of small pieces of cardboard with

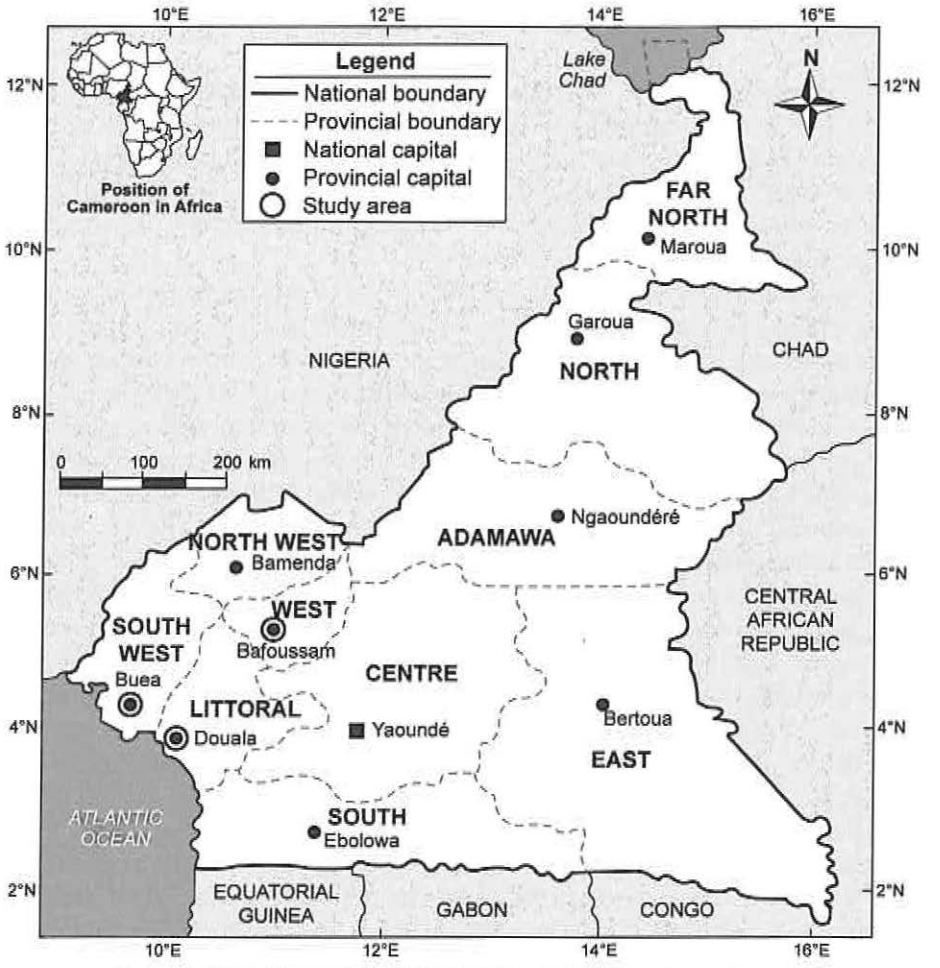

Figure 1. Map of Cameroon showing the cities of sample collection

commercially available scentless glue, laid in corners of various sections of the house. In some cases, these traps were baited with a small piece of juicy meat, or consisted of a container with a bit of beer.

Roaches trapped/collected were preserved in small glass vials containing a 70% alcohol solution and transported to the laboratory at the University of Buea for identification. Identification followed the criteria laid in the CD edition by Kunkel (1998) of Shelford (1908, 1910a, b) and in identification guidelines available online (http://entnemdept.ifas.ufl.edu/choate/blattaria_new.pdf). Some roaches were preserved dry in vials at -70 °C for future attempts at detecting any pathogens they could be harbouring.

The prevalence of household infestation (general and for each individual roach species) was assessed. The sign test (non parametric test) was used to determine if there was any difference between the prevalence of home infestation with roaches (Zar, 1984). The relative spatial distribution of species was assessed. Some habits of the roaches were equally determined (nocturnal v. diurnal).

Results

All people interviewed (including very young children, 2 and 3 years old) knew what a cockroach is, and would give a rather fair description of it. A house infestation of 86.5% was observed, which appeared almost homogeneous in the cities surveyed with 88.2% in Bafoussam, 90.4% in Buea and 84.7% in Douala ($X \geq 3$, $n = 3$, $P = 0.3125$). Data from the questionnaire agreed with trapping observations.

The German cockroach *Blatella germanica* (L.) appears as the dominating species in the study area (Table 1), and this trend could extend nationwide, or even to neighbouring countries as trapping in common transportation vehicles (buses and taxis) to and from other major cities showed that they were equally heavily infested with *B. germanica*, and may have played (and may still play) a major role in the spread of the pest through the country (Dickmu and Fokam, in preparation). *Periplaneta americana* immediately followed as second most prevalent and abundant species with a prevalence of 46.6%. The adult people interrogated pointed at *B. germanica* as a new roach that seems to have become conspicuous only in recent years. They spoke of it as a "replacement of adult roaches by very numerous smaller ones", and would recognise it as a "younger" *P. americana*. As such there seems to be a displacement of *P. americana* by *B. germanica* from preferred habitats such as kitchens, dining areas and modern toilets (in main houses) to bedrooms and pit toilets (outdoors). *Blatella germanica* seemed to appreciate living among onions in storage and could be found in thousands in/under palm-woven baskets containing this product (over 2000 individuals of various stages in many cases). Furthermore, the German cockroach was also the only species that was pointed at as diurnal, that we observed active in homes (especially kitchens and dining areas), and that could also be easily lured out of hiding during the day (less than 10 minutes with small piece of spicy and juicy food). It further appeared adapted to a wide range of micro-environmental conditions, and was found in many instances active in fridges where temperatures are as low as 4 °C.

Blatta orientalis, a cosmopolitan species and a serious pest elsewhere, seems to pose just a marginal problem in Cameroon. The Madeira cockroach *Leucophaea maderae* Fabricius was trapped in homes in Buea and Douala, but appears to be an incidental indoor inhabitant and lives very close to doors and windows that

Table 1. Species distribution of cockroach pests of homes in three major cities of Cameroon

Cockroach species	Prevalence of home infestation (%)						Total (%)	
	Bafoussam		Buea		Douala			
Blatta orientalis	28	(05.6)	2	(00.8)	68	(06.8)	98	(05.6)
Blatella germanica	303	(60.6)	71	(28.4)	629	(62.9)	1003	(57.3)
Periplaneta americana	169	(33.8)	150	(60.0)	496	(49.6)	815	(46.6)
Leucophaea maderae	00	(00.0)	32	(12.8)	55	(05.5)	87	(05.0)
Total	441	(88.2)	226	(90.4)	847	(84.7)	1514	(86.5)

open to the outside. They would scarcely venture far into homes, and would live mainly in dark, cool corners under bags or among stored tubers. They were, however, attracted to empty beer or wine glasses and bottles.

Just about 12.5% of the persons interviewed did not know what cockroaches feed on. The knowledgeable ones listed crumbs and wasted food, food (poorly stored), human and animal wastes and fabrics and acknowledged that better household/neighbourhood hygiene would be useful in reducing, if not the prevalence of household infestation, at least the intensity of infestation (number of cockroaches per home). Seventy-one percent (71.1%) of people (against 28.9%) declared that cockroaches disturbed them, especially in destroying valuable pieces of clothing and fabrics (57.4%), spoiling foodstuff (55.0%), and causing allergies (15.3%, of which 4.6% have been clinically confirmed). Many confirmed the embarrassment of having cockroaches parade their home or the seats of their car, especially in the presence of guests.

Despite this, roaches are just never contained (39.8%), or inefficiently combated by attempting to smash them with feet or a broom (40%), and are just incidentally controlled chemically, when mosquitoes (the most serious arthropod problem) are targeted. About 68.0% of the study population was aware of (and could name at least one) the diseases that could be transmitted by cockroaches (amoebiasis, typhoid fever, tuberculosis and leprosy).

Discussion

Cockroaches exhibited an amazing prevalence of infestation of homes in Cameroon. This may have increased tremendously with the economic crisis of the 1980–1990s that resulted in drastic reduction in the hygiene levels of many homes. Municipal Hygiene Services became slack in educating populations (as a part of the fight against 'major endemic diseases'), implementing their routine inspection of compounds for cleanliness and enforcing the prevailing regulation against defaulters to the cleanliness campaigns. Refuse disposal and pick up services were abandoned resulting in the piling up of garbage heaps that provide(d) virtually unlimited breeding sites not only for various cockroaches including *P. americana*, but also rats and flies.

The lack of previous data on cockroach infestations in homes in sub-Saharan Africa as a whole and Cameroon in particular makes it impossible to assess the alleged spatial displacement of *P. americana* by *B. germanica*. However, it is obvious from field observations that in houses where both species co-occur, *B. germanica* occupies what would appear as preferred infestation sites for roaches (kitchen, eating areas), or would be present in much higher numbers than *P. americana*. The latter is pushed to 'less convenient places', especially pit toilets and septic tanks outdoors that expose them more to 'hazards' from the environment, including predation. This can surely be accounted for by the much smaller individual size of these roaches that would make them require less food and as such, larger populations can co-inhabit in relatively small areas and live on little food. Moreover, they are able to easily find hiding places in tiny crevices and escape both physical and chemical attempts at exterminating them. This is further enhanced by a greater reproductive ability (larger reproductive rate and shorter generation time).

Cockroaches are not only a cosmetic problem (many people dread the eventuality of having them fall off their clothes in public, or moving around on the seats of their luxury cars), but they also pose a direct threat to health (Miller and Peters, 2004; Pai *et al.*, 2004, 2005). They adulterate food or food products with their faeces and defensive secretions, physically transport and often harbour pathogenic organisms (as can be expected with their movements from human waste to food or to areas where foodstuff is handled), and may cause severe allergic responses. In extremely heavy infestations they have been reported to bite humans and feed on food residues on the faces of sleeping humans (Litonjua *et al.*, 2001; Miller and Peters, 2004; Oishi *et al.*, 2004). In addition, some scientists suggest that German cockroach infestations may cause psychological stress in humans and that the stigma associated with infestations alters human behaviour (Botella *et al.*, 2005). Cockroach phobia is a widespread and well-studied phenomenon. For example, people with infested houses do less entertaining, and avoid the kitchen at night for fear of encountering cockroaches. This feeling was also encountered in our study as a number (18.4%) of adults of both sexes declared that they feared cockroaches. Although most people associate cockroach infestations with poor sanitary conditions, they make little effort to eradicate them from their houses, mainly because of their low economic status and low level of education.

Sensitivity to cockroaches in Cameroon certainly follows the worldwide trend of 23–60% (Arlian, 2002), but many cases may be going unsuspected because of the ignorance of affected people, lack of proper diagnostic/testing facilities and poverty that would proscribe consultation/laboratory testing. Reaction to cockroaches could account for some of the cases of asthma, as there seems to be a particular association between cockroach allergens and asthma (especially early childhood asthma) and also in cases of rhinitis and dermatitis. Cockroach allergens found on their body, shed body and egg cases, saliva and faeces (Bernton and Brown, 1964) are usually present in settled dust allergens, rather than air, as the particles are large and do not remain airborne unless disturbed (Miller and Peters, 2004).

The real economic importance of cockroaches as urban pests would be quite difficult to quantify because of their great versatility, broad scope of damage caused and lack of baseline data on their pest status in Cameroon in particular and in Africa as a whole. Yet, this seems to be a prerequisite to the design of appropriate measures to abolish this nuisance, or at least contain it within limits acceptable to the public (Bennett and Owen, 1986; Rust *et al.*, 1995). This survey unveils a hitherto unrecognised problem and stresses the need to investigate the real public health and economic importance of these insects to plan for efficient strategies to combat them.

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Potency of Two Isolates of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) against the Larvae of *Heteronychus licas* (Coleoptera: Scarabidae) Under Laboratory Conditions

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Abstract

The potency of two isolates of *M. anisopliae* (Metschin) Sorokin were evaluated against the larvae of black maize beetle *Heteronychus licas* Klug in the laboratory at 15 and 28 °C and RH of 55–70% at the University of Zimbabwe in 2000. Exposure of larvae to the fungus was achieved through incubating them in plastic vials with soil infected with the fungus suspended in oil and in water at concentrations ranging from 1.4×10^4–1.4×10^8 conidia/ml. Fungal infection reduced feeding activity and mortality was observed in all concentrations. Conidia concentration, temperature, and formulation had marked effects on mortality ($P < 0.05$) but isolate type had no significant effect ($P = 0.926$). Oil formulations were in general more effective than the water formulations. The most potent treatment at 15 °C was the oil suspension of isolate IMI350394 ($LC_{50} = 3.13 \times 10^4$ conidia/ml) with LT_{50}s of 9.2 weeks and less than 2 weeks at the lowest (1.4×10^4 conidia/ml) and highest concentrations (1.4×10^8 conidia/ml), respectively. At 28 °C the most potent treatment was the oil formulation of IMI098376 ($LC_{50} = 8.37 \times 10^4$ conidia/ml) with LT_{50}s of 9.5 weeks and less than 2 weeks at the lowest concentration and highest concentrations, respectively. Field trials of the oil formulations are recommended.

Key words: concentration, conidia, larvae, lethal, formulation, isolate, mortality, potency, biological control

Introduction

The gains brought about by the discovery and use of pesticides, firstly the organochlorines in the 1940s and later the organophosphates, carbamates, pyrethroids and others in the years that followed, which were hailed by many entomologists as the ultimate victory against insect pests, were short lived. Within short periods of time, insect resistance to, first the organochlorines and then to many other insecticides was reported. To date, the ever increasing resistance of pests to pesticides, inducement of secondary pest species, crop loss and plant hardening due to accumulative pesticide phytotoxicity and concern about harm to non-target species and environmental pollution has reinvigorated scientists' search for solutions in an integrated complex of control measures (Burges, 1981).

The perennial problem with chemical control in terms of cost, hazardousness and efficacy have led researchers in the sugar industry in Zimbabwe to turn their efforts at evaluating biological control using entomopathogenic

microorganisms. The first candidate was *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes). This is a promising agent for controlling insect pests because of its wide geographical and broad host range (Mazodze, pers. commun.). Biological control using this fungus, apart from being environmentally friendly, is likely to be self-perpetuating (Mazodze and Zvoutete, 1999). In addition, this fungus has no mammalian toxicity (Ward and Soper, 1981; and Jevanand and Kannan, 1995).

The black maize beetle (BMB) *Heteronychus licas* (Coleoptera: Scarabaeidae) is indigenous to Africa and is an important pest in tropical regions, causing damage to crops such as maize, sugarcane, tobacco and cereal crops through feeding on the roots and buried stems (Cackett, 1992).

In Zimbabwe, *H. licas* is endemic to the Southeast lowveld, where it is considered the most important insect pest of sugarcane (Cackett, 1992; Mazodze, 1997). Yield losses in severely affected fields are estimated at 40–50 tonnes cane per hectare (Mazodze and Zvoutete, 1999). Cackett (1992) has described the life cycle of *H. licas* in sugarcane under local conditions.

The rationale for carrying out this research was to compare the efficacy of two isolates of *M. anisopliae* isolated from different geographical areas, one from Australia (isolate IMI098376) and a local one, IMI350395 (isolate IMI350394), on larvae of BMB from the Southeast lowveld of Zimbabwe. Both isolates were originally isolated from *H. licas*.

Little has been done on the differential virulence of isolates of the same species when applied in different carrier materials (formulations). This study thus also aimed to explore the effects of water and oil based formulations on the virulence of these two isolates. Biological control using this fungus would be ideal in the sugar industry where continuous irrigation keeps the soil moist and the air relatively humid and thus suitable for establishment and perpetuation of the fungal pathogen.

Materials and Methods

The experiment was carried out at the University of Zimbabwe, Zimbabwe in 2000.

Sources and rearing of H. licas *larvae*

Heteronychus licas larvae were collected from ZSAES in Chiredzi in March, and kept in flowerpots. The flowerpots were filled with soil also from ZSAES. The flowerpots contained pieces of cut cane stems and bagasse as food for the larvae and kept at ambient humidity and a summer day length (about 12 hrs light and 12 hrs darkness).

Sources of M. anisopliae *isolates*

Isolate IMI098376 was obtained from the Centre for Agriculture and Biosciences International (CABI) and isolate IMI350394 was obtained from the Zimbabwe Sugar Association Experiment Station (ZSAES) in Chiredzi, Zimbabwe. Both

isolates were streaked onto PDA and incubated at 28 °C in darkness for maximum mycelial growth and sporulation. For positive identification of the fungus, slides were prepared from *in vitro* material of the fungus in distilled water and examined immediately under the microscope.

Determination of Spore Viability

Germination tests were carried out to determine spore viability. The mycelia were scrapped using a sterile blade and suspended in a 0.003M KH_2PO_4 solution containing 0.02 Triton X-100 (octyl phenoxy polethoxyethanol) (Hall, 1976) and filtered through sterile nylon chiffon (4 threads/mm) to remove the debris and dissociate the conidia balls. A Neubar's haemocytometer was used to count the conidia. At least 100 conidia were counted per replicate using a compound microscope and showered onto water agar, covered with a cover slip and incubated at 28 °C. Germination counts were made before extensive growth of germ tubes. The germination tests were conducted on different several days. The tests were repeated with oil.

Preparation of conidia suspensions

The surfaces of 10-day-old cultures of each isolate were scrapped using blades to harvest both conidia and hyphal debris. The mixture was suspended in 0.003 KH_2PO_4 solution containing 0.02 Triton X-100 and filtered through sterile nylon chiffon (4 threads/mm) to remove the debris and dissociate the conidia balls (confirmed by microscopic examination) (cf Feng and Johnson, 1990). Conidia concentration was ascertained with a haemocytometer under a microscope. The required concentrations ranging from 1.4×10^4 to 1.4×10^8 conidia/ml were made by serial dilution with either sterile water or sunflower oil (Panol oil).

Laboratory Bioassays

The bioassays were performed under aseptic conditions. Sterile plastic vials, each measuring 6 cm in height and 4 cm in diameter with holes in the bottom and top lid for radiation and drainage were used. The larvae were selected at random for bioassay to keep variation to a minimum. The larvae were gently removed from the flowerpots using sterile blunt forceps to avoid inadvertent cuticle damage, which would make them more prone to infection by fungi. Each vial contained the following: heat-treated soil (sterilised at 105 °C for 24 hrs before use) or non-treated soil for the control, moisture [supplied as distilled water (5 ml) at the start and periodically as necessary], one live larva (second or third stage larva) and food was provided as fresh bagasse (10 g per vial) (sterilised in 10% potassium hypochlorite). The soil used was from a *H. licas* infested sugarcane plot at ZSAES. There were 16 vials per treatment. The treatments were as follows: a control (without fungus) plus 10 ml distilled water and, for each of the two isolates (IMI350394 and IMI098376), 5 ml of conidia in water with conidia concentrations of about 1.4×10^4, 1.4×10^5, 1.4×10^6, 1.4×10^7 and 1.4×10^8 conidia/ml added to heat sterilised soil in each vial and incubated at 15 °C and RH of 55 to 70% and a photoperiod of about 11 hr. Each treatment

was replicated three times. This was repeated with the conidia suspended in oil.

The whole procedure was repeated and the vials incubated at 28 °C and the same humidity and photoperiod. The following mycotic effects were considered: site of host penetration by the fungus, mycelial growth on the insect body, anatomical changes in the host (colour, size) and the place where death takes place (under soil or above). Beetle larvae mortality was assessed 14 days after inoculation and thereafter every seven days for a total of 16 weeks. The assessment periods were based on earlier findings that the fungus is slow acting against the beetle with LT_{50} of between 5 and 6 weeks (Mazodze, 1997). Beetle larvae mortalities in the fungus-inoculated treatments were corrected for mortalities in the control using Abbott's formula (Abbott, 1925) given below:

$$\%\,Mortality = \frac{x - y \times 100}{x}$$

Where x is the percentage mortality in the untreated control and y is the percentage survival in the treated insects. The results of the bioassays were recorded as cumulative percentage mortality as well as total mortality for each concentration.

Confirmation of cause of death

Dead beetle larvae were inspected for the presence of *M. anisopliae* fungal growth (white mycelia and conidia) and recorded as mycosed (death caused by fungus). Larva cadavers not showing the fungus were surface sterilised for 2 min in 0.2% mercuric chloride, then for 2 min in 70% ethanol, then placed on PDA in Petri dishes and incubated at 28 °C. Incubation was done for 10 days during which the cadavers were monitored for fungal growth. Any fungal growths were scrapped and slides made for microscopic examination and those showing the fungus recorded. The proportion of larvae mycosed (confirmed by eye and after incubation) was calculated and recorded for each concentration.

Data analysis

Mortality data for each fungal isolate were Probit transformed and linearly regressed against concentration to determine LC_{50} (lethal concentration which kills 50% of the larvae) and against time (weeks) to determine LT_{50}s (time taken for 50% of the larvae to die) for all effective concentrations with the Probit procedure (SAS, 1995). Analysis of variance (ANOVA) was performed to find out if there are any marked differences in mean mortalities between isolates, temperature and formulations. Proportion analyses were used to compare the relative effectiveness of the different treatments on the mortality of the larvae.

Results

Metarhizium anisopliae was successfully cultured on PDA. It was observed to produce yellowish-green colonies. Microscopic examination of the fungus

showed the presence of conidiogenous cells borne at the apices of broadly branched, densely intertwined conidiophores that form a hymenium. Green conidia occurred in chains at the apical ends of conidiogenous cells and these were cylindrical in shape with rounded ends. The average spore viabilities were 85 and 84.3% in water and oil, respectively.

Observed feeding behaviour showed that feeding was reduced prior to death as indicated by the decrease in bagasse eaten just prior to death as compared to control treatments. Dead larvae turned pitch black. Mortality was recorded in all treatments where fungal conidia were added. There were a lot of variations in lethal time and the concentrations required to kill 50% of the population. In all treatments, percentage mycoses were observed to be slightly lower than percentage mortality.

Lethal time (LT_{50}s)

The LT_{50}s for the effective concentrations (concentrations which achieved 50% mortalities) are shown in Tables 1 and 2. A general decrease in LT_{50} with increase in conidia concentration was noted (Tables 1 and 2). Variations in LT_{50}s were also noted between isolates, temperature and suspension medium (formulation) (Tables 1 and 2).

Table 1. The LT_{50}s (in weeks) of two isolates of *Metarhizium anisopliae* against *Heteronychus licas* at levels of concentrations from 0 to 1.4 x 10^8 conidia/ml, at 15 °C

Isolate	Formulation	0	1.4 x 10^4	1.4 x 10^5	1.4 x 10^6	1.4 x 10^7	1.4 x 10^8
IMI098376	Water	–	–	10	3.2	2.5	<2
	Oil	–	–	9.4	4.7	3.2	2.8
IMI350394	Water	–	–	–	5.5	4.7	<2
	Oil	–	9.2	8.5	4.5	3.2	<2

– LT_{50} not achieved in time of observation.

Table 2. The LT_{50}s (in weeks) of two isolates of *Metarhizium anisopliae* against *Heteronychus licas* at levels of concentrations from 0 to 1.4 x 10^8 conidia/ml, at 28 °C

Isolate	Formulation	0	1.4 x 10^4	1.4 x 10^5	1.4 x 10^6	1.4 x 10^7	1.4 x 10^8
IMI098376	Water	–	–	6.8	2.1	2	<2
	Oil	–	9.5	7.2	4.1	2	<2
IMI350394	Water	–	–	9.8	2	2	<2
	Oil	–	–	6.2	5.4	4.6	2.3

– LT_{50} not achieved in time of observation.

Both isolates, irrespective of formulation, were effective against the larvae at the highest concentration (1.4 x 10^8 conidia/ml) at both temperatures (LT_{50} <3 weeks) (Tables 1 and 2). However, at the lowest concentration (1.4 x 10^4 conidia/ml), the oil formulation of isolate IMI350394 was the most effective treatment at 15 °C (LT_{50} = 9.2 weeks) and isolate IMI098376, also in oil was the

most effective treatment at 28 °C (LT_{50} = 9.5 weeks) (Tables 1 and 2). Regardless of all the variables, the lowest LT_{50} for larvae at the lowest concentration was 9.2 weeks and at the highest concentration it was less than 2 weeks. The water formulations of both isolates at 28 °C tended to perform better with an increase in concentration than the oil formulations.

Lethal concentration (LC_{50}s)

All concentration levels for both isolates were able to induce mortalities but lower concentrations generally induced lower mortalities. There was a general increase in beetle larvae mortality with increase in spore concentration.

The LC_{50} of the two isolates against *H. licas* larvae is shown Tables 3 and 4. IMI350394 (LC_{50} = 3.13 x 10^4 conidia/ml) and IMI098376 (LC_{50} = 8.5 x 10^4 conidia/ml) both in oil formulation were the most potent treatments against the larvae at 15 °C and 28 °C respectively (Tables 3 and 4). The oil formulations of both isolates at both temperatures were more potent than the water formulations (Tables 3 and 4). Water formulation for both isolates tended to be more potent against the larvae at higher temperature (28 °C) than at lower temperature (15 °C) (Tables 3 and 4). However, oil formulations for isolates IMI098376 tended to be more potent at higher temperature than at lower temperature while those of IMI350394 tended to be more potent at a lower temperature and less potent at a higher temperature. On the overall the most potent treatment for the larvae was isolate IMI350394 in oil formulation at 15 °C (LC_{50} = 3.13 x 10^4 conidia/ml).

Table 3. Probit analysis of larvae mortality data obtained from bioassays using two isolates of *Metarhizium anisopliae* against *Heteronychus licas* at 15 °C

Isolate	Formulation	Slope	χ^2	df	p	LC_{50} (Conidia/ml) with 95% fiducial limits (range)
IMI098376	Water	0.29	31.37	3	<0.00001	5.3x10^5 (9.95 x 10^4–2.26 x 10^6)
	Oil	0.23	42.71	3	<0.00001	3.98x10^5 (1.97 x 10^4–2.89 x 10^6)
IMI350394	Water	0.23	62.48	3	<0.00001	1.35 x 10^6 (5.07 x 10^4–1.87 x 10^7)
	Oil	0.19	45.78	3	<0.00001	3.13 x 10^4 (8 x 10^2–3.69 x 10^6)

Table 4. Probit analysis of larvae mortality data obtained from bioassays using two isolates of *Metarhizium anisopliae* against *Heteronychus licas* at 28 °C

Isolate	Formulation	Slope	χ^2	df	p	LC_{50} (Conidia/ml) with 95% fiducial limits (range)
IMI098376	Water	0.19	41.40	3	<0.00001	1.88 x 10^5 (9.96 x 10^2–1.76 x 10^6)
	Oil	0.39	43.81	3	<0.00001	8.37 x 10^4 (8.09 x 10^4–3.58 x 10^5)
IMI350394	Water	0.23	49.51	3	<0.00001	2.14 x 10^5 (2.57 x 10^3–1.87 x 10^6)
	Oil	0.22	45.78	3	<0.00001	1.90 x 10^5 (1.58 x 10^2–1.16 x 10^6)

Variables Influencing Mortality

Analysis of variance (ANOVA) showed that larval mortality was significantly influenced by temperature ($P = 0.03$), the medium in which the fungal conidia were added ($P < 0.0001$), the concentration of conidia added ($P < 0.0001$), the interaction between temperature and concentration ($P = 0.030$) and the interaction between temperature and formulation ($P = 0.008$).

Analysis of the percentages mortalities using proportions was able to highlight differences between specific treatments. This analysis also showed that suspension medium and temperature had some influence on beetle mortality (Tables 5 to 7).

Influence of different media on the same isolate

Comparison of the mean percentage mortality revealed that on the overall vegetable oil was relatively more effective in enhancing fungal virulence than water (Table 5). Proportion analysis shows that for isolate IMI350394 the medium in which the spores were applied proved to have some influence on mortality on larvae at 15 °C but not at 28 °C. At 15 °C the oil suspension was more effective (mean mortality of 72.8%) than the water suspension (mean mortality 49.4%) ($\chi^2 = 12.11$, $P = 0.0005$). On the other hand there were no marked differences in mortality between water and oil for isolate IMI098376 for the larvae at both 15 and 28 °C.

Table 5. Mean percentage mortalities and mycoses (in brackets) induced by two isolates of *Metarhizium anisopliae* in different media at 15 °C

Isolate	Formulation	% Mean mortality
IMI098376	Water	57a ± 34.4 (47.8 ± 35.2)
	Oil	59a ± 30.5 (53.8 ± 29.1)
IMI350394	Water	49.4a ± 31.7 (43.0 ± 31.4)
	Oil	72.8b ± 22.8 (67.6 ± 23.6)

Means in the same column followed by the same letter are not significantly different at $P = 0.05$ (Proportion analysis).

Table 6. Mean percentage mortalities and mycoses (in brackets) induced by two isolates of *Metarhizium anisopliae* in different media at 28 °C

Isolate	Formulation	% Mean mortality
IMI098376	Water	62.8a ± 25.7 (57.6 ± 22.6)
	Oil	72.8a ± 31.9 (64.8 ± 33.8)
IMI350394	Water	63.2a ± 29.3 (59.4 ± 31.4)
	Oil	66.8a ± 27.6 (59.2 ± 24.4)

Means in the same column followed by the same letter are not significantly different at $P = 0.05$ (Proportion analysis).

Effects of the same medium on different isolate

At 15 and 28 °C water did not have any influence on the potency of IMI098376 and IMI350394 on the larvae (Tables 5 and 6). Oil on the other hand showed limited differential enhancements on isolate potency. This was observed on the larval mortality at 15 °C where IMI350394 was markedly more effective than isolate IMI098376 ($\chi^2 = 4.37$, P = 0.03) (Table 5).

Influence of isolate and temperature

There were no marked differences in larval mortality at both 15 and 28 °C between isolates IMI098376 and IMI350394 (Table 7). Temperature, however, had a differential influence on larval mortality. Isolate IMI098376 was more effective at 28 °C than at 15 °C ($\chi^2 = 4.39$, P = 0.02) whereas temperature had no effect on isolate IMI350394 (Table 7).

Table 7. Mean percentage mortality and mycoses (in brackets) induced by two isolates of *Metarhizium anisopliae* at 15 and 28 °C

Temperature (°C)	Isolate	% Mean mortality
15	IMI098376	58.3a ± 1.70 (50.8 ± 4.2)
	IMI350394	61.1ab ± 16.5 (55.3 ± 17.4)
28	IMI098376	67.8b ± 7.10 (61.2 ± 5.1)
	IMI350394	65ab ± 2.50 (59.3 ± 0.1)

Means in the same column followed by the same letter are not significantly different at P = 0.05 (Proportion analysis).

Discussion

The two isolates of *M. anisopliae* caused mycosis in *H. licas*, which was confirmed by direct microscopic observations and by culturing the infected larvae on PDA. These observations agree with those of (Mazodze, pers. commun.). The association of this fungus with the black maize beetle was further confirmed by finding infected beetles in the sugarcane field (Mazodze, pers. commun.). The initial stages of insect infection by entomopathogenic fungi include the penetration of cuticle (Hajek and Lager, 1994). Studies have shown that cuticle solubilisation and subsequent hyphal penetration occur by action of extracellular enzymes and acid metabolites (Bidochka and Khachatourians, 1991). Following cuticle penetration, the fungus proliferates within the insect body. Penetration is frequently followed by blackening which is a melanic reaction of the integument at the site of the infection probably caused by changes in the phenoloxidase activity caused by the fungus (Ferron, 1978).

Observation on food consumption indicates that there is a reduction in feeding and this is quite encouraging as this would mean that damage to sugarcane would be markedly reduced once the larvae are infected.

Maniania (1992) showed that susceptibility of insect pests to fungal pathogens depends upon the fungus species. In addition, different levels of pathogenicity were observed towards different insect pests, when strains were isolated from the same host or from soil (Maniania, 1992). Although the two isolates tested can infect *H. licas*, their virulence varied greatly depending on the treatment conditions. This study has shown that larval mortality showed marked dependence on temperature, concentration of conidia in inocula, the medium in which they were suspended (formulation), the interaction between temperature and conidia concentration and the interaction between temperature and formulation.

Temperature affects mortality by affecting both the fungus and the insect. Temperature influences the rate of growth through interfering with enzymatic activities; with the consequence that growth only occurs within a particular range of temperature within which there is an optimum. The optimum temperature for *M. anisopliae* is 28 °C and it is at this temperature that maximum virulence was expected as the fungus can easily grow and overcome the insect's resistance. At this temperature the insect's activity levels are quite high and the insect is likely to move around frequently and hence pick up the conidia. On the other hand low temperatures slow down fungal growth and insect activity and hence mortalities are expected to be low. The differential effect of temperature on the two formulations (water and oil) was quite apparent. The enhanced potency of the water formulation for both isolates and the oil formulation of IMI098376 at a higher temperature (28 °C) against the larvae point out to the fact that higher temperatures may increase both fungal growth and insect activity resulting in the insect picking up the conidia which are made to germinate and grow rapidly by the high temperatures. On the other hand, the enhanced potency of IMI350394 in oil formulation at low temperatures may suggest that, conidia of this isolate can adhere onto the insect's body much easier at low temperature.

It is surprising that the type of isolate had no marked influence on larval mortality. Hajek and Lager (1994) noted that infection of the host is achieved when the conidia recognise the host as susceptible. This ability to recognise the host varies between isolates (Hajek and Lager, 1994). Recognition is dependent on chemical and topographical signals. It is thus possible that the two isolates can recognise the larvae to the same degree.

Total mortalities were highly correlated to conidia concentration, being higher under higher conidia concentrations and lower under lower conidia concentrations. This ties in well with Ferron's (1978) observations. Earliest mortalities and 50% mortalities occurred much earlier at higher concentrations than lower concentrations. This means that when conidia concentration is high, the chances of insects being infected and the levels of infections are also high. One important component in fungal disease development is the attachment of the infective unit (conidia) to the insect's body which in turn is dependent on whether the insect comes into contact with the unit or not (Roberts, 1981). Various laboratory application rates have thus been recommended, concentrations ranging from 10^4 to 10^8 conidia/ml (Ferron, 1971). It is also possible that the fewer the conidia the longer it will take for them to achieve adequate growth to cause mycoses. Low spore concentrations may also not be able to overcome

the healthy insects' resistance mechanisms and thus the insects can survive the infection, with only the older and unhealthy ones succumbing to the infection.

Differences in mortalities between the media used can be attributed to differential level of conidia adhesion to the insect cuticle. Adhesion is facilitated by hydrophobic interactions between the mucus produced by the conidia and the lipids and waxes on the cuticle. Lipids and waxes make the epicuticle highly lipophilic and thus highly hydrophobic. Oil and water may thus contribute in different ways to this adhesion process, oil being a better facilitator than water, allowing the conidia to stick with ease on the insect body wall because of the presence of lipids and waxes on the cuticle surface, unlike those with a water film around them where a "water-off-the-duck's-back" problem has to be overcome. This differential facilitation by oil may thus explain the general increase in virulence of the suspensions of both isolates in oil and in particular the marked difference in potency of the suspension of IMI350394 in oil as compared to the suspension in water to the larvae. These results thus confirm Prior, Jollands and Le Patourels' (1988) observations that some hyphomycete fungi are more infectious when applied in oil rather than in water.

The results of the investigation show that *M. anisopliae* isolate IMI098376 and IMI350394 infect *H. licas* and cause death within reasonable time periods (LT_{50}s of less than 3 weeks at concentrations of 1.4×10^8 conidia/ml) at temperatures between 15 °C and 28 °C and high humidities. These are the conditions prevailing in the Southeast lowveld of Zimbabwe. Infection results in reduced feeding and this means that cane damage is reduced once the larvae are diseased thus offering a potent protection to the sugarcane. It is important that field trials are done to determine the effectiveness of the isolates under field conditions in oil formulation.

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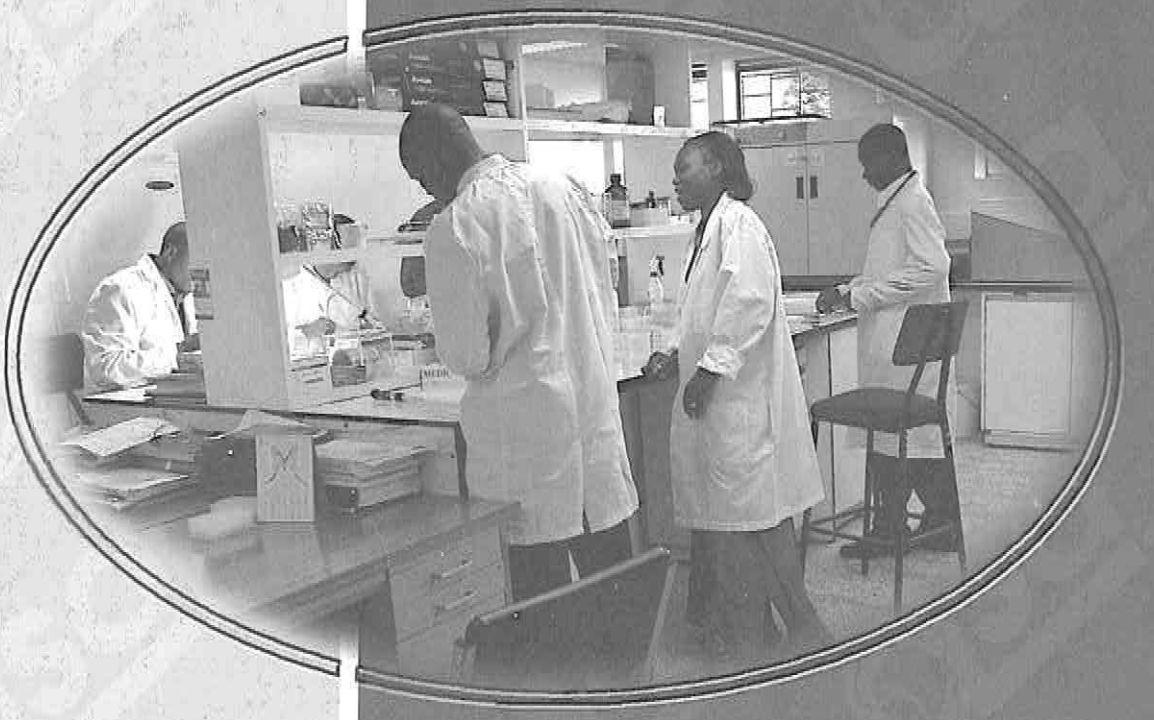

Annexes

ANNEX 1:
Programme

African Regional Postgraduate Programme in Insect Science (ARPPIS)

Silver Jubilee, 1983–2008

a collaborative Initiative of African universities in partnership with *icipe*

and

ARPPIS Scholars' Association (ASA)

Celebrate 25 years of ARPPIS

***icipe*'s Headquarters, Duduville Complex, Nairobi, Kenya**
25–28 November 2008

Plan of Activities involving the *icipe* Governing Council and ASA Scientific Conference

Overall Purpose of the Special GC/Alumni Interactive Session

Based on a 25-year historical perspective of ARPPIS, evaluate the performance of the programme and suggest ways of enhancing its capability to deliver relevant, quality research training that produces globally competitive graduates, who are committed and able to contribute effectively to resolving food and health insecurity in Africa.

General Objective:

Reach a consensus on the appropriate strategies needed to ensure effective delivery of quality graduates committed and able to take up research leadership in Africa.

Specific Objectives:

- Suggest measures for attaining an acceptable level of sustainability;
- Identify critical institutional strengthening needs of ARPPIS participating universities required to upgrade their research and training contribution to the programme;
- Provide ideas on the modernisation of the training curriculum of ARPPIS; and
- Ensure active participation and continued input from the alumni body.

Plan of Activities of the GC/Alumni Interactive and Scientific Symposium

DAY 1: Tuesday 25 November 2008

0800–0900 hrs Registration *(Dr E. Ndhine and Ms L. Igweta)*

SESSION 1

Introduction to the Scientific Symposium

Sessional Chair: Dr B. Torto/Prof. R. Maranga
Rapporteur: Dr C. Matoka

0900–0930 hrs Introduction of guests and Opening Remarks *(Prof. J. B. Okeyo-Owuor—ASA Chairman)*

0930–0940 hrs Welcome Address *(Prof. C. Borgemeister—Director General, icipe)*

0940–1010 hrs Key Note Address *(Prof. J. Ayertey—Department of Crop Science, University of Ghana, Legon)*

1010–1030 hrs Health Break

SESSION 2

Human, Animal and Plant Health Care for Sustainable Natural Resources Utilisation

Sessional Chair: Prof. S. Kyamanywa
Rapporteurs: Dr F. Demas/Dr S. Ould Ely

1030–1100 hrs ***Lead Paper:*** A reflection on 4Hs paradigm and development in Africa *(Dr Hans Herren—Former Director General, icipe)*

1100–1115 hrs ***Paper 1:*** Potential of soil injected liquid ammonium nutrition in crop pest management *(Charles Matoka)*

1115–1130 hrs ***Paper 2:*** Tsetse and wild hosts: Lessons from waterbuck on how to protect cattle from tsetse and sleeping sickness *(Nicholas Gikonyo)*

1130–1145 hrs ***Paper 3:*** Laboratory evaluation of larvicidal and growth regulatory activities of neem (*Azadirachta indica* A. Juss) seed and leaf extracts on *Anopheles arabiensis* Patton (Diptera: Culicidae) mosquito *(Habte Tekie)*

1145–1200 hrs ***Paper 4:*** Predation on Macrotermitinae termites subfamily by *Pachycondyla analis* ants *(Abdullahi A. Yusuf)*

1200–1215 hrs ***Paper 5:*** Effect of bioactive constituents of termite resistant plants on *Macrotermes* termites *(Bekele Jembere)*

1215–1230 hrs ***Paper 6:*** Potency of endod (*Phytolacca dodecandra*) crude extracts against mosquito vectors in the Lake Victoria basin, Kenya *(J. B. Okeyo-Owuor)*

1230–1300 hrs Plenary

1300–1400 hrs LUNCH

SESSION 3

Arthropods Eco-technology Adoption and Utilisation for Sustainable Development in Africa

Sessional Chair: Dr Getachew Tikubet
Rapporteur: Dr Esther Kioko

1400–1430 hrs ***Lead Paper:*** Arthropods eco-technology utilisation and development *(Suresh K. Raina)*

1430–1445 hrs ***Paper 7:*** Monitoring wild silkmoth, *Gonometa postica* Walker, abundance, host plant diversity and distribution in Imba and Mumoni woodlands in Mwingi, Kenya *(Ken Fening Okwae)*

1445–1500 hrs ***Paper 8:*** Woolly whitefly: A guest invasive alien insect pest of citrus fruits in Ethiopia *(Emana Getu)*

1500–1515 hrs ***Paper 9:*** Knowledge based approach for connecting ecological models to field data *(Henri Tonnang)*

1515–1530 hrs ***Paper 10:*** West Nile Virus: A re-emerging global health threat *(John Davies-Cole)*

1530–1545 hrs ***Paper 11:*** Suppression of tsetse populations and trypanosomiasis infection of cattle *(Getachew Tikubet)*

1545–1630 hrs Plenary

1630–1730 hrs Health Break

1730–2130 hrs Official Opening of the Symposium
- Tour of exhibition area
- Keynote Address *(Dr Michael Smalley, Director General, AMREF and past Coordinator of ARPPIS)*
- Cocktail Reception

DAY 2: Wednesday 26 November 2008

SESSION 4: GC/ARPPIS/ASA Interaction 1

Perspectives on the Mandate, Programme Design and Achievements of ARPPIS

Chair: Dr Ian Gordon, *icipe*'s Board of Training and Postgraduate Studies (IBTPS)
Rapporteur: Dr Vitalis Musewe

0830–0835 hrs	Introduction of ARPPIS / ASA (Dr Ian Gordon)
0835–0855 hrs	ARPPIS: The planning, design and implementation of an innovative institutional partnership to build human capital to solve Africa's problems *(Prof. Zbigniew T. Dabrowski, Former Coordinator, ARPPIS)*
0855–0915 hrs	ARPPIS: Reflections on accomplishments, outstanding issues and future challenges *(Prof. Ahmed Hassanali, Scientist Emeritus and former Head of Behavioural and Chemical Ecology Department)*
0915–0935 hrs	What ARPPIS has meant to me: Perspectives of a past beneficiary *(Prof. Pen-Mogi Nyeko, Vice Chancellor, Gulu University)*
0935–1035 hrs	Discussions
1035–1100 hrs	Health Break

SESSION 5: GC/ARPPIS/ASA Interaction 2

Defining the Role of ARPPIS in the Future: Strategic Implications, Opportunities and Challenges

Chair: Prof. Idah Sithole-Niang (Member, *icipe* Governing Council)

1100–1130 hrs	Global trends and strategic directions of capacity strengthening and what that suggests for ARPPIS activities in Africa *(Professor Arnold van Huis, Chair of Programme Committee of icipe's GC)*
1130–1200 hrs	Why ARPPIS must make a difference in Africa: Perspectives from an ARPPIS tripartite partnership (African Universities,

icipe and the alumni) *(Prof. Onesmo ole-MoiYoi, Chair, Kenyatta University Council)*

1200–1245 hrs ***Moderator:*** *Prof. Christian Borgemeister, Chair, ARPPIS Academic Board and Director General, icipe*

Focused discussions resulting in targeted recommendations on the way forward for ARPPIS based on the following key strategic issues:

CAPACITY AND POTENTIAL

1. Will ARPPIS, as designed and implemented, continue to deliver on its objectives?
   If not, why? What should be done?
2. Will ARPPIS continue to meet its stakeholders' expectations by building relevant capacity?
   If not, why? What should be done?
3. Will the ARPPIS model achieve sustainability?
   If not, why? What should be done?
4. Should ARPPIS broaden its reach and scope?
   If yes, why? How and with whom?
5. What is the role of alumni in ARPPIS sustainability?
6. ASA the Genesis and Beyond the 21st Century

1245–1300 hrs Closing remarks at the end of the GC/Alumni interactive session

1300–1400 hrs LUNCH BREAK

SESSION 6

Climate Change and Management of Arthropod Pests and Vectors

Sessional Chair: Dr D. Dakouo
Rapporteur: Dr K. Kambona

1400–1430 hrs ***Lead Paper:*** Climate change and effects on arthropods management *(Director, African Academy of Sciences [AAS])*

1430–1445 hrs ***Paper 12:*** Climate change in Sahel: Impacts on adaptation strategies in agricultural sector *(Serigne Kandji)*

1445–1500 hrs ***Paper 13:*** Field evaluation of food attractants and traps for the invasive fruit fly, *Bactrocera invadens* (Diptera: Tephritidae), in Kenya *(Ivan Rwomushana)*

1500–1515 hrs ***Paper 14:*** Controlling malaria vectors in areas of insecticides resistance in Central and West Africa: Does the spillage of petroleum products in *Anopheles* breeding sites have an impact on the pyrethroid resistance? *(Djouaka Rousseau)*

1515–1530 hrs	***Paper 15:*** The genetic diversity of Ghanaian populations of the whitefly *Bemisia tabaci* from molecular and ecological evidence *(Bonaventure Aman)*
1530–1545 hrs	***Paper 16:*** Biological control and integrated management of vegetable pests *(Ayalew Gashawbeza)*
1545–1615 hrs	Plenary
1615–1700 hrs	Health Break and Poster Presentations
Poster 1	Potential of soil injected liquid ammonium nutrition in crop pest management *(Charles Matoka)*
Poster 2	Effects of processing, storage time and infestation of *Araeacerus fasciculatus* (Degeer) (Coleoptera: Anthribidae) on chemical components of dried yam chips in Ghana *(Danjuma Salemon)*
Poster 3	Characterisation of odorant binding proteins from *Glossina* spp. (Diptera: Glossinidae) *(Steven R. Nyanjom)*
Poster 4	Host choice and multiple blood-feeding behaviour of malaria vectors, and other anophelines in Mwea rice scheme, Kenya *(Simon M. Muriu)*
Poster 5	Responses of *Rhipicephalus appendiculatus* (ixodid ticks) to different plant (*Calpurnia aurea*) extracts *(Paulin Nana)*
Poster 6	Population dynamics of *Bactrocera invadens* (Diptera: Tephritidae) in Kenya and its response to temperature *(Ivan Rwomushana)*
Poster 7	Biological cost of heavy metal resistance in *Anopheles gambiae sensu stricto* (Diptera: Culicidae) *(Paul Mireji)*
Poster 8	The oviposition performance of the African wild silkmoth, *Gonometa postica* Walker (Lepidoptera: Lasiocampidae) on different substrates *(Ken Okwae Fening)*
Poster 9	Distribution of chemo- and mechanoreceptors on antennae and maxillae of *Busseola fusca* stemborer larvae *(Gerald Juma)*
Poster 10	Do semiochemicals mediate defense behaviour in *Macrotermes michaelseni* termite–*Metarhizium anisopliae* fungus interactions? *(David Mburu)*
Poster 11	Household cockroaches: Emergence of German cockroach *Blatella germanica* as a serious urban pest in Cameroon *(Eric Fokam)*

Poster 12	The abundance and distribution of the Mediterranean fruit fly, *Ceratitis capitata* in late Valencia citrus orchards in Ghana *(Felix Appiah)*
Poster 13	Potency of two isolates of *Metarhizium anisopliae* against *Heteronychus licas* black maize beetle larvae *(Caston Makaka)*
Poster 14	What options for land use intensification? Findings from the belowground biodiversity project *(Anne Akol)*
Poster 15	Model utility: Impact of an exotic parasitoid, *Diadegma semiclausum*, on the diamondback moth, *Plutella xylostella (Henri Tonnang).*

DAY 3: Thursday 27 November 2008

SESSION 7

Arthropods Knowledge Integration and Dissemination for Community Livelihood in Africa

Sessional Chair: Dr Charles Maranga
Rapporteurs: Dr J. J. Randriamananaro/Dr A. Mabveni

0830–0900 hrs	***Lead Paper:*** Extension and arthropods knowledge dissemination in Africa *(Ephraim Mukisira)*
0900–0915 hrs	***Paper 17:*** Role of community involvement in bean integrated pest management (IPM) technology dissemination and adoption by smallholder farmers in sub-Saharan Africa *(Eliaineny Minja)*
0915–0930 hrs	***Paper 18:*** Lessons learned in IPM implementation: Cases from West and Central Africa *(Dona Dakouo)*
0930–0945 hrs	***Paper 19:*** Crop production and pest management practices in Kenya's Lake Victoria basin: Subsistence farmers' perceptions *(Arop Leek Deng)*
0945–1000 hrs	***Paper 20:*** Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) *(Hassane Mahamat)*
1000–1030 hrs	Plenary
1030–1045 hrs	Health Break

SESSION 8

ASA Business Session

Sessional Chair: Suleman Okech
Rapporteur: Dr J. Ogwang

1045–1130 hrs	AGM Reports *(Chair, Secretary, Treasurer and Chair TRO Foundation)*
1130–1230 hrs	Elections
1230–1250 hrs	Resolutions and way forward
1250–1300 hrs	Closing Remarks (Dr Vitalis Musewe)
1300–1305 hrs	Vote of Thanks (Dr Anne Akol)
1305–1400 hrs	LUNCH
1400–1700 hrs	Optional Tour *(Nairobi Park, Museum, Giraffe centre)*

DAY 4: Friday 28 November 2008 — Departure of participants

ANNEX II:
List of Participants

	Name/ Institution	Department/ Faculty	Area of Specialisation	Contact Address
1.	Simon Muriu – *icipe*	Human Health	Malaria–Vector Control	P.O. Box 31208-0600, Nairobi Tel: 020-8632000 Cell 0722-476117 E-mail: smuriu@icipe.org, E-mail: muriusm@yahoo.com
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3.	Prof. Fredrick Wanjala – Moi University	Biological Sciences	Entomology	P.O. Box 1125 Eldoret Tel: 0721-558386
4.	Dr John Githure – *icipe*	Human Health	Malaria	P.O. Box 30772-00100 Nairobi Tel: 020-8632000 Cell: 0722-727126 E-mail: jgithure@icipe.org
5.	Penninah Aloo-Obudho – Kenyatta University	Zoology	Aquatic Sciences	Tel: 020-810901 Cell: 0725-024640 E-mail: aloopennina@yahoo.com
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