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Chidawanyika, Frank

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EFFECTS OF DROUGHT ON THE PRODUCTION OF ELECTROPHYSIOLOGICALLY ACTIVE BIOGENIC VOLATILES IMPORTANT FOR CEREAL PEST MANAGEMENT

Frank Chidawanyika

Thesis submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfillment of the requirements for the degree PhD Science

Johannesburg, September 2014

Declaration

I, the undersigned, hereby declare that the work presented in this dissertation is my own original work and that I have not previously in its entirety, or in part, submitted it at any university or institution for a degree.

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List of supervisors

Promoter:

Prof. Frances D. Duncan
University of the Witwatersrand, South Africa

Co-promoters:

Prof. Zeyaur R. Khan and Dr. Charles A.O. Midega
International Centre of Insect Physiology and Ecology, *icipe*, Kenya

Prof. Toby J.A. Bruce
Rothamsted Research, United Kingdom

Abstract

Drought has the potential to reset trophic interactions within natural and managed ecosystems. I tested how drought stress in maize and companion plants that are used in cereal agroecosystems of Kenya affect oviposition preference, larval feeding, and development of the spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae). Five host species were tested (all Poaceae): maize (*Zea mays* L.), Napier grass (*Pennisetum purpureum* Schumach), signal grass [*Brachiaria brizantha* (A. Rich) Stapf], *Brachiaria* cv 'Mulato', and molasses grass [*Melinis minutiflora* (Beauv.)]. Under periods of water deficit, maize was oviposited on as much as under control unstressed maize in both choice and no-choice experiments. Similarly, larval leaf damage was not significantly different in drought-stressed and unstressed maize. However in Napier and signal grasses, oviposition occurred less on drought-stressed than on unstressed corresponding individuals of the same species. Oviposition acceptance and foliar damage remained low in both drought-stressed and unstressed molasses grass and Mulato. Larval survival and development remained high in drought-stressed maize, but not in Napier, signal, and molasses grass and Mulato, where survival and development were low in both drought-stressed and unstressed plants. Drought stress resulted in increased total plant volatile emission in Napier and signal grasses. This significant change in total volatile emission of the plants upon stress was not present in molasses grass, Mulato II, Silverleaf and Greenleaf *Desmodium* despite variable changes in the quantities and qualities of particular constituent compounds among all the plant species during water deficit. In wind tunnel bioassays, volatile organic compounds (VOCs) from irrigated Napier and signal grasses were more attractive to *C. partellus* than the treatment plants. However, volatiles from molasses grass, Mulato II, Silverleaf and Greenleaf *Desmodium* were never preferred by the gravid moths in both irrigated and drought stressed

conditions. These changes in chemical constituents of the plant VOCs also elicited behavioural responses in parasitoids. Drought-stressed Napier grass attracted gravid *Cotesia sesamiae* parasitoids. Other companion plant species such as molasses grass and Mulato which constitutively emit these terpenes remained attractive to the parasitoids even under periods of drought. I attribute this differential preference of the moths to the constitutive and/or induced emission of key terpenoid compounds in plants that were tested. The induction of terpenoid compounds was coupled with a reduction in the amount of green leaf volatiles such as (*Z*)-3-hexenyl acetate in Napier and signal grasses. Taken together, the results suggest that control of stemborer pests using stimulo-deterrent diversion tactics remains possible under periods of environmental change.

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"The illiterate of the 21st century will not be those who cannot read and write, but those who cannot learn, unlearn, and relearn" Alvin Toffler

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CHAPTER 1

General introduction

“It's obvious that the key problem facing humanity in the coming century is how to bring a better quality of life - for 8 billion or more people - without wrecking the environment entirely in the attempt”- E.O Wilson

1.1 Ecology and evolution of plant-insect interactions

Diametric paradigms of the coevolution between plants and insects exist due to the differences in the nature of their interactions. Between plants and insect herbivores, the relationship can best be viewed as an evolutionary 'arms race'. As plants have developed a number of strategies to avoid destructive consequences of feeding herbivorous insects, so have the insects developed counter offensive strategies in an attempt to maximise resource utilisation at both spatial and temporal scales. Such reciprocal evolution of two or more interacting species in response to selective pressures they exert on each other is referred to as coevolution (Elrich & Raven, 1964; Thompson, 2005). Between insects and plants, coevolution is believed to have existed for over a million years resulting in the wide diversity of plants and insects we see today (Fordyce, 2010; Janz, 2011). In turn, such diversity shapes the structure and function of ecological communities within various ecosystems (Futuyma, 2005). Mutualistic coevolution occurs when the interacting species evolve to enhance their interactions such as is the case with plants and their insect pollinators which reap nectar rewards (Kawakita & Kato, 2009; Landry, 2010; Hembry et al., 2013). Depending on the species in question however, such mutual relationships may either be facultative or obligate.

Another form of coevolution is antagonistic and results in the reduction of the fitness of one species by another as experienced in the case of plants and insect herbivores. In my study both types of the interactions were investigated in the form of insect herbivory on plants (antagonistic) and plant indirect defence through parasitoids (mutualistic) under drought stress in both cases.

1.2 Insect adaptations to plant defence

Insect adaptations include; behavioural, genetic and biochemical mechanisms. For example, Desurmont et al. (2011) demonstrated that the viburnum leaf beetle *Pyrrhalta viburni* changes its oviposition strategy by choosing to oviposit on infested plants in geographically isolated hosts as a behavioural local adaptation to plant defence. Insects have also developed mechanisms to survive or evade plant toxic defensive compounds. Upon detection through olfactory, contact or visual means, insects can evade toxic plants or parts of the plant through acquired experience (learning) or hereditary means (Chapman, 2003; Nealis & Nault, 2005; Després et al., 2007). In *Arabidopsis thaliana, Helicoverpa armigera* avoided eating near plant parts which had been induced to express defensive secondary metabolites in preference of the distal parts (Perkins et al., 2012).

Other insects have been shown to actively manipulate plant defence before attack (Sarmento et al., 2011). For example, the cabbage *looper Trichoplusiani* deactivates host plant defence by cutting trenches and feeding at the distal end, thus preventing plant defensive compounds from reaching the area of feeding (Dussourd, 2003). In addition, monarch caterpillars were shown to be able to deactivate the defence of milkweed *Asclepias curassavica* L. (Asclepiadaceae) by cutting the midrib or petiole of the leaf before feeding (Helmus & Dussourd, 2005). Herbivorous insects have also evolved metabolic mechanisms to suppress toxic compounds. This is achieved by the insect`s ability to up regulate detoxification enzymes that neutralise the threat of plant toxins have been widely reported (Stephens et al., 2000; Ciagnfrona et al., 2002; Després et al., 2007; Schmidt et al., 2010)**.** In some cases, insects detoxify their bodies by to excrete ingested plant toxins or shed the toxins with exuvia during moulting (Zagrobelny et al., 2004).

1.3 Plant defence mechanisms and adaptation to insect herbivory

Plants employ direct and indirect mechanisms to protect themselves against insect herbivores. The direct strategy relies on evolutionary antagonistic features (See Fig. 1.1) which include mechanical and chemical defence mechanisms that directly affect the herbivore (Roades, 1983; Khan et al., 2010). Mechanical defense mechanisms in the form of physical barriers such as waxes, resins and tough surfaces can be important constitutive forms of defence. In addition, these mechanical defences can also be in the form structural features such as trichomes, hairs and thorns (Fernandes, 1994; Lucas et al., 2000) which often discourage insect herbivore settlement on the plant. Direct chemical strategies are mostly anti-nutritive (deterrent) and/or toxic (Duffey & Stout, 1996; Chen, 2008).

Whilst anti-nutritive traits limit food supply in both pre- and post –ingestion, toxicity involves physical and chemical disruptions that harm the insect. For example, glucosinolate compounds present in *Brasssica* spp. are growth inhibitors which also serve as deterrents to many herbivores (Halkier & Gershenzon, 2006). In addition, protease inhibitors which can be expressed constitutively or induced upon insect attack have been attributed as a key strategy for defence in many plant species (Jongsma & Bolter, 1997; Jongsma & Beekwilder, 2011). Another form of direct defence in plants is manifested by the presence of specialised hairy surfaces known as glandular trichomes. These glandular trichomes can have multiple functional roles including enhancement of water economy under drought stress and defence against herbivores (Gonzales et al., 2008). For defence against insect herbivory, the glandular trichomes can secrete secondary metabolites which can also have toxic or deterrent properties (Boughton et al., 2005; Peiffer et al., 2009; Glas et al., 2012). Even though the direct defence

mechanisms have often been broadly divided into two categories (mechanical and chemical), there is evidence of interaction of both to maximise defence against herbivory in many plant species (Becerra et al., 2001; Rasmann & Agrawal, 2009; Ballhorn et al., 2013).

Indirect means of plant defence relies on the emission of volatile blends that attract the natural enemies of the herbivorous pests such as parasitoids (Gouinguene & Turlings, 2002) or entomopathogenic nematodes which attack root herbivores (Rasmann et al., 2011). In some plant species, the volatiles attracting the natural enemies are only produced after herbivory and are referred to as herbivore induced plant volatiles (HIPVs) (Kessler & Baldwin, 2001; Bruinsma et al., 2010). This is different from constitutive means of defense where plants are always in defence mode even during the absence of the herbivorous organisms.

The ability to activate defence when needed (inducible defence) is of adaptive significance as this helps plants to avoid metabolic costs associated with continuously activating defence even in the absence of herbivore attack (Agrawal, 1999; Purrington, 2000; Cipollini et al., 2003). In some circumstances, neighbouring plants can even prime their defence in response to volatile emission by attacked neighbouring plants in anticipation of future attack (Rodriguez-Saona & Frost, 2010). Such priming enables plants to respond more rapidly and efficiently to future exposure to herbivore stress when compared to plants without prior exposure (Conrath et al., 2006). Some plant species are also known to recruit entomopathogenic nematodes (EPNs) which defend them against below-ground attack by root herbivores. For example, maize roots attacked by corn rootworm *Diabrotica virgifera* release (*E*)-β-caryophelene which attracts EPNs which happen to be natural enemies of *D. virgifera* (Rasmann & Turlings, 2007).

There is also increasing evidence that such below-ground trophic interactions impact other interactions happening above ground on the same plant (Waddle et al., 2003; Bezemer

& van Dam, 2005; Rasmann & Turlings, 2007; Kaplan et al., 2008; Dematheis et al., 2012; Soler et al., 2012). In agriculture, understanding such multitrophic interactions provides excellent opportunities for developing environmentally benign methods for pest control. By manipulating the chemical ecology of agroecosystems, desirable modifications in the behaviour of pests and their natural enemies can be achieved.

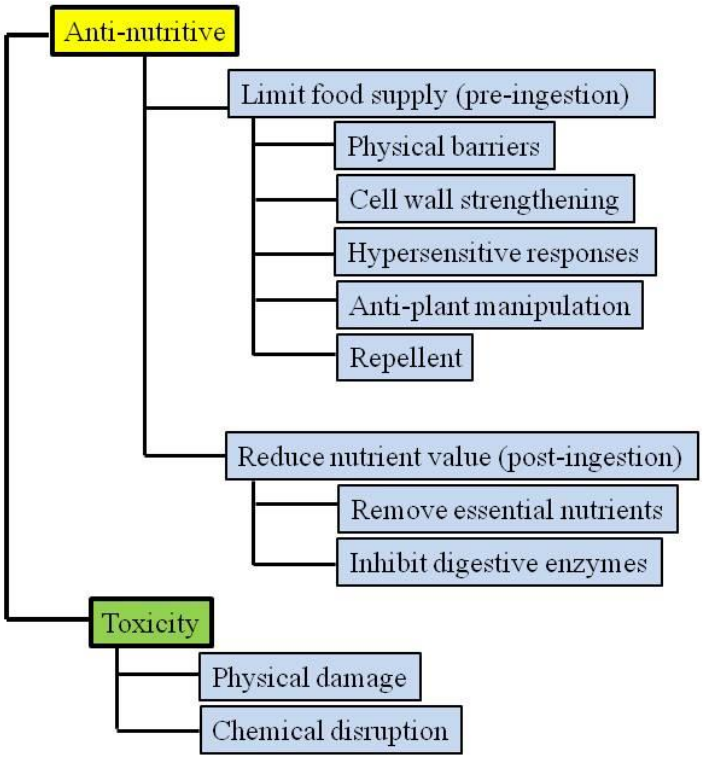


Figure 1.1: Summary of direct defence strategies employed individually and/or synergistically against herbivorous insects. (Redrawn from Chen, 2008).

1.4 Plant responses to abiotic stress

Environmental factors such as drought, extreme temperatures, salinity and rising CO_2 pose a number of challenges to plant growth and productivity. To cope with such abiotic challenges, plants often respond with adjustment to several key physiological, cellular and molecular processes resulting in changes in gene expression, protein and metabolite production (Ahuja et al., 2010). The manner in which an abiotic stress occurs may influence the plant`s response to abiotic stress. Severe stress may result in drastic changes in the plant`s physiological processes, where as mild stress offers the chance for a plant to acquire tolerance through acclimation. Thus it is of importance to take note of the severity and duration of plant exposure to stress when determining their responses to abiotic stress. Some of the responses of plants to different abiotic stressors with special reference to plant volatile production were reviewed by Niinemets (2010).

Generally, abiotic stressors in plants often elicit generic responses in the form of reactive oxygen species (ROS) which serve as stress signalling molecules and initiators of plant defence reactions (Apel & Hirt, 2004; Vickers et al., 2009a). The accumulation of ROS triggers production of isoprenoid VOCs which have antioxidant properties (Vickers et al., 2009b), regardless of the particular abiotic factor which elicited ROS production. It is therefore sometimes difficult to distinguish the contribution of individual abiotic stress factors to plant volatile production responses where multiple stress factors are occurring simultaneously. But in all cases, accumulation of ROS results in plant damage, if left uncontrolled, as a result of the oxidation of essential biological compounds such as nucleic acids, proteins and lipids which results in cell and plant necrosis. In the following segment of this section, a discussion is given of the types of morphological and physiological responses that are elicited by drought in plants.

Drought stress can be defined as any prolonged soil moisture deficit which may result in the reduction of the fitness of the plant. Plants facing drought respond via a number of mechanisms from the cell level up to the organ level of the plants including adjustments in the physiological processes. For example, leaves of drought stressed plants tend to have their surface area reduced (Taiz & Zeiger, 2006) before adjustment of the stomatal aperture or even closure in order to keep the water potential constant (Tardieu & Simmoneau, 1998; Gouinguené & Turlings, 2002). The stomatal closure is generally regarded as a direct response to the production of abscisic acid during moisture stress. In addition to this, moisture stress may lead to reduction in photosynthetic rates, increased leaf wax position and increased root growth (Taiz & Zeiger, 2006). Another consequence of changing climates is rising temperature which can trigger the heat shock response which is characterised by production of molecular chaperones known as heat shock proteins (Hsps) which serve to avoid aggregation of cellular proteins (Krishna, 2003).

1.4.1 Specific responses to drought and heat stress in plants

In response to the increasing quantities of ROS, as a result of abiotic stress, plant species are hypothesised to protect themselves by production of isoprenoid compounds which have antioxidant properties (Vickers et al., 2009a, b). Isoprene compounds have also been shown to be of importance in protection of plant photosynthesis under thermal stress (Sharkey & Yeh, 2001; Sharkey et al., 2008). The mechanisms used to achieve the protection have been reviewed by Sharkey et al (2008). The isoprenoid compounds are variable and can be produced through the cystosolic mevalonic acid (MVA) pathway or plastidic 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways (Rohmer, 1999; Lange et al., 2000; Vickers et al., 2009a). A typical isoprenoid carbon skeleton is derived from blocks of 5-carbon units which can be built into hemiterpenes (C_5), monoterpenes(C_{10}), diterpenes(C_{20}), tetraterpenes (C_{40}),

in the plastids or sesquiterpeses (C_{15}) and triterpenes(C_{30}) which form in the cytosol (Vickers et al., 2009a, b).

Drought stress has also been known to alter the emission of VOCs in plants. For instance, an increase of 5 to 310 times by volume in emission of a variety of hexanal compounds from potted drought stressed apple plants was reported by Ebel et al. (2006). However, the changes of emission of the volatile profiles largely depend on the type of water loss. Short term water loss results in decrease and increase in sesquiterpene and monoterpene emission respectively in a Mediterranean species. However, long term water loss appeared to decrease the production of all terpenoids in the same species (Ormeno, et al., 2007; Yuan et al., 2009).

In black poplar plants *Populas nigra* L., severe drought stress resulted in the reduction of isoprene emission (Fortunati et al., 2008). The changes in volatile emission as a result of drought stress or heat is therefore dependant on a number of factors including the plant species, VOCs in question and the season in which the stress event occurs (Llusià et al., 2006; Yuan et al., 2008). It is therefore difficult to make generic predictions on how volatile profiles will be affected in an ecosystem as a result of changing climate. Hence this project aims to investigate the chemical changes occurring in cereal agroecosystems as a result of drought their subsequent impact on tritrophic interactions.

1.5 Phenotypic plasticity of insect olfactory guided behaviour and plant responses to environmental variation

Phenotypic plasticity, which is the range of phenotypes a single genotype can express in response to variable environmental conditions (Nicotra et al., 2010), is one of the most important plant factors that may determine how a plant species may respond to abiotic stress. Such plasticity may be adaptive, if it confers increased fitness to a particular genotype in response to the stress which in turn ensures survival in otherwise unfavourable environments.

In addition, the responses of plants to abiotic stressors may be influenced by the plant growth stage e.g. vegetative or flowering as the metabolite requirements or investments in growth often differ across different stages. The plant`s health status with regards to biotic pressures (pests and diseases) may also be key in giving variable responses to a particular abiotic stress.

Olfaction plays a key role in the location of food, mating partners and oviposition sites. However, several factors influence the responsiveness, thus plasticity, of the insects to various odour objects in the environment. For example, the nature of the volatile chemicals and physiological status of the insects (age, gender, feeding and mating status) may influence the responses of insects to odours. In addition, environmental factors such as temperature and photoperiod are known to influence the responsiveness of insects to odour space (Anton et al., 2007).

Several mechanisms mediate the plasticity of insect olfactory guided behaviour. In most Lepidopteran male moths, for example, sensitivity to pheromones only occurs upon full maturity of sex organs (Anton et al., 2007). Upon mating, gravid moths are also known to become more sensitive to host-plant odours upon reaching physiological maturity for oviposition (Anton et al., 2007; Saveer et al., 2012). In *Lobesia botrana* female moths, such increased sensitivity to host-plant odours has been proved to be triggered soon after mating in preparation for oviposition (Anton et al., 2007; Masante-Roca et al., 2007). Apart from mating status, age differentially affects the response of insects to plant odours with insects of the same species using the same odour composition differently because of age (Anton et al., 2007 and references therein).

1.6 Major cereal crops in sub-Saharan Africa and their production constraints

Despite cereals being an important component for attaining food security in sub-Saharan Africa (SSA), production remains constrained by both biotic and abiotic factors. Since the onset of the Green Revolution pioneered by Borlaug and others a few decades ago

(Borlaug, 2007), production of maize (*Zea mays* L.) has moderately increased in Africa. Even though these yield benefits of Green Revolution in SSA are lower (1 t/ha) compared to other parts of the world such as South Asia (2.4 t/ha), Latin America (3.2 t/ha), East Asia and the Pacific (4.5 t/ ha) (World Development Report, 2008), cereals have become staple crops. In particular for countries lying in the eastern and southern parts of the African continent (Minja, 1990) where maize has become a staple crop due to access to improved varieties suited to specific regions resulting in better yield (Banziger et al., 2006; Borlaug, 2007). In 2001, for instance, Kenya had a per capita consumption rate of over 94kg, with Malawi, Zambia and Zimbabwe at over 100kg (Aquino et al., 2001). Such consumption rates confirmed maize as the highest cereal crop in those countries.

Sorghum (*Sorghum bicolar* L.) is also a key food source even in marginal areas, where maize will not thrive, making it a viable option for most poor and food insecure communities in Africa. Hence, sorghum cultivation has been adopted by a number of communities in African countries including Ethiopia, Kenya, Somalia and Sudan where drought frequently occurs. Apart from provision of animal and human feed, sorghum has been a subject of industrial research as a raw material for bio-energy and malting/beer brewing industries (Rooney and Waniska, 2000). Among a diverse range of millet species, pearl millet (*Pennisetum glaucum* L.) and finger millet (*Eleusine coracana* L.) have been an important alternative cereal food source in sub-Saharan Africa with equal or superior protein content than maize or sorghum (Taylor et al., 2006). Millets are also able to withstand harsh conditions such as drought or poor nutrient soils, making them ideal for cultivation in marginal areas. However, they possess high fat content which makes them difficult to store in the long term without compromising quality (Taylor et al., 2006). This has resulted in their limited adoption in industrial and domestic roles as compared with other cereals such as maize and sorghum.

Wheat has also served as an important crop in the sub-Saharan countries. However, despite the increasing consumption rate of wheat in many African countries, production of the crop at both commercial and household level remains very low due to its high demands in production costs when compared with other cereal crops (Morris & Byerlee, 1993). In addition, wheat variety development in terms of drought resistance also lags behind other cereals making it not feasible for production for farmers without irrigation facilities. However, this has not been the case with rice where significant strides have been made in development of varieties that are grown in dry land ecology under the New Rice for Africa (NERICA) programme (Atera et al., 2011). The NERICA programme uses conventional breeding methods of interspecific hybridisation of African rice *Oryza glaberrima* (Steud) and Asian rice *Oryza sativa* (L.) to develop new lines that are more tolerable or resistant to harsh environmental conditions (Atera et al., 2011; Balasubramanian et al., 2007). As a result, production of rice in sub-Saharan Africa has significantly increased. However, consumption still remains significantly higher than production levels resulting in the continent to rely heavily on imports.

Due to similar socio-economic and geographic factors of smallholder farmers in SSA, the main cereal production problems are almost always generic. First, farmers in most rural populations are resource poor and often struggle to access fertilisers and good quality seed, which are mandatory requirements for obtaining high yields in most cereals such as maize. Moreover, the demand for soil nutrient alternatives in most parts of SSA where crops such as maize are grown is very high due to poor nutrient soils that are mainly under smallholder farmers. Second, farming systems in Africa are increasingly under pressure from abiotic stress such as drought and extreme temperatures due to changing climates (El-Beltagy & Madkour, 2012). To counter this, efforts are underway to develop water efficient or drought tolerant crops with varying degrees of success (Banziger et al., 2006).

Third, cereal crops of SSA succumb to a wide range of biotic constraints. Lepidoptera pests such as *Busseola fusca* (Fuller) and the invasive *Chilo partellus* (Swinhoe) have been reported as highly destructive (Seshu-Reddy, 1998) with the potential of causing up to 88 % yield loss if left uncontrolled (Kfir et al., 2002). Apart from insect pests, some farmers in Africa also have a bigger challenge in parasitic weeds in the genus *Striga* (Khan et al., 2010). By using the soil nutrients and moisture intended for the crop *Striga* weeds retard crop growth and even result in 100 % yield loss (Fig. 1.2). Due to the expensive costs required for control using methods such as inorganic pesticides, stemborer control remains a challenge for many resource poor farmers.

Last, farmers also face further biotic challenges in crop diseases. For example, most maize varieties in SSA Africa are prone to both vector borne and non-vector borne diseases such as Grey Leaf Spot (GLS), Maize Streak Virus (MSV) and Rust (Thottapilly et al., 1993; Okori et al., 2004; Crous et al., 2006; Shepherd et al., 2007). More recently, maize varieties in some parts of Kenya have been reported to succumb to latest deadly diseases in the region such as Maize chlorotic mottle virus (MCMV) and Maize lethal necrosis disease (MLND) (Wangai et al., 2012). Even though there are some resistant varieties, wheat in SSA often succumbs to the stem rust fungus (Singh et al., 2011). Sorghum and millet varieties of Africa are also vulnerable to a diverse range of fungal and virus diseases as reviewed by Mtisi and McLaren (2000). Despite variable success in combating some of the biotic stressors affecting cereal production in Africa, challenges still remain in some areas for smallholder farmers. Furthermore, these plant pests and diseases respond to favourable climates and therefore have the capacity to increase their geographic range under changing climates. It is therefore vital that studies are carried to understand the impact of climate change factors such as drought may play on the population dynamics and geographic range of these antagonistic biotic agents.



Figure 1.2: Stunted growth as a result of parasitic weeds in the genus *Striga*.

1.7 Model system for the study

1.7.1 Push-pull system for control of stemborers

The push-pull (Fig. 1.3) uses knowledge of plant chemistry and insect behaviour to manipulate agro-ecosystems in a manner that is unfavourable to Lepidopteran pests whilst simultaneously promoting crop yield through reduced pest damage. Specifically selected companion plants are grown within and around the main crop (Khan et al., 2010) depending on their chemical properties which affect only the arthropod pests or also add to the agro-ecosystems by nitrogen fixation, weed suppression, palatability as fodder or even harvestable product for human consumption. Companion plants grown within the main crop should have the capacity to repel or deter insect pests from the main crop whilst the perimeter plants act as

the trap using attractive volatile blends which pull the pests from the main crop (Cook et al., 2007). This attraction of insects is not by chance but through detection of specific semiochemicals (Nordlund & Lewis, 1976; Dicke & Sabelis, 1988) in their specific proportions (Bruce et al., 2005). This is also true for plant defence mechanisms which repel potential herbivorous insects (Hardie et al., 1994; Khan et al., 2010).

In the push-pull model *Pennisetum purperum* (Schumach), which is commonly called Napier grass, is used as the attractive trap plant (Khan et al., 2000) because of its ability to produce more quantities of the attractive, mostly hexanal, compounds than sorghum or maize (Birkett et al., 2006) when the moths are most active (Chamberlain et al., 2006; Khan et al., 2010). The attractive green leaf volatiles which increase in the *P. purperum* are (E)-2-hexanal, (Z)-3-hexen-1-ol and (Z)-3-hexynyl acetate to approximately 100-fold in the first hour of scotophase (Chamberlain et al., 2006). The time of increase in volatiles is crucial as it coincides with the gravid moths` peak of activity as they search for oviposition sites (Päts, 1991).

Molasses grass, *Melinis minutiflora,* can also be used as an intercrop in push-pull as it exhibits desirable direct and indirect defence traits. Volatile blends emitted by this plant species are essential in both the repulsion of the female stemborer moths and attraction of the female parasitoid *C. sesamiae* Cameron (Hymenoptera: Braconidae) (Khan et al., 1997a, 2000). Chemical compounds critical for this role have been identified as (*E*)-ocimene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, β-caryophyllene, humulene and α-terpinolene (Khan et al., 1997; 2000, Picket et al., 2006). The ocimene and nonatriene compounds are also known to be produced by maize in response to insect herbivory (Turlings et al., 1990; 1995) and the latter group of compounds have since been demonstrated to be responsible for the signalling of increased parasitoid activity within the main crop (Khan et al., 1997). The *Desmodium* species can also be used as intercrop within this push-pull model as they carry similar

stemborer-repulsive and parasitoid-attractive properties (Khan et al., 2000; Midega et al., 2009).

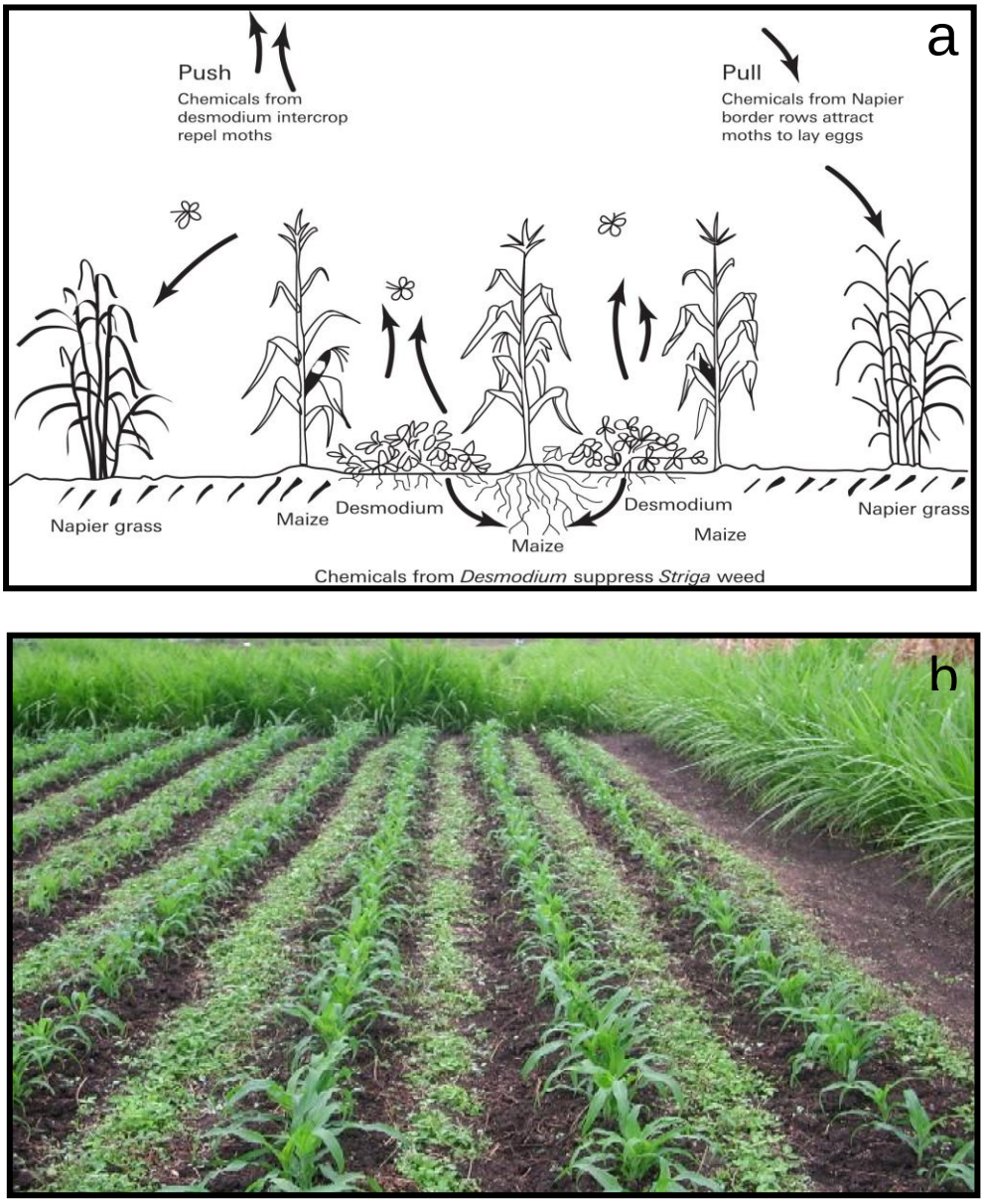


Figure 1.3: (a) Schematic diagram showing the mechanistic basis of the push-pull system (a) and (b) picture of a typical push-pull plot during the vegetative phase of a maize crop

Since its inception in Kenya, the push-pull has enjoyed widespread adoption and currently has over 75 000 households utilising it in East African region alone (Amudavi et al., 2008; Khan et al., 2008a; b). In addition to suppression of stemborers and *Striga*, many farmers who adopted the push-pull have reported a number of agronomic benefits including improved soil fertility and increment in grain yield by 2 t ha^{-1} (Khan et al., 2008a). The push-pull has also presented additional benefits to farmers in provision of fodder thereby boosting their milk productivity (Khan et al., 2008b), significantly higher returns in revenue, land and labour as opposed to their conventional production practices (Khan et al., 2008b; De Groote et al., 2010). By addressing SSA farmers' production constraints in pest-weed management, soil fertility improvement and livestock feed provision; the push-pull offers an environmentally sustainable way of farming with clear cut economic benefits to the farmers.

1.7.2 Bioecology of Chilo partellus

The biology of *C. partellus* is generally similar to many stemborers (Fig. 1.4). Gravid *C. partellus* lay eggs on the leaves of the host plant in batches of 10-80 eggs (Harris, 1962; 1990). A single female has the capacity to lay up to 400 eggs (Bosque-Pérez & Schulthess, 1998) in her life-time. Duration of incubation of the eggs is influenced by relative humidity and ambient temperature, incubation time is significantly reduced with increasing temperatures from 22 to 32 °C and relative humidity of up to 80 % (Usua, 1968). Similarly, larval development is also temperature dependant with a minimum and maximum threshold of 22 and 32 °C respectively (Usua, 1968). Newly hatched larvae will feed on the funnel leaves creating a characteristic damage pattern before they drill into the stems or migrate to neighbouring plants (Harris, 1962; Bosque-Pérez & Schulthess, 1998). After a development time of 26 to 33 days, the larvae may enter diapause to evade harsh environmental conditions

for up to 6 months (Harris, 1962). However, populations of *C. partellus* do not diapause in warm low-lying areas (van Hamburg, 1976; Way & Kfir, 1997; Berger, 1981). Diapausing individuals pupate at the onset of rains before they emerge as adult moths after 10-12 days (Bosque-Pérez & Schulthess, 1998). The adult moths are mostly sedentary during the day (resting on debris and plant surfaces) but become active at night and hence are classified as nocturnal (Kfir, 1998). It is also during the night time that moths mate.

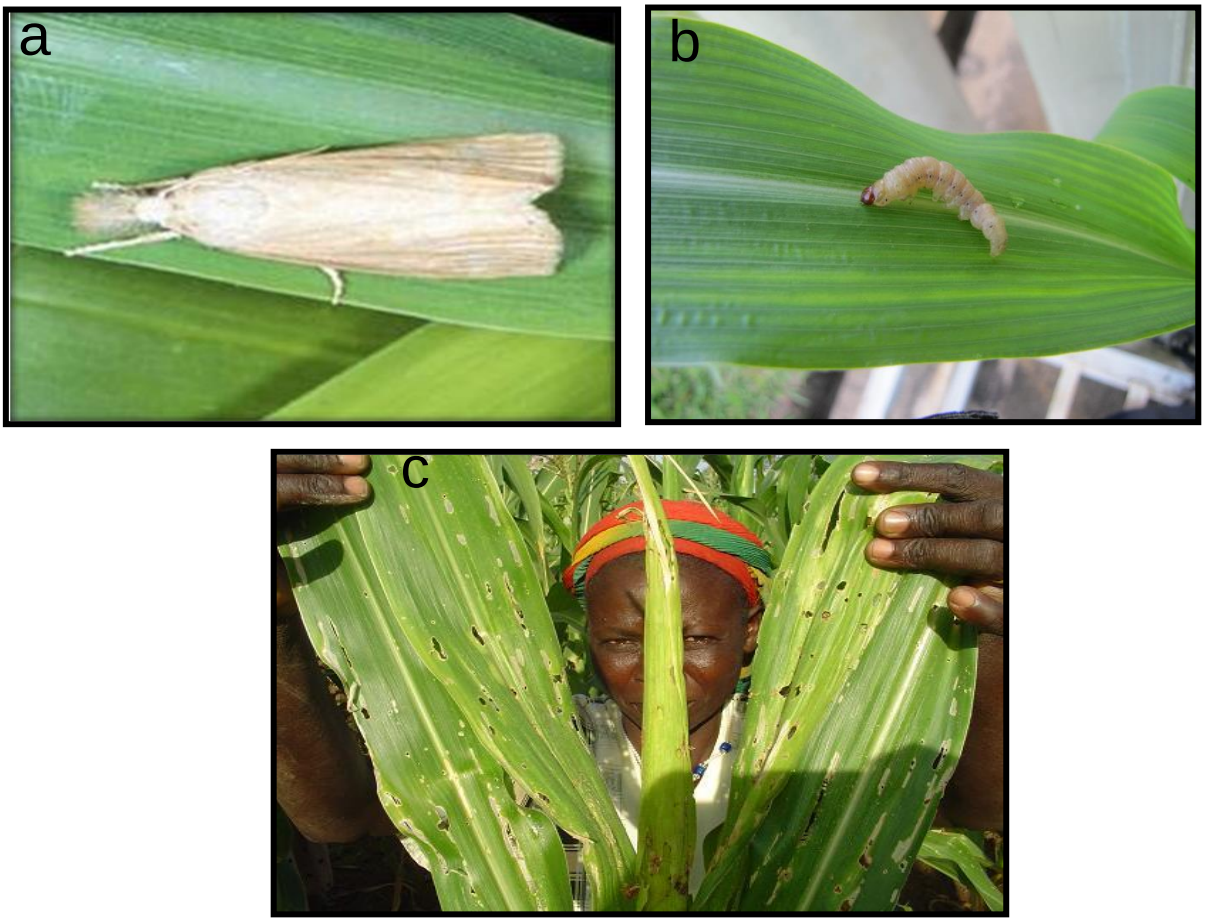


Figure 1.4: Adult (a) and larval (b) *Chilo partellus* and typical foliar damage inflicted by larvae on a maize crop (c)

1.7.3 Bioecology of Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae)

Cotesia sesamiae (Fig. 1.5) is a gregarious larval endoparasitoid species of the lepidopteran stemborer complex in Africa. Because of its wider geographic distribution and abundance (Kfir, 1995; Overholt et al., 1994), *C. sesamiae* is a very important species as it can be incorporated into biological and integrated pest management programmes. Upon emergence, adults mate before gravid females attack mid to late instar larvae depositing between 40-60 eggs per clutch (Potting et al., 1997; Mochiah et al., 2001; Gohole, 2003). Thereafter, eggs hatch and develop inside the host larvae until fully grown larvae emerge out of the dying host to form cocoons. Host location by gravid parasitoids is achieved by utilisation of a number of cues including HIPVs (Tamiru et al., 2011; 2012) and frass at shorter distances (Ngi-song & Overholt, 1997; Potting et al., 1997; Gohole, 2003). At 25 °C complete life cycle from egg to adult takes approximately 18 days (Ngi-song et al., 1995) whilst adult longevity is restricted to a range from 2 to 10 days (Mochiah et al., 2001).



Figure 1.5: Picture of an adult larval parasitoid *Cotesia sesamiae*

1.8 Problem statement and justification of the study

Plant VOCs play a crucial role as infochemicals for deception and deterrence in the push-pull system. However, the advent of abiotic stressors due to climate variability may bring changes to the volatile emission profiles with potential cascading effects to the ecological functions of the VOCs. The push-pull system for stemborer control relies on stemborer-repellent compounds such as *(E)*-β-ocimene and *(E)*-4,8,-dimethyl-1,3,7-nonatriene, from *Desmodium* genus and molasses grass which lure parasitoids of the stemborers. In addition to this, perimeter trap plants such as Napier grass play an important role by emitting volatiles that are more attractive to the female stemborers than maize (Khan et al., 1997a; 2000; 2010).

The perception of the volatile compounds for host or non-host recognition by moths or parasitoids is very specific in most instances (Bruce & Pickett, 2011). Usually, blends carrying specific proportions of compounds elicit particular behavioural responses from the insects and any changes in the composition of the blend proportion or components may result in failure to elicit a behavioural response or alternative behavioural responses (Bruce et al., 2010; Bruce & Pickett, 2011). The context in which insects perceive volatile cues is of importance, for example Webster et al. (2010) found that volatile components occurring together as a blend functioned as host cues and were attractive but when they were presented individually they were repellent. However, some insects are able to adjust their host location behaviour through behavioural plasticity in the form of learning (Bruce & Picket, 2011).

For the push-pull technique, it is not known how stemborers and their parasitoids will respond to any stress-induced volatile emission. The current project therefore has direct implications for how the efficacy of the current companion plants used in the push-pull may be affected by drought stress in context of their volatile emission profiles and how the insects

will interpret the cues. The project also has direct relevance to how the push-pull may be adopted in drier and hotter agro-ecological zones.

Apart from insights essential for the push-pull, investigations done in this project may generate novel science literature on how climate change factors may influence insect multi-trophic interactions in agro-ecosystems. The study may therefore answer important questions in evolutionary biology of both plants and insects. This approach may give a bigger picture of how climate change may affect ecosystems as compared to most empirical studies which focus on one model organism. Furthermore, the study will also show how plant indirect defence against herbivorous pests may be affected by abiotic stressors. The findings will therefore contribute to knowledge on sustainable pest management and adaptive strategies for small holder farmers in the face of changing climates.

1.9 Overall goal of the project

The overall goal of the project is to assess changes in wild grasses and legume volatile emission, brought about by abiotic stress, and their effects on the behaviour and biology of stemborers and their parasitoids.

1.9.1 Hypotheses of the study

1. Abiotic stress does not change the quality and quantity of plant volatiles important for direct and indirect plant defence
2. Changes in quality and quantity of plant VOCs as a result of abiotic stress do not influence stemborer and parasitoid behaviour
3. Emission of VOCs in response to abiotic stress does not vary across different species

1.9.2 Objectives of the study

1. To assess oviposition preference and larval development in various host plant species under drought stress
2. To establish the effects of drought on total (quantitative assessments) and quality (qualitative assessments) of electrophysiologically active VOCs
3. To establish how differences in plant VOCs quantity and quality, as result of drought, may influence behaviour of moths and parasitoids
4. To identify drought tolerant companion plants for use in push-pull technology for dry and hot areas

CHAPTER 2

Biologically based methods for pest management in agriculture under changing climates: challenges and future directions

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2.0 Introduction

Anthropogenic climate change has the potential to significantly influence the biology of all organisms, but particularly ectotherms (Deutsch et al., 2008; Huey et al., 2009; Dillon et al., 2010) hence the evidence for a shift in ectotherm distribution in response to climate change (Parmesan et al., 2003; Colwell et al., 2008). While climate change is often associated with global mean annual temperature increases (IPCC, 2007), likely favoring winter survival of insect pests, it is also coupled with increasing frequency and severity of extreme temperatures (Easterling et al., 2000; Frich et al., 2002) that may modify predictions of insect population dynamics (Kiritani et al., 2006; Musolin et al., 2007, Denlinger & Lee, 2010). Short-term thermal fluctuations can be particularly stressful to small insects because their body temperatures are typically in equilibrium with ambient temperatures (Stevenson, 1985). Hence, insects must be able to cope physiologically or compensate behaviourally with such changes in ambient temperature, both on spatial and temporal scales (Denlinger & Lee, 2010; Chown & Nicholson, 2004).

Physiologically, insects can adjust thermal tolerance over the short term, a phenomenon typically termed 'hardening' (Bowler, 2005; Lagerspetz, 2006). Over the longer term, thermal tolerance may be altered through acclimation in the laboratory or acclimatization in the field, and generally is a response to changes in environmental mean temperatures with e.g., season (Huey & Berrigan, 1996; Hoffmann et al., 2003). Hardening typically yields reversible physiological changes while acclimation and acclimatization can produce either reversible or irreversible physiological changes depending on the trait or whether developmental or maternal stages were exposed to temperature variation. Lagerspetz (2006), Hoffmann et al. (2003); Fischer et al. (2003) and Martinat (1987) emphasized the

importance of incorporating short-term undesirable weather transients (e.g., cold spells) in determining insect population dynamics. Nevertheless, previous studies suggest considerable complexity when attempting to predict effects of transient weather patterns on insects' physiology or life-history traits (Lafferty et al., 2009; Nyamukondiwa & Terblanche 2010; Marshall & Sinclair, 2010).

Biological control is a method of controlling pests that relies on predation, parasitism, herbivory and other natural mechanisms and can be an important component of integrated pest management (IPM) programs (Hoy, 1994). However, the efficacy of biological control using natural enemies depends on a complex but delicate relationship between natural enemies and their insect pest hosts whose balance can be offset by a changing climate. Environmental factors (e.g., temperature) directly affects the survival, development, reproduction and dispersal of pest insects and thus their potential biogeography and biotic potential (Cammel & Knight, 1992; Southwood et al., 2000). It is well known that temperature fluctuations are the major factors affecting insect biology, activity and distribution of natural enemies in agro-ecosystems (Duale, 2005; Sorribas et al., 2012). Moreover, several studies have indicated climate change affects several life history parameters e.g., generation time, fecundity, sex ratios and lifespan of parasitoids and their natural enemies (Liu et al., 1995; Kalyebi et al., 2005). Similarly, temperature extremes may reduce insect survival, reduce fecundity and retard natural enemies' development (Hance et al., 2007).

Several studies have documented the likely effects of a changing climate on insect pest-natural enemies' interactions (Sorribas et al., 2012; Porter et al., 1991; Cannon et al., 1998; Estay et al., 2009). Similarly, by assessing physiological traits of thermal tolerance and water balance (Klok & Chown, 2001), it was shown that global climate change may affect the phenology of *Paractora dreuxi* (Diptera: Helcomyzidae) (Seguy). This review aims at

examining the likely effects of climate change on insect biological control and how increased global temperatures will require adaptive management strategies to cope with changing status of insects and their natural enemies. I discuss how changes in global climate factors such as temperature increase will impact on i) insect-natural enemy-host plant interactions, ii) insect/plant diversity iii) population growth/abundance iv) effectiveness of crop protection technologies, with special emphasis to biological control using natural enemies. This information has critical implications for sustainable pest management and food security, especially for developing countries in Africa, where food security is an urgent challenge (Sasson, 2012).

2.1 Biologically-Based Pest Control Methods

Reducing public health and environmental risks associated with chemical pesticide use is of growing concern in developed and developing countries (Lynch, 1998). This has motivated the call for the adoption of biologically based IPM systems, an essential step towards reducing risks associated with the use of highly toxic pesticides (Lynch, 1998). Biological control can be classified into three basic categories namely conservation, classical and augmentation (Lockwood & Ewen, 1997; van Lenteren, 2011). First, conservation biological control (CBC) involves the deliberate human practice aimed at promoting the survival and activity of natural enemies at the expense of pest populations (Eilenberg et al., 2001). For example, ecological strips consisting of selected non-crop plants can be deliberately created to provide food sources and overwintering shelters as well as protect local natural enemies from pesticide disturbances thereby enhancing CBC as successfully shown in cereals, cabbages, and fruit orchards (Landis et al., 2000; Jonsson et al., 2008).

Plant indirect defense mechanisms which rely on volatiles that attract natural enemies after pest damage, herbivore induced plant volatiles (HIPVs), have also been exploited to

increase the activity of parasitoids in CBC systems (Jonsson et al., 2008; Khan et al., 2008). In New Zealand and Australia the "attract and reward" is another form of the CBC approach where the use of HIPVs is combined with measures that increase key resources needed by natural enemies such as flowering plants in the commercial crop (Khan et al., 2008). More recently, another form of CBC which combines stimulo-deterrent diversion tactics and conservation of parasitoids, through careful habitat manipulation has been shown to be effective against stem borers on sorghum *Sorghum bicolor* L. (Moench) and maize *Zea mays* (L.) (Cook et al., 2007). Habitat manipulation has also been used to increase nectar availability, which increases the fecundity and longevity of some parasitoids (Idris & Grafius, 1995; Idris & Grafius, 1997; Johanowicz & Mitchell, 2000), thereby increasing the efficacy of the program. For example, flowers are planted into commercial brassicaceous crops to enhance suppression of the diamondback moth, *Plutella xylostella* (L.) by its natural enemies (Talekar & Shelton, 1993).

Second, classical biological control (BC) involves collection of natural enemies from their area of origin and releasing them in the new area where their host was introduced accidentally (van Lenteren, 1997; Perdikis et al., 2011). This is of particular importance when the introduced pest species has no known alternative parasitoids indigenous to the area. However, the efficacy of a classical biological control will depend on the newly released parasitoids to successfully establish populations that can compete in the new environment. For example in the USA, the alien yellow starthistle *Centaurea solstitialis* (L.) has been successfuly controlled by insect natural enemies such as *Bangasternus orientalis* (Carpiomont), *Eusternopus villosus* (Boheman), *Urophora sirunaseva* (Hering) and *Chaetorellia succinea* (Costa) that attack the seed head (Gutierrez et al., 2008).

Last, augmentative biological control (ABC) is the periodic release of large numbers of mass-reared natural enemies with the aim of supplementing natural enemy populations or

flooding (*i.e*., inundating) pest populations with natural enemies (van Lenteren, 1997; Colliera & van Steenwyk, 2004). It is commercially deployed in various cropping systems worldwide and two forms of ABC are distinguished namely the inundative approach and the seasonal inoculative method (van Lenteren, 1997; Lomer et al., 2001). In the inundative release method, the BC agent is collected, mass-reared and released periodically in large numbers as for example a biotic insecticide to achieve immediate pest control in crops where viable breeding populations of the natural enemy are not possible (van Lenteren, 1997). This approach has been successfully applied in sugarcane for control of the sugarcane borer *Diatraea saccharalis* (F.) in Latin America (Macedo, 2000). Other examples include the inundative release of common green lacewings *Chrysoperla carnea* (Stephens) to suppress *Erythroneura variabilis* (Beamer) and *E. elegantula* (Osborn) in vineyards (Daane et al., 1996); and the release of *Trichogramma* spp for control of lepidopteran pests in vegetables, corn, rice, other cereals and cotton in Russia, China, SE Asia, Mexico and South America (Koch, 1996).

The seasonal inoculative approach differs from inundative method in that it is deployed in short-term crops, the production season of which is not longer than one year and where multiple pest generations occur (van Lenteren, 1997). The aim of the method is to obtain both immediate pest control as well as a build-up of the biological control agent population over the entire duration of the same production season (van Lenteren, 1997). Examples of the successful deployment of this technique include the release of *Trissolcus basalis* (Wollaston) for the control of *Nezara viridula* (L.) in Brazil; biological control of soybean stink bugs by inoculative releases of *T. basalis* (Corréa-Ferreira, 2011); control of the citrus blackfly, *Aleurocanthus woglumi* (Ashby) by inoculative releases of *Eretmocerus serius* (Silvestri) and *Amitus hesperidium* (Silvestri) in Cuba, Costa Rica, Mexico and Panama (van Lenteren, 1997); and the inoculation with *Metarhizium flavoviride*

(Gams and Rozyspal) or *Verticillium lecanii* (Zimmerman) for the control of locusts *Schistocerca gregaria* (Forskal) (Fargues et al., 1997) and grasshoppers (Jonsson et al., 1988).

2.2 Challenges in biologically-based pest management in relation to climate change

Alterations in physiology and population dynamics, as a result of climate change, will bring new arrangements to levels of biological organization and ultimately ecological interactions in various species. In most geographic locations, shifts in climates may result in novel environmental conditions which are not only likely able to reduce the fitness but also deplete the quality and quantity of resources (e.g. food habitat) available for arthropod communities thereby threatening their existence in those areas. To counter this, arthropod populations facing unfavorable conditions may respond through either physiological or behavioural compensation (Chown & Nicholson, 2004; Chown et al., 2011), at both individual and population level, to better compete in the new environment; or they may migrate to new and favorable locations (Parmesan et al., 2008). However, due to differences in the capacity to respond to various abiotic stressors and resource availability, fitness levels and dominance of various individual populations, they will be threatened, resulting in new species composition per locality, possibly dominated by the most adaptive ones (Marshall & Sinclair, 2010). Moreover, such modifications by living organisms, for better survival in life threatening environmental conditions, have already been hypothesized to pose new problems to them (Zachariassen, 1996).

In turn, the resultant failure to successfully compete in the stressful conditions may lead to different species composition in both pests and their natural enemies. For agriculture, such changes are of importance as they create new structures in original pest abundance, emergence of formerly secondary to primary pests (Cammell & Knight, 1992), colonization

of new areas which were previously unfavorable (Cannon, 1998; Parmesan et al., 1999) and more importantly the modifications of habitats (Chown et al., 2011; Nair et al., 2003; Webb et al., 2005; Markarieva & Gorshkov, 2007). This may lead to reduction in numbers of natural enemies and parasitoids in the agro-ecosystem. Consequently, the changes may result in reduction in the efficacy of biological control due to alterations in the predator-prey relationships or lack thereof. In this section, an overview of challenges in biological control as a result of climate-induced changes in pest or parasitoid biology and general habitat modifications is discussed.

2.2.1 Habitat fragmentation and natural enemy diversity or abundance

It is widely known that crop surroundings play a crucial role in the conservation of natural enemies and parasitoids (Johnsson et al., 2008; Nicholls et al., 2000; Wilby & Thomas, 2002; Dyer, 2005). However, in most agro-ecosystems, much attention is paid to the crop (e.g., planting, irrigation fertilization) as compared with the peripheral environment. Such an approach has not only ensured perpetual optimal crop growth, but dependable hosts for pests as well. Through elaborate efforts that ensure crop growth and survival, insect pest herbivory has been indirectly guaranteed. However, this is contrary to the micro environment faced by natural enemies dwelling in the periphery where the natural habitats may not be receiving similar attention resulting in their numbers being reduced.

One consequence of changing climates may be habitat fragmentation of living organisms (Deutsch et al, 2008; Dyer, 2005; Opdam & Wascher, 2004). Thus, losses in suitable habitats will threaten the biodiversity and mere existence of organisms (Fahrig, 2003) including natural enemies or predators and parasitoids important for pest control in agro-ecosystems (Letourneau et al., 2011). Consequently, the reduction or extinction of natural enemy populations will permit a pest build up, if unchecked, or over-reliance on

alternative tactics for pest control which may be unsustainable, environmentally unfriendly and deplored by the consumers.

2.2.2 Insect biology and physiology in relation to environmental change

Temperature affects a range of biochemical and physiological processes and, along with water availability, is probably the major environmental factor affecting insect population dynamics at either the individual or population level (Chown & Nicholson, 2004, Cossins & Bowler, 1987; Angilletta, 2009). At individual level, it has already been shown that factors such as temperature play a key role in determining insect fitness (Basson et al., 2011), field performance (Kristensen et al., 2008, Chidawanyika & Terblanche, 2011) and survival (Chidawanyika & Terblanche, 2010). However, because of variability in response to thermal stress, for instance, which might be introduced by age, gender, ontogeny (Bowler & Terblanche, 2008) and the species in question, mismatches in development and activity between pests and parasitoids may occur with cascading effects on the efficacy of biological control programs.

Use of mass-reared parasitoids in augmentative efforts may be a challenge as well since the insects being introduced into novel environments, which might be stressful, may perform poorly (Sørenson et al., 2012). Hence, in scenarios where pests perform better than their corresponding indigenous parasitoids under stress, the efficacy of a biological program will be dramatically reduced. However, some parasitoid species in the wild may benefit from increasing winter temperatures. As discussed by Hance et al. (2007 and references therein), exposure to cold temperature in juvenile parasitoids results in reduced longevity of the adults (Porter et al., 1991). Furthermore, cold exposure of adult parasitoids in the family *Scelionidae* reduces their longevity even after returning to optimal or warmer temperatures (Porter et al., 1991; Robinet & Roques, 2010). Low temperatures during development are also known to

cause deformations and low fecundity in parasitoids. Basing on these limitations posed by low temperatures, a rise in winter temperatures may become beneficial to biologically-based pest management strategies for these specific parasitoid species, through improvements in their fecundity, development and longevity. At a population level, rising temperatures result in reduced generation time, rapid population growth and sometimes increased geographical ranges depending on resource availability (Denlinger & Lee, 2010; Chown & Nicholson, 2004). However, such a positive correlation in temperature and population increase in pests is not uniform across different species even when facing similar conditions. This results in the asynchrony of life cycles of pests and parasitoids or general reduction in populations of parasitoids required to effectively suppress pest populations. Such an asynchrony will create a temporal shortage or extinction of food resources for parasitoids whilst crop pest phenology is in line with the crop cycle. This consequently exerts pressure on the cropping system, especially in the cases of specialist parasitoids (Stacey, 2003).

Furthermore, some pests may increase their invasion potential in relation to their ability to deal with changing climates either through phenotypic plasticity or variation in basal tolerance (Janzen, 1967; Ghalambor et al., 2007, Chown et al., 2007, Nyamukondiwa et al., 2010). A classic example is the invasive maize stemborer *Chilo partellus* (Swinhoe), which was first introduced to Africa accidentally in Malawi but managed to establish itself in several African countries, becoming more destructive than the indigenous species in some instances (Sithole, 1990). Whilst the first introduction of *C. partellus* into Africa may have not been due to climate change, it has become apparent that its further establishment in several African regions follows a distinct pattern, which may be partly influenced by both climate and altitude (Ong`amo et al., 2006). Such pest dynamics increase pressure on the already-strained predator and parasitoid populations.

2.2.3 Chemical ecology and tritrophic interactions in agroecosystems

Climate induced changes in plant factors will affect quality and quantity of resources available for the insects resulting in variable direct and indirect consequences on the development times, size and fitness of both pests and parasitoids (Thomson et al., 2010). This may thus offset predation and parasitism (Thomson et al., 2010), which sometimes reduces the efficacy of biological control programs. Perhaps another dimension of plant physiology which is likely to be modified, due to climate change, with a resultant impact on biological control is their secondary metabolism with a resultant impact on indirect defense mechanisms. It has already been shown that some plant species emit specific volatiles in response to elicitors in the saliva or secretions (during oviposition) of particular foraging herbivores, which call for natural enemies and parasitoids of the herbivores in question (Hilker & Meiners, 2006).

Other plant species, when attacked, have also been shown to emit volatile compounds which warn neighboring plants to prime their defense in advance in a phenomenon referred to as 'eavesdropping' (Rodriguez-Saona & Frost, 2010). Such volatiles have since been generally called herbivore induced plant volatiles (HIPVs) (Rodriguez-Saona & Frost, 2010). The HIPVs have been regarded as having evolutionary significance in that their production is only switched on when needed as opposed to constitutive mechanisms, which are always switched on. The latter may hence be 'wasteful' in terms of plant resource investment. However, recent studies have shown that plants undergoing abiotic stress respond by production of volatile isoprenoid compounds, perhaps, to avoid oxidative damage as a result of accumulation of reactive oxygen species in plants undergoing abiotic stress (Vickers et al., 2009a, b).

Isoprene compounds have since been shown to have the capacity to repel other specialist parasitoids, such as *Diadegma semiclausum* (Hellen) (Gershenzon, 2008;

Loivamäki et al., 2008), and to influence herbivore feeding decisions (Laothawornkitkul et al., 2008a). It is therefore clear that isoprene production, as a result of abiotic stress, may influence plant-insect interactions in different agro-ecosystems. However, little is known of how the plants will prioritize their defense, in terms of volatile emission, when faced with biotic and multiple abiotic stressors associated with climate change.

For biological control, changes in volatile composition important for defense may result in failure for parasitoids to locate their host as some may require specific volatile blends in order to perform the desired functions (Bruce & Pickett, 2011). Moreover, it has been shown that environmental stressors such as temperature impact on fitness, olfactory perception (Riveron et al., 2009) and ultimately the ability to of the insects to track their hosts (Thomson et al., 2010). Therefore, if such changes occur in the parasitoids, biological control programs using natural enemies will be rendered less effective.

2.2.4 Complexity in the outcome of climate change impacts on natural enemy abundance and population dynamics

While it has increasingly become clear that the climate is changing (IPCC, 2007), an accurate prediction of the consequent effects on species distribution remains a daunting task. In most ecosystems, baseline species distribution, before any change in climates, is determined by a host of interactive factors between abiotic and biotic factors of the species in question. However, bioclimatic models used to predict future species prediction have often omitted or failed to account for all of the important factors resulting in them being questionable (Pearson & Dawson, 2003; Wiens et al., 2009). In these models, behavioural, dispersal mechanisms and inter-specific interactions, which can be made by living organisms in changing climates, have often been neglected (Davis et al., 1998; Heikinnen et al., 2006) or assumptions which bring uncertainties to the models have often been used (Wiens et al., 2009). Such lack of availability of reliable tools for prediction are a challenge to farmers, in

particular, those who currently use biological control as their main insect pest control tactic. Availability of reliable tools will not only boost the confidence in users of the model, but also avert catastrophes in pest management due to over-reliance on flawed models.

Apart from bioclimatic models, resource constraints and challenges in experimental design have made empirical elucidation of the ecologically relevant behavioural and biological responses to climate change difficult. As a result, most inferences on the outcome of climate change are made from ecophysiological studies based on a single as opposed to multiple abiotic factors acting on different species. This is done despite increasing evidence of the differences in vulnerability or responses exhibited by some organisms when facing multiple as opposed to single stressors (Mikkelsen et al., 2008). Possibly, through cross-tolerance (Patori & Foyer, 2002) or additive effects of different stressors, such differences arise and may be species specific. It can therefore sometimes be misleading to rely on the inferences from the single-factor approach even though they serve as an important baseline indicator for individual physiological limits to stress tolerance. Such challenges bring new complications to farmers who are in principal, supposed to rely on accurate data and predictions in order to carry adequate planning and implementation of their crop protection strategies.

2.3 Future directions

2.3.1. Environmental stress biology, evolutionary resilience and ecologically relevant measures for organism response to changes in climates

Insect population dynamics can be strongly influenced by adaptive behaviours and traits (Hoffmann et al., 2012). Furthermore, insects/natural enemy extinction events, colonization rates and demographic rates are influenced by organisms' adaptation in habitat selection, life history traits, niche breadth and dispersal behaviour (Hanski & Mononen,

2011). Recent studies suggest that in most cases, adaptive traits (e.g., thermal tolerance), significantly affect demographic dynamics and hence shape species distributions and population dynamics under a changing climate (Sørenson et al., 2012; Kellermann et al., 2009, Hoffmann, 2010). Similarly, in insects, low genetic variation in thermal adaptation can limit population growth and increase extinction risks in organisms living closer to their critical thermal limits (Patori & Foyer, 2002; Chown et al., 2010). However, although it is generally accepted that climate is changing, mitigating and coping with these effects remains an unresolved challenge (Parmesan, 2006; Williams et al., 2008). In order to maintain or even improve biological control using natural enemies in a changing climate, several adaptive management strategies need to be implemented to cope with the changing status of insects and their natural enemies.

First and foremost, experimental protocols investigating the likely probable effects of climate change on insect-natural enemy interactions needs to be highly accurate. Although it has been documented that laboratory determined physiological traits (e.g., thermal tolerance) closely approximates their ability to cope with the stress under natural environments and are good indices of species fitness facing climate change (Deutsch et al., 2008), in most cases, there are ranging debates over the ecological relevance of experimental protocols used. For example, in thermal biology, previous studies have indicated that the methodological approach employed to determine an insect's thermal tolerance can affect the types of insights that can be gained, and ecological relevance, of these thermal limits (Chown & Nicholson, 2004; Lutterschmidt & Hutchison, 1997). I therefore suggest that experimental protocols predicting climate change effects on insect population abundance should incorporate ecologically relevant measures of fitness traits (e.g., temperature tolerance) that are likely to occur in the species' natural environment (Terblanche et al., 2011).

Moreover, forecasting efforts for insects' responses to climate change has almost exclusively focused on the variation in mean temperatures (Deutsch et al., 2008; Crozier & Dwyer, 2006). However, it is expected that the magnitude and severity of temperature variances and extremes may also increase under future scenarios (Easterling et al., 2000) with concomitant reduction in ectotherm fitness (Hoffmann, 2010; Somero, 2009). From a functional perspective, how changes in means and variances of temperature might affect the basal, phenotypic plasticity of temperature tolerance as well as life-history traits of ectotherms, remain poorly elucidated but are critical for predicting physiological responses in the wild (Marshall & Sinclair, 2010). I suggest that detailed analyses of changes in both means and variability of temperature for both pests and their natural enemies are a critical component of accurate forecasting of insect/natural enemy population-level responses to climate change. Furthermore, some other environmental stressors other than temperature may also impact synergistically or antagonistically on ectotherm fitness in a changing climate (Terblanche et al., 2011). I therefore suggest that experimental protocols investigating ectotherm fitness in a changing climate should also incorporate other factors to evaluate possible interaction effects across factors on species' fitness related responses (Crozier & Dwyer, 2006). In addition to interactive effects of physical factors, biotic factors such as parasitism or chemical ecology of various organisms in an interactive multitrophic system may be affected resulting in distortions in predator-prey relationships. In such a case, the fate of some species` populations in multitrophic systems will not be directly determined by the changes in climate on its population but by a species that is high or low in their food chain. Hence, wherever possible, experimental protocols that inform predictive models for changing climates should incorporate tests of other species, which might be of importance in the agro-ecosystems.

The ability of organisms to mount physiological responses to variation in temperature at different time-scales may be an important component of insect/natural enemy persistence and thus efficacy of biological control under climate change scenarios (Angilletta, 2009; Hoffmann et al., 2010; Hanski et al., 2010). From a theoretical standpoint, rapid evolution of thermal tolerance traits/phenotypes and their plasticity has been predicted (Angilletta, 2009, Ghalambor et al., 2007). Some studies indicated that if plasticity has an additive genetic basis, plasticity levels might evolve and likely contribute to evolutionary adaptation (Ghalambor et al., 2007). Phenotypic plasticity may buffer organisms upon introduction to novel and thermally unfavorable environments (Nyamukondiwa et al., 2010) and thus ensure survival when facing climatic stress (Chown et al., 2007). However, current experimental protocols and crop protection specialists are ignoring the role of evolutionary processes in designing ways to protect and even improve biodiversity under global climate change (Somero, 2009; Terblanche et al., 2010; Hendry et al., 2011). I therefore propose that biological insect pest management programs using natural enemies should aim at developing resilient agro-ecosystems that maintain species' and populations' evolutionary potential (Hoffmann et al., 2011; Sgró et al., 2010). Pörtner and Farrell (2008) point out that this may be possible through improvement in genetic diversity and processes that encourage continuous *in situ* evolutionary adaptation. In agriculture, use of evolutionary processes to manage biotic interactions can be of importance as discussed in Thrall et al. (2011). Through the help of artificial selections in crops, parasitoids or any other biological agents used to control may even be enhanced to gain competitive advantage over the pests intended for control when facing a selection pressure such as heat or drought. Such an approach is not new in agriculture as it has already succeeded in crop breeding, domestication of animals and pesticide resistance management (Hendry et al., 2011; Thrall et al., 2011). Hence if given the

investment priority in research, some pests may be managed even better under changing climates.

Recent studies have proved that phenotypic plasticity can be used to enhance field performance of mass reared insects released for pest management (Kristensen et al., 2008; Chidawanyika et al., 2011). I also argue that by manipulating thermal performance of mass-reared predators and parasitoids through acclimation, field performance in augmentative programs may at least be temporarily improved and hence improve pest management programs (Sørenson et al., 2012). Similarly, studies emphasize that pest management programs should incorporate valuable information acquired from studies of the evolutionary biology of thermal performance (Kristensen et al., 2008; Chidawanyika & Terblanche, 2011; Crandal, 2009). Likewise, this approach may be critical for improving efficacy of biological control programs in the face of climate change.

2.3.2 Monitoring, ecological assessments and ecosystem management

Apart from predictive models, regular monitoring and ecological assessments might be an important tool to accurately expose the impact of climate change on the distribution and abundance of pest and natural enemy populations. Whilst predictive models will be important in forecasting (Berry et al., 2002; Pearson et al., 2002) their predictive power is often limited by assumptions, which may lack ecological considerations (Pearson & Dawson, 2003; Guisan & Thuiller, 2005). Hence ecological assessments and monitoring will generate huge ecological data sets that may be overlooked by physiological studies or predictive models. Such ecological factors are likely to give more valuable information of how agents of biological control will interact under changing climates.

However, as the case with predictive models, assessments of various systems will require reliable ecological indicators in order to avoid making erroneous conclusions. These ecological indicators will serve as tools that portray the structure, function and composition

of the ecosystems (Dale & Suzanne, 2001) or trends that will be happening over time (Cairns et al., 1993) within and around the crops. Ecological indicators will therefore serve as early warning tools for detecting deficits in management strategies inflicted by climate change whilst also giving a database of various ecological transitions, which can be correlated to climate events. In turn, management strategies can be implemented earlier before extensive damage. However, as outlined by Dale & Suzanne, (2001) use of ecological indicators can be problem if (i) the ecological indicators lack scientific integrity (ii) choice of indicators is confounded in management and (iii) the assessments are based on a small number of indicators. To counter this, concerted efforts between, scientists, farmers and policy makers should exist. Therefore, scientists should develop ecological assessment tools that are accurate but simplified to enable usage by a broad range of users. With adequate data collection from researchers, government agencies and farmers (at their local level) using well designed assessment tools, important conclusions can be drawn on the status of habitats, species composition and abundance in various regions.

Where parasitoid populations will be high but activity is reduced due to fragmented habitats, farmers can increase parasitoid attack on pests by use of companion cropping and indirect plant defence. Such is the case with the push-pull strategy used in the control of stemborers, which increases parasitisation of insect pests, by their parasitoids, through manipulation of the agro-ecosystem to lure parasitoids directly into the cropping system (Khan et al., 2008; Cook et al., 2007). Augmentative strategies can also be used to boost parasitoid populations whilst using techniques such as acclimation to improve activity in variable environments (Kristensen et al., 2008; Chidawanyika et al., 2011; Sørenson et al., 2012). Moreover, biological control can also be enhanced through feeding natural enemies with honey. This phenomenon has been shown to improve fecundity and longevity of hymenopteran parasitoids (Cook et al., 2007; Idris et al., 1997; Johanowicz et al., 1997).

Hence identification for particular tactics and sometimes in combination will be important in tackling pests biologically under changing climates.

2.4 Conclusion

In conclusion, I propose that integrating physiology, population dynamics and climate mapping shows great promise for making robust predictions of the potential effects of global climate change on biodiversity (Hartley et al., 2010). Thus, to better elucidate the link between climate change, biodiversity and its impacts on biological control using natural enemies, a fruitful area of future studies would be in developing mechanistic physiological approaches (and ones that consider ecological factors) to understanding climate change effects on insect biology, biodiversity and population dynamics. Furthermore, there is significant evidence that species are evolving with climate change (Bradshaw & Holzapfel, 2006; Hoffmann & Daborn, 2007). Hence future predictions of climate change effects on insect biodiversity should incorporate evolutionary potential (Hoffmann & Sgró, 2011; Sgró, et al., 2010, Kearney et al., 2009).

CHAPTER 3

Oviposition acceptance and larval development of Chilo partellus stemborers in drought-stressed wild and cultivated grasses of East Africa*

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3.0 Introduction

In recent years, the effects of global climate change (GCC) on ecosystem structure and function have become a matter of increasing concern. One consequence of GCC events is the increased frequency and intensity of plant abiotic stressors such as drought due to decreased or erratic rainfall (Hoerling & Kumar, 2003; Christensen et al., 2007) and increased growing season temperatures (Burke et al., 2009). However, the interaction of plants with their abiotic environment is not static as they often respond via a myriad of mechanisms that can be physiological, morphological, and/or biochemical (Gershenzon, 1984; Chaves et al., 2003; Vickers et al., 2009a, b; Gutbrodt et al., 2011). Being autotrophs that support diverse organisms, plant responses induced by extreme abiotic environments may have cascading effects up the various trophic levels within the ecosystem. For example, drought stress has been shown to have the potential to change the way plants interact with herbivorous insects through modifications of plant traits such as resistance to insects (Mody et al., 2009), nutritive value (Mattson & Haack, 1987; Hale et al., 2005; Mody et al., 2007), and amounts of constitutive chemical defence compounds (Hale et al., 2005; Gutbrodt et al., 2012a).

Three major hypotheses have been advanced to explain and/or predict the relationships that may exist between plants and insects under drought stress. First, the plant stress hypothesis (White, 1969) which supports the idea that drought stress improves the development of phytophagous insects through the increase in available free amino acids in plant tissues as a result of hydrolysed foliar proteins (Brodbeck & Strong, 1987; Huberty & Denno, 2004) and reduction in defence compounds (Rhoades et al., 1979). Second, the plant vigour hypothesis (Price, 1991) which posits that healthy, more vigorous plants are more desirable for insect herbivores than drought stressed ones because of higher nutrient content,

osmotic potential in shoots, and lower rates of abscission (Price, 1991; Preszler & Price, 1995; Cornelissen et al., 2008; Tariq et al., 2012).Third, the pulsed stress hypothesis (Huberty & Denno, 2004) which postulates that the performance of phloem feeders is enhanced under intermittently (pulsed) water stressed plants, perhaps due to the rise in the amount of available nitrogen and turgor pressure during recovery (Tariq et al., 2012).

Although there are several studies in support of each of the hypotheses, there are also several others that refute some of them (discussed in Huberty & Denno, 2004; Tariq et al., 2012). From the foregoing, it is clear that drought stress may either enhance or reduce a plant's susceptibility to herbivorous insects (Vallat et al., 2005; Meyer et al., 2006) and potentially determine intraspecific food choices as demonstrated by Neto et al. (2012). More recently, plant responses to abiotic stress have been reported to include changes in quality and quantity of emitted volatile organic compounds (VOCs) for protection against stress-related damages (Gershenzon & Dudareva, 2007; Vickers et al., 2009a, b; Yuan et al., 2009; Niinemets, 2010; Holopainen & Gershenzon, 2010). However, such compounds have also been cited as playing various ecological roles in plant-insect interactions (Gershenzon & Dudareva, 2007; Laothawornkitkul et al., 2008a, b; Loivamäki et al., 2008). Hence, abiotic stressors have the potential to reshape chemically mediated trophic interactions in both natural and managed ecosystems.

Despite such findings and the knowledge that specific blends are important for insect host location (reviewed by Bruce & Pickett, 2011), very few studies have directly explored how the changes induced by abiotic stress, such as drought, may affect insect host preference both for agricultural crops and natural hosts. Most studies focus primarily on one crop of interest, for example, Seagraves et al. (2011) showed that *Orius insidiosus* (Say.) preferred to lay more eggs on drought-stressed *Phaseolus vulgaris* (L.) Indeed, investigations of plant-insect interactions under stress often give valuable information on the integrity of the

phenological associations of the host and herbivore (e.g., van Asch et al., 2007; 2010). However, one ecological aspect which may not be accounted for by such a single plant species approach is the consequences of abiotic stress in terms of relative suitability of different potential host plants for generalist herbivores. In agriculture, understanding such relationships is of importance as it indicates the future vulnerability of agroecosystems and the suitability of pest management strategies.

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) is a highly destructive polyphagous pest in cereal crops in East and Southern Africa. It feeds on the leaves of plants before burrowing deep into the stem, compromising the plant's fitness and productivity and in severe cases causing death (Kfir et al., 2002). In East Africa the 'push-pull' system has been used to suppress the pest with considerable success through manipulation of trophic interactions in the agroecosystem (Cook et al., 2007; Hassanali et al., 2008). This system uses Napier grass *Pennisetum purpureum* Schumach (Poaceae) as a perimeter 'pull' or trap plant because of its ability to attract stemborer oviposition away from the maize *Zea mays* (L.) (Poaceae) crop, while providing limited support for the development of the larvae (van den Berg, 2006; Khan et al., 2006, 2007; Cook et al., 2007; Midega et al., 2010). The second integrated component of the strategy involves stemborer-repellent plants such as *Desmodium* spp. (Fabaceae) or molasses grass [*Melinis minutiflora* (Beauv.) (Poaceae)] as intercrops with the cereals.

To address how environmental stressors may reshape the trophic interactions, I investigated how water deficits may impact the crops used in the 'push-pull' system and wild grasses. Hence, my objectives in this project were to assess the differences (1) in oviposition acceptence of *C. partellus* in wild and cultivated grass under optimal and drought stress conditions, and (2) in larval growth and development in the wild and cultivated grasses under optimal and drought stress conditions. This was done with a goal of selecting plants that

exhibit desirable traits compatible with the 'push-pull' companion cropping system even under drought stress for possible use in drier regions.

3.1 Materials and methods

3.1.1 Study site

The studies were conducted at the International Centre of Insect Physiology Thomas Odhiambo Campus (ITOC) situated in Mbita Point, western Kenya (0°25'S, 34°12'E). In this area (1 200 m above sea level), cereal stemborers are a major constraint to cultivation of maize. Mean annual temperature is 27 °C, annual precipitation averages 900 mm but with frequent occurrence of extended periods of water deficit that induce drought stress responses in plants.

3.1.2 Study organisms

The *C. partellus* moths and larvae used in these studies were taken from a colony maintained at ITOC using an artificial diet as described by Onyango & Ochieng-Odero (1994). In all experiments, newly hatched larvae were used without any prior exposure to the test plants. Five test plant species were used: Napier grass variety 'bana', signal grass *Brachiaria brizantha* (A. Rich) Stapf, *Brachiaria* cv Mulato [a hybrid developed by International Centre for Tropical Agriculture (CIAT), Columbia], molasses grass, and a maize variety (WH505) (all Poaceae). All plants were planted in perforated 5 l plastic buckets filled with vertisol soil from the fields at Mbita Point ITOC. Napier grass, obtained from a stock sourced from Kenya Agricultural Research Institute (KARI) as described in Khan et al. (2006), was planted using root splits. Similarly, signal and molasses grass were planted from root splits obtained from wild collections from Mbita Point fields of the ITOC, whereas Mulato was grown from seeds.

3.1.3 Oviposition assays

Two-way choice tests

Drought stress response in treatment plants was induced using modifications of methods adapted from Efeoğlu et al. (2009). At 3 weeks after planting (ca. 2 weeks after seedling emergence), treatment plants were denied water for up to 10-13 days whilst control plants were adequately watered by watering to field capacity once each day during that period. Two-way choice tests were then carried out following modifications of protocols as described in Midega et al. (2010). Four independent pairs of drought-stressed and control plants per species were placed in individual cages (40 × 40 × 80 cm) before four gravid moths were introduced into each cage (Fig. 3.1). This set-up was repeated 3 times under similar ambient conditions to allow for comparison as a pooled dataset from 12 independent pairs for each species and treatment. The plants were placed in the corners of the cage, the moths were released in the centre of the base of the cage. A moistened wad of cotton wool was placed in the cages to allow for feeding of the moths. Moths were then allowed to oviposit overnight under natural ambient conditions. The following morning, leaf parts carrying eggs were excised to enable counting of eggs under a binocular microscope at times 6.5 magnification. Here, choice was regarded as the preferential oviposition by the insects in a cage when given differently treated plants (Singer, 1996; Midega et al., 2010).



Figure 3.1: Set-up of cages and the pairs of plants (1 drought stressed and 1 control) used for the 2-way choice test of *Chilo partellus* (Swinhoe) (Lepidoptera: Tortricidae) bioassays

No-choice tests

The same experimental procedure as described above was used but with only one plant per cage. For each species, four plants were used per treatment and the set-up was replicated 3 times.

3.1.4 Larval survival assays

Larval survival on whole plants

A full factorial design was used where plant species acted as the independent categorical predictor with two levels (drought stressed and irrigated control). After 3 weeks of planting under adequate watering (once every day to soil field capacity), eight plants from each species were randomly partitioned into two categories; drought stress (treatment) and the

control groups. Four plants of each species from both treatment and control categories were randomly and individually placed into wooden-framed netted cages placed on tables as above. These tables had their legs dipped in moats at all times to avoid any predators coming into the cages.

Drought stress in treatment plants was induced by withholding watering for 10-13 days whilst control plants maintained their watering schedule. Ten newly hatched larvae were then placed in the whorl section of each caged plant (treatment and control) using a soft camel-hair paint brush to allow for development on the plants. Upon larval infestation, treatment plants were watered once to avoid them reaching permanent wilting point (after ca. 10-13 days) as determined in preliminary trials. However, control plants were watered to field capacity once every 2 days to ensure adequate moisture just as prior to the infestations. Thereafter, larvae were allowed to feed and develop on individual plants in netted cages throughout the experiment. As most *C. partellus* eggs are known to hatch in the morning (Leuschner, 1990; Khan et al., 2006), all infestations were conducted in the morning to mimic natural conditions. After 15 days of larval feeding, visual assessments were carried out to assess the degree of leaf damage as it gives a good indication of the infestation and sometimes level of acceptability of a host plant (Leuschner, 1990; Khan et al., 2006). The score was scaled from 1-5 where 1 was assigned for individual plants with little/no visual damage and 5 for those with the most damage. Prior to collection of the data, visual scores were first callibrated by tracing leaves for each species and treatment on a graph paper to estimate herbivore-damaged area following Blundell & Peart (2000). In order to maintain consistency, all visual scores were done by the same person and were closely correlated to measured values.

All the caged plants were then dissected in order to obtain data on the number and life-stage of the surviving larvae (Fig. 3.2). These dissected plants were replaced by

individual whole plants of the same species, age, and watering/stress regime which were then infested by all the surviving larvae in each cage to allow the completion of their development. After a further 15 days of larval development on the new host plant replacements, plants were dissected as above. This experiment was repeated 3 times with four replications per species (n = 12 for each treatment and control). In all cases, larvae were considered to have survived after showing locomotory motion upon mild prodding with a soft brush.



Figure 3.2: Surviving larvae of *Chilo partellus* (Lepidoptera: Crambidae) (Swinhoe) recovered after plant dissection during survival and life stage assessments

Larval survival on excised whorls of the plants

Laboratory assays were carried out to assess the rate of survival and development of *C. partellus* larvae on drought stressed and control grass species. At 4 weeks after planting, plant stems with leaves and sheaths attached (whorl) were cut from each plant species from both

treatment and control plants and placed in transparent Kilner jars (20 cm high, 8 cm diameter) in a dark room in the laboratory. The whorls of the plants from the same species and treatment were randomly arranged in a jar and the set-up was replicated 20 times. Ten newly hatched larvae were then placed into each of the jars using a fine camel-hair brush. The whorls of the plants were approximately 15 cm long and 5 cm in diameter (Khan et al., 2006) to provide adequate material for the larvae to feed on. Perforated lids covered with a wire mesh were used to tightly secure the openings in the jars. After 5 days, larvae were retrieved and their life stages were determined. The surviving larvae were introduced to new freshly cut whorls and stems for mature larvae of corresponding plant species under the same watering regimes. Feeding and collection of data in this manner, every 5 days, for the same individuals continued until conclusion of the experiment after 40 days where the majority of the surviving larvae had pupated.

3.2 Statistical analyses

All statistical analyses were performed using R (R Development Core Team, 2010). One-way analysis of variance (ANOVA) using a generalised linear model was used to test the differences in percentage of larval survival, feeding scores, and no-choice oviposition traits (total number of eggs, number of clutches, and number of eggs per clutch) in all plant species. Survival percentages were first arcsine transformed. Tukey's studentized range test was used to separate means at the threshold level of $\alpha = 0.05$. In all the plant species, a Student's t-test was used to determine differences in the numbers of eggs and egg clutches within species of drought-stressed vs. control plants.

3.3 Results

3.3.1 Oviposition assays

Two-way choice tests

In dual choice tests between individual drought-stressed and control plants of the same species, *C. partellus* laid significantly more eggs on control Napier and signal grass (Table 3.1). However, there was no significant difference in the total number of eggs laid on treated and control plants for maize, Mulato, and molasses grass (Table 3.1).

Table 3.1 Mean (± SE) number of eggs per plant laid by *Chilo partellus* in a choice test between control and drought-stressed plants of the same species. For each plant species and treatment, a total of 48 moths were tested in groups of 4 moths per cage.

Plant species	Control	Drought-stressed	t	P
Maize	218.3 ± 35.9	187.1 ± 25.6	0.4	0.70
Napier grass	380 ± 33.8	123.9 ± 22.5	5.3	<0.001
Signal grass	323.2 ± 63.9	134.2 ± 28.5	4.1	0.001
Mulato grass	16.4 ± 4.7	18.7 ± 4.8	0.9	0.38
Molasses grass	9.4 ± 3.0	8.8 ± 2.7	0.1	0.88

No-choice tests

Among the control plants, i.e., those not exposed to drought stress, *C. partellus* moths oviposited significantly more eggs on Napier and signal grass than on the other test plants (Table 3.2). Based on the number of eggs oviposited per plant, Napier and signal grass were most accepted for egg laying by the moths, followed by maize, whereas Mulato and molasses grass were least accepted. However, this order of acceptance changed markedly under drought stress where maize received significantly more oviposition in terms of eggs

compared to the other test plants (Table 3.2). When plants were drought stressed, more than twice as many eggs were laid on maize than on Napier or signal grass (Table 3.2). Thus, drought stressed maize remained relatively acceptable for the moths, whereas Mulato and molasses grass remained least acceptable. The number of clutches did not differ among drought-stressed maize and Napier and signal grass, but it was significantly lower in Mulato and molasses grass (Table 3.2).

Table 3.2 Mean (± SE) number of eggs, clutches, and eggs per clutch laid by *Chilo partellus* (Lepidoptera: Crambidae) (Swinhoe) on single control (C) and drought-stressed (D) plants in no-choice tests

Treatment	No. eggs	No. clutches	No. eggs per clutch
Maize (C)	126 ± 19.7b	3.75 ± 0.5a	31.3 ± 3.6a
Maize (D)	186 ± 52.7b	4.4 ± 0.9a	19.3 ± 2.5b
Napier grass (C)	233.3 ± 20.5a	3.6 ± 0.3a	37.8 ± 4.4a
Napier grass (D)	68.2 ± 16.7c	2.3 ± 0.5a	20.2 ± 2.4b
Signal grass (C)	222.8 ± 54.3a	4.8 ± 0.7a	32.6 ± 3.1a
Signal grass (D)	71.9 ± 16.1c	2.2 ± 0.4a	19.6 ± 3.1b
Mulato (C)	20.5 ± 13d	2.9 ± 0.6a	12.2 ± 1.2c
Mulato (D)	20.3 ± 22d	1.1 ± 0.7b	14.2 ± 4.2c
Molasses grass (C)	25.8 ± 7.8d	1.2 ± 0.3b	11.4 ± 4.1c
Molasses grass (D)	1.1 ± 0.8d	0.2 ± 0.1b	11.4 ± 3.2c
$F_{9,111}$	7.39	6.1	8.4
P	<0.001	<0.001	<0.001

Means within a column followed by the same letter are not significantly different (Tukey's studentized range test: P>0.05).

3.3.2 Larval survival and development

Cage experiments

After 14 and 28 days of larval infestation, there were significant differences in percentage larval survival between drought-stressed and non-stressed plants of the various species, but in both periods and both treatments survival rates were significantly higher in maize than in the other plants (Table 3.3). Pair-wise comparisons indicated no significant within-species differences in percentage larval survival between drought-stressed vs. control plants at both 14 and 28 days.

Table 3.3 Mean (± SE) survival (%) of insectary-reared *Chilo partellus* (Lepidoptera: Crambidae) (Swinhoe) larvae after 14 and 28 days feeding on control (C) and drought-stressed (D) plants

Treatment	14 days	28 days
Maize (C)	42.1 ± 0.9a	31.9 ± 1.5a
Maize (D)	47.5 ± 2.5a	36.2 ± 2.6a
Napier grass (C)	22.4 ± 3.3b	4.6 ± 2.4b
Napier grass (D)	11.1 ± 3.9bc	2.2 ± 2.2b
Signal grass (C)	0 ± 0c	0 ± 0b
Signal grass (D)	0 ± 0c	0 ± 0b
Mulato (C)	11.8 ± 3.8bc	0 ± 0b
Mulato (D)	1.8 ± 1.7c	0 ± 0b
Molasses grass (C)	0 ± 0c	0 ± 0b
Molasses grass (D)	0 ± 0c	0 ± 0b
$F_{9,118}$	63.82	102.49
P	<0.001	<0.001

Means within a column followed by the same letter are not significantly different (Tukey's studentized range test: P>0.05).

Significantly larger portions of drought-stressed and control maize leaves were fed on by *C. partellus* larvae than of those from the other test plants (Fig. 3.3). Feeding scores of drought-stressed vs. control plants did not differ across species (Fig. 3.3), indicating that drought stress did not affect larval feeding in any of the species.

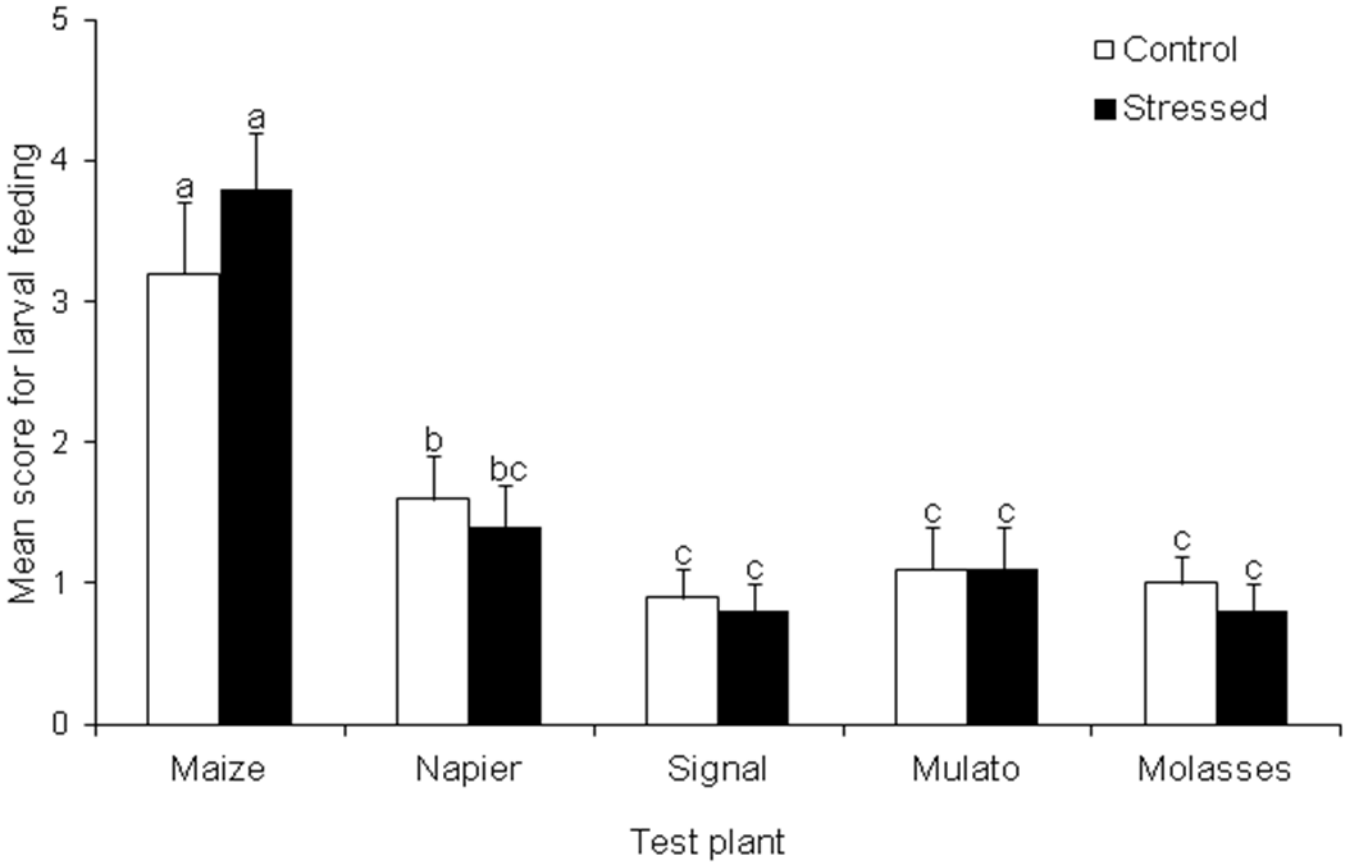


Figure 3.3 Mean (+ SE) score for leaf feeding by *Chilo partellus* (Lepidoptera: Crambidae) (Swinhoe) larvae 28 days after infestation in both drought-stressed and control plants; 1 = minimal or no feeding, 5 = heavy feeding. Means capped with different letters are significantly different (Tukey's studentized range test: $P<0.05$).

Larval survival and development (laboratory experiments)

Molasses and signal grass did not support larval survival beyond 20 days, whereas Mulato and Napier grass did not support larval survival beyond 30 days under drought stress (Fig. 3.4). The larvae were most successful on maize plants regardless of the treatment as was the case with cage experiments.

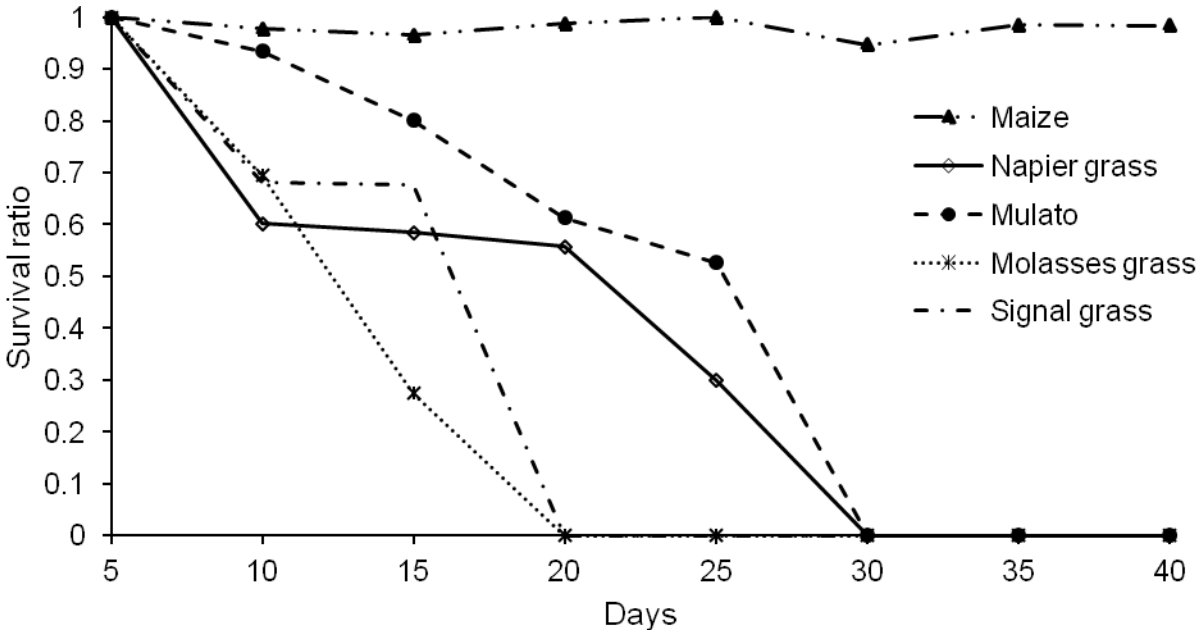


Figure 3.4 Survival of *Chilo partellus* (Lepidoptera: Crambidae) (Swinhoe) in wild and cultivated grasses expressed as the ratio of survival on drought stressed grass to survival on non-stressed control grass of the same species. Steepness of the curves shows the drop in survival of the larvae in treated plants relative to control at any particular time.

3.4 Discussion

Due to the limited capacity of long-distance dispersal in many lepidopteran neonates (Showler, 2002; Showler & Moran, 2003), adult oviposition choice is of crucial importance as it directly determines the initial available resources and potential threat from predators for

the offspring (Zalucki et al., 2002). This is even more so due to the rapid changes in climatic conditions that have been shown to modify plant characteristics (Gutbrodt et al., 2012a, b). Gravid moths are therefore likely to be presented with unique decision-making challenges when searching for a host plant during the prevalence of plant abiotic stress.

The current study indicated that gravid *C. partellus* moths accepted drought-stressed Napier and signal grass plants less than well-watered control plants for oviposition. On the other hand, oviposition on drought-stressed and control maize plants was not different in both choice and no-choice tests. The outcome of drought stress on insect oviposition varies across insect-plant systems. For example, Showler & Castro (2010) reported that drought-stressed sugar cane *Saccharum* spp. hybrids were preferred by the Mexican rice borer *Eoreuma loftini* (Dyar) to adequately watered sugarcane, perhaps, as they speculated, due to the increased amounts of dry leaf tissue and free essential amino acids that they found in the stems of the drought-stressed plants. In addition, drought stress has been associated with increased carbohydrate levels because of their important role in plant osmotic adjustment (Schubert et al., 1995; Showler, 2013). Such carbohydrates (often in the form of soluble sugars) and free amino acids can occur on the leaf surfaces of plants (Fiala et al., 1990; Soldaat et al., 1996). Consequently, these phylloplane compounds can determine host acceptance by phytophagous insects, upon detection by contact chemoreceptors on the antenna, tarsi, proboscis, and ovipositor of the moths (Städler, 1984; Blaney & Simmonds, 1988; Ramaswamy, 1988; Hora & Roesingh, 1999; Calatayud et al., 2008; Showler & Castro, 2010). It was demonstrated by Derridj et al. (1986) that elevated sugar levels in maize resulted in higher preference for oviposition in the European corn borer. Hence, my results are likely to be based on changes in the quality and quantity of attractive or repellent VOCs (see Birkett et al., 2006; Chamberlain et al., 2006; Bruce et al., 2010; Khan et al., 2010), together with contact cues that are used by the moths to select a host.

Molasses grass, which has been used to repel *C. partellus* in a push-pull system under optimum rainfall conditions (Khan et al., 1997), and Mulato remained unattractive under control and drought-stress conditions, being the least accepted of all test plants. They thus represent potential repellent companion plants for management of *C. partellus* in drier agro-ecologies. However, the chemistry of Mulato and behavioural responses of *C. partellus* needs to be studied to confirm this.

Drought also induces several genetically determined plastic morphological responses, including changes in plant height, leaf size and texture and curling of plant leaves, as a result of water loss from tissues (O'Toole & Cruz, 1980; Hsiao et al., 1984; Chaves et al., 2003). Even though I did not quantify morphological responses in my study, it was apparent that these responses varied among the tested species. As such morphological changes are widely known to influence host acceptance in Lepidoptera (Thompson & Pellmyr, 1991; Renwick & Chew, 1994; Zalucki et al., 2002; Midega et al., 2010; Showler & Castro, 2010), it is likely that they also influenced oviposition selection in my study.

Larval survival was poor in all drought-stressed and unstressed grasses in contrast to maize where larval survival remained unaffected by drought. Such poor survival in the non-stressed attractive Napier grass has already been attributed to the sticky sap that arrests larval locomotion, together with the poor nutritional content of the species (Khan et al., 2007, 2010). Furthermore, drought stress neither improved survival of larvae nor altered feeding scores on Napier grass. This suggests that Napier grass remains an unsuitable host for *C. partellus* larval growth and development under drought stress. Even though there was some indication of larval leaf feeding in signal grass, larval survival was not supported in either case.

The current results have direct implications for the push-pull system. Under drought stress, *C. partellus* favoured oviposition or feeding in the maize plants ahead of all the wild

grasses, including Napier and signal grass. This implies that Napier and signal grass may not be effective perimeter 'pull' plants under dry conditions. Mulato and molasses grass stayed unattractive even under drought-stress conditions. This calls for careful selection of perimeter trap and intercrop plants for use in a push-pull system in areas where water deficits are prevalent. Under field trials, Mulato has been identified as a potentially suitable perimeter plant by acting as a barrier that reduces crop attack just as effectively as plots surrounded by Napier grass (Khan et al., 2014). In addition, Mulato remains robust and withstands extended drought conditions relative to the other test plants, including Napier grass.

In conclusion, the current study demonstrated that maternal choices in *C. partellus* that impact offspring performance can be influenced by drought stress. Surprisingly, I found that insects made better choices under drought stress because they laid fewer eggs on Napier and signal grasses which are not good hosts for larval development. It is of importance to consider such behavioural traits in order to understand how stress may reshape plant-insect relations at short and long time scales. A critical component of this should be a determination of the effect of drought stress on VOCs and subsequent impact on stemborer oviposition preferences. Such an understanding may form the basis of climate-smart adaptation strategies of biology-based methods for pest control under changing climates.

CHAPTER 4

Acute drought stress induces and augments associational resistance to insect herbivore attack in the “push-pull” system for stemborer control*

*Chapter formatted and will be submitted for publication

4.0 Introduction

Plant volatile organic compounds (VOCs) play an important role as cues for host location in herbivorous insects. For gravid insects searching for oviposition sites, plant VOCs can be good indicators of diverse information about the suitability of the host (Bruce et al., 2005; Reisenman et al., 2013; Späthe et al., 2013; Wäschke et al., 2014). However, plant abiotic stressors, such as drought, often alter plant secondary metabolism (Atkinson & Urwin, 2012; DeLucia et al., 2012) with potential induction of changes in quality and quantity of VOCs (Vickers et al., 2009a, b; Holopainen & Gershenzon, 2010). Such changes in the composition and properties of the plant VOCs, regardless of the causal agent, may influence odour mediated host preferences of insects (Bruce et al., 2010; Bruce & Pickett, 2011). Under changing climates and environments, this can be a concern when developing push-pull pest management strategies that rely on specific blends of plant VOCs to repel pests, attract them to trap crops and attract their natural enemies (Cook et al., 2007).

In East Africa, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) remains one of the highly destructive polyphagous pests in the production of the staple maize (*Zea mays* L.) and strategic small grain cereals. Damage by the pest is achieved through larval feeding on the leaves and stems resulting in 20-80% economic loss (Kfir et al., 2002). Effective control strategies for such stemborer pests have been either elusive or expensive for small scale farmers in sub-Saharan Africa. To help combat the pests in a cost effective manner, the push-pull system was developed by the International Centre of Insect Physiology and Ecology (ICIPE), Kenya and Rothamsted Research. This push-pull system relies on on-farm biodiversity to confer associational resistance (Sholes, 2008; Jactel et al., 2011; Plath et al., 2012; Zakir et al., 2013a) which is achieved by intercropping crops with the pests` non-host plants simultaneously with wild their hosts in the borders of the crop plot. In its conventional

form, the push-pull system consists of maize that is intercropped with *Desmodium* spp. whilst bordered by Napier grass (Khan et al., 2010). Volatile organic compounds produced by *Desmodium* plants repel stemborers whilst Napier grass attracts them away from the maize plots. Control of the pests is then further enhanced by high oviposition rates on the perimeter grasses which function as a trap crop and suppress larval growth and mobility (Khan et al., 2006).

The advent of climate change, however, poses new challenges to the push-pull system through factors such as drought. Napier grass, for example, has relatively high water requirements. Even though it has a deep rooted system which can enable survival under periods of drought stress, little is known about its volatile emission profiles under stress. Such is the case with other current and candidate companion plants for the push-pull system. Six key compounds that were identified as important for host seeking gravid moths in the push-pull system were only identified under optimal conditions (Khan et al., 2000; Birkett et al., 2006). A gap in knowledge relating to how the plant VOC emission profile changes under different abiotic stressors therefore exists.

In a recent study, I found that host acceptance by gravid moths changed between drought-stressed and control host plants (Chidawanyika et al., 2014). Apart from contact cues, such changes may also be attributed to changes in volatile emission profiles and subsequent trophic interactions. For example in maize, abiotic stress induced by heavy metal poisoning was shown to prime the plants to produce more herbivore induced plant volatiles (HIPVs) upon insect herbivory (Winter et al., 2012). In other studies, Gouinguene and Turlings (2002) showed that abiotic factors such as temperature, soil and atmospheric humidity, and fertilization rate influence VOC production in corn plants with potential impact on subsequent trophic interactions. Given this background, I investigated how drought stress

affected the chemically-mediated interaction between *C. partellus* and companion plants of the push-pull system. Specifically, I asked if 1) drought stress changes volatile emission profiles of current and potential grasses of the push-pull system 2) drought-induced changes in volatile emission profiles elicit differential behavioural and electrophysiological responses in *C. partellus*.

4.1 Materials and methods

4.1.1 Insects

An artificial diet described by Onyango and Ochieng-Odero (1994) was used to rear the *C. partellus* moths that were used in these studies. In order to control for age related differences in the electrophysiological responses and wind tunnel bioassays, only 1-2 day old gravid moths were used.

4.1.2 Plants

Napier grass variety 'bana', molasses grass, signal grasses *Brachiaria brizantha* (A. Rich) Stapf and *Brachiaria* cv. Mulato II [products from 3-way cross of *B. ruziziensis x B. decumbens x B. brizantha* (all Poaceae)], together with two legume species, *Desmodium uncinatum* (Jacq.) (hereafter called Silverleaf *Desmodium*) and *Desmodium intortum* Mill.) Urb) (hereafter called Greenleaf *Desmodium*) were used in the study. All plant species were planted in vertisol soil that was held in perforated 5 litre plastic buckets under natural conditions (c. 25°C, 65% RH; 12L: 12D). Root splits of stock plants from Kenya Agricultural Research Institute (KARI) were used for planting Napier grass as in Khan et al. (2006). Similarly, root splits from collections of signal grass and molasses grass held at the ICIPE Mbita Point ITOC field station were used for planting experimental specimens for these species. Mulato II and all *Desmodium* species were propagated using seeds. At 3 weeks after planting (ca. 2 weeks after seedling emergence) under optimal conditions, plants from

all species were randomly partitioned into two groups; control and treatment. Control plants had adequate water by watering to soil field capacity once a day whilst those of the treatment were denied water for 10-13 days as described in Chapter 3.

4.1.3 Volatile collection

Volatiles were collected from intact control and treated plants by headspace sampling (Fig. 3.1) (Tamiru et al., 2011). Polyethyleneterephthalate (PET) bags (volume 3.2 L, ~12.5 mm thickness) were cleaned by preheating at 150°C for 30 mins before fitting Swagelock inlet and outlet ports. They were then used to cover the plants after fitting adsorbent filters, Porapak Q (0.05 g, 60 / 80 mesh; Supelco), in the outlet ports. Charcoal-filtered air was pumped through the inlet ports into the bags at a rate of 600 mL min^{-1} and air was drawn out through the outlet ports at rate of 400 mL min^{-1}. Each entrainment lasted for 48 hrs for all plants after the initial start at 2 hrs after scotophase. Upon completion of entrainment, elution of the samples from the Porapak Q into sample vials was done using 0.5 mL dichloromethane.



Figure 3.1: Picture showing set-up for headspace collection of plant volatile samples

4.1.4 Laboratory wind tunnel

The wind tunnel was situated in a white environmentally controlled room set at 26 ± 2°C, ~2 lux light intensity and 55 ± 5 % RH, a wind tunnel set-up was established following Curkovic and Brunner (2006) and Curkovic et al. (2009). The working arena of the tunnel was formed by transparent Plexiglas (polymethyl methacrylate) material that was 2.7 m long, 70 cm wide and 70 cm high. An electric fan (40 cm diameter, Donkin Fans Co., South Africa) drew air through the tunnel at a rate of approximately 45 cm s^{-1}. The air that was drawn through the tunnel then exited from the room via an outlet to avoid contamination.

4.1.5 Bioassay protocol and behavioural observations

Plant odour was delivered in the tunnel using discs of filter paper (~ 2cm diameter each) that were individually mounted onto a platform opposite the fan near the exit of the tunnel. Prior to that, these discs were first moistened by adding 10 µl aliquots of collected volatile samples using micropipettes (Drummond 'microcap', Drummond Scientific Co. Broomall, PA, USA). Gravid moths were individually placed on a stand on the downwind side opposite the odour source. Each moth was assayed only once and thereafter discarded upon completion of observations lasting 10 min. Behavioural responses of the moths were scored based on their mere flight towards and actual contact with the surface of the odour source. For all volatile samples, 15 replications were done where each moth was regarded as a replicate. In all cases, surfaces of the glassware platforms where the volatile component and the insects were placed were first cleaned with 70 % ethanol before being heated at 100 °C for 30 minutes prior to assaying any replicate. Surfaces of the wind tunnel were similarly cleaned with 70 % ethanol, and allowed to dry, but without the oven heating, before each assay.

4.1.6 Coupled Gas chromatography-mass spectrometry (GC-MS)

A capillary gas chromatography column (HP1, 50m length, 0.25mm i.d., 0.25 µm film thickness) (Fig. 4.2) coupled directly to a mass spectrometer (VG Autospec, Fisons Instruments, Machester, UK) was used to analyse aliquots of the headspace samples. Oven temperature was programmed to remain at 50 °C for 3 mins before ramping up to 200 °C at 7 °C min^{-1} and then further heated to 300 °C at 100 °C min^{-1} where it was held constant for 2 min. Ionization was achieved by electron impact (70 Ev, 250 °C). Comparison of retention indices and mass spectra of authentic standards with those of the samples was used to identify compounds.



Figure 4.2: Picture showing equipment used for gas chromatography analysis of plant volatile samples

4.1.7 Chemicals

Commercially sourced synthetic standards; decanal, decane, *R)*-Linalool, (E)-(1R,9S)-caryophyllene, eugenol, α-Pinene, methyl salicylate and (Z)-3-hexenyl acetate were purchased from Avocado Research Chemicals and Sigma Aldrich. Following Leopold (1990), (E)-4,8-Dimethyl-1,3,7-nonatriene (DMNT) (> 98%) and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (> 98%) were synthesised through the oxidation of corresponding aldehydes in geraniol and (E,E)-farnesol respectively then followed by a Wittig methylenation. High purity (E)-Ocimene (>95% by GC) was synthesised in a two step protocol. First, 3-methyl-3-sulpholene was functionalised using 1-bromo-3-methylbut-2-ene (Chou et al., 1984) and then followed by extrusion of sulphur dioxide using lithium aluminium hydride (Gaoni, 1977).

4.1.8 Electroantennogram (EAG) recordings

Coupled GC-electroantennography (GC-EAG) was carried out using antennae of female *C. partellus* and the headspace samples of plants. EAG recordings were made using Ag–AgCl glass electrodes filled with saline solution [composition as in Maddrell (1969), but without glucose]. An antenna was excised and suspended between the two electrodes. The tip of the terminal part of the antenna was removed to ensure a good contact. Signals were passed through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and analysed using a customised software package (Syntech). The GC-EAG system, in which the effluent from the GC column is simultaneously directed to the antennal preparation and the GC detector, has been described previously (Wadhams, 1990). Separation of the volatiles was achieved on a GC (Agilent Technologies, 6890N) equipped with a cold on-column

injector and a FID using a HP-1 column (50 m, 0.32 mm ID, 0.52 lm film thickness). The oven temperature was maintained at 30 °C for 2 mins and then ramped up at 15 °C min^{-1} to 250 °C. The carrier gas was helium. Outputs from the EAG amplifier and the FID were monitored simultaneously and analysed using the Syntech software package. Peaks eluting from the GC column were judged to be active if they elicited EAG activity in three or more of five coupled runs.

4.2 Statistical analysis

All statistical analyses were performed in R programme (R Foundation for statistical computing, 2010). A Chi-square test was used to determine the differences in i) the number of flights towards and ii) actual contact with the odour source among control and treatment samples of the same species. Student *t*-tests were done to test significant differences in the means of the quantities of the EAG active compounds that were emitted by drought-stressed and irrigated plants of each species. Total amounts of eluted compounds from the plant samples were analysed as normalized peak area units based on the GC-MS analysis. Differences between the volatiles from drought stressed and irrigated plants of each species were tested using the Mann-Whitney *U*-tests. Thereafter, the sequential Bonferroni method was carried out to correct for any potential family wise error rate (Holm, 1979)

4.3 Results

4.3.1 Wind tunnel behavioural responses

Gravid moths were more attracted to odour samples from irrigated Napier grass (χ^2 = 7.033, d.f. = 1, P = 0.008) and signal grass (χ^2 = 4.887, d.f. = 1, P = 0.031) than their respective drought stressed individuals as indicated by the number of moths that contacted the odour source (Table 4.1). However, there was no immediate discrimination in the initial flight

response towards the odour source between control and treatment plants of both Napier grass (χ^2 = 0.682, d.f. = 1, P = 0.408) and signal grass (χ^2 = 1.429, d.f. = 1, P = 0.232). Odour samples from both treatment and control molasses grasses, Mulato II, Silverleaf and Greenleaf *Desmodium* were not attractive as shown by the low responses in both the initial flight behaviour towards odour and actual contact with the odour source for all samples (Table 4.1).

Table 4.1: Wind-tunnel behavioural responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) to volatile samples taken from various plant species that were either optimally irrigated or drought stressed. Moths were used only once and assayed individually for 10 minutes.

			Towards odour source		Contact with odour source	
Plant Species	df	N	χ^2	P-value	χ^2	P-value
Napier grass	1	30	0.682	0.408	7.033	0.008
Molasses grass	1	30	0.37	0.53	0.025	0.543
signal grass	1	30	1.429	0.232	4.887	0.031
Mulato II	1	30	2.727	0.098	1.154	0.273
Silverleaf *Desmodium*	1	30	1.132	0.343	0.391	0.71
Greenleaf *Desmodium*	1	30	1.126	0.283	0.411	0.68

4.3.2 Identification of electrophysiologically active compounds

Gravid *C. partellus* moths detected a total of 24 different electrophysiologically active compounds from EAG analysis of the samples of drought-stressed and irrigated plants of the 6 plant species (Fig. 4.3). Nineteen of these active compounds were characterised by either GC-MS analyses or coinjection on both polar (HP-1) and non-polar (DB-wax) columns guided by the Kovat indices of the synthetic standards. Traces of GC-EAG responses of gravid *C. partellus* moths to drought-stressed Napier, molasses, signal grasses and Mulato II are shown in Fig. 4.3. Within each species, most compounds either appeared in both drought-stressed and irrigated plants or were not detected at all in both the 2 sets of plants (Table 4.2). Some of these compounds were common to all the plant species and treatments. There was a significant increase in the emission of DMNT in both drought stressed Napier and signal grasses (Table 4.2; Fig. 4.3). However, there was no significant difference in the emission of DMNT between drought-stressed and irrigated molasses grass, Mulato II and the two plants species of the genus *Desmodium*.

Drought stress resulted in the increase of (*E*)-ocimene in molasses and signal grasses, and Greenleaf *Desmodium*. However, there was no such significant difference in the emission of (*E*)-ocimene between drought-stressed and irrigated Silverleaf *Desmodium*. No (*E*)-ocimene was detected in Napier grass and Mulato II. There was an increase of the emission of (*R*) linalool in drought-stressed Napier, molasses and signal grasses, Mulato II but not in Silverleaf *Desmodium* where there was no significant difference. In all the plant species, the acute drought stress generally elicited increased emission of particular compounds and overall total volatile emission (Fig. 4.4). However, in Napier grass, there was a significant reduction in the emission of Z3HA and methyl salicylate following drought stress (Table 2. Fig. 4.4)

Table 4.2: Mean emission rates of electrophysiologically active compounds that were emitted by grasses and legumes under irrigated and drought stressed conditions. Mean emission rates are given as ng plant^{-1} h^{-1} (± s.e.). In all cases 5 plants per species per treatment (n = 5) were entrained for volatile collections where 'nd' denotes compounds which were not detected in the respective plant volatiles.

	Napier grass				molasses grass			
Compound	Drought stressed	irrigated	t	P	Drought stressed	irrigated	t	P
6-methyl-5hepten-2-one	nd	nd	nd	nd	nd	nd	nd	nd
benzaldehyde	nd	nd	nd	nd	16.2 (± 2.34)	14.6 (± 0.8)	1.86	0.45
(Z)-3-hexenyl acetate	trace	1.7 (± 0.3)	3.12	0.02	nd	nd	nd	nd
(E)-ocimene	nd	nd	nd	nd	23.6 (± 6.65)	8.65 (± 4.35)	5.21	< 0.001
(R)-linalool	32 (± 11.35)	25 (± 8.1)	5.64	< 0.001	34.14 (± 12.48)	9.82 (± 3.68)	6.34	< 0.001
DMNT	78.1 (± 32.12)	16 (± 5.24)	6.2	< 0.001	68.32 (± 18.21)	47.85 (± 12)	1.14	0.72
methyl salicylate	trace	16.34 (± 6.4)	4.76	0.001	nd	nd	nd	nd
decanal	nd	nd	nd	nd	nd	nd	nd	nd
α-pinene	1.8 (± 0.6)	1.6 (± 0.4)	2.44	0.41	nd	nd	nd	nd
eugenol	6.2 (± 1.4)	1.2 (± 0.6)	4.55	0.02	nd	nd	nd	nd
α-murolene	3.88 (± 1.2)	2.46 (± 0.68)	1.96	0.59	nd	nd	nd	nd
(E)-caryophyllene	nd	nd	nd	nd	nd	nd	nd	nd
TMTT	trace	2.12 (± 0.6)	4.37	0.04	nd	nd	nd	nd
Total	121.98 (± 43.1)	66.42 (± 22.1)	16.1	< 0.001	142.26 (± 69.12)	80.92 (± 47.63)	5.93	0.88

Table 4.2 (continued)

	Silverleaf *Desmodium*				Greenleaf *Desmodium*			
Compound	Drought stressed	irrigated	t	P	Drought stressed	irrigated	t	P
6-methyl-5-hepten-2-one	nd	nd	nd	nd	nd	nd	nd	nd
benzaldehyde	nd	nd	nd	nd	nd	nd	nd	nd
(Z)-3-hexenyl acetate	8.14 (± 4.66)	6.98 (± 3.33)	2.44	0.73	1.3 (± 0.3)	4.33± (1.24)	2.89	< 0.001
(E)-ocimene	2.54 (± 0.98)	1.78 (± 0.6)	4.28	0.82	45.3 (± 12)	21.3 (± 6.24)	2.64	< 0.001
(R)-linalool	0.6 (± 0.1)	2.1 (± 0.7)	5.63	< 0.001	nd	nd	nd	nd
DMNT	16.34 (± 4.24)	11.76 (± 3.2)	1.67	0.48	7.43 (± 2.88)	6.4 (± 2.7)	0.4	< 0.001
methyl salicylate	22.12 (± 9.21)	16.44 (± 5.2)	8.3	0.02	22.12 (± 9.21)	18.4 (± 5.2)	8.3	0.57
decanal	nd	nd	nd	nd	nd	nd	nd	nd
α-pinene	nd	nd	nd	nd	nd	nd	nd	nd
eugenol	1.6 (± 0.25)	2.45 (± 1.21)	2.33	0.62	3.6 (± 1.22)	2.95 (± 0.96)	3.72	0.48
α-murolene	nd	nd	nd	nd	nd	nd	nd	nd
(E)-caryophyllene	nd	nd	nd	nd	nd	nd	nd	nd
TMTT	nd	nd	nd	nd	4.26 (± 1.6)	1.3 (± 0.4)	4.77	0.02
Total	51.34 (± 19.63)	41.51 (± 11.1)	3.31	0.45	84.01 (± 58.5)	51.72 (± 32.14)	4.21	0.83

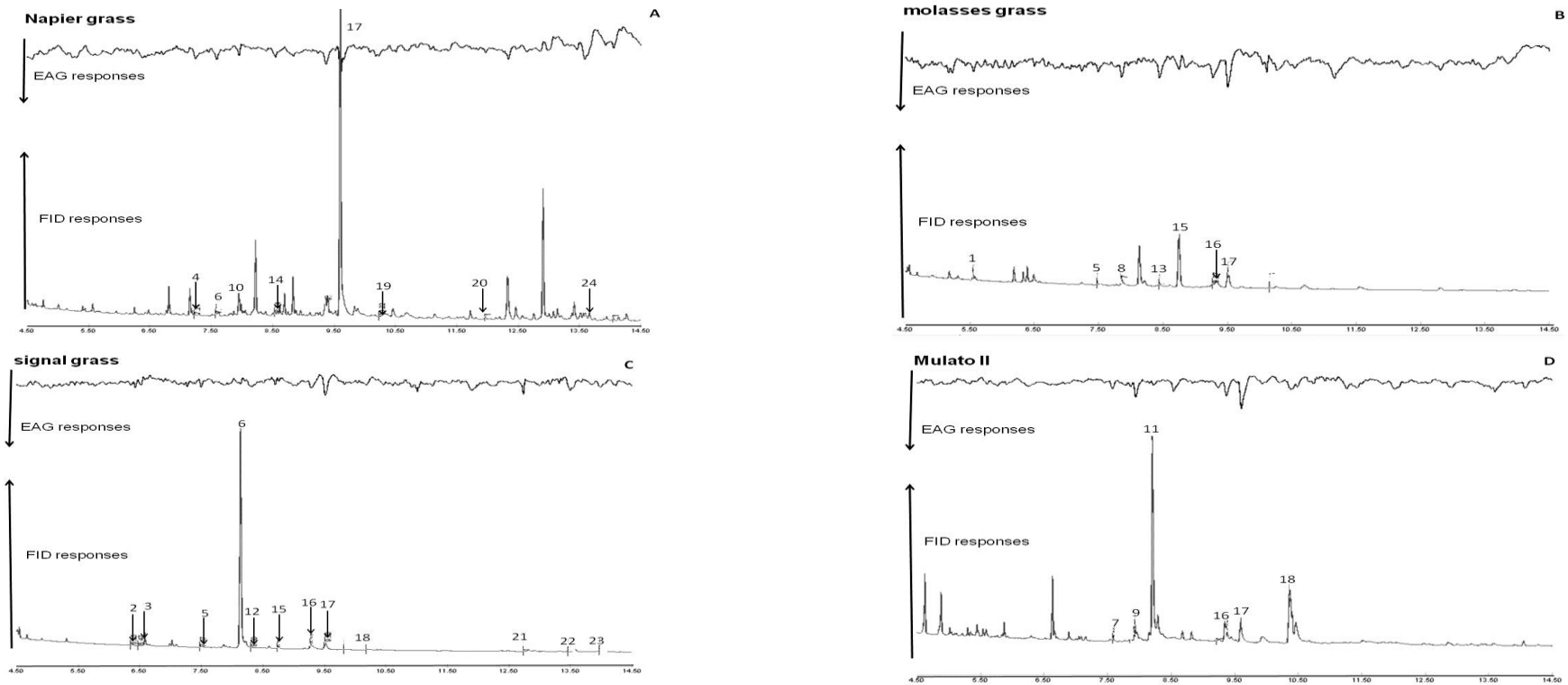


Figure 4.3: GC-EAG responses of gravid *Chilo partellus* (Swinhoe) (Lepidoptera Crambidae) to volatiles from drought stressed grasses of the push-pull system. Represented compounds are 1, Z-3-hexenal; 2, ethylbenzene; 3, M-xylene; 4, butyl isothioayanate; 5, benzaldehyde; 6, α-pinene; 7, Z-2-heptenal; 8, trans2-ethyl-2-hexen-1-ol; 9, 6-methyl-5-hepten-2-one; 10, Z-2-octan-01-ol; 11, Z-3-hexen-1-ol acetate; 12, decane; 13, benzeneacetaldehyde; 14, cyclohexanone; 15, *E*-ocimene; 16, linalool; 17, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT);18, methyl

salicylate; 19, 1-nonanol; 20, eugenol; 21, caryophyllene; 22, β-bisbolene; 23, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); 24, α-muurolene.

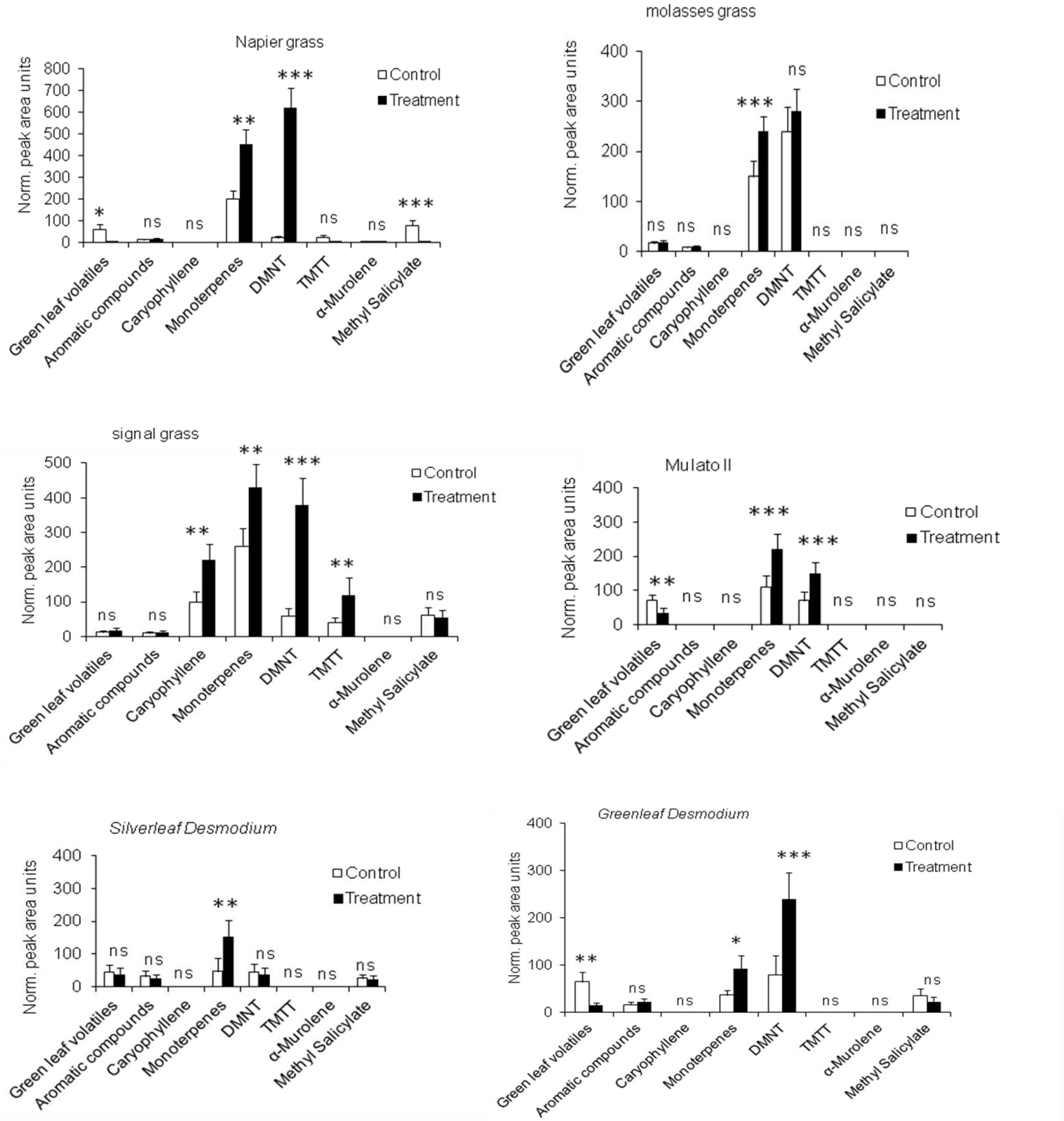


Figure 4.4: Quantitative emission rates of volatile compounds by Napier grass, molasses grass and signal grasses, Mulato II and, Silverleaf and Greenleaf *Desmodium* under irrigated and drought stress conditions.

4.4 Discussion

My study suggests that drought stress alters the emission of plant VOCs with subsequent impact on trophic interactions. In wind tunnel bioassays, volatile samples of drought-stressed Napier and signal grasses, wild hosts of *C. partellus*, were not as attractive to the moths when compared with those from the irrigated plants (Table 1). These changes in moth preferences can be explained by the increased emission of monoterpenes such as (*E*) ocimene, (*R*) linalool and the hormotepene DMNT in drought-stressed Napier and signal grasses. These terpenes have already been reported to be of importance in mediating plant-insect interactions in various studies. For example, upon perception of insect herbivore attack, plants signal metabolic events that result in increased emission, among other VOCs, of DMNT, and sesquiterpenes (*E*) caryophyllene and TMTT (Gouinguene et al., 2001; 2003; Degen et al., 2012; Tamiru et al., 2011; 2012). In most cases, such herbivore induced plant volatiles (HIPVs) serve as recruitment cues of egg and larval parasitoids as an indirect defence mechanism (Tumlinson et al., 1993; Schnee et al., 2006; Degenhardt, 2009; Dicke & Baldwin, 2010; Tamiru et al., 2011; 2012) and can also be exploited by hyperparasitoids (Poelman et al., 2012)

Conversely, HIPVs elicited by feeding larvae have been demonstrated to be repellents of conspecific adult female moths searching for oviposition sites (De Moraes et al., 2001; Allmann et al., 2013; Zakir et al., 2013b). Such ability of plant HIPVs to repel searching gravid moths has been regarded as a way of the plant to prevent further insect colonisation. However, other reports have regarded the oviposition non-preference of HIPVs as a way by which gravid insects can maximise progeny fitness potential through avoidance of intraspecific competition and increased risk of predation (Rodriguez-Saona & Frost, 2010). The present study gives evidence that may inadvertently support both postulations. First, the

increased emission of VOCs similar to HIPVs in drought-stressed Napier and signal grasses prevents any further stress due to insect herbivore damage thereby improving the fitness potential of the plant in such circumstances. Second, *C. partellus* moths may have evolved to detect VOCs produced by plants under abiotic stress, which may lead to plant mortality, as cues to avoid poor quality hosts for maximisation of offspring performance. In my previous study, oviposition preference and acceptance was reduced in Napier and signal grasses. Furthermore larval performance was poor in drought stressed Napier and signal grasses (Chidawanyika et al., 2014). Even though the larval survival was also poor in the more attractive irrigated Napier and signal grasses, the non-preference of VOCs from drought stressed plants of these species show how insect herbivores may use cues from plants to select the ideal host. Through acute drought stress these modifies emissions of plant VOCs among natural and managed grassland communities may offer windows of for the plant to escape insect herbivore attack. However, it is not clear how much such an escape of herbivore attack actually contributes to the plants` typical Darwinian fitness upon resumption of watering since these periodic abiotic stressors also affect fitness traits such as reproduction and dispersal, and longevity.

Apart from quantitative changes in VOC emissions, drought stress could have influenced the quality of the VOCs resulting in differential responses of the moths in the wind tunnel bioassays. In previous studies, Bruce et al. (2010) showed that an increased ratio of Z3HA to minor component VOCs due to reduction in its emission rates by signal grass upon insect oviposition resulted in reduced oviposition preference by *C. partellus* moths. In the present study, drought-stressed Napier and signal grasses had a dramatic reduction in the emission of Z3HA compared to the corresponding irrigated plants (Table 2; Fig. 2). Even though I did not test for the specific responses of the moths to the synthetic standards at different ratios, relative changes in proportions of Z3HA in the formerly attractive Napier and

signal grasses could have influenced moth behavioural responses in my study. The reduction in emission of Z3HA and other green leaf volatiles during drought stress was also present in other species such as Mulato and Greenleaf *Desmodium* (Table 2, Fig. 2) Even though the latter is not known as a host of *C. partellus*, this marked reduction in Z3HA relative to other compounds may have contributed to the non-preference by the moths. Furthermore, other non-hosts such as molasses grass and both *Desmodium* species which constitutively emit the repulsive DMNT had similar emission profiles even under drought stress (Table 2; Fig. 3) (Khan et al., 1997a). In some case, drought stress appeared to result in an increase in the emission of DMNT thereby augmenting their repulsive nature against the gravid *C. partellus* moths.

The mechanisms and functional ecological role played by plant VOCs that are induced by abiotic stress remains an area of active debate (Holopainen, 2004; 2011; Niinemets, 2010; Blande et al., 2014; Loreto et al., 2014). Terpenoids are one major group of emitted plant VOCs upon the perception of stress due to abiotic stressors (Gershenzon & Dudareva, 2007; Sharkey et al., 2008; Vickers et al., 2009a; Sharkey & Monson, 2014). In my study, most of the plant species that I tested were either induced or maintained the emission of various terpernoids followed by a reduction in other groups of compounds such as green leaf volatiles (Table 2; Fig. 2). Even though production of plant volatiles may have metabolic costs for the plant (Sharkey et al., 2008; Neilson et al., 2013), the prioritised emission of terpenes during drought stress in my study indicates that they may therefore be of importance in relieving plants undergoing abiotic stress. My findings are also similar to those reported by Gouingerne and Turlings (2001). In their study, a decrease in soil humidity resulted in an increase in the emission of the terpenes DMNT and (*E*) β farnesene in corn plants. However, unlike the case in my study, they also reported an increase in the green leaf

volatile Z3HA. Therefore even though the majority of the responses to abiotic stress may be generic, some remain species specific.

The present study also has direct implications to the push-pull system and may help clarify the mechanistic basis of ecological interactions therein under environmental change. First, the ability of formerly 'pull' or attractive plants of the push-pull system to act as repellents through increased emission of mostly terpenoids under abiotic stress is a form of phenotypic plasticity under environmental variability. The plasticity of Napier grass shown in the present study may therefore allow the species to perform dual roles of attracting and repelling moths under optimal and drought stress conditions respectively. In the latter, however, the increased repellent effect by both the peripheral and intercrop may have been the key to efficacy of the push-pull against stemborers during periodic water deficits. Periodic drought events of between 10 to 15 days within a crop growing season are common in east Africa where the push-pull system is currently practised (Chidawanyika et al., 2014). However, the push-pull has been successful in suppressing crop attack using Napier grass as a 'pull' companion plant under such conditions. Furthermore, collected VOCs of Napier and signal grasses under drought stress were attractive to the larval parasitoid *C. sesamiae (unpublished data).* This attraction is based on the increase in already identified compounds that are constitutively emitted by companion plants such as molasses grass (Khan et al., 1997) and HIPVs in maize landraces Tamiru et al. 2011; 2012) with subsequent increased parasitisation of the pests.

Second, the more drought tolerant Mulato II which constitutively emits DMNT also emitted the compound in even larger quantities under drought stress (Table 2; Fig. 2). This induction of increased DMNT emission was not present in with molasses grass, Silverleaf and Greenleaf *Desmodium* as there was no difference in their constitutive emission of DMNT

under irrigated and drought stress conditions. In molasses grass and Greenleaf *Desmodium* however, there was a significant increase in the constitutive emission of another terpene (*E*) ocimene under drought stress conditions. This was not the case with Mulato II and Silverleaf *Desmodium*. These variations in the emission profiles of the plant species in response to stress may have been a result of a variety of factors (Mittler, 2006; Niinemets, 2010). Even though all the plants were exposed to similar periods of water deficit, differences in VOC emission responses, apart from genetic limitations, may also have been due to the relative severity (mild *vs* severe) of the stress to a particular plant species. Furthermore, differences in the perception, transduction and propagation of the stress signals across the different species could have contributed to the differences in the emission profiles (see discussions in Niinemets 2010). However, my observations during the study showed differences in the plants to cope with stress as some already showed signs of wilting. This may have also been due to the high metabolic cost of the emission of the terpenoids itself as they are known to be more expensive to produce per unit weight when compared to other biogenic VOCs (Gershenzon, 1994; Paré & Tumlinson, 1999). Furthermore, terpenes are also an important source of olefinic compounds which are precursors of phytotoxic products (Monson & fall, 1989; Buchbauer et al., 1994). This phytoxicity often occurs when the terpenoids accumulate as they become pollutants comprising of photooxidants and ozone (Paré & Tumlinson, 1999). However, this phytoxicity has mostly been demonstrated in trees and not grasses (e.g. Monson & Fall, 1989; Buchbauer et al., 1994). It is therefore not clear if this form of phytotoxicity occurred in my study system for the plants which showed more phenotypic signs of stress coincidentally emitted more induced terpenes.

Selection of plants for more resilient plants in the push-pull should therefore incorporate agronomic performance of the companion plants in addition to volatile emission profiles. Such agronomic performance and volatile emission based selection of companion

plants are already showing high potential in drier areas of Kenya. Under field conditions the replacement of Napier grass as the perimeter plant with the more drought resilient Mulato in the push-pull system significantly reduces pest damage even in drier environments (Khan et al., 2014). Last, the behavioural responses of the moths to the VOCs of the known repellent plants remained the same under variable emission profiles from irrigated and drought conditions (Table 4.1). Therefore, the protection of crops in the push-pull system under drought conditions remains possible through the associational resistance offered by non-host plants and the induced production of pest repellent terpenoids in peripheral companion plants such as Napier and signal grasses. Furthermore, this associational resistance may be further augmented by the increased pest parasitism due to increased attraction of parasitoids by the same terpene compounds (Chapter 5).

In conclusion, the study demonstrates that abiotic stressors can affect plant ecological interactions through alterations in volatile emission profiles. However such responses to abiotic stressors or lack thereof, are species specific. Even though I did not do any transcriptional analysis, there were generic responses of some grasses in production of terpenes in response to abiotic stressors as is the case upon insect herbivory. This shows that there is some form of cross-talk in the signaling pathways of plants under biotic and abiotic stressors to potentially maximise on defense mechanisms when faced with multiple stressors, as is the norm under field conditions (see discussions in Fujita et al., 2006; Atkinson & Urwin, 2012).

CHAPTER 5

Behavioural and electrophysiological responses of Cotesia sesamiae to drought stressed companion plants of the “push-pull” system*

5.0 Introduction

The modification of habitats in pest control programmes can dramatically improve the activity and efficacy of natural enemies against insect pests. One such pest control programme benefitting from increased activity of natural enemies is the 'push-pull' system currently used in East Africa for suppression of stemborer pests (Pickett et al., 2014). In this pest control system, intercropped companion plants such as molasses grass have been shown to increase the activity of parasitoids (Khan et al., 1997). Pivotal to these multitrophic interactions are plant volatile organic compounds (VOCs) which possess differential attractive and repulsive appeal to the parasitoids and pests respectively (Cook et al., 2007; Khan et al., 2014). Consequently, there is improved protection of the crop through both increased parasitism of the juvenile pests, due to enhanced host location, and repulsion of the gravid adults.

Climate induced environmental changes however pose both direct and indirect threats to arthropods at different spatial and temporal scales. At a course scale, global changes may result in changes in arthropod geographic distribution, community composition and abundance, and general ecosystem function (Easterling et al., 2000; Bale et al., 2002; Parmesan, 2006; Hance et al., 2007; Walther 2010; Chidawanyika et al., 2012). Finer scale impacts may include within-species and organism-level alterations in physiology, phenology and longevity or survival times (Chidawanyika & Terblanche 2010; Thomson et al., 2010). Furthermore, plastic and genetic responses at both organism and species level may influence *in situ* adaptation and performance of insects` various traits (e.g. flight, body size, mating) (Chown et al., 2010; Chidawanyika & Terblanche 2011, Nyamukondiwa et al., 2011).

For insect herbivores, the impact of changing climates may be determined by the responses of the plants to climate change factors such as drought and heat (Bauerfeind & Fischer, 2013a; b; Chidawanyika et al., 2014; Leingärtner et al., 2014). Drought stress and

heat may alter plant quality through changes in N concentration and leaf C:N ratios (Zvereva & Kozlov, 2006; Sardans et al., 2008) and reduction in concentration of plant secondary defence compounds (Gutbrodt et al., 2011; 2012a, b). Herbivorous insects may opportunistically benefit from such periodic changes in plant quality due to environmental change (Behmer, 2009; Gutbrodt et al., 2011; Bauerfeind & Fischer, 2013a). In similar comparative studies, English-Loeb et al. (1997) showed that feeding the performance of herbivorous *Spodoptera exigua* on tomato leaf tissue was reduced on the drought-stressed plants contrary to the optimally irrigated due to increased concentration of defence compounds in the stressed plants. The consequences of interactive plant and insect relationships under environmental stress are therefore highly species specific and are often difficult to predict.

Other plant responses that can be induced by environmental change may be transcriptomatic, cellular and physiological with subsequent alterations of hormone-regulated biosynthetic pathways in the plant (Ahuja et al., 2010; Atkinson & Urwin, 2012). Such changes in biosynthetic pathways may affect the quality and quantity of produced ecologically relevant plant VOCs (Dicke & Loreto, 2009). For insects, changes in components of blends of VOCs may affect their olfactory perception with subsequent impact on the potential interactive encounters within and across different species and trophic levels (Bruce et al., 2005; Bruce & Pickett, 2011). Plant volatile-mediated indirect defence against insect herbivory is one area which may be affected by such a mechanism under environmental change. Even though it is hypothesised that abiotic stressors may induce production of VOCs which act as anti oxidative agents protecting against oxidative damage (Peñuelas & Staudt, 2009; Vickers et al., 2009a), changes in quantity and quality of the VOCs may limit the attraction of natural enemies and parasitoids. In incidences of pest outbreaks, plant productivity may therefore be limited due to compromised defence against

herbivory even when there is sufficient VOC mediated protection against abiotic stress induced oxidative damage.

It is therefore important to understand the consequence of such environmental changes on plant-insect interactions as they may have direct implications on crop performance in agroecosystems. For example, Tariq et al. (2013) reported a change in volatile emission profiles due to interactive effects of root herbivory and drought stress with a subsequent reduced pest parasitism by parasitoids. Such studies show the potency that environmental changes and abiotic stressors have in reshaping the structure and function of ecosystems. However, many studies focus on the changes in the ecosystem structure and composition (e.g. Yang et al., 2011). Many aspects of environmental change on insects have focused on changes in geographic distribution (e.g. Parmesan et al., 1999; Parmesan, 2006), plasticity to thermal variability (e.g. Kingsolver et al., 2004; Terblanche & Chown, 2006; Chidawanyika & Terblanche, 2011; Cooper et al., 2012) and responses to changes in plant nutritional quality (e.g. Neto et al., 2012; Gutbrodt et al., 2011; 2012; Bauerfeind & Fischer, 2013a; Chidawanyika et al., 2014). However, for plant quality and the outcome of plant-insect interactions under environmental change, much of this focus has been on herbivore-plant interactions. Very few studies focus on the direct interactions of plants and natural enemies or parasitoids of insect herbivores. This is despite the knowledge that the impact of changing climates on interactive natural enemies and/ parasitoids may be mediated by the initial responses, or lack thereof, of their host species (including plants) to climate induced stress (Hance et al., 2007).

For the push-pull system, responses of various endoparasitoids to volatiles from intact companion plants have previously been tested exclusively under optimal conditions (e.g. Gohole et al., 2003). Here, I investigated the impact of acute drought stress on the emission of ecologically relevant VOCs and their subsequent effect on the electrophysiological and

behavioural responses of larval parasitoids. I hypothesised that; i) acute drought stress does not change the volatile emission profiles of legumes and grasses used in the push-pull system ii) drought induced changes in emission profiles of VOCs do not affect the behavioural and electrophysiological responses of *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae) parasitoids. *Cotesia sesamiae* parasitoids are an important biological control agent for cereal stemborer pests in farming systems of sub-Saharan Africa (Gohole et al., 2003). If left uncontrolled, yield losses of up to 80% in strategic crops such as maize (*Zea mays* L.) have been reported (Kfir et al., 2002). Through parasitisation of the pests during the larval stage where crop damage occurs, *C. sesamiae* is an important component of biologically based control programmes targeting Lepidopteran stemborers.

5.1 Materials and Methods

5.1.1 Study site

The studies were carried out at the International Centre of Insect Physiology Thomas Odhiambo Campus (ITOC) situated at Mbita Point (1 200m above sea level), western Kenya (0°25`S, 34°12`E). Despite relatively high annual rainfall (average 900mm), periodic regimes of water deficits that may induce acute drought stress responses in plants are highly prevalent in this region.

5.1.2 Insects and plants

All *C. sesamiae* insects for the study were sourced from a colony maintained at ITOC. These parasitoids were reared on a stemborer larvae diet following methods previously described by Overholt et al. (1994). Adult parasitoids had access to 20% honey: water for feeding. In all experiments, day old mated females with no previous encounter with plants were used. Six plant species that were used as test plants comprised of 2 legumes and 4 grass species which

were planted in perforated 5 l buckets. Vertisol soils were used for the planting as previously described in Chidawanyika et al. (2014). *Desmodium intortum* and *D. uncinatum* were planted using purchased seeds from Kenya seed company, Nairobi Kenya. Napier grass variety 'bana', molasses and signal grasses *Brachiaria brizantha* (A. Rich) Stapf, were all grown from root splits. *Brachiaria* cv Mulato [a hybrid developed by International Centre for Tropical Agriculture (CIAT), Columbia] was planted from seeds.

5.1.3 Plant treatment and volatile collection

Treatment plants were prepared by withdrawing irrigation for 10-13 days at 3 weeks after planting. In the same interim, control plants received optimal irrigation as was the case soon after planting (see Chidawanyika et al., 2014). Thereafter, a headspace sampling protocol previously described by Agelopoulos et al. (1999) was used to entrain the volatiles. In brief, leaves of the treatment and control plants in their different levels of herbivory were enclosed in 3.2 litre polyethyleneterephthalate (PET) bags (~12.5 mm thick) previously heated to 150 °C before use. Charcoal filtered air was then pumped and drawn through Swagelock inlet and outlet ports fitted on the bag at a rate of 600 and 400 mL min^{-1} respectively for 48 hrs. Porapak Q (0.05 g, 60/80 mesh; Supelco) inserted in all the outlet ports was responsible for the actual collection of the volatiles which upon retrieval were eluted using 0.5 mL of dichloromethane. Thereafter, eluted samples were kept in vials for further use in future bioassays, electrophysiological and chemical analyses. Each of these treatments and their control were replicated 5 times.

5.1.4 Electroantennogram (EAG) Recording

GC-EAG responses to samples of plant volatile compounds from treated or control plants were recorded using previously described methods (Tamiru et al., 2011; 2012). An excised head capsule of the female *C. sesamiae* was mounted on an indifferent electrode. EAG recordings were then done by Ag–AgCl glass electrodes that were filled with glucose-free saline solution (Maddrell, 1969). For each run, an antenna was inserted into the tip of the electrode using micromanipulators. To ensure good contact, the tip of the terminal part of each antenna was used in the bioassays was removed to ensure a good contact. Signals from the electrophysiological responses were passed through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and recorded using specialized software package (Syntech). The GC-EAG system where the effluent from the GC column is simultaneously channelled to the antennal preparation and the GC detector, has been described previously (Wadhams, 1990). A GC (Agilent Technologies, 6890N) equipped with a cold on-column injector and a FID using a HP-1 column (50 m, 0.32 mm ID, 0.52 lm film thickness) was used for separation of the volatiles. Helium was used as the carrier gas for the odours. For each GC run, the oven temperature was maintained at 30 °C for 2 mins and then ramped up at 15 °C min^{-1} to 250 °C. The outputs from the amplifier and the FID were then monitored simultaneously and analysed using the Syntech software package. For all plant samples, at least 5 replications were done where peaks eluting from the GC column were only deemed to be active if they elicited EAG activity in at least three runs.

5.1.5 Four arm olfactometer bioassay

A perspex four-arm olfactometer (Pettersson 1970) previously by Tamiru eta l., (2011) was used to test the response of parasitoids to the volatiles. Aliqouts of the headspace samples (10 µl) were applied using a micropipette (Drummond 'microcap', Drummond Scientific Co. Broomall, PA, USA.) to a piece of filter paper (4x 25) before being inserted in each arm of the olfactometer. Air was drawn into the four arms at a rate of 260 mL min^{-1}. Naïve gravid female parasitoids were individually placed in the central chamber of the olfactometer using custom-made glass tubing. Orientation of the arms in each test was such that control solvent-carrying arms were opposite each other as was the case with the ones with the headspace samples being assayed. This arrangement would allow a choice test between volatiles from the treatments and their respective controls for each plant species by comparing time spent in different regions of the olfactometer using 'Olfa' software (F. Nazzi, Udine, Italy). Each of these experiments was replicated 12 times with new parasitoids and clean olfactometers each time. The response of the parasitoids to the headspace samples was further verified using authentic standards of synthetic compounds at a dosage of 1 µg/10µL. A synthetic compound was applied in one of the arms whilst the rest acted as blank controls carrying solvent only. Furthermore, a blend of the synthetic headspace comprising of similar ratios and concentration as in the attractive headspace samples was tested.

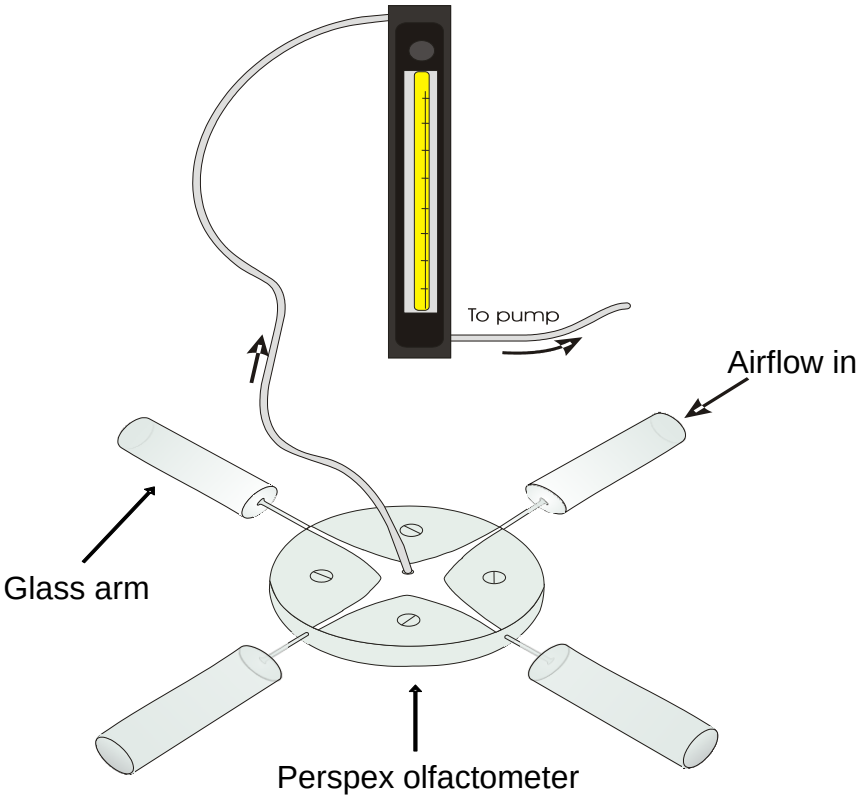


Figure 5.1: Schematic diagram of the four-arm olfactometer that was used to assay for behavioural responses in *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae).

5.2 Statistical analyses

Data from the four-arm olfactometer bioassays were treated as compositional and first converted into proportions and logratio transformations before comparisons were done using analysis of variances (ANOVAs). Significant difference of the means were tested using Fisher`s LSD test.

5.3 Results

5.3.1 Olfactometer behavioural responses to head space samples

Parasitoid behavioural responses The gravid *C. sesamiae* females were more attracted to the volatile samples from drought stressed Napier grass compared to the irrigated ones ($F_{1,38}$ = 8.96, P = 0.001). The attractiveness of volatiles from molasses grass and Mulato were not different at both drought or irrigated conditions (molasses grass: $F_{1,36}$ = 3.2, P = 0.63; Mulato: $F_{1,36}$ = 4.3, P = 0.77). Similarly, there was no significant difference between the responses to volatiles from drought stressed signal grass ($F_{1,36}$ = 3.66, P = 0.81). Volatiles from drought stressed Silverleaf *Desmodium* and its control elicited no significant differences in the behavioural responses of the parasitoids $F_{1,38}$ = 4.34, P = 0.002. However, volatiles from drought stressed Greenleaf *Desmodium* were more attractive to the parasitoids compared to their corresponding optimally irrigated plants ($F_{1,38}$ = 6.87, P = 0.002).

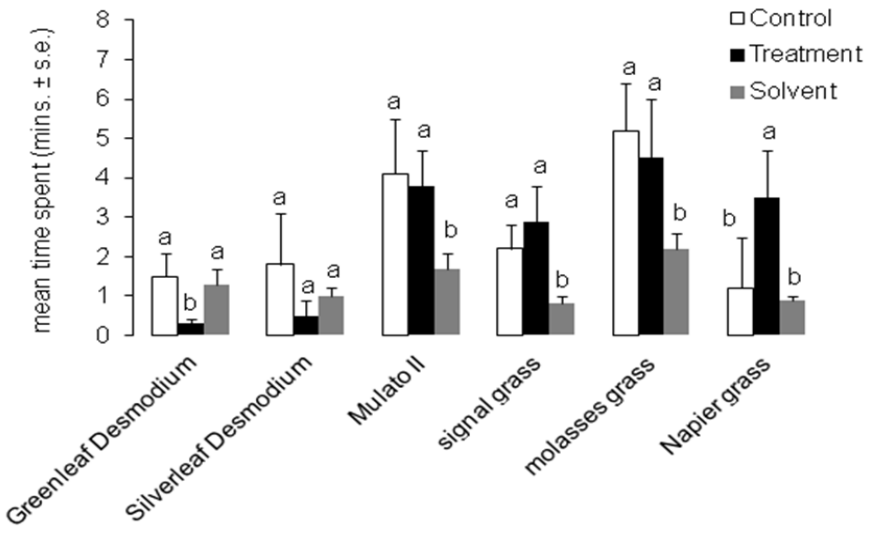
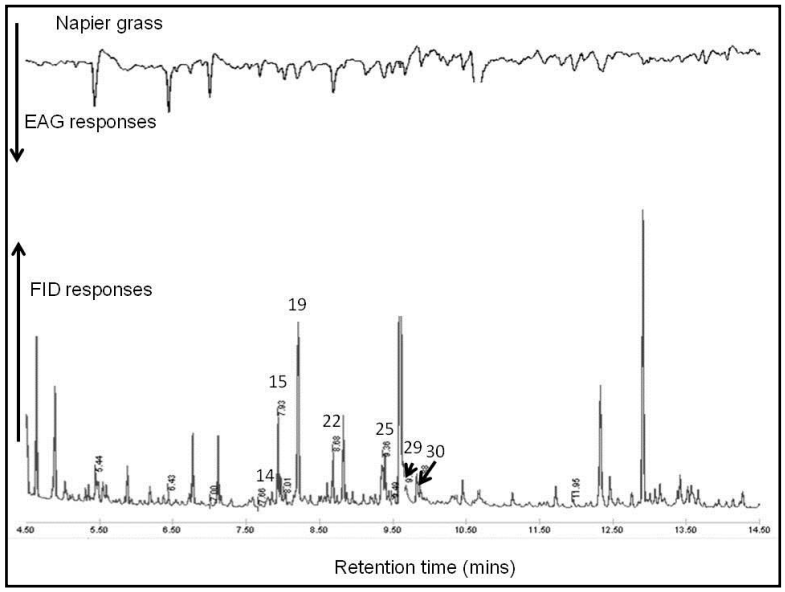
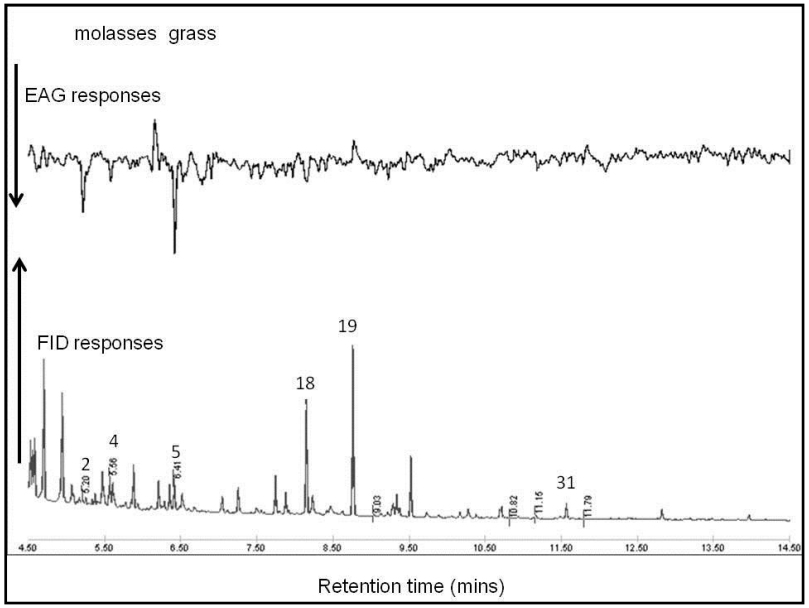


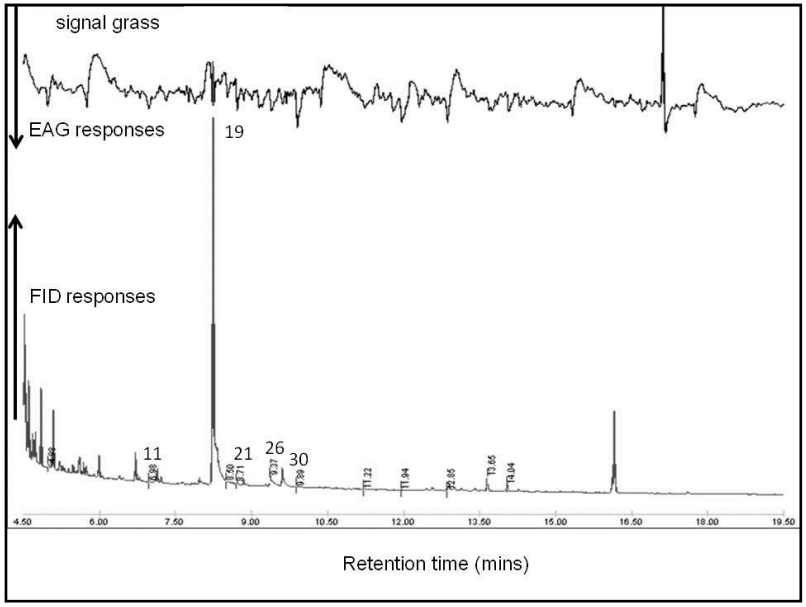
Figure 5.2: Responses of *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) parasitoids to plant volatiles from drought stressed and irrigated grasses and legumes of the push-pull system. Each parasitoid was assayed for 12 minutes in a four-arm olfactometer.

5.3.2 Identification of attractive compounds

Samples of the drought stressed grasses were subjected to GC-EAG analysis. Gravid *C. sesamiae* insects detected 36 compounds from all the drought stressed grasses. Of these detected compounds, only 5 were unidentified. In four arm olfactometer bioassays all individual compounds were tested for their attractiveness to *C. sesamiae* using 1 µg dosages of authentic standards. Ocimene, Linalool, DMNT and TMTT were more attractive when compared with blank (solvent) controls. Z-3-hexen-1-ol acetate, α-pinene, caryophyllene, indole and eugenol were not attractive at this dosage.


Napier grass
EAG responses
FID responses
19
15
22
25
29
30
14
Retention time (mins)


molasses grass
EAG responses
FID responses
19
18
4
2
5
31
Retention time (mins)


signal grass
EAG responses
19
FID responses
11
21
26
30
Retention time (mins)

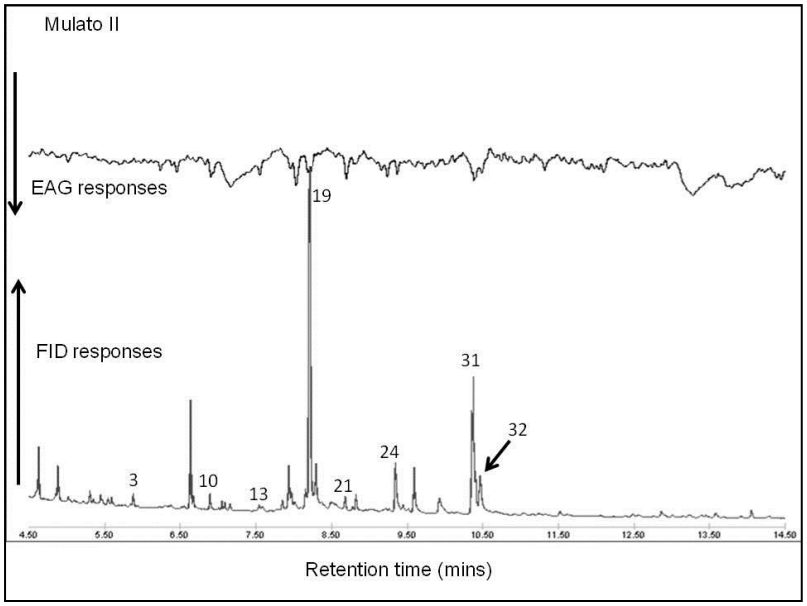

Mulato II
EAG responses
19
FID responses
31
32
24
3
10
13
21
Retention time (mins)

Figure 5.3: Electrophysiological responses of *Cotesia sesamiae* to volatiles from drought stressed grasses of the push-pull system. Represented compounds are listed below:

1, methyl propanoic acid; 2, toluene; 3, (1*E*)-2-methyl butanal oxime; 4, *unidentified* ; 5, 3 methyl butanoic acid; 6, 3-hexen-1-ol (*Z*); 7, (1Z) methyl butanal oxime; 8, trans2-ethyl-2-hexen-1-ol; 9, ethyl benzene; 10, heptanal; 11, (*Z*) 3-hexen-1-ol acetate; 12, dimethyl pyrazine; 13, α-pinene; 14, methyl-3-pentenoic acid; 15, *E*-ocimene; 16, linalool; 18, methyl salicylate; 19, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT); 20,p cymene; 21, limonene; 22, methyl benzoate; 23, nonanal; 24, *unidentified*, 25, 2-decanone, 26, dimethyl benzal dehyde 26, methyl salicylate; 27, decanal; 28; *unidentified*; 29, indole; 30, verbanol; 31, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); 32, α-muurolene.

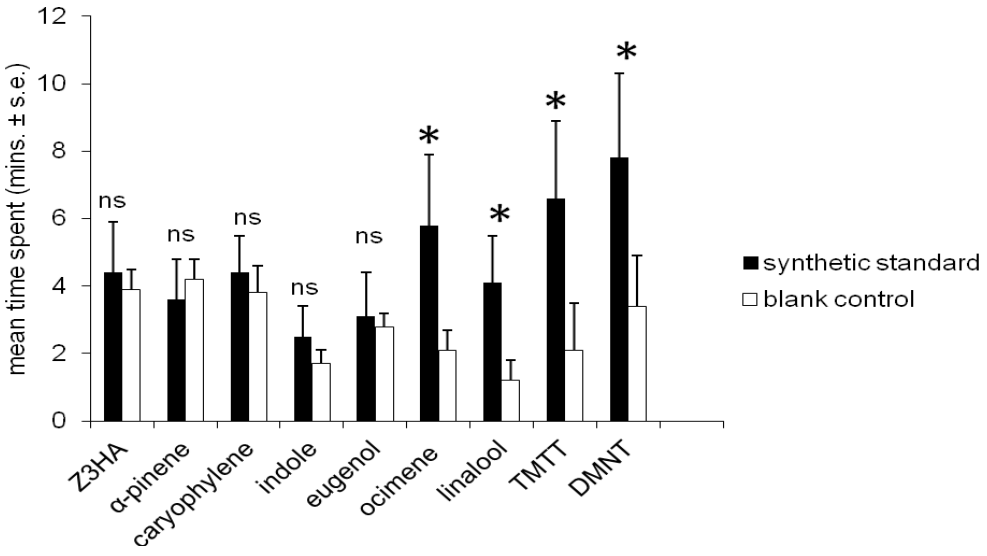


Figure 5.4: Behavioural responses of *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) to synthetic standards. For all compounds, bioassays were done using a dosage of 1µg/10µl in a 4 arm Perspex olfactometer.

5.4 Discussion

In the current study, acute drought stress resulted in variable changes in quantity and quality of the VOCs of grasses and legumes that were tested as outlined in chapter 4. These differential changes in chemical properties of the various VOCs elicited behavioural and electrophysiological responses in the parasitoid *C. sesamiae*. In Napier grass the increase in the amount of DMNT in drought stressed plants resulted in increased attraction of *C. sesamiae.* However this attraction of the parasitoids to drought stressed plants in signal grass was not significantly different from control (irrigated) plants despite having a significant increase in the emission of DMNT. This further supports a report by Bruce and Pickett (2011) that odour blends need to be of the correct mixes in order to elicit specific behavioural responses. Hence despite plant VOCs carrying known attractive compounds such as DMNT,

they may not always be attractive to parasitoids if they are not in certain relative proportions of the blend (see discussions in Bruce & Pickett 2011).

Volatiles from drought stressed molasses grass, Mulato II and Greenleaf *Desmodium* remained attractive to the gravid *C. sesamiae* parasitoids. This attraction can be attributed to the stable emission of attractive terpenes under drought-stress. However in molasses grass and Mulato II there was no significant difference in the attraction of the parasitoids between control and treatment plants as the plants were attractive in both control and treatment conditions.

The above results therefore suggest that the manipulation of agroecosystems for pest control is possible under drier conditions. However, the results should be accepted with caveats as I did not test for actual parasitism of pests under field conditions. Field tests to determine if this parasitoid attraction to plant volatiles under drought stress results in increased pest parasitism. In other studies, Tariq et al. (2013) reported that parasitoids preferred odour from well watered plants as drought resulted in reduced emission of volatiles. Drought alone resulted in reduced percentage parasitism of aphids by parasitoids by 40-55% (Tariq et al., 2013). Future studies should endeavour to investigate how drought may impact the actual pest predation by parasitoids in the push-pull system.

CHAPTER 6

Conclusions and recommendations

6.0 Concluding remarks

In conclusion, drought differentially affects plant responses with subsequent impact on insect host preferences and performance:

- Gravid moths did not accept laying eggs on drought stressed Napier and Signal grasses due to the changes in volatile quantity and quality in the host plants (Chapters 3 and 4). Even though larval development remained poor in the species after stress, Napier and signal grasses may not be as effective in the push-pull if they are used as the attractive trap plants during periods of water deficits.
- The onset of drought/water deficit by plants generally results in an increase in emission of plant VOCs. In particular the emission of terpenoids which may play a role in relieving plants from oxidative damage increases.
- Such terpenoids also play a role in plant-insect interactions of the push-pull by acting as repellents to *Chilo partellus* moths and attractants to the larval parasitoid *Cotesia sesamiae* (Chapters 4 and 5).

The current studies are however in no way exhaustive, several interesting questions stem from the current findings. For example, the capacity of the respective repulsion and attraction of moths and parasitoids by the terpenoids under field conditions is yet to be tested. Further studies, may consider testing the role of such terpenes from companion plants such as Napier and signal grasses and the efficacy they may confer to the push-pull in drier environments.

Furthermore, the attraction of parasitoids even when they may be no larval prey present may present long term evolutionary problems in host location for the parasitoids. Ideally, insect-host relationship will be strengthened when a particular odour is associated with a reward (larvae for oviposition in this instance). Most hymenopteran organisms such as

parasitoids are credited for their high capacity of associative learning. Further comparative studies may help unravel such associative learning behavior in *C. sesamiae* and potential changes that may be brought by rapid behavioural evolution under environmental stress. Such findings may also be used to manipulate insect behaviour for increased efficacy of the push-pull system in drier environments.

In addition, the current study just focused on how the insects respond to plant-mediated environmental change. However, environmental factors such as temperature variability can directly influence insect performance (e.g. Chidawanyika et al., 2010, 2011). Hence to get more insight on the impact of climate change on the push-pull system, future studies may also investigate how direct impacts of climate on insect physiology and ecology can influence the trophic interactions of the push-pull system.

7.0 REFERENCES

Agelopoulos NG, Hooper AM, Maniar SP, Pickett JA & Wadhams LJ (1999) A novel approach for isolation of volatile chemicals released by individual leaves of a plant *in situ*. *Journal of Chemical Ecology* 25: 1411-1425.

Agrawal AA, Tuzun S & Bent E (1999) Induced plant defence: evolution of induction and adaptive phenotypic plasticity. in *Inducible plant defenses against pathogens and herbivores: Biochemistry, ecology, and agriculture* American Phytopathological Society Press, St. Paul, MN. USA pp 251-268.

Ahuja I, de Vos, RCH, Bones AM & Hall RD (2010) Plant molecular stress responses face climate change. *Trends in Plant Science* 15: 664-674.

Allmann S, Späthe A, Bisch-Knaden et al. (2013) Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. *eLife* 2: e00421. DOI: 10.7554/eLife.00421.

Amudavi DM, Khan ZR, Wanyama JM, Midega CAO, Pittchar J, Hassanali A & Pickett JA (2008) Evaluation of farmers' field days as a dissemination tool for push-pull technology in Western Kenya. *Crop Protection* 28: 225-235.

Angilletta MJ (2009) Thermal Adaptation. In *A Theoretical and Empirical Synthesis*; Oxford University Press: Oxford, UK.

Apel K & Hurt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* 55: 373–399.

Aquino PF, Calvo CR & Flores D (2001) Selected maize statistics. In P L. Pingali, eds., *CIMMYT 1999-2000 world maize facts and trends. Meeting world maize needs: technological opportunities and priorities for the public sector. CIMMYT*, Mexico, D. F. Pages 45-57.

Atkinson NJ & Urwin P (2012) The interaction of plant biotic and abiotic stresses: from genes to field. *Journal of Experimental Botany* 63: 3523-3543.

Bale JS, Masters GJ, Hodgkinson D et al. (2002) Herbivory in global climate change research: direct effects of rising temperatures on insect herbivores. *Global Change Biology* 8: 1-16.

Banziger M, Setimela PS, Hodon D & Vivek B (2006) Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. *Agricultural Water Management* 80: 212-224.

Basson CH, Nyamukondiwa C & Terblanche JS (2011) Fitness costs of rapid cold-hardening in *Ceratitis capitata*. *Evolution* 66: 296–304.

Battisti A, Stastny M, Buffo E & Larson S (2006) A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly *Global Change Biology* 12: 662-671.

Bauerfeind SS & Fischer K (2013a) Increased temperature reduces herbivore host-plant quality. *Global Change Biology* 19: 3272-3282.

Bauerfeind SS & Fischer K (2013b) Targeting the right trait: The relative suitability of a host plant depends on the herbivore trait considered and ambient temperature. *Basic and Applied Ecology* 14: 555-564.

Behmer ST (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology* 54: 165-187.

Berger A (1981) *Biological control of the spotted stalkborer, Chilo partellus* (Swinhoe) *in maize by using the bacteria Bacillus thuringiensis. Rep. Inst. Nacional de Investigaáao Agronomica.Proj. UNDP/FAO MOZ/75/009*, Maputo, Mozambique.

Berry PM, Dawson TP, Harrison PA & Pearson RG (2002) Modelling potential impacts of climate change on the bioclimatic envelope of species in Britain and Ireland. *Global Ecology and Biogeography* 11: 453–462.

Birkett MA, Chamberlain K, Khan ZR, Pickett JA, Toshova T, Wadhams LJ & Woodcock CM (2006) Electrophysiological responses of the Lepidopterous stemborers *Chilo partellus* and *Busseola fusca* to volatiles from wild and cultivated host plants. *Journal of Chemical Ecology* 32: 2475-2487.

Blande JD, Holopainen JK & Niinemets Ü (2014) Plant volatiles in polluted atmospheres: stress responses and signal degradation. *Plant, Cell and the Environment* 37: 1892-1904.

Blaney WM & Simmonds MSJ (1988) Food selection in adult and larvae of three species of Lepidoptera: a behavioural and electrophysiological study. *Entomologia Experimentalis et Applicata* 49: 111-121.

Blundell AG & Peart DR (2000) High abscission rates of damaged expanding leaves: field evidence from seedlings of a Bornean rain forest tree. *American Journal of Botany* 87: 1693-1698.

Borlaug N (2007) Feeding a hungry world. *Science* 318: 359.

Bowler K (2005) Acclimation, heat shock and hardening. *Journal of Thermal Biology* 30: 125–130.

Bowler K & Terblanche JS (2008) Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? *Biological Reviews* 83: 339–355.

Bosque-P´erez NA & Schulthess F (1998) Maize: West and Central Africa. In Polaszek, ed. African Cereal Stemborers Economic Importance, Taxonomy, Natural Enemies and Control CAB International. pp. 11–27.

Bradshaw WE & Holzapfel CM (2006) Evolutionary response to rapid climate change. *Science* 312: 1477–1478.

Brodbeck B & Strong D (1987) Amino acid nutrition of herbivorous insects and stress to host plants. *Insect Outbreaks: Ecological and Evolutionary Perspectives* (ed. by P Barbosa & JC Schultz), pp. 347–364. Academic Press, New York, NY, USA.

Bruce TJA, Wadhams LJ & Woodcock CM (2005) Insect host location: a volatile situation. *Trends in Plant Science* 10: 269-274.

Bruce TJA & Pickett JA (2007). Plant defence signalling induced by biotic attacks. *Current Opinion in Plant Biology* 10: 387-392.

Bruce TJA, Midega CAO, Birkett MA, Pickett JA & Khan ZR (2010) Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. *Biology Letters* 6: 314-317.

Bruce TJA & Pickett JA (2011) Perception of plant volatile blends by herbivorous insects-finding the right mix. *Phytochemistry* 72: 1606-1611.

Bruinsma M, van Loon JJA & Dicke M (2010) Increasing insight into induced plant defense mechanisms using elicitors and inhibitors. *Plant Signaling and Behavior* 5: 271-274.

Buchbauer G, Jirovetz L, Wasicky M & Nikiforov A (1994) Comparative investigation of Douglas fir headspace samples, essential oils, and extracts (needles and twigs) using GC-FID and GC-FTIR-MS. *Journal of Agriculture and Food Chemistry* 42: 2852–2854.

Burke MB, Lobell DB & Guarino L (2009) Shifts in African crop climates by 2050, and the implications for crop improvement and genetic resources conservation. *Global Environmental Change* 19: 317-325.

Cairns J, McCormick PV & Niederlehner BR (1993) A proposed framework for developing indicators of ecosystem health. *Hydrobiologia* 236: 1–44.

Cammell ME & Knight JD (1992) Effects of climatic change on the population dynamics of crop pests. *Advances in Ecological Research* 22: 117–162.

Cannon RJC (1998) The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biology* 4: 785–796.

Calatayud P-A, Guénégo H, Ahuya P, Wanjoya A, Le Rü B et al. (2008) Flight and oviposition behaviour of the African stemborer, *Busseola fusca*, on various host plant species. *Entomologia Experimentalis et Applicata* 129: 348-355.

Chamberlain K, Khan ZR, Pickett JA, Toshova T & Wadhams LJ (2006) Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chilo partellus* and *Busseola fusca*. *Journal of Chemical Ecology* 32: 565-577.

Chaves MM, Maroco JP & Pereira JS (2003) Understanding plant responses to drought: from genes to the whole plant. *Functional Plant Biology* 30: 239-264.

Chidawanyika, F & Terblanche JS (2010) Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of Insect Physiology* 57: 108–117.

Chidawanyika, F & Terblanche JS (2011) Costs and benefits of thermal acclimation for codling moth, ***Cydia pomonella*** (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. *Evolutionary Applications* 4: 534–544.

Chidawanyika F, Midega CAO, Bruce TJA, Duncan F, Pickett JA & Khan ZR (2014) Oviposition acceptance and larval development of *Chilo partellus* stemborers in

drought-stressed wild and cultivated grasses of East Africa. *Entomologia Experimentalis et Applicata* 151: 209-217.

Chou T, Tso H & Chang L (1984) Stereoselective one-step syntheses of trans ocimene and alpha farnesene. *Journal of the Chemical Society, Chemical Communications* 20: 1323-1324.

Chown SL & Nicolson SW (2004) *Insect Physiological Ecology: Mechanisms and Patterns*; Oxford University Press: Oxford, UK.

Chown SL & Terblanche JS (2007) Physiological diversity in insects: Ecological and evolutionary contexts. *Advances in Insect Physiology* 33: 50–152.

Chown SL, Slabber S, McGeoch MA, Janion C & Leinaas HP (2007) Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society of London B* 274: 2661–2667.

Chown SL, Hoffmann AA, Kristensen T, Angilletta M & Strenseth N (2010) Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* 43: 3–15.

Chown SL, Sørenson JG & Terblanche JS (2011) Water loss in insects: An environmental change perspective. *Journal of Insect Physiology* 57: 1070–1084.

Christensen JH, B Hewitson, A Busuioc, A Chen, X Gao et al. (2007) Regional Climate Projections. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (ed. by S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt M Tignor & HL Miller), pp. 847-940. Cambridge University press, Cambridge, UK.

Colliera T & van Steenwyk R (2004) A critical evaluation of augmentative biological control. *Biological Control* 31: 245–256.

Colwell RK, Brehm G, Cardelus CL, Gilman AC & Longin JT (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* 322: 258–261.

Conrath U, Beckers GJM, Flors V, et al. (2006). Priming: Getting ready for battle. *Molecular Plant-Microbe Interactions* 19: 1062-1071.

Cook SM, Khan ZR & Pickett JA (2007) The use of 'push-pull' strategies in integrated pest management. *Annual Review of Entomology* 52: 375-400.

Cooper BS, Tharp JM, Jernberg II & Angilletta MJ (2012) Developmental plasticity of thermal tolerances in temperate and subtropical populations of *Drosophila melanogaster Journal of Thermal Biology* 37: 211-216.

Cornelissen T, Wilson FG & Vasconcellos-Neto J (2008) Size does matter: variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos* 117: 1121-1130.

Corrêa-Ferreira BS (1993) Utilização do parasitóide de ovos Trissolcus basalis (Wollaston) no controle de percevejos da soja. In *Circular Técnica 11*; Embrapa-CNPSo: Londrina, Brazil, pp. 40.

Corrêa–Ferreira, BS & Moscardi F (2011) Biological control of soybean stink bugs by inoculative releases of *Trissolcus basalis*. *Entomologia Experimentalis et Applicata* 79: 1–7.

Cossins A & Bowler K (1987) *Temperature Biology of Animals*; Chapman and Hall: London, UK.

Crandal K (2009) A multifacted approach to species conservation. *Animal Conservation* 12: 105–106.

Crozier L & Dwyer G (2006) Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. *American Naturalist* 167: 853–866.

Curkovic T & Brunner JF (2006) Evaluation of permethrin for attracticide development against *Choristoneura rosaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae) males. *Crop Protection* 25: 973-976.

Curkovic T, Brunner JF & Landolt PJ (2009) Field and laboratory responses of male leaf roller moths, *Choristoneura rosaceana* and *Pandemis pyrusana*, to pheromone concentrations in an attracticide paste formulation. *Journal of Insect Science* 9: 1-14.

Daane KM, Yokota GY, Zheng Y & Hagen KS (1996) Inundative release of common green lacewings (Neuroptera: Chrysopidae) to suppress *Erythroneura variabilis* and *E. elegantula* (Homoptera: Cicadellidae) in vineyards. *Environmental Entomology* 25: 1224–1234.

Dale VH & Suzanne CB (2001) Challenges in the development and use of ecological indicators. *Ecological Indicators* 1: 3–10.

Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B & Wood S (1998) Making mistakes when predicting shifts in species range in response to global warming. *Nature* 391: 783–786.

Degen T,Bakalovic N, Bergvinson D & Turlings TCJ (2012) Differential performance and parasitism of caterpillars on maize inbred lines with distinctly different herbivore-induced volatile emissions *PLOS ONE* 7: e47589.

Degenhardt J (2009) Indirect defense responses to herbivory in grasses. *Plant Physiology* 149: 96-102.

DeLucia EH, Nabity PD, Zavala JA & Berenbaum MR (2012) Climate change: resetting plant-insect interactions. *Plant Physiology* 160: 1677-1685.

De Moraes CM, Mescheer MC & Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature* 410: 577-580.

De Groote H, Vanlauwe B, Rutto E, Odhiambo GD, Kanampiu F & Khan ZR (2010) Economic analysis of different options in intergrated pest and soil fertility management in maize systems of Western Kenya using categorical data analysis. *Agricultural Systems* 103: 233-244.

Denlinger D & Lee RE (2010) *Low Temperature Biology of Insects*; Cambridge University Press: Cambridge, UK.

Derridj S, Fiala V & Jolivet E (1986) Increase of European corn borer (*Ostrinia nubilalis*) oviposition induced by treatment of maize plants with maleic hydrazide: role of leaf carbohydrate. *Entomologia Experimentalis et Applicata* 41: 305-310.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC & Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the USA* 105: 6668–6672.

Dicke M & Baldwin I (2010) The evolutionary context of herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science* 15: 167-175.

Dicke M & Loreto F (2009) Induced plant volatiles: from genes to climate change. *Trends in Plant Science* 15: 115-117.

Dicke M & Sabelis MW (1988) How plants obtain predatory mites as bodyguards. *Netherlands Journal of Zoology* 38: 148-165.

Dillon ME, Wang G & Huey RB (2010) Global metabolic impacts of recent climate warming. Nature 476: 704–707.

Duale AH (2005) Effect of temperature and relative humidity on the biology of the stem borer parasitoid *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae) for the management of stem borers. *Environmental Entomology* 34: 1–5.

Dudareva N, Pichersky E & Gershenzon J (2004) Biochemistry of plant volatiles. *Plant Physiology* 135: 1893–1902.

Dyer JM (2005) Implications of habitat fragmentation on climate change-induced forest migration. *Professional Geographer* 46: 449–459.

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: Observations, modelling, and impacts. *Science* 289: 2068–2074.

Ebel RC, Mattheis JP & Buchanan DA (2006) Drought stress of apple trees alters leaf emissions of volatile compounds. *Physilogia Plantarum* 93: 709-712.

Efeoğlu B, Ekmekçi Y & Çiçek N (2009) Physiological responses of three maize cultivars to drought stress and recovery. *South African Journal of Botany* 75: 34-42.

Eilenberg J, Hajek A & Lomer C (2001) Suggestions for unifying the terminology in biological control. *Biological Control* 46: 387–400.

El-Beltagy, A. Madkour, M. (2012). Impact of climate change on arid lands agriculture. *Agriculture and Food Security* 1: 3-15.

English-Loeb G, Stout MJ & Duffey SS (1997) Drought stress in tomatoes: changes in plant chemistry and potential nonlinear consequences for insect herbivores. *Oikos* 79: 456-468.

Estay SA, Lima M & Labra FA (2009) Predicting insect pest status under climate change scenarios: combining experimental data and population dynamics modelling. *Journal of Applied Entomology 133*: 491–499.

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology Evolution and Systematics* 34: 487–515.

Fargues J, Ouedraogo A, Goettel MS & Lomer CJ (1997) Effect of temperature, humidity and inoculation method on susceptibility of *Schistocerca gregaria* to *Metarhizium flavoviride*. *Biocontrol Science and Technology 7*: 345–356.

Fiala V, Glad C, Martin M, Jolivet E & Derridj S (1990) Occurrence of soluble carbohydrates on the phylloplane of maize (*Zea mays* L.): variations in relation to leaf heterogeneity and position on the plant. *New Phytologist* 115: 609-615.

Fischer K, Eenhoorn E, Bot ANM, Brakefield PM & Zwaan BJ (2003) Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. *Proceedings of the Royal Society of London B* 270: 2051–2056.

Frich P, Alexander LV, Della-Marta P, Gleason B, Haylock M, Tank AMK & Peterson T (2002) Observed coherent changes in climatic extremes during the second half of the twentieth century. *Climate Research* 3: 193–212.

Fujita M, Fujita Y, Noutoshi Y et al. (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology* 9: 436-442.

Fortunati A, Barta C, Brilli F, Centritto M, Zimmer I, Scnitzler JP & Loreto F (2008) Isoprene emission is not temperature dependent during and after severe drought-stress: a physiological and biochemical analysis. *Plant Journal* 55: 687–697.

Gaoni Y (1977) Lithium aluminium hybride promoted extrusion of SO2 from sulfolenes and sulfolene-adducts. 1, 3-Dienes, 3-chloro- and 3,3 –dichloro-1,4-di-enes and skipped polyenes. *Tetrahedron Letters* 11: 947-950.

Gershenzon J (1984) *Phytochemical adaptations to stress. Recent Advances in Phytochemistry*, Vol. 18 (ed. by BN Timmermann, C Steelink & FA Loewus), pp. 273-320. Plenum Press, New York, NY, USA.

Gershenzon J (1994) Metabolic costs of terpenoid accumulation in higher plants. *Journal of Chemical Ecology* 20: 12811328.

Gershenzon J & Dudareva N (2007) The function of terpene natural products in the natural world. *Nature Chemical Biology* 3: 408-414.

Gershenzon J (2008) Insects turn up their noses at sweating plants. *Proceedings of the National Academy of Sciences of the USA* 105: 17211–172212.

Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ & Wang G (2007) Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative Comparative Biology* 46: 5–17.

Gohole LS (2003) Enhancing Foraging Behaviour of Stemborer Parasitoids: Role of non-Host Plant *Melinis minutiflora*. *PhD Thesis, Wageningen University, Netherlands*. ISBN: 90-5808-800-6.

Gohole LS, Overholt WA, Khan ZR, Pickett JA & Vet LEM (2003) Effects of molasses grass, *Melinis minutiflora*Volatiles on the foraging behaviour of the cereal stemborer parasitoid, *Cotesia sesamiae. Journal of Chemical Ecology* 29: 731-745.

Gouinguené SP, Turlings TCJ & Degen T (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* 11: 9-16.

Gouinguené SP & Turlings TC (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology* 129: 1296-1307.

Gouinguené SP, Alborn H & Turlings TCJ (2003) Induction of volatile emissions in maize by different larval instars of *Spodoptera littoralis*. *Journal of Chemical Ecology* 29: 145-162.

Guisan A & Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters* 8: 993–1009.

Gutbrodt B, Mody K & Dorn S (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* 120: 1732-1740.

Gutbrodt B, Dorn S & Mody K (2012a) Drought stress affects constitutive but not induced herbivore defence resistance in apple plants. *Arthropod-Plant Interactions* 6: 171-179.

Gutbrodt B, Dorn S, Unsicker SB & Mody K (2012b) Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry. *Chemoecology* 22: 101-111.

Gutierrez AP, Ponti L, d'Oultremont T & Ellis CK (2008) Climate change effects on poikilotherm tritrophic interactions. *Climate Change* 87: S167–S192.

Hale BK, Herms DA, Hansen RC, Clausen TP & Arnold D (2005) Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid induced resistance of poplar to two lymantriid defoliators. *Journal of Chemical Ecology* 31: 2601-2620.

Hance T, van Baaren J, Vernon P & Boivin G (2007) Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology* 52: 107–126.

Hanski I & Mononen, T (2011) Eco-evolutionary dynamics of dispersal in spatially heterogeneous environments. *Ecology Letters* 14: 1025–1034.

Hardie J, Isaacs R, Pickett JA, Wadhams LJ & Woodcock CM (1994) Methyl salicylate and (-)-(1*R*, 5S)-myrtenal are plant derived repellents of black bean aphid, Aphis fabae Scop. (Homoptera: Aphididae). *Journal of Chemical Ecology* 20: 2847-2855.

Harris KM (1962) Lepidopterous stem borers of cereals in Nigeria. *Bulletin of Entomological Research* 53:139–71.

Harris KM (1990) Bioecology and *Chilo* species. *International Journal of Tropical Insect Science* 11:467–77.

Hartley S, Krushelnycky PD & Lester PJ (2010) Integrating physiology, population dynamics and climate to make multi-scale predictions for the spread of an invasive insect: The Argentine ant at Haleakala National Park, Hawaii. *Ecography* 33: 83–94.

Hassanali A, Herren H, Khan ZR, Pickett JA & Woodcock CM (2008) Integrated pest management: the push-pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. *Philosophical Transactions of the Royal Society* B 363: 611-621.

Heikinnen RK, Luoto M, Araujo MB, Virkkala R, Thuiller W & Sykes MT (2006) Methods and uncertainties in bioclimatic envelope modelling under climate change. *Progress in Physical Geography* 6*:* 1–27.

Hendry AP, Kinnison MT, Heino M, et al. (2011). Evolutionary principles and their practical applications. *Evolutionary Applications* 4: 159–183.

Hilker M & Meiners T (2006) Early herbivore alert: insect eggs induce plant defence. *Journal of Chemical Ecology* 32 : 1379–1397.

Hoerling M & Kumar A (2003) The perfect ocean for drought. *Science* 299: 691-694.

Hoffmann AA, Sørensen JG & Loeschcke V (2003) Adaptation of *Drosophila* to temperature extremes: Bringing together quantitative and molecular approaches. *Journal of Thermal Biology* 28: 175–216.

Hoffmann AA & Daborn PJ (2007) Towards genetic markers in animal populations as biomonitors for human-induced environmental change. *Ecology Letters* 10: 63–76.

Hoffmann AA (2010) Physiological climatic limits in *Drosophila*: patterns and implications. *Journal of Experimental Biology* 213: 870–880.

Hoffmann AA & Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature* 470: 479–485.

Hoffmann AA Chown SL & Clusella-Trullas S (2012) Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology* 27: 934-949.

Holm S (1979) A simple sequential rejective multiple test procedure. *Scandinavian Journal of Statistics* 6: 65-77.

Holopainen JK (2004) Multiple functions of induced plant volatiles. *Trends in Plant Science* 9: 529-533.

Holopainen JK & Gershenzon J (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science* 15: 176-184.

Holopainen JK (2011) Can forest trees compensate for stress-generated growth loses by induced production of volatile compounds. *Tree Physiology* 31: 1356-1377.

Hora KH & Roesingh P (1999) Oviposition in *Yponomeuta cagnagellus*: the importance of contact cues for host plant acceptance. *Physiological Entomology* 24: 109-120.

Hoy MA (1994) Parasitoids and Predators in Management of Arthropod Pests. In *Introduction to Insect Pest Management*; Metcalf RL, Luckman WH, Eds.; John Wiley Sons: New York, NY, USA.

Hsiao TC, O'Toole JC, Yambao EB & Turner NC (1984) Influence of osmotic adjustment on leaf rolling and tissue death in rice. *Plant Physiology* 75: 338-341.

Huberty AF & Denno RF (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology* 85: 1383-1398.

Huey RB & Bennett AF (1990) Physiological adjustments to fluctuating thermal environments: An ecological and evolutionary perspective. In *Stress Proteins in Biology and Medicine*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, pp. 37–59.

Huey RB & Berrigan D (1996) Testing evolutionary hypothesis of acclimation. In *Phenotypic and Evolutionary Adaptation to Temperatures*; Johnston IA & Bennett AF Eds.; Cambridge University Press: Cambridge, UK, pp. 205–237.

Huey RB & Pascual M (2009) Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* 90: 1715–1720.

Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Pérez H.J.A & Garland T Jr (2009) Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society of London B* 467 276: 1939–1948.

Idris AB & Grafius E (1995) Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth, *Plutella xylostella*, (Lepidoptera: Plutellidae). *Environmental Entomology* 24: 1726–1735.

Idris AB & Grafius E (1997) Nectar-collecting behavior of *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Plutellidae) *Environmental Entomology* 26: 114–120.

Im ES, Jung IW & Bae DH (2011) The temporal and spatial structures of recent and future trends in extreme indices over Korea from a regional climate projection. *International Journal of Climate Research* 31: 72–86.

IPCC. *Climate Change* (2007): *The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Solomon, S., Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, Eds; Cambridge University Press: Cambridge, UK.

Jactel H, Birgersson G & Andersson S (2011) Non-host volatiles mediate associational resistance to the pine processionary moth. *Oecologia* 166: 703-711.

Janzen DH (1967) Why mountain passes are higher in tropics. *American Naturalist* 101: 233–249.

Johanowicz D & Mitchell ER (2000) Effects of sweet alyssum flowers on the longevity of the parasitoid wasps *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Florida Entomologist* 83: 41–47.

Jonsson DL Huang HC & Harper AM (1988) Mortality of grasshoppers (Orthoptera: Acrididae) inoculated with a Canadian isolate of the fungus *Verticillium lecanii*. *Journal of Invertebrate Pathology* 52: 335–342.

Jonsson M, Wratten SD, Landis DA & Gurr GM (2008) Recent advances in conservation biological control of arthropods by arthropods. *Biological Control* 45: 172–175.

Kalyebi A, Sithanantham S, Overholt WA, Hassan SA & Mueke JM (2005) Parasitism, longevity and progeny production of six indigenous Kenyan trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) at different temperature and relative humidity regimes. *Biocontrol Science and Technology* 15: 255–270.

Kearney M, Porter WP, Williams C, Ritchie S & Hoffmann AA (2009) Integrating biophysical and evolutionary theory to predict climatic impacts on species' ranges: The dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology* 23: 528–538.

Kellermann V, Heerwaarden B, Sgró CM & Hoffmann AA (2009) Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* 325: 1244–1246.

Kessler A & Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.

Kfir R (1995) Parasitoids of the African stem borer, *Busseola fusca* (Lepidoptera: Noctuidae), in South Africa. *Bulletin of Entomological Research* 85: 369–377.

Kfir R (1997) Competitive displacement of Busceola fusca (Lepidoptera: Noctuidae) by Chilo partellus (Lepidoptera: Pyralidae. *Annals of the Entomological Society of America* 90: 619-624.

Kfir R (1998). Maize and grain sorghum: southern Africa, p. 29-37 *in*: Polaszek A. (ed.), *African Cereal Stem Borers: Economic Importance, Taxonomy, Natural Enemies and Control*, CABI International.

Kfir R, Overholt WA, Khan ZR & Polaszek A (2002) Biology and management of economically important lepidopteran cereal stem borers in Africa. *Annual Review of Entomology* 47: 701-731.

Khan ZR, Chilishwa P, Ampong-Nyarko K, Smart LE, Polaszek A, Wandera J, Mulaa MA (1997a) Utilisation of wild gramineous plants for the management of cereal stemborers in Africa. *International Journal of Tropical Insect Science* 17: 143-150.

Khan ZR, Ampong-Nyarko K, Chiliswa P et al. (1997b) Intercropping increases parasitism of pests. *Nature* 388: 631-632.

Khan ZR, Pickett JA, van den Burg, J, Wadhams LJ & Woodcock CM (2000) Exploiting chemical ecology and species diversity. Stemborer and Striga control for maize and sorghum in Africa. *Pest Management Science* 56: 957-962.

Khan ZR, Hassanali A, Overholt W, Khamis TM, Hooper AM, Pickett JA, Wadhams LJ & Woodcock CM (2002) Control of Striga hermonthica by intercropping with *Desmodium* spp. and the mechanism defined as allelopathic. *Journal of Chemical Ecology* 28: 1871-1885.

Khan ZR, Midega CAO, Hutter NJ, Wilkins RM & Wadhams LJ (2006) Assessment of the potential of Napier grass (*Pennisetum purpureum*) varieties as trap plants for management of *Chilo partellus. Entomologia Experimentalis et Applicata* 119: 15-22.

Khan ZR, Midega CAO, Wadhams LJ, Pickett JA & Mumuni A (2007) Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push-pull strategy. *Entomologia Experimentalis et Applicata* 124: 201-211.

Khan ZR, Midega CAO, Njuguna EM, Amudavi DM, Wanyama JM & Pickett JA (2008a) Economic performance of the 'push–pull' technology for stemborer and *Striga* control in smallholder farming systems in western Kenya. *Crop Protection* 27: 1084-1097.

Khan ZR, Amudavi DM, Midega CAO, Wanyama JM & Pickett JA (2008b) Farmers' perceptions of a 'push–pull' technology for control of cereal stemborers and *Striga* weed in western Kenya. *Crop Protection* 27: 976-987.

Khan ZR, James DG, Midega CAO & Pickett JA (2008c) Chemical ecology and conservation biological control. *Biological Control* 45: 210–224.

Khan ZR, Midega CAO, Bruce TJA, Hooper AM, Pickett JA (2010) Exploiting phytochemicals for developing the push-pull crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany* 61: 4185-4196.

Khan ZR, Midega CAO, Pittchar JO, Murage AW, Birkett MA et al. (2014) Achieving food security for one million sub-Saharan African poor through push-pull innovation by 2020. *Philosophical Transactions of the Royal Society* B 369: 20120284.

Kingsolver JG, Ragland GJ & Shlichta JG (2004) Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* 58: 1521–1529.

Kiritani K (2006) Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. *Population Ecology* 48: 5–12.

Klein Koch CA (1996) Proyectos de control biológico en curso en Ecuador. In *M.C. Zapater El Control Biológico en América Latina*; IOBC: Buenos Aires, Argentina, pp. 41–47.

Klok CJ & Chown SL (2001)Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *Journal of Insect Physiology* 47: 95–101.

Krishna P (2003) Plant responses to heat. In: *Topics in Current Genetics* volume 4: Springer-Verlag Berlin Heidelberg pp. 73-101.

Kristensen TN, Hoffmann AA, Overgaard J, Sørensen JG, Hallas R & Loeschcke V (2008) Costs and benefits of cold acclimation in field-released Drosophila. *Proceedings of the National Academy of Sciences of the USA* 105: 216–221.

Laffert KD (2009) The ecology of climate change and infectious diseases. *Ecology* 90: 888–900.

Lagerspetz KYH (2006) What is thermal acclimation? *Journal of Thermal Biology* 31: 332–336.

Landis DA, Wratten SD & Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175–201.

Landry C (2012) Mighty Mutualisms: The Nature of Plant-pollinator Interactions. *Nature Education Knowledge* 3: 37.

Lange BM, Rujan T, Martin W & Croteau R (2000) Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. *Proceedings of the National Academy of Science USA* 97: 13172–13177.

Laothawornkitkul J, Paul ND, Possell M, Mullineaux PM, Hewitt CN & Taylor JE (2008a) The role of isoprene in insect herbivory. *Plant Signaling and Behavior* 3: 1141-1142.

Laothawornkitkul J, Paul ND, Vickers CE, Possellm M, Taylor JE et al. (2008b) Isoprene emissions influence herbivore feeding decisions. *Plant, Cell and Environment* 31: 1410-1415.

Leingärtner A, Hoiss B, Krauss J & Steffan-Dewenter I (2014) Combined effects of extreme climatic events and elevation on nutritional quality and herbivory of Alpine plants. *PLOS ONE* 9(4): e93881

Letourneau DK, Armbrecht I, Rivera BS, et al. (2011) Does plant diversity benefit agroecosystems? A synthetic review. *Ecological Applications* 21: 9–21.

Leuschner K (1990) A review of laboratory and field screening procedures for *Chilo partellus. International Journal of Tropical Insect Science* 11: 627-638.

Llusià J, Peñuelas J, Alessio GA & Estiarte MA (2006) Seasonal contrasting changes of foliar concentrations of terpenes and other volatile organic compound in four dominant species of a Mediterranean shrubland submitted to a field experimental drought and warming. *Physiologia Plantarum* 127: 632–649.

Liu SS, Zhang GM & Zhu J (1995) Influence of temperature variations on rate development in insects analysis of case studies from entomological literature. *Annals of the Entomological Society of America* 88: 107–119.

Lockwood JA & Ewen AB (1997) Biological control of rangeland grasshoppers and locusts. In *The Bionomics of Grasshoppers, Katydids and their Kin*; Gangwere, S.K., Muralingan, M.C., Muralingan, M., Eds.; CABI International: Wallingford, UK, pp. 421–442.

Loivamäki M, Mumm R, Dicke M & Schnitzler J-P (2008a) Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proceedings of the National Academy of Sciences of the USA* 105: 17430–17435.

Loivamäki M, Mumm R, Dicke M & Schnitzler JP (2008b) Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proceedings of the National Academy of Science USA* 105: 17430–17435.

Lomer CJ, Bateman RP, Johnson DL, Langewald J & Thomas M (2001) Biological control of locusts and grasshoppers. *Annual Review of Entomology 46*: 667–702.

Loreto F, Dicke M, Schnitzler J-G & Turlings TSJ (2014) Plant volatiles and the environment. *Plant, Cell and the Environment* 37: 1905-1908.

Lutterschmidt WI & Hutchison VH (1997) The critical thermal maximum: Data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology 75:* 1553–1560.

Lynch S (1998) Measuring progress in the transition to biologically-based IPM. Proceedings of the OECD/FAO Workshop on Integrated Pest Management and Pesticide Risk Reduction, Neuchâtel, Switzerland, 28 June–2 July 1998.

Macedo N (2000) Método de criação do parasitóide Cotesia flavipes (Cameron). In *Controle, Biológico de Pragas: Produção massal e controle de qualidade*; Bueno, V.H.P., Ed.; Editora UFLA: Lavras, Minas Gerais, Brazil, pp. 161–174.

Maddrell SHP (1969) Secretion by the Malphigian tubules of Rhodinus. The movement of ions and water. *Journal of Experimental Biology* 51:71-79.

Markarieva AM & Gorshkov VG (2007) Biotic pump of atmospheric moisture as driver of the hydrological cycle on land. *Hydrological Earth Systems Science.* 11: 1013–1033.

Marshall KE & Sinclair BJ (2010) Repeated stress exposure results in a survival-reproduction trade-off in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B* 277: 963–969.

Martinat PJ (1987) The Role of Climatic Variation and Weather in Forest Insect Outbreaks. In *Insect Outbreaks*; Barbosa, P., Schultz, J.C., Eds.; Academic Press: San Diego, CA, USA, pp. 241–268.

Mattson WJ & Haack RA (1987) The role of drought in outbreaks of plant-eating insects. *Bioscience* 37: 110-118.

Meyer ST, Roces F & Wirth R (2006) Selecting the drought stressed: effects of plant stress on intraspecific and within plant herbivory patterns of the leaf-cutting ant *Atta colombica. Functional Ecology* 20: 973–984.

Midega CAO, Khan ZR, van den Berg, J, Ogol CKPO, Bruce TJ & Pickett JA (2009) Non-target effects of the 'push–pull' habitat management strategy: parasitoid activity and soil fauna abundance. *Crop Protection* 28: 1045–1051.

Midega CAO, Khan ZR, Pickett JA & Nylin S (2010) Host plant selection behaviour of *Chilo partellus* and its implication for effectiveness of a trap. *Entomologia Experimentalis et Applicata* 138: 40-47.

Mikkelsen TN, Beier C, Jonasson S et al. (2008) Experimental design of multifactor climate change experiments with elevated CO_2 warming and drought: the CLIMAITE project. *Functional Ecology* 22: 185–195.

Minja EM (1990) Management of *Chilo spp*. infesting cereals in eastern Africa. *International Journal of Tropical Insect Science* 11: 489-499.

Mittler R (2006) Abiotic stress, the field environment and stress combination. *Trends in Plant Science* 11: 15-19.

Mochiah BM, Ngi-song AJ, Overholt WA & Botchey M (2001) Host suitability of four cereal stemborers (Lepidoptera: Crambidae, Noctuidae) for different geographic populations of *Cotesia sesamiae* (Cameron) (Hymenopter: Braconidae) in Kenya. *Biological Control* 21: 285-292.

Mody K, Unsicker SB & Linsenmair KE (2007) Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. *Ecology* 88: 1012-1020.

Mody K, Eichenberger D & Dorn S (2009) Stress magnitude matters: different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology* 34: 133-143.

Mohamed HM, Khan ZR, Overholt WA & Elizabeth DK (2004) Behaviour and biology of *Chilo partellus* (Lepidoptera: Pyralidae) on maize and wild gramineous plants. *International Journal of Tropical Insect Science* 24(4): 287-297.

Monson RK & Fall R (1989) Isoprene emission from aspen leaves. *Plant Physiology* 90: 267-274.

Musolin DH (2007) Insects in a warmer world: ecological, physiological and life history responses of true bugs (Heteroptera) to climate change. *Global Change Biology 13*: 1565–1585.

Mustin K, Benton TG, Dytham C & Travis JMJ (2009) The dynamics of climate-induced range shifting; perspectives from simulation modeling. *Oikos* 118: 131–137.

Nair US; Lawton RO; Welch RM; Pielke RA Sr. (2003) Impact of land use on Costa Rican tropical montane cloud forests: Sensitivity of cumulus cloud field characteristics to lowland deforestation. *Journal of Geophysical Research* 108 (D7): 4206.

Neislon EH, Goodger JQD, Woodrow IE & Møller BL (2013) Plant chemical defence: at what cost? *Trends in Plant Science* 18: 250-258.

Neto JDR, Pinho BX, Meyer ST, Wirth R & Leal IR (2012) Drought stress drives intraspecific choice of food plants by *Atta* leaf cutting ants. *Entomologia Experimentalis et Applicata* 144: 209-215.

Ngi-Song AJ, Overholt WA & Ayertey JN (1995) Suitability of African gramineous stemborers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). *Environmental Entomology* 24: 978-984.

Ngi-Song AJ & Overholt WA (1997) Host location and acceptance of *Cotesia flavipes* (Cameron) and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), parasitoids of African stemborers: Role of frass and other cues. *Biological Control* 9: 136-142.

Nicholls CI, Parrella M & Altieri MA (2000) Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agriculture and Forest Entomology* 2: 107–113.

Nicotra AB, Atkin OK, Bonser SP, et al. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15: 684-692.

Niinemets Ü (2010) Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in Plant Science* 15: 145-153.

Nyamukondiwa C, Kleynhans E & Terblanche JS (2010a) Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology* 35: 565–575.

Nyamukondiwa C & Terblanche JS (2010b) Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: thermal history affects short term responses to temperature. *Physiological Entomology* 35: 255–264.

Nyamukondiwa C, Terblanche JS, Marshall KE & Sinclair BJ (2011) Basal cold but not heat tolerance constraints plasticity among *Drosophila* species (Diptera: Drosophilidae). *Journal of Evolutionary Biology* 24: 1927-1938.

Onyango FO & Ochien-Odero JPR (1994) Continuous rearing of the maize stemborer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* 73: 139-144.

Ong'amo GO, Le Rü BP, Dupas S, Moyal P, Calatayud PA & Silvain JF (2006) Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. *Ann. de la Société Entomol. de France* 42: 171–177.

Opdam P & Wascher D (2004) Climate change meets habitat fragmentation: Linking landscape and biogeographical scale levels in research and conservation. *Biological Conservation* 117: 285–297.

Ormeno E, Mévy JP, Vila B, Bousquet-Mélou A, Greff S, Bonin G & Fernandez C (2007) Water deficit stress induces different monoterpene and sesquiterpene emission changes in Mediterranean species. Relationship between terpene emissions and plant water potential. *Chemosphere* 67: 276–284.

O'Toole JC & Cruz RT (1980) Response of leaf water potential, stomatal resistance and leaf rolling to water stress. *Plant Physiology* 65: 428-432.

Overholt WA, Ogedah K & Lammers PM (1994) Distribution and sampling of *Chilo partellus* (Lepidoptera: Pyrllidae) in maize and sorghum on the Kenya coast. *Bulletin of Entomological Research* 84: 67-68.

Overholt WA, Ngi-Song AJ, Omwega, CO et al. (1997) A review of the introduction and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in East Africa for biological control of cereal stemborers. *International Journal of Tropical Insect Science* 17:19–35.

Paré PW & Tumlinson JH (1999) Plant volatiles as defense against insect herbivores. *Plant Physiology* 121: 325-332.

Parmesan C, Ryrholm N, Stefanescu C et al. (1999) Pole ward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* 399: 579-583.

Parmesan C & Yohe GA (2003) globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics 37*: 637–669.

Patori GM & Foyer CH (2002) Common components, networks, and pathways of cross-tolerance to stress. The central role of "Redox" and abscisic acid–mediated controls. *Plant Physiology* 129: 460–468.

Päts P (1991) Activity of *Chilo partellus* (Lepidoptera: Pyralidae): eclosion, mating and oviposition time. *Bulletin of Entomological Research* 81: 93-96.

Pearson RG, Dawson TP, Berry PM & Harrison PA (2002) Species: A spatial evaluation of climate impact on the envelope of species. *Ecological Modelling* 154: 289–300.

Pearson RG & Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*12: 361–371.

Peñuelas JR & Staudt (2009) BVOCs and global change. *Trends in Plant Science* 15: 133-144.

Perdikis D, Fantinou A, Lykouressis D (2011) Enhancing pest control in annual crops by conservation of predatory Heteroptera. *Biological Control* 59: 13–21.

Pickett JA, Aradottir GI, Birkett MA et al. (2014) Delivering sustainable crop protection systems via seed: exploiting natural constitutive and inducible defence pathways. *Philosophical Transactions of the Royal Society* B 369: 1639 20120281.

Plath M, Dorn S, Riedl J, Barrios H & Mody K (2012) Associational resistance and associational susceptibility: specialist herbivores show contrasting responses to tree stand diversification. *Oecologia* 169: 477-487.

Poelman EH, Bruinsma M, Zhu F et al. (2012) Hyperparasitoids use herbivore induced plant volatiles to locate their parasitoid host *PLOS Biology* 0(11): e1001435. doi:10.1371/journal.pbio.1001435.

Porter JH, Parry ML & Carter TR (1991) The potential effects of climate change on agricultural insect pests. *Agricultural and Forest Meteorology* 57: 221–240.

Pörtner HO & Farrell AP (2008) Physiology and climate change. *Science* 322: 690–692.

Potting RPJ, Overholt WA, Osae-Danso FO & Takasu K (1997) Foraging behaviour and life history of the stemborer parasitoid *Cotesia flavipes. Journal of Insect Behaviour* 10: 1-29.

Preszler RW & Price PW (1995) A test of plant vigor, plant-stress and plant genotype effects on leaf oviposition and performance. *Oikos* 74: 485-492.

Price PW (1991) The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244-251.

R Development Core Team (2011) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramaswamy SB (1988) Host finding by moths: sensory modalities and behaviours. *Journal of Insect Physiology* 34: 235-249.

Reisenman CE, Riffell JA, Duffy K, Pesque A, Mikles D & Goodwin B (2013) Species-specific effects of herbivory on the oviposition behavior of the moth *Manduca sexta*. *Journal of Chemical Ecology* 39: 76-89.

Renwick JAA & Chew FS (1994) Oviposition behaviour in Lepidoptera. *Annual Review of Entomology* 39: 377-400.

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. *Herbivores: Their Interaction with Secondary Metabolites* (ed. by GA Rosenthal & DH Janzen), pp. 3-54. Academic Press, New York, NY, USA.

Riveron J, Boto T & Alcorta E (2009) The effect of environmental temperature on olfactory perception in Drosophila melanogaster. *Journal of Insect Physiology* 55: 943–951.

Roades DF (1983) Herbivore population dynamics and plant chemistry. In: Denno RF, McClure MS, eds. *Variable Plants and Herbivores in Natural and Managed Systems*. New York: Academic Press, 155–220.

Robinet C & Roques A (2010) Direct impacts of recent climate warming on insect populations. *Integrative Zoology*: 132–142.

Rodriguez-Saona CR, Frost CJ (2010) New evidence for a multi-functional role of herbivore-induced plant volatiles in defense against herbivores. *Plant Signaling and Behavior* 5: 58-60.

Rohmer, M. (1999). The discovery of a mevalonate-independent pathway for isoprenoidbiosynthesis in bacteria, algae and higher plants. *Natural Products Reports* 16: 565–574.

Rooney LW & Waniska RD (2000) Sorghum food and industrial utilization, in *Sorghum: Origin, History, Technology, and Production*. C.W. Smith and R.A. Frederiksen (eds.), John Wiley and Sons,Inc., New York, pp. 689-729.

Sardans J, Pennulas J, Estiarte M & Prieto P (2008) Warming and drought alter C and N concerntration, allocation and accumulation in a Mediterranean shrubland. *Global Change Biology* 14: 2304-2316.

Sasson A (2012) Food security for Africa. An urgent global challenge. *Agricuture and Food Security* 1: 2.

Schnee C, Köllner TG, Held M, Turlings TCJ, Gershenzon J & Degenhardt J (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proceedings of the National Academy of Sciences of the USA* 103: 1129-1134.

Schubert S, Serraj R, Plies-Blazer E & Mengel K (1995) Effect of drought stress on growth, sugar concerntrations and amino acid accumulations in N-fixing alfalfa (*Medicago sativa*). *Journal of Plant Physiology* 146: 541-546.

Seagraves MP, Riedell WE & Lundgren JG (2011) Oviposition preference for water-stressed plants in *Orious insidious* (Hemiptera: Anthocoridae). *Journal of Insect Behaviour* 24: 132-143.

Seshu–Reddy KV (1998) Maize and sorghum: East Africa. In: *African Cereal Stemborers. Economic Importance, Taxonomy, Natural Enemies.* Ed. Polasszek, A, CAB International.

Sgró CM, Lowe AJ & Hoffmann AA (2010) Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications* 4: 326–337.

Sharkey TD & Yeh S (2001) Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 52: 407-436.

Sharkey TD, Wiberley AE & Donohue AR (2008) Isoprene emission from plants: why and how. *Annual Review of Botany* 101: 5–18.

Sharkey TD & Monson RK (2014) The future of isoprene emission from leaves, canopies and landscapes. *Plant, Cell and Environment* 37: 1727-1740.

Sholes ODV (2008) Effects of associational resistance and host density on woodland insect herbivores. *Journal of Animal Ecology* 77: 16-23.

Showler AT (2002) Effects of water deficit stress, shade, weed competition and kaolin particle film on selected foliar free amino acid accumulations in cotton *Gossypium hirsutum* (L.). *Journal of Chemical Ecology* 28: 631-651.

Showler AT (2013) Water deficit stress – host plant nutrient accumulations and associations with phytophagous arthropods. Abiotic Stress – *Plant Responses and Applications in Agriculture* (ed. by K Vahdat & C Leslie), pp. 388-410. Intech Publishers, Rijeka, Croatia.

Showler AT & Castro BA (2010) Influence of drought stress on Mexican rice borer (Lepidoptera: Crambidae) oviposition preference in sugarcane. *Crop Protection* 29: 415-421.

Showler AT & Moran PJ (2003) Effects of drought-stressed cotton, *Gossypium hirsutum* L., on beet armyworm, *Spodoptera exigua* (Hübner), oviposition, and larval feeding preferences and growth. *Journal of Chemical Ecology* 29: 1997-2011.

Singer MC (1986) The definition and measurement of oviposition preference in plant-feeding insects. *Insect-Plant Interactions* (ed. by JR Miller & TA Miller), pp 65-69. Springer, New York, NY, USA.

Sithole SZ (1990) Status and control of the Stem Borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in Southern Africa. *International Journal of Tropical Insect Science* 11: 481-488.

Soldaat LL, Boutin J-P & Derridj S (1996) Species specific composition of free amino acids on the leaf surface of four *Senecio* species. *Journal of Chemical Ecology* 22: 1-12.

Soler R, Van der Puttin WH, Harvey JA, Vet LEM, Dicke M & Bezemer TM (2012) Root herbivore effects on aboveground multitrophic interactions: patterns, processes and mechanisms. *Journal of Chemical Ecology* 38: 755-767.

Somero GN (2009) The physiology of climate change: how potentials for acclimatisation and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology* 213: 912–920.

Sørenson JG, Addison MF & Terblanche JS (2012) Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. *Crop Protection* 38: 87–94.

Sorribas J, van Baaren J, Garcia-Marí F (2012) Effects of climate on the introduction, distribution and biotic potential of parasitoids: Applications to biological control in California red scale. *Biological Control* 62: 103-112.

Southwood TRE & Henderson PA (2000) Ecological Methods. In *Blackwell Science*; Oxford, USA.

Späthe A, Reinecke A, Olsson SB, Kesavan S, Knaden M & Hansson BS (2013) Plant species- and status-specific odorant blends guide oviposition choice in the moth *Manduca sexta*. *Chemical Senses* 38: 147-159.

Stacey D (2003) Climate and biological control in organic crops. *International Journal of Pest Management 49*: 205–214.

Städler E (1984) Contact chemoreception. *Chemical Ecology of Insects* (ed. WJ Bell & RT Cardé), pp 3-35. Sinauer, Sunderland, MA, USA.

Stevenson RD (1985) The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *American Naturalist* 3: 362–386.

Taiz L & Zeiger E (2006) *Plant Physiology*. 3rd edition Sinauer Associates, Inc.

Talekar NS & Shelton AM (1995) Biology, ecology and management of diamondback moth. *Annual Review of Entomology* 38: 275–302.

Tamiru A, Bruce TJA, Woodcock CM et al. (2011) Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. *Ecology Letters* 14: 1075-1083.

Tardieu F & Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* 49: 419–432.

Tariq M, Wright DJ, Rossiter JT & Staley JT (2012) Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. *Agricultural and Forest Entomology* 14: 177-185.

Tebaldi C & Sansó B (2009) Joint projections of temperature and precipitation change from multiple climate models: A hierarchical Bayesian approach. *Journal of the Royal Statistical Society A* 172: 83–106.

Terblanche JS & Chown SL (2006) The relative contributions of developmental plasticity and adult acclimation to physiological variation in tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae) *Journal of Experimental Biology* 209: 1064–1073.

Terblanche JS, Nyamukondiwa C & Kleynhans E (2010) Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). *Entomologia. Experimentalis et Applicata* 137: 304–315.

Terblanche JS, Hoffmann AA, Mitchell KA, Rako L, Le Roux PC & Chown SL (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology* 214: 3713–3725.

Thompson JN & Pellmyr O (1991) Evolution of oviposition behaviour and host preference in Lepidoptera. *Annual Review of Entomology* 36: 65-89.

Thomson LJ, Macfadyen S & Hoffmann AA (2010) Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control* 52: 296–306.

Thrall PH, Oakeshott JG, Fitt G et al. (2011) Evolution in agriculture: The application of evolutionary approaches to the management of biotic interactions in the agro-ecosystems. *Evolutionary Applications* 4: 200–215.

Tomozeiu R, Pavan V, Cacciamani C & Amici M (2006) Observed temperature changes in Emilia-Romagna: mean values and extremes. *Climate Research* 31: 217–225.

Tumlinson JH, Lewis WJ & Vet LEM (1993) How parasitic wasps find their hosts. *Scientific American* 268: 100-106.

Usua EJ (1968) Effects of varying populations of *Busceola fusca* larvae on growth and yield of maize. *Journal of Economic Entomology* 61: 375-376.

Vallat A, Gu H & Dorn S (2005) How rainfall, relative humidity and temperature influences volatile emissions from apple trees in situ. *Phytochemistry* 66: 1540-1550.

van Asch M, van Tienderen PH, Holleman LJM & Visser ME (2007) Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology* 13: 1596-1604.

van Asch M, Julkunen-Tiito R & Visser ME (2010) Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology. *Functional Ecology* 24: 1103-1109.

van den Berg J (2006) Oviposition preference and larval survival of *Chilo partellus* (Lepidoptera: Pyralidae) on Napier grass (*Pennisetum purpureum*) trap crops. *International Journal of Pest Management* 52: 39-44.

van Lenteren JC (2011) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *Biological Control* 57: 1–20.

Vickers CE, Gershenzon J, Lerdau MT & Loreto F (2009a) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology*: 283-291.

Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J et al. (2009b) Isoprene synthesis protects transgenic plants from oxidative stress. *Plant, Cell and Environment* 32: 520-531.

Wadhams LJ (1990) The use of coupled gas chromatography: electrophysiological techniques in the identification of insect pheromones. In: *Chromatography and Isolation of Insect Hormones and Pheromones* (eds McCaffery, A.R. & Wilson, I.D.) Plenum Press, New York, pp. 281–288.

Walther G-R, Post E, Convey P et al. (2002) Ecological responses to recent climate change. *Nature* 416: 389-395.

Wäschke N, Hardge K, Hancock C, Hilker M, Obermaier E, Meiners T (2014) Habitats as complex odour environments: how does plant diversity affect herbivore and parasitoid orientation? *PLOS ONE* 9(1): e85152. doi:10.1371/journal.pone.0085152.

Webb TJ, Woodward FJ, Hannah L & Gaston KJ (2005) Forest cover-rainfall relationships in a biodiversity hotspot: the Atlantic forest of Brazil. *Ecological Applications* 15:1968–1983.

Webster B, Bruce TJA, Pickett JA & Hardie J (2010) Volatiles functioning as host cues in a blend become non-host cues when presented alone to the black bean aphid. *Animal Behaviour* 79: 451-457.

White TCR (1969) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* 50: 905-909.

White TCR (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90–105.

Wiens JA, Stralberg D, Jongsomjit D, Howell CA & Snyder MA (2009) Niches, models, and climate change: Assessing the assumptions and uncertainties. *Proceedings of the National Academy of Sciences of the USA* 106: 19729–19736.

Wilby A, Thomas MB (2002) Are the ecological concepts of assembly and function of biodiversity useful frameworks for understanding natural pest control? *Agriculture Forest Entomology* 4: 237–243.

Williams SE, Shoo LP, Isaac JL, Hoffmann AA & Langham G (2008) Towards and integrated framework for assessing the vulnerability of species to climate change. *PLOS Biology* 6: e325, doi:10.1371/journal.pbio.0060325.

Winter TR, Borkowski L, Zeier J & Rostas M (2012) Heavy metal stress can prime for herbivore- induced plant volatile emission. *Plant, Cell and Environment* 35: 1287-1298.

World Bank (2008) *Agriculture for Development*, World Development Report 2008.

Yang Z, Powell JR, Zhang C & Du G (2012) The effect of environmental and phylogenetic drivers on community assembly in an alpine meadow community. *Ecology* 93: 2321-2328.

Yuan JS, Himanen SJ, Holopainen JK, Chen F & Neal Stewart Jr. C (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. *Trends in Ecology and Evolution* 24: 323-331.

Zachariassen KE (1996) The water conserving physiological compromise of desert insects. *European Journal of Entomology* 93: 359–367.

Zakir A, Sadek MM, Bengtsson M, Hansson BS, Witzgall P, Anderson P (2013a) Herbivore-induced plant volatiles provide associational resistance against an ovipositing herbivore. *Journal of Ecology* 101: 410-417.

Zakir A, Bengtsson M, Sadek MM et al. (2013b) Specific response to herbivore-induced *de novo* synthesized plant volatiles provides reliable information for host plant selection in a moth. *Journal of Experimental Biology* 216: 3257-3263.

Zalucki MP, Clarke AR & Malcolm SB (2002) Ecological and behaviour of first instar larval Lepidoptera. *Annual Review of Entomology* 47: 361-393.

Zvereva EL & Kozlov MV (2006) Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a meta-analysis. *Global Change Biology* 12: 27-41.

8.0 Appendices

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Article

Biologically Based Methods for Pest Management in Agriculture under Changing Climates: Challenges and Future Directions

Frank Chidawanyika [1,†], Pride Mudavanhu [2] and Casper Nyamukondiwa [3,*]

[1] Global Change and Sustainability Research Institute, School of Animal, Plant and Environmental Sciences, Faculty of Science, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa; E-Mail: fchidawanyika@icipe.org

[2] Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa; E-Mail: mudavanhu@sun.ac.za

[3] Department of Earth and Environmental Sciences, Faculty of Science, Botswana International University of Science and Technology (BIUST). Private Bag BO 041 Bontleng, Gaborone, Botswana

[†] Current address: International Centre of Insect Physiology and Ecology (ICIPE), Habitat Management Programme, Plant Health Division, P.O. Box 30, Mbita Point Research Station, Mbita Point, Kenya

* Author to whom correspondence should be addressed;
E-Mail: nyamukondiwac@biust.ac.bw; Tel.: +267-392-6909; Fax: +267-392-6904.

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Abstract: The current changes in global climatic regimes present a significant societal challenge, affecting in all likelihood insect physiology, biochemistry, biogeography and population dynamics. With the increasing resistance of many insect pest species to chemical insecticides and an increasing organic food market, pest control strategies are slowly shifting towards more sustainable, ecologically sound and economically viable options. Biologically based pest management strategies present such opportunities through predation or parasitism of pests and plant direct or indirect defense mechanisms that can all be important components of sustainable integrated pest management programs. Inevitably, the efficacy of biological control systems is highly dependent on natural enemy-prey interactions, which will likely be modified by changing climates. Therefore, knowledge of how insect pests and their natural enemies respond to climate variation is of fundamental importance in understanding biological insect pest management under global

climate change. Here, we discuss biological control, its challenges under climate change scenarios and how increased global temperatures will require adaptive management strategies to cope with changing status of insects and their natural enemies.

Keywords: climate change; integrated pest management; insect population dynamics

1. Introduction

Anthropogenic climate change has the potential to significantly influence the biology of all organisms, but particularly ectotherms [1–3]; hence the evidence for a shift in ectotherm distribution in response to climate change [4,5]. While climate change is often associated with global mean annual temperature increases [6], likely favoring winter survival of insect pests, it is also coupled with increasing frequency and severity of extreme temperatures [7–12] that may modify predictions of insect population dynamics [13–15]. Short-term thermal fluctuations can be particularly stressful to small insects because their body temperatures are typically in equilibrium with ambient temperatures [16–18]. Hence, insects must be able to cope physiologically or compensate behaviorally with such changes in ambient temperature, both on spatial and temporal scales [15,19,20]. Physiologically, insects can adjust thermal tolerance over the short term, a phenomenon typically termed 'hardening' [21,22]. Over the longer term, thermal tolerance may be altered through acclimation in the laboratory or acclimatization in the field, and generally is a response to changes in environmental mean temperatures with e.g., season [23,24]. Hardening typically yields reversible physiological changes while acclimation and acclimatization can produce either reversible or irreversible physiological changes depending on the trait or whether developmental or maternal stages were exposed to temperature variation [22,24–26]. Martinat (1987) emphasized the importance of incorporating short-term undesirable weather transients (e.g., cold spells) in determining insect population dynamics [27]. Nevertheless, previous studies suggest considerable complexity when attempting to predict effects of transient weather patterns on insects' physiology or life-history traits [28–30].

Biological control is a method of controlling pests that relies on predation, parasitism, herbivory and other natural mechanisms and can be an important component of integrated pest management (IPM) programs [31]. However, the efficacy of biological control using natural enemies depends on a complex but delicate relationship between natural enemies and their insect pest hosts whose balance can be offset by a changing climate. Environmental factors (e.g., temperature) directly affects the survival, development, reproduction and dispersal of pest insects and thus their potential biogeography and biotic potential [32,33]. It is well known that temperature fluctuations are the major factors affecting insect biology, activity and distribution of natural enemies in agro-ecosystems [34,35]. Moreover, several studies have indicated climate change affects several life history parameters e.g., generation time, fecundity, sex ratios and lifespan of parasitoids and their natural enemies [36,37]. Similarly, temperature extremes may reduce insect survival, reduce fecundity and retard natural enemies' development [38].

Several studies have documented the likely effects of a changing climate on insect pest-natural enemies' interactions [35,39–41]. Similarly, by assessing physiological traits of thermal tolerance and water balance, [42] it was shown that global climate change may affect the phenology of *Paractora dreuxi* Seguy (Diptera: Helcomyzidae). This review aims at examining the likely effects of climate change on insect biological control and how increased global temperatures will require adaptive management strategies to cope with changing status of insects and their natural enemies. We discuss how changes in global climate factors such as temperature increase will impact on [1] insect-natural enemy-host plant interactions, [2] insect/plant diversity [3] population growth/abundance [4] effectiveness of crop protection technologies, with special emphasis to biological control using natural enemies. This information has critical implications for sustainable pest management and food security, especially for developing countries in Africa, where food security is an urgent challenge [43].

2. Biologically-Based Pest Control Methods

Reducing public health and environmental risks associated with chemical pesticide use is of growing concern in developed and developing countries [44]. This has motivated the call for the adoption of biologically based IPM systems, an essential step towards reducing risks associated with the use of highly toxic pesticides [44].

Biological control can be classified into three basic categories namely conservation, classical and augmentation [45,46]. First, conservation biological control (CBC) involves the deliberate human practice aimed at promoting the survival and activity of natural enemies at the expense of pest populations [47]. For example, ecological strips consisting of selected non-crop plants can be deliberately created to provide food sources and overwintering shelters as well as protect local natural enemies from pesticide disturbances thereby enhancing CBC as successfully shown in cereals, cabbages, and fruit orchards [48,49].

Plant indirect defense mechanisms which rely on volatiles that call for natural enemies after pest damage, herbivore induced plant volatiles (HIPVs), have also been exploited to increase the activity of parasitoids in CBC systems [49,50]. In New Zealand and Australia the "attract and reward" is another form of the CBC approach where the use of HIPVs is combined with measures that increase key resources needed by natural enemies such as flowering plants in the commercial crop [50]. More recently, another form of CBC which combines stimulo-deterrent diversion tactics and conservation of parasitoids, through careful habitat manipulation has been shown to be effective against stem borers on sorghum *Sorghum bicolor* L. (Moench) and maize *Zea mays* L. [51]. Habitat manipulation has also been used to increase nectar availability, which increases the fecundity and longevity of some parasitoids [52–54], thereby increasing the efficacy of the program. For example, flowers are planted into commercial brassicaceous crops to enhance suppression of the diamondback moth, *Plutella xylostella* (L.) by its natural enemies [55].

Second, classical biological control (BC) involves collection of natural enemies from their area of origin and releasing them in the new area where their host was introduced accidentally [46,56]. This is of particular importance when the introduced pest species has no known alternative parasitoids indigenous to the area. However, the efficacy of a classical biological will depend on the newly

released parasitoids to successfully establish populations that can compete in the new environment. For example in the USA, the alien yellow starthistle *Centaurea solstitialis* [L] has been successfuly controlled by insect natural enemies such as *Bangasternus orientalis* [Carpiomont], *Eusternopus villosus* (Boheman), *Urophora sirunaseva* (Hering) and *Chaetorellia succinea* (Costa) that attack the seed head [57].

Last, augmentative biological control (ABC) is the periodic release of large numbers of mass-reared natural enemies with the aim of supplementing natural enemy populations or flooding (*i.e.*, inundating) pest populations with natural enemies [46,58]. It is commercially deployed in various cropping systems worldwide and two forms of ABC are distinguished namely the inundative approach and the seasonal inoculative method [46,59]. In the inundative release method, the BC agent is collected, mass-reared and released periodically in large numbers as for example a biotic insecticide to achieve immediate pest control in crops where viable breeding populations of the natural enemy are not possible [46]. This approach has been successfully applied in sugarcane for control of the sugarcane borer *Diatraea saccharalis* (F) in Latin America [60]. Other examples include the inundative release of common green lacewings *Chrysoperla carnea* (Stephens) to suppress *Erythroneura variabilis* (Beamer) and *E. elegantula* (Osborn) in vineyards [61]; and the release of *Trichogramma* spp for control of lepidopteran pests in vegetables, corn, rice, other cereals and cotton in Russia, China, SE Asia, Mexico and South America [62].

The seasonal inoculative approach differs from inundative method in that it is deployed in short-term crops, the production season of which is not longer than one year and where multiple pest generations occur [46]. The aim of the method is to obtain both immediate pest control as well as a build-up of the biological control agent population over the entire duration of the same production season [46]. Examples of the successful deployment of this technique include the release of *Trissolcus basalis* (Wollaston) for the control of *Nezara viridula* (L) in Brazil [63]; biological control of soybean stink bugs by inoculative releases of *T. basalis* [64]; control of the citrus blackfly, *Aleurocanthus woglumi* (Ashby) by inoculative releases of *Eretmocerus serius* (Silvestri) and *Amitus hesperidium* (Silvestri) in Cuba, Costa Rica, Mexico and Panama [46]; and the inoculation with *Metarhizium flavoviride* (Gams and Rozyspal) or *Verticillium lecanii* (Zimmerman) for the control of locusts *Schistocerca gregaria* (Forskal) [65] and grasshoppers [66].

3. Challenges in Biologically-Based Pest Management in Relation to Climate Change

Alterations in physiology and population dynamics, as a result of climate change, will bring new arrangements to levels of biological organization and ultimately ecological interactions in various species. In most geographic locations, shifts in climates may result in novel environmental conditions which are not only likely able to reduce the fitness but also deplete the quality and quantity of resources (e.g., food habitat) available for arthropod communities thereby threatening their existence in those areas. To counter this, arthropod populations facing unfavorable conditions may respond through either physiological or behavioral compensation [20,67], at both individual and population level, to better compete in the new environment; or they may migrate to new and favorable locations [68]. However, due to differences in the capacity to respond to various abiotic stressors and resource availability, fitness levels and dominance of various individual populations, they will be threatened,

resulting in new species composition per locality, possibly dominated by the most adaptive ones [30]. Moreover, such modifications by living organisms, for better survival in life threatening environmental conditions, have already been hypothesized to pose new problems to them [69].

In turn, the resultant failure to successfully compete in the stressful conditions may lead to different species composition in both pests and their natural enemies. For agriculture, such changes are of importance as they create new structures in original pest abundance, emergence of formerly secondary to primary pests [32], colonization of new areas which were previously unfavorable [40,68] and more importantly the modifications of habitats [67,70–72], which may lead to reduction in numbers of natural enemies and parasitoids in the agro-ecosystem. Consequently, the changes may result in reduction in the efficacy of biological control due to alterations in the predator-prey relationships or lack thereof. In this section, an overview of challenges in biological control as a result of climate-induced changes in pest or parasitoid biology and general habitat modifications is discussed.

3.1. Habitat Fragmentation and Natural Enemy Diversity or Abundance

It is widely known that crop surroundings play a crucial role in the conservation of natural enemies and parasitoids [49,73–75]. However, in most agro-ecosystems, much attention is paid to the crop (e.g., planting, irrigation fertilization) as compared with the peripheral environment. Such an approach has not only ensured perpetual optimal crop growth, but dependable hosts for pests as well. Through elaborate efforts that ensure crop growth and survival, insect pest herbivory has been indirectly guaranteed. However, this is contrary to the micro environment faced by natural enemies dwelling in the periphery where the natural habitats may not be receiving similar attention resulting in their numbers being reduced.

One consequence of changing climates may be habitat fragmentation of living organisms [1,76,77]. Thus, losses in suitable habitats will threaten the biodiversity and mere existence of organisms [78] including natural enemies or predators and parasitoids important for pest control in agro-ecosystems [75]. Consequently, the reduction or extinction of natural enemy populations will permit a pest build up, if unchecked, or over-reliance on alternative tactics for pest control which may be unsustainable, environmentally unfriendly and deplored by the consumers.

3.2. Insect Biology and Physiology in Relation to Environmental Change

Temperature affects a range of biochemical and physiological processes and, along with water availability, is probably the major environmental factor affecting insect population dynamics at either the individual or population level [20,79,80]. At individual level, it has already been shown that factors such as temperature play a key role in determining insect fitness [81], field performance [82,83] and survival [84]. However, because of variability in response to thermal stress, for instance, which might be introduced by age, gender, ontogeny [85] and the species in question, mismatches in development and activity between pests and parasitoids may occur with cascading effects on the efficacy of biological control programs. Use of mass-reared parasitoids in augmentative efforts may be a challenge as well since the insects being introduced into novel environments, which might be stressful, may perform poorly [86]. Hence, in scenarios where pests perform better than their corresponding indigenous parasitoids under stress, the efficacy of a biological program will be dramatically reduced.

However, some parasitoid species in the wild may benefit from rising winter temperatures. As discussed by Hance *et al.* (2007 and references therein), exposure to cold temperature in juvenile parasitoids results in reduced longevity of the adults [39]. Furthermore, cold exposure of adult parasitoids in the family Scelionidae reduces their longevity even after returning to optimal or warmer temperatures [39,87]. Low temperatures during development are also known to cause deformations and low fecundity in parasitoids. Basing on these limitations posed by low temperatures, a rise in winter temperatures may become beneficial to biologically-based pest management strategies for these specific parasitoid species, through improvements in their fecundity, development and longevity. At a population level, rising temperatures result in reduced generation time, rapid population growth and sometimes increased geographical ranges depending on resource availability [15,20]. However, such a positive correlation in temperature and population increase in pests is not uniform across different species even when facing similar conditions. This results in the asynchrony of life cycles of pests and parasitoids (or general reduction in populations of parasitoids required to effectively suppress pest populations. Such an asynchrony will create a temporal shortage or extinction of food resources for parasitoids whilst crop pest phenology is in line with the crop cycle. This consequently exerts pressure on the cropping system, especially in the cases of specialist parasitoids [88].

Furthermore, some pests may increase their invasion potential in relation to their ability to deal with changing climates either through phenotypic plasticity or variation in basal tolerance [89–92]. A classic example is the invasive maize stemborer *Chilo partellus* (Swinhoe), which was first introduced to Africa accidentally in Malawi but managed to establish itself in several African countries, becoming more destructive than the indigenous species in some instances [93]. Whilst the first introduction of *C. partellus* into Africa may have not been due to climate change, it has become apparent that its further establishment in several African regions follows a distinct pattern, which may be partly influenced by both climate and altitude [94]. Such pest dynamics increase pressure on the already-strained predator and parasitoid populations.

3.3. Chemical Ecology and Tritrophic Interactions in Agroecosystems

Climate induced changes in plant factors will affect quality and quantity of resources available for the insects resulting in variable direct and indirect consequences on the development times, size and fitness of both pests and parasitoids [95]. This may thus offset predation and parasitism [95], which sometimes reduces the efficacy of biological control programs.

Perhaps another dimension of plant physiology which is likely to be modified, due to climate change, with a resultant impact on biological control is their secondary metabolism with a resultant impact on indirect defense mechanisms. It has already been shown that some plant species emit specific volatiles in response to elicitors in the saliva or secretions (during oviposition) of particular foraging herbivores, which call for natural enemies and parasitoids of the herbivores in question [96]. Other plant species, when attacked, have also been shown to emit volatile compounds which warn neighboring plants to prime their defense in advance in a phenomenon referred to as 'eavesdropping' [97]. Such volatiles have since been generally called herbivore induced plant volatiles (HIPVs) [97]. The HIPVs have been regarded as having evolutionary significance in that their production is only switched on when needed as opposed to constitutive mechanisms, which are always

switched on. The latter may hence be 'wasteful' in terms of plant resource investment. However, recent studies have shown that plants undergoing abiotic stress respond by production of volatile isoprenoid compounds, perhaps, to avoid oxidative damage as a result of accumulation of reactive oxygen species in plants undergoing abiotic stress [98]. Isoprene compounds have since been shown to have the capacity to repel other specialist parasitoids, such as *Diadegma semiclausum* Hellen [99,100], and to influence herbivore feeding decisions [101]. It is therefore clear that isoprene production, as a result of abiotic stress, may influence plant-insect interactions in different agro-ecosystems. However, little is known of how the plants will prioritize their defense, in terms of volatile emission, when faced with biotic and multiple abiotic stressors associated with climate change.

For biological control, changes in volatile composition important for defense may result in failure for parasitoids to locate their host as some may require specific volatile blends in order to perform the desired functions [102]. Moreover, it has been shown that environmental stressors such as temperature impact on fitness, olfactory perception [103] and ultimately the ability to of the insects to track their hosts [95]. Therefore, if such changes occur in the parasitoids, biological control programs using natural enemies will be rendered less effective.

3.4. Complexity in the Outcome of Climate Change Impacts on Natural Enemy Abundance and Population Dynamics

While it has increasingly become clear that the climate is changing [6], an accurate prediction of the consequent effects on species distribution remains a daunting task. In most ecosystems, baseline species distribution, before any change in climates, is determined by a host of interactive factors between abiotic and biotic factors of the species in question. However, bioclimatic models used to predict future species prediction have often omitted or failed to account for all of the important factors resulting in them being questionable [104,105]. In these models, behavioral, dispersal mechanisms and inter-specific interactions, which can be made by living organisms in changing climates, have often been neglected [106,107] or assumptions which bring uncertainties to the models have often been used [105]. Such lack of availability of reliable tools for prediction are a challenge to farmers, in particular, those who are currently or plan to use biological control as their main insect pest control tactic. Availability of reliable tools will not only boost the confidence in users of the model, but also avert catastrophes in pest management due to over-reliance on flawed models.

Apart from bioclimatic models, resource constraints and challenges in experimental design have made empirical elucidation of the ecologically relevant behavioral and biological responses to climate change difficult. As a result, most inferences on the outcome of climate change are made from ecophysiological studies based on a single as opposed to multiple abiotic factors acting on different species. This is done despite increasing evidence of the differences in vulnerability or responses exhibited by some organisms when facing multiple as opposed to single stressors [108]. Possibly, through cross-tolerance [109] or additive effects of different stressors, such differences arise and may be species specific. It can therefore sometimes be misleading to rely on the inferences from the single-factor approach even though they serve as an important baseline indicator for individual physiological limits to stress tolerance. Such challenges bring new complications to farmers who are in

principal, supposed to rely on accurate data and predictions in order to carry adequate planning and implementation of their crop protection strategies.

4. Future Directions

4.1. Environmental Stress Biology, Evolutionary Resilience and Ecologically Relevant Measures for Organism Response to Changes in Climates

Insect population dynamics can be strongly influenced by adaptive behaviors and traits [110]. Furthermore, insects/natural enemy extinction events, colonization rates and demographic rates are influenced by organisms' adaptation in habitat selection, life history traits, niche breadth and dispersal behavior [111]. Recent studies suggest that in most cases, adaptive traits (e.g., thermal tolerance), significantly affect demographic dynamics and hence shape species distributions and population dynamics under a changing climate [86,112,113]. Similarly, in insects, low genetic variation in thermal adaptation can limit population growth and increase extinction risks in organisms living closer to their critical thermal limits [109,114]. However, although it is generally accepted that climate is changing, mitigating and coping with these effects remains an unresolved challenge [115,116]. In order to maintain or even improve biological control using natural enemies in a changing climate, several adaptive management strategies need to be implemented to cope with the changing status of insects and their natural enemies.

First and foremost, experimental protocols investigating the likely probable effects of climate change on insect-natural enemy interactions needs to be highly accurate. Although it has been documented that laboratory determined physiological traits (e.g., thermal tolerance) closely approximates their ability to cope with the stress under natural environments and are good indices of species fitness facing climate change [1], in most cases, there are ranging debates over the ecological relevance of experimental protocols used. For example, in thermal biology, previous studies have indicated that the methodological approach employed to determine an insect's thermal tolerance can affect the types of insights that can be gained, and ecological relevance, of these thermal limits [20,117]. We therefore suggest that experimental protocols predicting climate change effects on insect population abundance should incorporate ecologically relevant measures of fitness traits (e.g., temperature tolerance) that are likely to occur in the species' natural environment [118].

Moreover, forecasting efforts for insects' responses to climate change has almost exclusively focused on the variation in mean temperatures [1,119]. However, it is expected that the magnitude and severity of temperature variances and extremes may also increase under future scenarios [7,12] with concomitant reduction in ectotherm fitness [113,120]. From a functional perspective, how changes in means and variances of temperature might affect the basal, phenotypic plasticity of temperature tolerance as well as life-history traits of ectotherms, remain poorly elucidated but are critical for predicting physiological responses in the wild [30,121]. We suggest that detailed analyses of changes in both means and variability of temperature for both pests and their natural enemies are a critical component of accurate forecasting of insect/natural enemy population-level responses to climate change. Furthermore, some other environmental stressors other than temperature may also impact synergistically or antagonistically on ectotherm fitness in a changing climate [118]. We therefore

suggest that experimental protocols investigating ectotherm fitness in a changing climate should also incorporate other factors to evaluate possible interaction effects across factors on species' fitness related responses [119]. In addition to interactive effects of physical factors, biotic factors such as parasitism or chemical ecology of various organisms in an interactive multitrophic system may be affected resulting in distortions in predator-prey relationships. In such a case, the fate of some species' populations in multitrophic systems will not be directly determined by the changes in climate on its population but by a species that is high or low in their food chain. Hence, wherever possible, experimental protocols that inform predictive models for changing climates should incorporate tests of other species, which might be of importance in the agro-ecosystems.

The ability of organisms to mount physiological responses to variation in temperature at different time-scales may be an important component of insect/natural enemy persistence and thus efficacy of biological control under climate change scenarios [80,110,111]. From a theoretical standpoint, rapid evolution of thermal tolerance traits/phenotypes and their plasticity has been predicted [80,90]. Some studies indicated that if plasticity has an additive genetic basis, plasticity levels might evolve and likely contribute to evolutionary adaptation [90]. Phenotypic plasticity may buffer organisms upon introduction to novel and thermally unfavorable environments [92] and thus ensure survival when facing climatic stress [91]. However, current experimental protocols and crop protection specialists are ignoring the role of evolutionary processes in designing ways to protect and even improve biodiversity under global climate change [120–122]. We therefore propose that biological insect pest management programs using natural enemies should aim at developing resilient agro-ecosystems that maintain species' and populations' evolutionary potential [123,124]. Pörtner and Farrell (2008) point out that this may be possible through improvement in genetic diversity and processes that encourage continuous *in situ* evolutionary adaptation [125]. In agriculture, use of evolutionary processes to manage biotic interactions can be of importance as discussed in [126]. Through the help of artificial selections in crops, parasitoids or any other biological agents used to control may even be enhanced to gain competitive advantage over the pests intended for control when facing a selection pressure such as heat or drought. Such an approach is not new in agriculture as it has already succeeded in crop breeding, domestication of animals and pesticide resistance management [122,126]. Hence if given the investment priority in research, some pests may be managed even better under changing climates.

Recent studies have proved that phenotypic plasticity can be used to enhance field performance of mass reared insects release for pest management [82,83]. We also argue that by manipulating thermal performance of mass-reared predators and parasitoids through acclimation, field performance in augmentative programs may at least be temporarily improved and hence improve pest management programs [86]. Similarly, [86] studies emphasize that pest management programs should incorporate valuable information acquired from studies of the evolutionary biology of thermal performance [82,83,127]. Likewise, this approach may be critical for improving efficacy of biological control programs in the face of climate change.

4.2. Monitoring, Ecological Assessments and Ecosystem Management

Apart from predictive models, regular monitoring and ecological assessments might be an important tool to accurately expose the impact of climate change on the distribution and abundance of pest and

natural enemy populations. Whilst predictive models will be important in forecasting [128,129], their predictive power is often limited by assumptions, which may lack ecological considerations [104,130,131]. Hence ecological assessments and monitoring will generate huge ecological data sets that may be overlooked by physiological studies or predictive models. Such ecological factors are likely to give more valuable information of how agents of biological control will interact under changing climates.

However, as the case with predictive models, assessments of various systems will require reliable ecological indicators in order to avoid making erroneous conclusions. These ecological indicators will serve as tools that portray the structure, function and composition of the ecosystems [132] or trends that will be happening over time [133] within and around the crops. Ecological indicators will therefore serve as early warning tools for detecting deficits in management strategies inflicted by climate change whilst also giving a database of various ecological transitions, which can be correlated to climate events. In turn, management strategies can be implemented earlier before extensive damage. However, as outlined by [132] use of ecological indicators can be problem if (i) the ecological indicators lack scientific integrity (ii) choice of indicators is confounded in management and (iii) the assessments are based on a small number of indicators. To counter this, concerted efforts between, scientists, farmers and policy makers should exist. Therefore, scientists should develop ecological assessment tools that are accurate but simplified to enable usage by a broad range of users. With adequate data collection from researchers, government agencies and farmers (at their local level) using well designed assessment tools, important conclusions can be drawn on the status of habitats, species composition and abundance in various regions.

Where parasitoid populations will be high but activity is reduced due to fragmented habitats, farmers can increase parasitoid attack on pests by use of companion cropping and plant indirect defense. Such is the case with the push-pull strategy used in the control of stemborers, which increases parasitisation of insect pests, by their parasitoids, through manipulation of the agro-ecosytem to lure parasitoids directly into the cropping system [50,51]. Augmentative strategies can also be used to boost parasitoid populations whilst using techniques such as acclimation to improve activity in variable environments [82,83,86]. Moreover, biological control can also be enhanced through feeding natural enemies with honey. This phenomenon has been shown to improve fecundity and longevity of hymenopteran parasitoids [52–54]. Hence identification for particular tactics and sometimes in combination will be important in tackling pests biologically under changing climates.

5. Conclusion

In conclusion, we propose that integrating physiology, population dynamics and climate mapping shows great promise for making robust predictions of the potential effects of global climate change on biodiversity [134]. Thus, to better elucidate the link between climate change, biodiversity and its impacts on biological control using natural enemies, a fruitful area of future studies would be in developing mechanistic physiological approaches (and ones that consider ecological factors) to understanding climate change effects on insect biology, biodiversity and population dynamics. Furthermore, there is significant evidence that species are evolving with climate change [135,136].

Hence future predictions of climate change effects on insect biodiversity should incorporate evolutionary potential [123,124,137].

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References

1. Deutsch, C.A.; Tewksbury, J.J.; Huey, R.B.; Sheldon, K.S.; Ghalambor, C.K.; Haak, D.C.; Martin, P.R. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci.USA* **2008**, *105*, 6668–6672.
2. Huey, R.B.; Deutsch, C.A.; Tewksbury, J.J.; Vitt, L.J.; Hertz, P.E.; Pérez, H.J.A.; Garland, T., Jr. Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. London B 467* **2009**, *276*, 1939–1948.
3. Dillon, M.E.; Wang, G.; Huey, R.B. Global metabolic impacts of recent climate warming. *Nature* **2010**, *476*, 704–707.
4. Parmesan, C.; Yohe, G. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **2003**, *421*, 37–42.
5. Colwell, R.K.; Brehm, G.; Cardelus, C.L.; Gilman, A.C.; Longino, J.T. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* **2008**, *322*, 258–261.
6. IPCC. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L., Eds.; Cambridge University Press: Cambridge, UK, 2007.
7. Easterling, D.R.; Meehl, G.A.; Parmesan, C.; Changnon, S.A.; Karl, T.R.; Mearns, L.O. Climate extremes: Observations, modelling, and impacts. *Science* **2000**, *289*, 2068–2074.
8. Frich, P.; Alexander, L.V.; Della-Marta, P.; Gleason, B.; Haylock, M.; Tank, A.M.K.; Peterson, T. Observed coherent changes in climatic extremes during the second half of the twentieth century. *Clim. Res.* **2002**, *3*, 193–212.
9. Battisti, A.; Stastny, M.; Buffo, E.; Larson, S. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Glob. Change Biol.* **2006**, *12*, 662–671.
10. Tomozeiu, R.; Pavan, V.; Cacciamani, C.; Amici, M. Observed temperature changes in Emilia-Romagna: Mean values and extremes. *Clim. Res.* **2006**, *31*, 217–225.
11. Tebaldi, C.; Sansó, B. Joint projections of temperature and precipitation change from multiple climate models: A hierarchical Bayesian approach. *J. R. Stat. Soc.* **2009**, *172*, 83–106.
12. Im, E.S.; Jung, I.W.; Bae, D.H. The temporal and spatial structures of recent and future trends in extreme indices over Korea from a regional climate projection. *Int. J. Clim.* **2011**, *31*, 72–86.

13. Kiritani, K. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. *Pop. Ecol.* **2006**, *48*, 5–12.
14. Musolin, D.H. Insects in a warmer world: ecological, physiological and life history responses of true bugs (Heteroptera) to climate change. *Glob. Change Biol.* **2007**, *13*, 1565–1585.
15. Denlinger, D.L.; Lee, R.E. *Low Temperature Biology of Insects*; Cambridge University Press: Cambridge, UK, 2010.
16. Stevenson, R.D. The relative importance of behavioural and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am. Nat.* **1985**, *3*, 362–386.
17. Huey, R.B.; Bennett, A.F. Physiological adjustments to fluctuating thermal environments: An ecological and evolutionary perspective. In *Stress Proteins in Biology and Medicine*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 1990; pp. 37–59.
18. Chown, S.L.; Terblanche, J.S. Physiological diversity in insects: Ecological and evolutionary contexts. *Adv. Insect Physiol.* **2007**, *33*, 50–152.
19. Huey, R.B.; Pascual, M. Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* **2009**, *90*, 1715–1720.
20. Chown, S.L.; Nicolson, S.W. *Insect Physiological In Ecology: Mechanisms and Patterns*; Oxford University Press: Oxford, UK, 2004.
21. Bowler, K. Acclimation, heat shock and hardening. *J. Therm. Biol.* **2005**, *30*, 125–130.
22. Lagerspetz, K.Y.H. What is thermal acclimation? *J. Therm. Biol.* **2006**, *31*, 332–336.
23. Huey, R.B.; Berrigan, D. Testing evolutionary hypothesis of acclimation. In *Phenotypic and Evolutionary Adaptation to Temperatures*; Johnston, I.A., Bennett, A.F., Eds.; Cambridge University Press: Cambridge, UK, 1996; pp. 205–237.
24. Hoffmann, A.A.; Sørensen, J.G.; Loeschcke, V. Adaptation of *Drosophila* to temperature extremes: Bringing together quantitative and molecular approaches. *J. Therm. Biol.* **2003**, *28*, 175–216.
25. Fischer, K.; Eenhoorn, E.; Bot, A.N.M.; Brakefield, P.M.; Zwaan, B.J. Cooler butterflies lay larger eggs: Developmental plasticity versus acclimation. *Proc. R. Soc. London B* **2003**, *270*, 2051–2056.
26. Terblanche, J.S.; Chown, S.L. The relative contributions of developmental plasticity and adult acclimation to physiological variation in tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae) *J. Exp. Biol.* **2006**, *209*, 1064–1073.
27. Martinat, P.J. The Role of Climatic Variation and Weather in Forest Insect Outbreaks. In *Insect Outbreaks*; Barbosa, P., Schultz, J.C., Eds.; Academic Press: San Diego, CA, USA, 1987; pp. 241–268.
28. Lafferty, K.D. The ecology of climate change and infectious diseases. *Ecology* **2009**, *90*, 888–900.
29. Nyamukondiwa, C.; Terblanche, J.S. Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: thermal history affects short term responses to temperature. *Physiol. Entomol.* **2010**, *35*, 255–264.
30. Marshall, K.E.; Sinclair, B.J. Repeated stress exposure results in a survival-reproduction trade-off in *Drosophila melanogaster*. *Proc. R. Soc. London B* **2010**, *277*, 963–969.

31. Hoy, M.A. Parasitoids and Predators in Management of Arthropod Pests. In *Introduction to Insect Pest Management*; Metcalf, R.L., Luckman, W.H., Eds.; John Wiley Sons: New York, NY, USA, 1994.
32. Cammell, M.E.; Knight, J.D. Effects of climatic change on the population dynamics of crop pests. *Adv. Ecol. Res.* **1992**, *22*, 117–162.
33. Southwood, T.R.E.; Henderson, P.A. *Ecological Methods*; Blackwell Science: Oxford, UK, 2000.
34. Duale, A.H. Effect of temperature and relative humidity on the biology of the stem borer parasitoid *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae) for the management of stem borers. *Environ. Entomol.* **2005**, *34*, 1–5.
35. Sorribas, J.; van Baaren, J.; Garcia-Marí, F. Effects of climate on the introduction, distribution and biotic potential of parasitoids: Applications to biological control in California red scale. *Biol. Control* **2012**, in press.
36. Liu, S.S.; Zhang, G.M.; Zhu, J. Influence of temperature variations on rate development in insects analysis of case studies from entomological literature. *Ann. Entomol. Soc. Am.* **1995**, *88*, 107–119.
37. Kalyebi, A.; Sithanantham, S.; Overholt, W.A.; Hassan, S.A.; Mueke, J.M. Parasitism, longevity and progeny production of six indigenous Kenyan trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) at different temperature and relative humidity regimes. *Biocontrol. Sci. Technol.* **2005**, *15*, 255–270.
38. Hance, T.; van Baaren, J.; Vernon, P.; Boivin, G. Impact of extreme temperatures on parasitoids in a climate change perspective. *Ann. Rev. Entomol.* **2007**, *52*, 107–126.
39. Porter, J.H.; Parry, M.L.; Carter, T.R. The potential effects of climate change on agricultural insect pests. *Agric. Forest Meteorol.* **1991**, *57*, 221–240.
40. Cannon, R.J.C. The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biol.* **1998**, *4*, 785–796.
41. Estay, S.A.; Lima, M.; Labra, F.A. Predicting insect pest status under climate change scenarios: combining experimental data and population dynamics modelling. *J. Appl. Entomol.* **2009**, *133*, 491–499.
42. Klok, C.J.; Chown, S.L. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, *Paractora dreuxi* (Diptera: Helcomyzidae). *J. Insect Physiol.* **2001**, *47*, 95–101.
43. Sasson, A. Food security in Africa: An urgent global challenge. *Agric. Food Sec.* **2012**, doi:10.1186/2048-7010-1-2.
44. Lynch, S. Measuring progress in the transition to biologically-based IPM. In Proceedings of the OECD/FAO Workshop on Integrated Pest Management and Pesticide Risk Reduction, Neuchâtel, Switzerland, 28 June–2 July 1998.
45. Lockwood, J.A.; Ewen, A.B. Biological control of rangeland grasshoppers and locusts. In *The Bionomics of Grasshoppers, Katydids and their Kin*; Gangwere, S.K., Muralingan, M.C., Muralingan, M., Eds.; CABI International: Wallingford, UK, 1997; pp. 421–442.
46. Van Lenteren, J.C. The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake. *BioControl* **2011**, *57*, 1–20.

47. Eilenberg, J.; Hajek, A.; Lomer, C. Suggestions for unifying the terminology in biological control. *BioControl* **2001**, *46*, 387–400.
48. Landis, D.A.; Wratten, S.D.; Gurr, G.M. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Ann. Rev. Entomol.* **2000**, *45*, 175–201.
49. Jonsson, M.; Wratten, S.D.; Landis, D.A.; Gurr, G.M. Recent advances in conservation biological control of arthropods by arthropods. *Biol. Control* **2008**, *45*, 172–175.
50. Khan, Z.R.; James, D.G.; Midega, C.A.O.; Pickett, J.A. Chemical ecology and conservation biological control. *Biol. Control* **2008**, *45*, 210–224.
51. Cook, S.M.; Khan, Z.R.; Pickett, J.A. The use of 'push-pull' strategies in integrated pest management. *Ann. Rev. Entomol.* **2007**, *52*, 375–400.
52. Idris, A.B.; Grafius, E. Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth, *Plutella xylostella*, (Lepidoptera: Plutellidae). *Environ. Entomol.* **1995**, *24*, 1726–1735.
53. Idris, A.B.; Grafius, E. Nectar-collecting behavior of *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Plutellidae) *Environ. Entomol.* **1997**, *26*, 114–120.
54. Johanowicz, D.; Mitchell, E.R. Effects of sweet alyssum flowers on the longevity of the parasitoid wasps *Cotesia marginiventris* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Florida Entomol.* **2000**, *83*, 41–47.
55. Talekar, N.S.; Shelton, A.M. Biology, ecology and management of diamondback moth. *Ann. Rev. Entomol.* **1993**, *38*, 275–302.
56. Perdikis, D.; Fantinou, A.; Lykouressis, D. Enhancing pest control in annual crops by conservation of predatory Heteroptera. *Biol. Control* **2011**, *59*, 13–21.
57. Gutierrez, A.P.; Ponti, L.; d'Oultremont, T.; Ellis, C.K. Climate change effects on poikilotherm tritrophic interactions. *Clim. Change* **2008**, *87*, S167–S192.
58. Colliera, T.; van Steenwyk, R. A critical evaluation of augmentative biological control. *Biol. Control* **2004**, *31*, 245–256.
59. Lomer, C.J.; Bateman, R.P.; Johnson, D.L.; Langewald, J.; Thomas, M. Biological control of locusts and grasshoppers. *Ann. Rev. Entomol.* **2001**, *46*, 667–702.
60. Macedo, N. Método de criação do parasitóide Cotesia flavipes (Cameron). In *Controle, Biológico de Pragas: Produção massal e controle de qualidade*; Bueno, V.H.P., Ed.; Editora UFLA: Lavras, Minas Gerais, Brazil, 2000; pp. 161–174.
61. Daane, K.M.; Yokota, G.Y.; Zheng, Y.; Hagen, K.S. Inundative release of common green lacewings (Neuroptera: Chrysopidae) to suppress *Erythroneura variabilis* and *E. elegantula* (Homoptera: Cicadellidae) in vineyards. *Environ. Entomol.* **1996**, *25*, 1224–1234.
62. Klein Koch, C.A. Proyectos de control biológico en curso en Ecuador. In *M.C. Zapater El Control Biológico en América Latina*; IOBC: Buenos Aires, Argentina, 1996; pp. 41–47.
63. Corrêa-Ferreira, B.S. Utilização do parasitóide de ovos Trissolcus basalis (Wollaston) no controle de percevejos da soja. In *Circular Técnica 11*; Embrapa-CNPSo: Londrina, Brazil, 1993; p. 40.

64. Corrêa–Ferreira, B.S.; Moscardi, F. Biological control of soybean stink bugs by inoculative releases of *Trissolcus basalis*. *Entomol. Exp. Appl.* **2011**, *79*, 1–7.
65. Fargues, J.; Ouedraogo, A.; Goettel, M.S.; Lomer, C.J. Effect of temperature, humidity and inoculation method on susceptibility of *Schistocerca gregaria* to *Metarhizium flavoviride*. *Biocontrol. Sci. Technol.* **1997**, *7*, 345–356.
66. Jonsson, D.L.; Huang, H.C.; Harper, A.M. Mortality of grasshoppers (Orthoptera: Acrididae) inoculated with a Canadian isolate of the fungus *Verticillium lecanii*. *J. Invertebr. Pathol.* **1988**, *52*, 335–342.
67. Chown, S.L.; Sørenson, J.G.; Terblanche, J.S. Water loss in insects: An environmental change perspective. *J. Insect Physiol.* **2011**, *57*, 1070–1084.
68. Parmesan, C.; Ryrholm, N.; Stefanescu, C.; Hill, J.K.; Thomas, C.D.; Descimon, H.; Huntley, B.; Kaila, L.; Kullberg, J.; Tammaru, T.; *et al*. Pole ward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **1999**, *399*, 579–583.
69. Zachariassen, K.E. The water conserving physiological compromise of desert insects. *Eur. J. Entomol.* **1996**, *93*, 359–367.
70. Nair, U.S.; Lawton, R.O.; Welch, R.M.; Pielke, R.A., Sr. Impact of land use on Costa Rican tropical montane cloud forests: Sensitivity of cumulus cloud field characteristics to lowland deforestation. *J. Geog. Res.* **2003**, doi:10.1029/2001JD001135.
71. Webb, T.J.; Woodward, F.J.; Hannah, L.; Gaston, K.J. Forest cover-rainfall relationships in a biodiversity hotspot: the Atlantic forest of Brazil. *Ecol. Appl.* **2005**, *15*, 1968–1983.
72. Markarieva, A.M.; Gorshkov, V.G. Biotic pump of atmospheric moisture as driver of the hydrological cycle on land. *Hyd. Earth Sys. Sci.* **2007**, *11*, 1013–1033.
73. Nicholls, C.I.; Parrella, M.; Altieri, M.A. Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agric. Forest Entomol.* **2000**, *2*, 107–113.
74. Wilby, A.; Thomas, M.B. Are the ecological concepts of assembly and function of biodiversity useful frameworks for understanding natural pest control? *Agric. Forest Entomol.* **2002**, *4*, 237–243.
75. Letourneau, D.K.; Armbrecht, I.; Rivera, B.S.; Lerma, J.M.; Carmona, E.J.; Daza, M.C.; Escobar, S.; Galindo, V.; Gutierrez, C.; Lopez, S.D.; *et al*. Does plant diversity benefit agroecosystems? A synthetic review. *Ecol. Appl.* **2011**, *21*, 9–21.
76. Dyer, J.M. Implications of habitat fragmentation on climate change-induced forest migration. *Prof. Geog.* **2005**, *46*, 449–459.
77. Opdam, P.; Wascher, D. Climate change meets habitat fragmentation: Linking landscape and biogeographical scale levels in research and conservation. *Biol. Conserv.* **2004**, *117*, 285–297.
78. Fahrig, L. Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. S.* **2003**, *34*, 487–515.
79. Cossins, A.; Bowler, K. *Temperature Biology of Animals*; Chapman and Hall: London, UK, 1987.
80. Angilletta, M.J. Thermal Adaptation. In *A Theoretical and Empirical Synthesis*; Oxford University Press: Oxford, UK, 2009.

81. Basson, C.H.; Nyamukondiwa, C.; Terblanche, J.S. Fitness costs of rapid cold-hardening in *Ceratitis capitata*. *Evolution* **2011**, *66*, 296–304.
82. Kristensen, T.N.; Hoffmann, A.A.; Overgaard, J.; Sørensen, J.G.; Hallas, R.; Loeschcke, V. Costs and benefits of cold acclimation in field-released Drosophila. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 216–221.
83. Chidawanyika, F.; Terblanche, J.S. Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. *Evol. Appl.* **2011**, *4*, 534–544.
84. Chidawanyika, F.; Terblanche, J.S. Rapid thermal responses and thermal tolerance in adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *J. Insect Physiol.* **2010**, *57*, 108–117.
85. Bowler, K.; Terblanche, J.S. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? *Biol. Rev.* **2008**, *83*, 339–355.
86. Sørenson, J.G.; Addison, M.F.; Terblanche, J.S. Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. *Crop Prot.* **2012**, *38*, 87–94.
87. Robinet, C.; Roques, A. Direct impacts of recent climate warming on insect populations. *Integr. Zool.* **2010**, *2*, 132–142.
88. Stacey, D. Climate and biological control in organic crops. *Int. J. Pest Manage.* **2003**, *49*, 205–214.
89. Janzen, D.H. Why mountain passes are higher in tropics. *Am. Nat.* **1967**, *101*, 233–249.
90. Ghalambor, C.K.; Huey, R.B.; Martin, P.R.; Tewksbury, J.J.; Wang, G. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr Comp. Biol.* **2007**, *46*, 5–17.
91. Chown, S.L.; Slabber, S.; McGeoch, M.A.; Janion, C.; Leinaas, H.P. Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc. R. Soc. London. Ser. B* **2007**, *274*, 2661–2667.
92. Nyamukondiwa, C.; Kleynhans, E.; Terblanche, J.S. Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecol. Entomol.* **2010**, *35*, 565–575.
93. Sithole, S.Z. Status and control of the Stem Borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in Southern Africa. *Int. J. Trop. Sci.* **1990**, *11*, 479–488.
94. Ong'amo, G.O.; Le Rü, B.P.; Dupas, S.; Moyal, P.; Calatayud, P.A.; Silvain, J.F. Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. *Ann. de la Société Entomol. de France* **2006**, *42*, 171–177.
95. Thomson, L.J.; Macfadyen, S.; Hoffmann, A.A. Predicting the effects of climate change on natural enemies of agricultural pests. *Biol. Control* **2010**, *52*, 296–306.
96. Hilker, M.; Meiners, T. Early herbivore alert: insect eggs induce plant defence. *J. Chem. Ecol.* **2006**, *32*, 1379–1397.
97. Rodriguez-Saona, C.R.; Frost, C.J. New evidence for a multi-functional role of herbivore-induced plant volatiles in defense against herbivores. *Plant Signaling Behav.* **2010**, *5*, 58–60.
98. Vickers, C.E.; Gershenzon, J.; Lerdau, M.T.; Loreto, F. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nat. Chem. Biol.* **2009**, *5*, 283–290.

99. Gershenzon, J. Insects turn up their noses at sweating plants. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17211–172212.
100. Loivamäki, M.; Mumm, R.; Dicke, M.; Schnitzler, J.P. Isoprene interferes with the attraction of bodyguards by herbaceous plants. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17430–17435.
101. Laothawornkitkul, J.; Paul, N.D.; Vicker, C.E.; Possell, M.; Taylor, J.E.; Mullineaux, P.M.; Hewitti, C.N. Isoprene emissions influence herbivore feeding decisions. *Plant Cell Environ.* **2008**, *31*, 1410–1415.
102. Bruce, T.J.A.; Picket, J.A. Perception of volatile blends by herbivorous insects-Finding the right mix. *Phytochemistry* **2011**, *72*, 1605–1611.
103. Riveron, J.; Boto, T.; Alcorta, E. The effect of environmental temperature on olfactory perception in Drosophila melanogaster. *J. Insect Physiol.* **2009**, *55*, 943–951.
104. Pearson, R.G.; Dawson, T.P. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecol. Biogeogr.* **2003**, *12*, 361–371.
105. Wiens, J.A.; Stralberg, D.; Jongsomjit, D.; Howell, C.A.; Snyder, M.A. Niches, models, and climate change: Assessing the assumptions and uncertainties. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 19729–19736.
106. Davis, A.J.; Jenkinson, L.S.; Lawton, J.H.; Shorrocks, B.; Wood, S. Making mistakes when predicting shifts in species range in response to global warming. *Nature* **1998**, *391*, 783–786.
107. Heikinnen, R.K.; Luoto, M.; Araujo, M.B.; Virkkala, R.; Thuiller, W.; Sykes, M.T. Methods and uncertainties in bioclimatic envelope modelling under climate change. *Prog. Phys. Geog.* **2006**, *6*, 1–27.
108. Mikkelsen, T.N.; Beier, C.; Jonasson, S.; Holmstrup, M.; Schmidt, I.K.; Ambus, P.; Pilegaard, K.; Michelsen, A.; Albert, K.; Andresen, L.C.; *et al.* Experimental design of multifactor climate change experiments with elevated CO_2 warming and drought: the CLIMAITE project. *Funct. Ecol.* **2008**, *22*, 185–195.
109. Patori, G.M.; Foyer, C.H. Common components, networks, and pathways of cross-tolerance to stress. The central role of "Redox" and abscisic acid–mediated controls. *Plant Physiol.* **2002**, *129*, 460–468.
110. Hoffmann, A.A.; Chown, S.L.; Clusella-Trullas, S. Upper thermal limits in terrestrial ectotherms: How constrained are they? *Funct. Ecol.* **2012**, in press.
111. Hanski, I.; Mononen, T. Eco-evolutionary dynamics of dispersal in spatially heterogeneous environments. *Ecol. Lett.* **2011**, *14*, 1025–1034.
112. Kellermann, V.; Heerwaarden, B.; Sgró, C.M.; Hoffmann, A.A. Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* **2009**, *325*, 1244–1246.
113. Hoffmann, A.A. Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* **2010**, *213*, 870–880.
114. Chown, S.L.; Hoffmann, A.A.; Kristensen, T.; Angilletta, M.; Strenseth, N. Adapting to climate change: a perspective from evolutionary physiology. *Clim. Res.* **2010**, *43*, 3–15.
115. Parmesan, C. Ecological and evolutionary responses to recent climate change. *Ann. Rev. Ecol. Evol. Syst.* **2006**, *37*, 637–669.

116. Williams, S.E.; Shoo, L.P.; Isaac, J.L.; Hoffmann, A.A.; Langham, G. Towards and integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* **2008**, doi:10.1371/journal.pbio.0060325.
117. Lutterschmidt, W.I.; Hutchison, V.H. The critical thermal maximum: Data to support the onset of spasms as the definitive end point. *Can. J. Zool.* **1997**, *75*, 1553–1560.
118. Terblanche, J.S.; Hoffmann, A.A.; Mitchell, K.A.; Rako, L.; Le Roux, P.C.; Chown, S.L. Ecologically relevant measures of tolerance to potentially lethal temperatures. *J. Exp. Biol.* **2011**, *214*, 3713–3725.
119. Crozier, L.; Dwyer, G. Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. *Am. Nat.* **2006**, *167*, 853–866.
120. Somero, G.N. The physiology of climate change: how potentials for acclimatisation and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **2009**, *213*, 912–920.
121. Terblanche, J.S.; Nyamukondiwa, C.; Kleynhans, E. Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). *Entomol. Exp. Appl.* **2010**, *137*, 304–315.
122. Hendry, A.P.; Kinnison, M.T.; Heino, M.; Day, T.; Smith, T.B.; Fitt, G.; Bergstrom, C.T.; Oakeshott, J.; Jorgensen, P.S.; Zalucki, M.P.; *et al.* Evolutionary principles and their practical applications. *Evol. Appl.* **2011**, *4*, 159–183.
123. Hoffmann, A.A.; Sgrò, C.M. Climate change and evolutionary adaptation. *Nature* **2011**, *470*, 479–485.
124. Sgrò, C.M.; Lowe, A.J.; Hoffmann, A.A. Building evolutionary resilience for conserving biodiversity under climate change. *Evol. Appl.* **2010**, *4*, 326–337.
125. Pörtner, H.O.; Farrell, A.P. Physiology and climate change. *Science* **2008**, *322*, 690–692.
126. Thrall, P.H.; Oakeshott, J.G.; Fitt, G.; Southerton, S.; Burdon, J.J.; Sheppard, A.; Russell, R.J.; Zalucki, M.; Heino, M.; Denison, F.; *et al.* Evolution in agriculture: The application of evolutionary approaches to the management of biotic interactions in the agro-ecosystems. *Evol. Appl.* **2011**, *4*, 200–215.
127. Crandal, K. A multifacted approach to species conservation. *Anim. Conserv.* **2009**, *12*, 105–106.
128. Berry, P.M.; Dawson, T.P.; Harrison, P.A.; Pearson, R.G. Modelling potential impacts of climate change on the bioclimatic envelope of species in Britain and Ireland. *Global Ecol. Biogeogr.* **2002**, *11*, 453–462.
129. Pearson, R.G.; Dawson, T.P.; Berry, P.M.; Harrison, P.A. Species: A spatial evaluation of climate impact on the envelope of species. *Ecol. Modell.* **2002**, *154*, 289–300.
130. Guisan, A.; Thuiller, W. Predicting species distribution: offering more than simple habitat models. *Ecol. Lett.* **2005**, *8*, 993–1009.
131. Mustin, K.; Benton, T.G.; Dytham, C.; Travis, J.M.J. The dynamics of climate-induced range shifting; perspectives from simulation modeling. *Oikos* **2009**, *118*, 131–137.
132. Dale, V.H.; Suzanne, C.B. Challenges in the development and use of ecological indicators. *Ecol. Indic.* **2001**, *1*, 3–10.
133. Cairns, J.; McCormick, P.V.; Niederlehner, B.R. A proposed framework for developing indicators of ecosystem health. *Hydrobiologia* **1993**, *236*, 1–44.

134. Hartley, S.; Krushelnycky, P.D.; Lester, P.J. Integrating physiology, population dynamics and climate to make multi-scale predictions for the spread of an invasive insect: The Argentine ant at Haleakala National Park, Hawaii. *Ecography* **2010**, *33*, 83–94.
135. Bradshaw, W.E.; Holzapfel, C.M. Evolutionary response to rapid climate change. *Science* **2006**, *312*, 1477–1478.
136. Hoffmann, A.A.; Daborn, P.J. Towards genetic markers in animal populations as biomonitors for human-induced environmental change. *Ecol. Lett.* **2007**, *10*, 63–76.
137. Kearney, M.; Porter, W.P.; Williams, C.; Ritchie, S.; Hoffmann, A.A. Integrating biophysical and evolutionary theory to predict climatic impacts on species' ranges: The dengue mosquito *Aedes aegypti* in Australia. *Funct. Ecol.* **2009**, *23*, 528–538.

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Oviposition acceptance and larval development of *Chilo partellus* stemborers in drought-stressed wild and cultivated grasses of East Africa

Frank Chidawanyika[1,2]*, Charles A.O. Midega[1], Toby J.A. Bruce[3], Frances Duncan[2], John A. Pickett[3] & Zeyaur R. Khan[1]

[1]*Habitat Management Programme, International Centre of Insect Physiology and Ecology, PO Box 30, Mbita Point, Kenya,* [2]*Global Change and Sustainability Research Institute, School of Animal Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, Johannesburg 2050, South Africa, and* [3]*Biological Chemistry and Crop Protection Department, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK*

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Abstract

Maternal host choices during oviposition by herbivorous insects determine the fitness of their offspring and may be influenced by environmental changes that can alter host-plant quality. This is of particular relevance to 'push-pull' cropping systems where host preferences are exploited to manage insect pest populations. We tested how drought stress in maize and companion plants that are used in these systems affect oviposition preference, larval feeding, and development of the spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae). Five host species were tested (all Poaceae): maize (*Zea mays* L.), Napier grass (*Pennisetum purpureum* Schumach), signal grass [*Brachiaria brizantha* (A. Rich) Stapf], *Brachiaria* cv. 'Mulato', and molasses grass [*Melinis minutiflora* (Beauv.)]. Under drought stress, maize experienced as much oviposition as control unstressed maize in choice and no-choice experiments. Similarly, larval leaf damage was not significantly different in drought-stressed vs. unstressed maize. In contrast, oviposition occurred less on drought-stressed than on unstressed Napier and signal grass. Oviposition acceptance and leaf damage remained low in both drought-stressed and unstressed molasses grass and Mulato. Larval survival and development remained high in drought-stressed maize, but not in Napier, signal, and molasses grass and Mulato, where survival and development were low in both drought-stressed and unstressed plants. Our results indicate that herbivore responses to drought-stressed plants depend on the plant species and that drought stress can change host preference and acceptance rankings. In particular, trap-crops such as Napier grass may not divert oviposition from the main maize crop under drought stress conditions.

Introduction

In recent years, the effects of global climate change (GCC) on ecosystem structure and function have become a matter of increasing concern. One consequence of GCC events is the increased frequency and intensity of plant abiotic stressors such as drought due to decreased or erratic rainfall (Hoerling & Kumar, 2003; Christensen et al., 2007); and increased growing season temperatures (Burke et al., 2009). However, the interaction of plants with their abiotic environment is not static as they often respond via a myriad of mechanisms that can be physiological, morphological, and/or biochemical (Gershenzon, 1984; Chaves et al., 2003; Vickers et al., 2009a,b; Gutbrodt et al., 2011). Being autotrophs that support diverse organisms, plant responses induced by extreme abiotic environments may have cascading effects up the various trophic levels within the ecosystem. For example, drought stress has been shown to have the potential to change the way plants interact with herbivorous insects through modifications of

*Correspondence: Frank Chidawanyika, Habitat Management Programme, International Centre of Insect Physiology and Ecology, ICIPE, PO Box 30, Mbita Point, Kenya. E-mail: fchidawanyika@icipe.org

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plant traits such as resistance to insects (Mody et al., 2009), nutritive value (Mattson & Haack, 1987; Hale et al., 2005; Mody et al., 2007), and amounts of constitutive chemical defence compounds (Hale et al., 2005; Gutbrodt et al., 2012a).

Three major hypotheses have been advanced to explain and/or predict the relationships that may exist between plants and insects under drought stress. First, the plant stress hypothesis (White, 1969) which supports the idea that drought stress improves the development of phytophagous insects through the increase in available free amino acids in plant tissues as a result of hydrolysed foliar proteins (Brodbeck & Strong, 1987; Huberty & Denno, 2004) and reduction in defence compounds (Rhoades, 1979). Second, the plant vigour hypothesis (Price, 1991) which posits that healthy, more vigorous plants are more desirable for insect herbivores than drought-stressed ones because of higher nutrient content, osmotic potential in shoots, and lower rates of abscission (Price, 1991; Preszler & Price, 1995; Cornelissen et al., 2008; Tariq et al., 2012). Third, the pulsed stress hypothesis (Huberty & Denno, 2004) which postulates that the performance of phloem feeders is enhanced under intermittently (pulsed) water stressed plants, perhaps due to the rise in the amount of available nitrogen and turgor pressure during recovery (Tariq et al., 2012).

Although there are several studies in support of each of the hypotheses, there are also several others that refute some of them (discussed in Huberty & Denno, 2004; Tariq et al., 2012). From the foregoing, it is clear that drought stress may either enhance or reduce a plant's susceptibility to herbivorous insects (Vallat et al., 2005; Meyer et al., 2006) and potentially determine intraspecific food choices as demonstrated by Neto et al. (2012). More recently, plant responses to abiotic stress have been reported to include changes in quality and quantity of emitted volatile organic compounds (VOCs) for protection against stress-related damages (Gershenzon & Dudareva, 2007; Vickers et al., 2009a,b; Yuan et al., 2009; Holopainen & Gershenzon, 2010; Niinemets, 2010). However, such compounds have also been cited as playing various ecological roles in plant-insect interactions (Gershenzon & Dudareva, 2007; Laothawornkitkul et al., 2008a,b; Loivamäki et al., 2008). Hence, abiotic stressors have the potential to reshape chemically mediated trophic interactions in both natural and managed ecosystems.

Despite such findings and the knowledge that specific blends are important for insect host location (reviewed by Bruce & Pickett, 2011), very few studies have directly explored how the changes induced by abiotic stress, such as drought, may affect insect host preference both for agricultural crops and natural hosts. Most studies focus primarily on one crop of interest, for example, Seagraves et al. (2011) showed that *Orius insidiosus* (Say) preferred to lay more eggs on drought-stressed *Phaseolus vulgaris* L. Indeed, investigations of plant-insect interactions under stress often give valuable information on the integrity of the phenological associations of the host and herbivore (e.g., van Asch et al., 2007, 2010). However, one ecological aspect which may not be accounted by such a single plant species approach is the consequences of abiotic stress in terms of relative suitability of different potential host plants for generalist herbivores. In agriculture, understanding such relationships is of importance as it indicates the future vulnerability of agro-ecosystems and the suitability of pest management strategies.

Chilo partellus Swinhoe (Lepidoptera: Crambidae) is a highly destructive polyphagous pest in cereal crops in East and Southern Africa. It feeds on the leaves of plants before burrowing deep into the stem, compromising the plant's fitness and productivity, and in severe cases causing death (Kfir et al., 2002). In East Africa, the 'push-pull' system has been used to suppress the pest with considerable success through manipulation of trophic interactions in the agro-ecosystem (Cook et al., 2007; Hassanali et al., 2008). This system uses Napier grass [*Pennisetum purpureum* Schumach (Poaceae)] as a perimeter 'pull' or trap plant because of its ability to attract stemborer oviposition away from the maize [*Zea mays* L. (Poaceae)] crop, while providing limited support for the development of the larvae (van den Berg, 2006; Khan et al., 2006, 2007; Cook et al., 2007; Midega et al., 2010). The second integrated component of the strategy involves stemborer-repellent plants such as *Desmodium* spp. (Fabaceae) or molasses grass [*Melinis minutiflora* (Beauv.) (Poaceae)] as intercrops with the cereals.

To address how environmental stressors may reshape the trophic interactions, we investigated how water deficits may impact the crops used in the 'push-pull' system and wild grasses. Hence, our objectives in this project were to assess the differences (1) in oviposition acceptance of *C. partellus* in wild and cultivated grass under optimal and drought stress conditions and (2) in larval growth and development in the wild and cultivated grasses under optimal and drought stress conditions. This was done with a goal of selecting plants that exhibit desirable traits compatible with the 'push-pull' companion cropping system even under drought stress for possible use in drier regions.

Materials and methods

Study site

The studies were conducted at the International Centre of Insect Physiology Thomas Odhiambo Campus (ITOC) situated in Mbita Point, western Kenya (0°25′S, 34°12′E).

In this area (1 200 m above sea level), cereal stemborers are a major constraint to cultivation of maize. Mean annual temperature is 27 °C, annual precipitation averages 900 mm but with frequent occurrence of extended periods of water deficit that induce drought stress responses in plants.

Study organisms

The *C. partellus* moths and larvae used in these studies were taken from a colony maintained at ITOC using an artificial diet as described by Onyango & Ochieng-Odero (1994). In all experiments, newly hatched larvae were used without any prior exposure to the test plants. Five test plant species were used: Napier grass variety 'bana', signal grass *Brachiaria brizantha* (A. Rich) Stapf, *Brachiaria* cv. Mulato (a hybrid developed by International Centre for Tropical Agriculture, Columbia), molasses grass, and a maize variety (WH505) (all Poaceae). All plants were planted in perforated 5-l plastic buckets filled with vertisol soil from the fields at Mbita Point ITOC. Napier grass, obtained from a stock sourced from Kenya Agricultural Research Institute as described in Khan et al. (2006), was planted using root splits. Similarly, signal and molasses grass were planted from root splits obtained from wild collections from Mbita Point fields of the ITOC, whereas Mulato was grown from seeds.

Oviposition assays

Choice tests. Drought stress response in treatment plants was induced using modifications of methods adapted from Ekmekçi & Çiçek (2009). At 3 weeks after planting, treatment plants were denied water for up to 10–13 days while control plants were adequately watered during that period. Choice tests were then carried out following modifications of protocols as described in Midega et al. (2010). Four independent pairs of drought-stressed and control plants per species were placed in individual cages (40 × 40 × 80 cm) before four gravid moths were introduced into each cage. This set-up was repeated 3× under similar ambient conditions to allow for comparison as a pooled dataset from 12 independent pairs for each species and treatment. The plants were placed in the corners of the cage, the moths were released in the centre of the base of the cage. A moistened wad of cotton wool was placed in the cages to allow for feeding of the moths. Moths were then allowed to oviposit overnight under natural ambient conditions. The following morning, leaf parts carrying eggs were excised to enable counting of eggs under a binocular microscope at 6.5× magnification. Here, choice was regarded as the preferential oviposition by the insects in a cage when given differently treated plants (Singer, 1986; Midega et al., 2010).

No-choice tests. The same experimental procedure as described above was used but with only one plant per cage. For each species, four plants were used per treatment and the set-up was replicated 3×.

Larval development assays

Larval survival and development (whole plants). After 3 weeks of planting and growth under adequate watering, eight plants from each species were randomly partitioned into two categories; drought stress (treatment) and the control groups. Four plants of each species from both treatment and control categories were randomly and individually placed into wooden-framed netted cages placed on tables as above. These tables had their legs dipped in moats at all times to avoid any predators coming into the cages.

Drought stress in treatment plants was induced as above by withholding watering for 10–13 days while control plants received adequate water. Ten newly hatched larvae were then placed in the whorl section of each caged plant (treatment and control) using a soft camel-hair paint brush to allow for development on the plants. Upon larval infestation, treatment plants were watered once to avoid them reaching permanent wilting point (after ca. 10–13 days) as determined in preliminary trials. However, control plants were watered once every 2 days to ensure adequate moisture just as prior to the infestations. Thereafter, larvae were allowed to feed and develop on individual plants in netted cages throughout the experiment. As most *C. partellus* eggs are known to hatch in the morning (Leuschner, 1990; Khan et al., 2006), all infestations were conducted in the morning to mimic natural conditions. After 15 days of larval feeding, visual assessments were carried out to assess the amount of leaf damage as it gives a good indication of the infestation and sometimes level of acceptability of a host plant (Leuschner, 1990; Khan et al., 2006). The score was scaled from 1 to 5, where 1 was assigned to individual plants with little/no visual damage and 5 to those with the most damage. Prior to collection of the data, visual scores were first calibrated by tracing leaves for each species and treatment on a graph paper to estimate herbivore-damaged area following Blundell & Peart (2000). To maintain consistency, all visual scores were done by the same person and were closely correlated with measured values.

All the caged plants were then dissected to obtain data on the number and life-stage of the surviving larvae. These dissected plants were replaced by individual whole plants of the same species, age, and watering/stress regime which were then infested by all the surviving larvae in each cage to allow the completion of their development. After a further 15 days of larval development on the new host plant

replacements, plants were dissected as above. This experiment was repeated 3× with four replications per species (n = 12 for each treatment and control). In all cases, larvae were considered to have survived after showing movement upon careful prodding with a soft brush.

Larval survival and development (excised leaves). Laboratory assays were carried out to assess the rate of survival and development of *C. partellus* larvae on drought-stressed and control grass species. At 4 weeks after planting, plant stems with leaves and sheaths attached (whorl) were cut from each plant species from both treatment and control plants, and placed in transparent Kilner jars (20 cm high, 8 cm diameter) in a dark room of the laboratory. The whorls were randomly arranged in each jar and the set-up was replicated 20×. Ten newly hatched larvae were then placed into each of the jars using a fine camel-hair brush. The whorls of the plants were ca. 15 cm long and 5 cm in diameter (Khan et al., 2006) to provide adequate material for the larvae to feed on. Perforated lids covered with a wire mesh were used to tightly secure the openings in the jars. After 5 days, larvae were retrieved and their life stages were determined. The surviving larvae were introduced to new freshly cut whorls and stems for mature larvae of corresponding plant species under the same watering regimes. Feeding and collection of data in this manner, every 5 days, for the same individuals continued until conclusion of the experiment after 40 days.

Statistical analysis

All statistical analyses were performed using R (R Development Core Team, 2011). One-way analysis of variance (ANOVA) using a generalised linear model was used to test the differences in percentage of larval survival, feeding scores, and no-choice oviposition traits (total number of eggs, number of clutches, and number of eggs per clutch) in all plant species. Survival percentages were first arcsine transformed. Tukey's studentized range test was used to separate means at the threshold level of $\alpha = 0.05$. In all the plant species, a Student's t-test was used to determine differences in the numbers of eggs and egg clutches within species of drought-stressed vs. control plants.

Results

Oviposition assay

Choice tests. In dual choice tests between individual drought-stressed and control plants of the same species, *C. partellus* laid significantly more eggs on control Napier and signal grass (Table 1). However, there was no significant difference in the total number of eggs laid on treated and control plants for maize, Mulato, and molasses grass (Table 1).

Table 1 Mean (± SE) number of eggs per plant laid by *Chilo partellus* in a within-species choice test between control and drought-stressed plants. For each plant species and treatment, a total of 48 moths were tested in groups of four moths per cage

Plant species	Control	Drought-stressed	t	P
Maize	218.3 ± 35.9	187.1 ± 25.6	0.4	0.70
Napier grass	380 ± 33.8	123.9 ± 22.5	5.3	<0.001
Signal grass	323.2 ± 63.9	134.2 ± 28.5	4.1	0.001
Mulato grass	16.4 ± 4.7	18.7 ± 4.8	0.9	0.38
Molasses grass	9.4 ± 3.0	8.8 ± 2.7	0.1	0.88

No-choice tests. Among the control plants, i.e., those not exposed to drought stress, *C. partellus* moths oviposited significantly more eggs on Napier and signal grass than on the other test plants (Table 2). Based on the number of eggs oviposited per plant, Napier and signal grass were most accepted for egg laying by the moths, followed by maize, whereas Mulato and molasses grass were least accepted. However, this in the order of acceptance changed markedly under drought stress, where maize received significantly more oviposition in terms of eggs compared to the other test plants (Table 2). When plants were drought stressed, more than twice as many eggs were laid on maize than on Napier or signal grass (Table 2).

Table 2 Mean (± SE) number of eggs, clutches, and eggs per clutch laid by *Chilo partellus* on single control (C) and drought-stressed (D) plants in no-choice tests

Treatment	No. eggs	No. clutches	No. eggs per clutch
Maize (C)	126 ± 19.7b	3.75 ± 0.5a	31.3 ± 3.6a
Maize (D)	186 ± 52.7b	4.4 ± 0.9a	19.3 ± 2.5b
Napier grass (C)	233.3 ± 20.5a	3.6 ± 0.3a	37.8 ± 4.4a
Napier grass (D)	68.2 ± 16.7c	2.3 ± 0.5a	20.2 ± 2.4b
Signal grass (C)	222.8 ± 54.3a	4.8 ± 0.7a	32.6 ± 3.1a
Signal grass (D)	71.9 ± 16.1c	2.2 ± 0.4a	19.6 ± 3.1b
Mulato (C)	20.5 ± 13d	2.9 ± 0.6a	12.2 ± 1.2c
Mulato (D)	20.3 ± 22d	1.1 ± 0.7b	14.2 ± 4.2c
Molasses grass (C)	25.8 ± 7.8d	1.2 ± 0.3b	11.4 ± 4.1c
Molasses grass (D)	1.1 ± 0.8d	0.2 ± 0.1b	11.4 ± 3.2c
$F_{9,111}$	7.39	6.1	8.4
P	<0.001	<0.001	<0.001

Means within a column followed by the same letter are not significantly different (Tukey's studentized range test: P>0.05).

Thus, drought-stressed maize remained relatively acceptable for the moths, whereas Mulato and molasses grass remained least acceptable. The number of clutches did not differ among drought-stressed maize and Napier, and signal grass, but it was significantly lower in Mulato and molasses grass (Table 2).

Larval survival and development

Cage experiments. After 14 and 28 days of larval infestation, there were significant differences in percentage larval survival between drought-stressed and non-stressed plants of the various species, but in both periods and both treatments survival rates were significantly higher in maize than in the other plants (Table 3). Pair-wise comparisons indicated no significant within-species differences in percentage larval survival between drought-stressed vs. control plants at both 14 and 28 days.

Significantly larger portions of drought-stressed and control maize leaves were fed on by *C. partellus* larvae than of those from the other test plants (Figure 1). Feeding scores of drought-stressed vs. control plants did not differ across species (Figure 1), indicating that drought stress did not affect larval feeding in any of the species.

Larval survival and development (laboratory experiments). Molasses and signal grass did not support larval survival beyond 20 days, whereas Mulato and Napier grass did not support larval survival beyond 30 days under drought stress (Figure 2). The larvae were most successful on maize plants regardless of the treatment as was the case with cage experiments.

Table 3 Mean (± SE) survival (%) of insectary-reared *Chilo partellus* larvae after 14 and 28 days feeding on control (C) and drought-stressed (D) plants

Treatment	14 days	28 days
Maize (C)	42.1 ± 0.9a	31.9 ± 1.5a
Maize (D)	47.5 ± 2.5a	36.2 ± 2.6a
Napier grass (C)	22.4 ± 3.3b	4.6 ± 2.4b
Napier grass (D)	11.1 ± 3.9bc	2.2 ± 2.2b
Signal grass (C)	0 ± 0c	0 ± 0b
Signal grass (D)	0 ± 0c	0 ± 0b
Mulato (C)	11.8 ± 3.8bc	0 ± 0b
Mulato (D)	1.8 ± 1.7c	0 ± 0b
Molasses grass (C)	0 ± 0c	0 ± 0b
Molasses grass (D)	0 ± 0c	0 ± 0b
$F_{9,118}$	63.82	102.49
P	<0.001	<0.001

Means within a column followed by the same letter are not significantly different (Tukey's studentized range test: P>0.05).

Discussion

Due to the limited capacity of long-distance dispersal in many lepidopteran neonates (Showler, 2002; Showler & Moran, 2003), adult oviposition choice is of crucial importance as it directly determines the initial available resources and potential threat from predators for the offspring (Zalucki et al., 2002). This is even more, so due to the rapid changes in climatic conditions that have been shown to modify plant characteristics (Gutbrodt et al., 2012a,b). Gravid moths are therefore likely to be presented with unique decision-making challenges when searching for a host plant during the prevalence of plant abiotic stress.

This study indicated that gravid *C. partellus* moths accepted drought-stressed Napier and signal grass plants less than well-watered control plants for oviposition. On the other hand, oviposition on drought-stressed and control maize plants was not different in both choice and no-choice tests. The outcome of drought stress on insect oviposition varies across insect-plant systems. For example, Showler & Castro (2010) reported that drought-stressed sugar cane *Saccharum* spp. hybrids were preferred by the Mexican rice borer, *Eoreuma loftini* (Dyar), to adequately watered sugarcane, perhaps, as they speculated, due to the increased amounts of dry leaf tissue and free essential amino acids that they found in the stems of the drought-stressed plants. In addition, drought stress has been associated with increased carbohydrate levels because of their important role in plant osmotic adjustment (Schubert et al., 1995; Showler, 2013). Such carbohydrates (often in the form of soluble sugars) and free amino acids can occur on the leaf surfaces of plants (Fiala et al., 1990; Soldaat et al., 1996). Consequently, these phylloplane compounds can determine host acceptance by phytophagous insects, upon detection by contact chemoreceptors on the antenna, tarsi, proboscis, and ovipositor of the moths (Städler, 1984; Blaney & Simmonds, 1988; Ramaswamy, 1988; Hora & Roesingh, 1999; Calatayud et al., 2008; Showler & Castro, 2010). It was demonstrated by Derridj et al. (1986) that elevated sugar levels in maize resulted in higher preference for oviposition in the European corn borer. Hence, our results are likely to be based on changes in the quality and quantity of attractive or repellent VOCs (see Birkett et al., 2006; Chamberlain et al., 2006; Bruce et al., 2010; Khan et al., 2010), together with contact cues that are used by the moths to select a host.

Molasses grass, which has been used to repel *C. partellus* in a push-pull system under optimum rainfall conditions (Khan et al., 1997), and Mulato remained unattractive under control and drought stress conditions, being the least accepted of all test plants. They thus represent

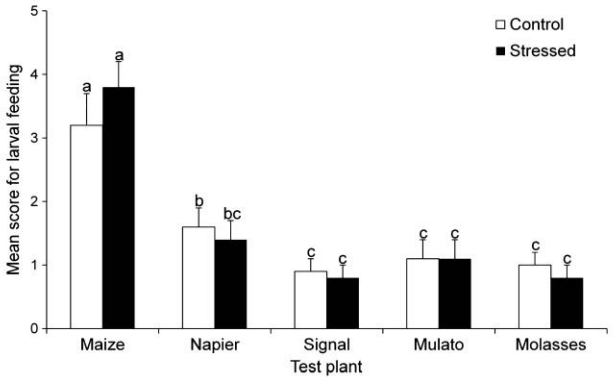


Figure 1 Mean (+ SE) score for leaf feeding by *Chilo partellus* larvae 28 days after infestation in both drought-stressed and control plants; 1 = minimal or no feeding, 5 = heavy feeding. Means capped with different letters are significantly different (Tukey's studentized range test: $P<0.05$).

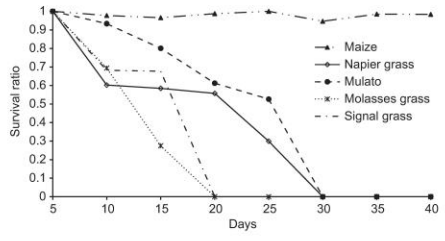


Figure 2 Survival of *Chilo partellus* in wild and cultivated grasses expressed as the ratio of survival on drought-stressed grass to survival on non-stressed control grass of the same species. Steepness of the curves shows the drop in survival of the larvae in treated plants relative to control at any particular time.

potential repellent companion plants for management of *C. partellus* in drier agro-ecologies.

Drought also induces several genetically determined plastic morphological responses, including texture changes and curling of plant leaves, as a result of water loss from tissues (O'Toole & Cruz, 1980; Hsiao et al., 1984; Chaves et al., 2003). Even though we did not quantify morphological responses in our study, it was apparent that these responses varied among the tested species. As such morphological changes are widely known to influence host acceptance in Lepidoptera (Thompson & Pellmyr, 1991; Renwick & Chew, 1994; Zalucki et al., 2002; Midega et al., 2010; Showler & Castro, 2010), it is likely that they also influenced oviposition selection in our study.

Larval survival and development was poor in all drought-stressed and unstressed grasses in contrast to maize where larval survival remained unaffected by drought. Such poor survival in the non-stressed attractive Napier grass has already been attributed to the sticky sap that arrests larval locomotion, together with the poor nutritional content of the species (Khan et al., 2007, 2010). Furthermore, drought stress neither improved survival of larvae nor altered feeding scores on Napier grass. This suggests that Napier grass remains an unsuitable host for *C. partellus* larval growth and development under drought stress. Even though there was some indication of larval leaf feeding in signal grass, larval development was not supported in either case.

The current results have direct implications for the push-pull system. Under drought stress, *C. partellus* favoured oviposition or feeding in the maize plants ahead of all the wild grasses, including Napier and signal grass. This implies that Napier and signal grass may not be effective perimeter 'pull' plants under dry conditions. Mulato and molasses grass stayed unattractive even under drought stress conditions. This calls for careful selection of perimeter trap and intercrop plants for use in a push-pull systems in areas where water deficits are prevalent. Under field trials, Mulato has been identified as a potentially suitable perimeter plant by acting as a barrier that reduces crop attack just as effectively as plots surrounded by Napier grass (Khan et al., 2014). In addition, Mulato remains robust and withstands extended drought conditions relative to the other test plants, including Napier grass.

In conclusion, this study demonstrated that maternal choices in *C. partellus* that impact offspring performance can be influenced by drought stress. Surprisingly, we found that insects made better choices under drought stress because they laid fewer eggs on Napier and signal grass, which are not good hosts for larval development. It is of importance to consider such behavioural traits to understand how stress may reshape plant–insect relations at short- and long-time scales. A critical component of this

should be a determination of the effect of drought stress on VOCs and subsequent impact on stemborer oviposition preferences. Such an understanding may form the basis of climate-smart adaptation strategies of biology-based methods for pest control under changing climates.

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References

van Asch M, van Tienderen PH, Holleman LJM & Visser ME (2007) Predicting adaptation of phenology in response to climate change, an insect herbivore example. Global Change Biology 13: 1596–1604.

van Asch M, Julkunen-Tiito R & Visser ME (2010) Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology. Functional Ecology 24: 1103–1109.

van den Berg J (2006) Oviposition preference and larval survival of *Chilo partellus* (Lepidoptera: Pyralidae) on Napier grass (*Pennisetum purpureum*) trap crops. International Journal of Pest Management 52: 39–44.

Birkett MA, Chamberlain K, Khan ZR, Pickett JA, Toshova T et al. (2006) Electrophysiological responses of lepidopterous stemborers *Chilo partellus* and *Busseola fusca* to volatiles from wild and cultivated host plants. Journal of Chemical Ecology 32: 2475–2487.

Blaney WM & Simmonds MSJ (1988) Food selection in adult and larvae of three species of Lepidoptera: a behavioural and electrophysiological study. Entomologia Experimentalis et Applicata 49: 111–121.

Blundell AG & Peart DR (2000) High abscission rates of damaged expanding leaves: field evidence from seedlings of a Bornean rain forest tree. American Journal of Botany 87: 1693–1698.

Brodbeck B & Strong D (1987) Amino acid nutrition of herbivorous insects and stress to host plants. Insect Outbreaks: Ecological and Evolutionary Perspectives (ed. by P Barbosa & JC Schultz), pp. 347–364. Academic Press, New York, NY, USA.

Bruce TJA & Pickett JA (2011) Perception of volatile blends by herbivorous insects: finding the right mix. Phytochemistry 72: 1605–1611.

Bruce TJA, Midega CAO, Birkett MA, Pickett JA & Khan ZR (2010) Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. Biology Letters 6: 314–317.

Burke MB, Lobell DB & Guarino L (2009) Shifts in African crop climates by 2050, and the implications for crop improvement and genetic resources conservation. Global Environmental Change 19: 317–325.

Calatayud P-A, Guénégo H, Ahuya P, Wanjoya A, Le Rü B et al. (2008) Flight and oviposition behaviour of the African stemborer, *Busseola fusca*, on various host plant species. Entomologia Experimentalis et Applicata 129: 348–355.

Chamberlain K, Khan ZR, Pickett JA, Toshova T & Wadhams LJ (2006) Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chilo partellus* and *Busseola fusca*. Journal of Chemical Ecology 32: 565–577.

Chaves MM, Maroco JP & Pereira JS (2003) Understanding plant responses to drought: from genes to the whole plant. Functional Plant Biology 30: 239–264.

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X et al. (2007) Regional climate projections. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (ed. by S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor & HL Miller), pp. 847–940. Cambridge University Press, Cambridge, UK.

Cook SM, Khan ZR & Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annual Review of Entomology 52: 375–400.

Cornelissen T, Wilson FG & Vasconcellos-Neto J (2008) Size does matter: variation in herbivory between and within plants and the plant vigor hypothesis. Oikos 117: 1121–1130.

Derridj S, Fiala V & Jolivet E (1986) Increase of European corn borer (*Ostrinia nubilalis*) oviposition induced by treatment of maize plants with maleic hydrazide: role of leaf carbohydrate. Entomologia Experimentalis et Applicata 41: 305–310.

Ekmekçi Y & Çiçek N (2009) Physiological responses of three maize cultivars to drought stress and recovery. South African Journal of Botany 75: 34–42.

Fiala V, Glad C, Martin M, Jolivet E & Derridj S (1990) Occurrence of soluble carbohydrates on the phylloplane of maize (*Zea mays* L.): variations in relation to leaf heterogeneity and position on the plant. New Phytologist 115: 609–615.

Gershenzon J (1984) Phytochemical adaptations to stress. Recent Advances in Phytochemistry, Vol. 18 (ed. by BN Timmermann, C Steelink & FA Loewus), pp. 273–320. Plenum Press, New York, NY, USA.

Gershenzon J & Dudareva N (2007) The function of terpene natural products in the natural world. Nature Chemical Biology 3: 408–414.

Gutbrodt B, Mody K & Dorn S (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. Oikos 120: 1732–1740.

Gutbrodt B, Dorn S & Mody K (2012a) Drought stress affects constitutive but not induced herbivore defence resistance in apple plants. Arthropod-Plant Interactions 6: 171–179.

Gutbrodt B, Dorn S, Unsicker SB & Mody K (2012b) Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry. Chemoecology 22: 101–111.

Hale BK, Herms DA, Hansen RC, Clausen TP & Arnold D (2005) Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid induced resistance of poplar to two lymantriid defoliators. Journal of Chemical Ecology 31: 2601–2620.

Hassanali A, Herren H, Khan ZR, Pickett JA & Woodcock CM (2008) Integrated pest management: the push-pull approach for controlling insect pests and weeds of cereals, and its potential for other agricultural systems including animal husbandry. Philosophical Transactions of the Royal Society B 363: 611–621.

Hoerling M & Kumar A (2003) The perfect ocean for drought. Science 299: 691–694.

Holopainen JK & Gershenzon J (2010) Multiple stress factors and the emission of plant VOCs. Trends in Plant Science 15: 176–184.

Hora KH & Roesingh P (1999) Oviposition in *Yponomeuta cagnagellus*: the importance of contact cues for host plant acceptance. Physiological Entomology 24: 109–120.

Hsiao TC, O'Toole JC, Yambao EB & Turner NC (1984) Influence of osmotic adjustment on leaf rolling and tissue death in rice. Plant Physiology 75: 338–341.

Huberty AF & Denno RF (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology 85: 1383–1398.

Kfir R, Overholt WA, Khan ZR & Polaszek A (2002) Biology and management of economically important stemborers in Africa. Annual Review of Entomology 47: 701–731.

Khan ZR, Chiliswa P, Ampong-Nyarko K, Smart LE, Polaszek A et al. (1997) Utilisation of wild gramineous plants for management of cereal stemborers in Africa. International Journal of Tropical Insect Science 17: 143–150.

Khan ZR, Midega CAO, Hutter NJ, Wilkins RM & Wadhams LJ (2006) Assessment of the potential of Napier grass (*Pennisetum purpureum*) varieties as trap plants for management of *Chilo partellus*. Entomologia Experimentalis et Applicata 119: 15–22.

Khan ZR, Midega CAO, Wadhams LJ, Pickett JA & Mumuni A (2007) Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stemborer (*Busseola fusca*) in a push-pull strategy. Entomologia Experimentalis et Applicata 124: 201–211.

Khan ZR, Midega CAO, Bruce TJA, Hooper AM & Pickett JA (2010) Exploiting phytochemicals for developing 'push-pull' crop protection strategy for cereal farmers in Africa. Journal of Experimental Botany 61: 4185–4196.

Khan ZR, Midega CAO, Pittchar JO, Murage AW, Birkett MA et al. (2014) Achieving food security for one million sub-Saharan African poor through push-pull innovation by 2020. Philosophical Transactions of the Royal Society B 369: 20120284.

Laothawornkitkul J, Paul ND, Possell M, Mullineaux PM, Hewitt CN & Taylor JE (2008a) The role of isoprene in insect herbivory. Plant Signaling and Behavior 3: 1141–1142.

Laothawornkitkul J, Paul ND, Vickers CE, Possellm M, Taylor JE et al. (2008b) Isoprene emissions influence herbivore feeding decisions. Plant, Cell and Environment 31: 1410–1415.

Leuschner K (1990) A review of laboratory and field screening procedures for *Chilo partellus*. Insect Science and Its Application 11: 627–638.

Loivamäki M, Mumm R, Dicke M & Schnitzler J-P (2008) Isoprene interferes with the attraction of bodyguards by herbaceous plants. Proceedings of the National Academy of Sciences of the USA 105: 17430–17435.

Mattson WJ & Haack RA (1987) The role of drought in outbreaks of plant-eating insects. BioScience 37: 110–118.

Meyer ST, Roces F & Wirth R (2006) Selecting the drought stressed: effects of plant stress on intraspecific and within plant herbivory patterns of the leaf-cutting ant *Atta colombica*. Functional Ecology 20: 973–984.

Midega CAO, Khan ZR, Pickett JA & Nylin S (2010) Host plant selection behaviour of *Chilo partellus* and its implication for effectiveness of a trap. Entomologia Experimentalis et Applicata 138: 40–47.

Mody K, Unsicker SB & Linsenmair KE (2007) Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. Ecology 88: 1012–1020.

Mody K, Eichenberger D & Dorn S (2009) Stress magnitude matters: different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. Ecological Entomology 34: 133–143.

Neto JDR, Pinho BX, Meyer ST, Wirth R & Leal IR (2012) Drought stress drives intraspecific choice of food plants by *Atta* leaf cutting ants. Entomologia Experimentalis et Applicata 144: 209–215.

Niinemets Ü (2010) Mild versus severe stress and BVOCs: thresholds, priming and consequences. Trends in Plant Science 15: 145–153.

Onyango FO & Ochieng-Odero JPR (1994) Continuous rearing of the maize stemborer *Busseola fusca* on an artificial diet. Entomologia Experimentalis et Applicata 73: 139–144.

O'Toole JC & Cruz RT (1980) Response of leaf water potential, stomatal resistance and leaf rolling to water stress. Plant Physiology 65: 428–432.

Preszler RW & Price PW (1995) A test of plant vigor, plant-stress and plant genotype effects on leaf oviposition and performance. Oikos 74: 485–492.

Price PW (1991) The plant vigor hypothesis and herbivore attack. Oikos 62: 244–251.

R Development Core Team (2011) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramaswamy SB (1988) Host finding by moths: sensory modalities and behaviours. Journal of Insect Physiology 34: 235–249.

Renwick JAA & Chew FS (1994) Oviposition behaviour in Lepidoptera. Annual Review of Entomology 39: 377–400.

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. Herbivores: Their Interaction with Secondary Metabolites (ed. by GA Rosenthal & DH Janzen), pp. 3–54. Academic Press, New York, NY, USA.

Schubert S, Serraj R, Plies-Blazer E & Mengel K (1995) Effect of drought stress on growth, sugar concerntrations and amino acid accumulations in N-fixing alfalfa (*Medicago sativa*). Journal of Plant Physiology 146: 541–546.

Seagraves MP, Riedell WE & Lundgren JG (2011) Oviposition preference for water-stressed plants in *Orious insidious* (Hemiptera: Anthocoridae). Journal of Insect Behavior 24: 132–143.

Showler AT (2002) Effects of water deficit stress, shade, weed competition and kaolin particle film on selected foliar free amino acid accumulations in cotton *Gossypium hirsutum* (L.). Journal of Chemical Ecology 28: 631–651.

Showler AT (2013) Water deficit stress – host plant nutrient accumulations and associations with phytophagous arthropods. Abiotic Stress – Plant Responses and Applications in Agriculture (ed. by K Vahdat & C Leslie), pp. 388–410. Intech Publishers, Rijeka, Croatia.

Showler AT & Castro BA (2010) Influence of drought stress on Mexican rice borer (Lepidoptera: Crambidae) oviposition preference in sugarcane. Crop Protection 29: 415–421.

Showler AT & Moran PJ (2003) Effects of drought-stressed cotton, *Gossypium hirsutum* L., on beet armyworm, *Spodoptera exigua* (Hübner), oviposition, and larval feeding preferences and growth. Journal of Chemical Ecology 29: 1997–2011.

Singer MC (1986) The definition and measurement of oviposition preference in plant-feeding insects. Insect-Plant Interactions (ed. by JR Miller & TA Miller), pp. 65–69. Springer, New York, NY, USA.

Soldaat LL, Boutin J-P & Derridj S (1996) Species specific composition of free amino acids on the leaf surface of four *Senecio* species. Journal of Chemical Ecology 22: 1–12.

Städler E (1984) Contact chemoreception. Chemical Ecology of Insects (ed. by WJ Bell & RT Cardé), pp. 3–35. Sinauer, Sunderland, MA, USA.

Tariq M, Wright DJ, Rossiter JT & Staley JT (2012) Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. Agricultural and Forest Entomology 14: 177–185.

Thompson JN & Pellmyr O (1991) Evolution of oviposition behaviour and host preference in Lepidoptera. Annual Review of Entomology 36: 65–89.

Vallat A, Gu H & Dorn S (2005) How rainfall, relative humidity and temperature influences volatile emissions from apple trees *in situ*. Phytochemistry 66: 1540–1550.

Vickers CE, Gershenzon J, Lerdau MT & Loreto F (2009a) A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nature Chemical Biology 5: 283–291.

Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J et al. (2009b) Isoprene synthesis protects transgenic plants from oxidative stress. Plant, Cell and Environment 32: 520–531.

White TCR (1969) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology 50: 905–909.

Yuan JS, Himanen SJ, Holopainen JK, Chen F & Stewart CN, Jr (2009) Smelling global climate change mitigation of function for plant volatile organic compounds. Trends in Ecology and Evolution 24: 323–331.

Zalucki MP, Clarke AR & Malcolm SB (2002) Ecology and behaviour of first instar larval Lepidoptera. Annual Review of Entomology 47: 361–393.

Appendix C: Manuscript for publication III

Acute drought stress induces and augments associational resistance to insect herbivore attack in the "push-pull" system for stemborer control

Frank Chidawanyika[12], Charles A.O. Midega[1], Toby J.A. Bruce[3], Christine Woodcock[3], John C. Caulfield[3], Frances D. Duncan[2], John A. Pickett[3] and Zeyaur R. Khan[1]

[1]Habitat Management Programme, International Centre of Insect Physiology and Ecology P.O. Box 30 Mbita Point, Kenya

[2]Global Change and Sustainability Research Institute, School of Animal Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa

[3]Biological Chemistry Department, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK

Abstract

Drought has the potential to reset trophic interactions. Using wind tunnel bioassays, we tested how drought-induced changes in the quantity and quality of volatile organic compounds (VOCs) from grasses and legumes of the "push-pull" system impacted the behavioural responses of gravid *Chilo partellus*. Drought stress resulted in increased total plant volatile emission in Napier and signal grasses. This significant change in total volatile emission of the plants upon stress was not present in molasses grass, Mulato II, Silverleaf and Greenleaf *Desmodium* despite variable changes in the quantities and qualities of particular constituent compounds among all the plant species during water deficit. Volatiles from irrigated Napier and signal grasses were more attractive to *C. partellus* than the treatment plants in wind tunnel bioassays. However, volatiles from molasses grass, Mulato II, Silverleaf and Greenleaf *Desmodium* were not preferred by moths in both irrigated and drought stressed conditions. We attribute this differential preference of the moths to the constitutive and/or induced emission of key terpenoid compounds in plants that were tested. In Napier and signal

26 grasses, this induction of terpenoid compounds was coupled with a reduction in the amount
27 of green leaf volatiles such as (*Z*)-3-hexenyl acetate which are known to be of importance in
28 the orientation of herbivorous insects. By induction and augmentation of such chemical
29 changes in hosts and non-host plants, drought stress confers associational resistance to crops
30 in the push-pull systems through non-preference by the moths.

31 **Key words:** Crambidae, environmental change, host location, insect-plant interactions, plant
32 volatiles, biological control

33 **Introduction**

34 Plant volatile organic compounds (VOCs) play an important role as cues for host location in
35 herbivorous insects. For gravid insects searching for oviposition sites, plant VOCs can be
36 good indicators of diverse information about the suitability of the host (Bruce et al. 2005;
37 Reisenman et al. 2013; Späthe et al. 2013; Wäschke et al. 2014). However, plant abiotic
38 stressors, such as drought, often alter plant secondary metabolism (Atkinson and Urwin 2012;
39 DeLucia et al. 2012) with potential induction of changes in quality and quantity of VOCs
40 (Vickers et al. 2009a and b; Holopainen and Gershenzon 2010). Such changes in the
41 composition and properties of the plant VOCs, regardless of the causal agent, may influence
42 odour mediated host preferences of insects (Bruce et al. 2010; Bruce and Pickett 2011).
43 Under changing climates and environments, this can be a concern when developing push-pull
44 pest management strategies that rely on specific blends of plant VOCs to repel pests, attract
45 them to trap crops and attract their natural enemies (Cook et al. 2007).

46 In East Africa, *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) remains one of the
47 highly destructive polyphagous pests in the production of the staple maize (*Zea mays* L.) and
48 strategic small grain cereals. Damage by the pest is achieved through larval feeding on the

49 leaves and stems resulting in 20-80% economic loss (Kfir et al. 2002). Effective control
50 strategies for such stemborer pests have been either elusive or expensive for small scale
51 farmers in sub-Saharan Africa. To help combat the pests in a cost effective manner, the push-
52 pull system was developed by the International Centre of Insect Physiology and Ecology
53 (ICIPE), Kenya and Rothamsted Research. This push-pull system relies on on-farm
54 biodiversity to confer associational resistance (Sholes 2008; Jactel et al. 2011; Plath et al.
55 2012; Zakir et al. 2013a) which is achieved by intercropping crops with the pests` non-host
56 plants simultaneously with wild their hosts in the borders of the crop plot. In its conventional
57 form, the push-pull system consists of maize that is intercropped with *Desmodium* spp. whilst
58 bordered by Napier grass (Khan et al. 2010). Volatile organic compounds produced by
59 *Desmodium* plants repel stemborers whilst Napier grass attracts them away from the maize
60 plots. Control of the pests is then further enhanced by high oviposition rates on the perimeter
61 grasses which function as a trap crop and suppress larval growth and mobility (Khan et al.
62 2006).

63 The advent of climate change, however, poses new challenges to the push-pull system
64 through factors such as drought. Napier grass, for example, has relatively high water
65 requirements. Even though it has a deep rooted system which can enable survival under
66 periods of drought stress, little is known about its volatile emission profiles under stress. Such
67 is the case with other current and candidate companion plants for the push-pull system. Six
68 key compounds that were identified as important for host seeking gravid moths in the push-
69 pull system were only identified under optimal conditions (Khan et al. 2000; Birkett et al.
70 2006). A gap in knowledge relating to how the plant VOC emission profile changes under
71 different abiotic stressors therefore exists.

72 In a recent study, we found that host acceptance by gravid moths changed between
73 drought-stressed and control host plants (Chidawanyika et al. 2014). Apart from contact cues,
74 such changes may also be attributed to changes in volatile emission profiles and subsequent
75 trophic interactions. For example in maize, abiotic stress induced by heavy metal poisoning
76 was shown to prime the plants to produce more herbivore induced plant volatiles (HIPVs)
77 upon insect herbivory (Winter et al. 2012). In other studies, Gouinguene and Turlings (2002)
78 showed that abiotic factors such as temperature, soil and atmospheric humidity, and
79 fertilization rate influence VOC production in corn plants with potential impact on
80 subsequent trophic interactions. Given this background, we investigated how drought stress
81 affected the chemically-mediated interaction between *C. partellus* and companion plants of
82 the push-pull system. Specifically, we asked if 1) drought stress changes volatile emission
83 profiles of current and potential grasses of the push-pull system 2) drought-induced changes
84 in volatile emission profiles elicit differential behavioural and electrophysiological responses
85 in *Chilo partellus*.

86 **Materials and Methods**

87 *Insects*

88 An artificial diet described by Onyango and Ochieng-Odero (1994) was used to rear the *C.*
89 *partellus* moths that were used in these studies. In order to control for age related differences
90 in the electrophysiological responses and wind tunnel bioassays, only 1-2 day old gravid
91 moths were used.

92 *Plants*

93 Napier grass variety 'bana', molasses grass, signal grasses *Brachiaria brizantha* (A. Rich)
94 Stapf and *Brachiaria* cv. Mulato II [products from 3-way cross of *B. ruziziensis x B.*

95 *decumbens x B. brizantha* (all Poaceae)], together with two legume species, *Desmodium*
96 *uncinatum* (hereafter called Silverleaf *Desmodium*) and *Desmodium intortum* (hereafter
97 called Greenleaf *Desmodium*) were used in the study. All plant species were planted in
98 vertisol soil that was held in perforated 5 litre plastic buckets under natural conditions (c.
99 25°C, 65% RH; 12L: 12D). Root splits of stock plants from Kenya Agricultural Research
100 Institute (KARI) were used for planting Napier grass as in Khan et al. (2006). Similarly, root
101 splits from collections of signal grass and molasses grass held at the ICIPE Mbita Point ITOC
102 field station were used for planting experimental specimens for these species. Mulato II and
103 all *Desmodium* species were propagated using seeds. After 3 weeks of growth under optimal
104 conditions, plants from all species were randomly partitioned into two groups; control and
105 treatment. Control plants had adequate irrigation whilst those of the treatment were denied
106 water for 10-13 days as described by Chidawanyika et al. (2014).

107 *Volatile collection*

108 Volatiles were collected from intact control and treated plants by headspace sampling
109 (Tamiru et al. 2011). Polyethyleneterephthalate (PET) bags (volume 3.2 L, ~12.5 mm
110 thickness) were cleaned by preheating at 150°C for 30 mins before fitting Swagelock inlet
111 and outlet ports. They were then used to cover the plants after fitting adsorbent filters,
112 Porapak Q (0.05 g, 60 / 80 mesh; Supelco), in the outlet ports. Charcoal-filtered air was
113 pumped through the inlet ports into the bags at a rate of 600 mL min^{-1} and air was drawn out
114 through the outlet ports at rate of 400 mL min^{-1}. Each entrainment lasted for 48 hrs for all
115 plants after the initial start at 2 hrs after scotophase. Upon completion of entrainment, elution
116 of the samples from the Porapak Q into sample vials was done using 0.5 mL
117 dichloromethane.

118

119 *Laboratory wind tunnel*

120 The wind tunnel was contained in a white environmentally controlled room set at 26 ± 2°C,
121 ~2 lux light intensity and 55 ± 5 % RH, a wind tunnel set-up was established following
122 Curkovic and Brunner (2006) and Curkovic et al. (2009). The working arena of the tunnel
123 was formed by transparent Plexiglas (polymethyl methacrylate) material that was 2.7 m long,
124 70 cm wide and 70 cm high. An electric fan (40 cm diameter, Donkin Fans Co., South
125 Africa) drew air through the tunnel at a rate of approximately 45 cm s^{-1}. The air that was
126 drawn through the tunnel then exited from the room via an outlet to avoid contamination.

127 *Bioassay protocol and behavioural observations*

128 Plant odour was delivered in the tunnel using discs of filter paper (~ 2cm diameter each) that
129 were individually mounted onto a platform opposite the fan near the exit of the tunnel. Prior
130 to that, these discs were first moistened by adding 10 µl aliquots of collected volatile samples
131 using micropipettes (Drummond 'microcap', Drummond Scientific Co. Broomall, PA, USA).
132 Gravid moths were individually placed on a stand on the downwind side opposite the odour
133 source. Each moth was assayed only once and thereafter discarded upon completion of
134 observations lasting 10 min. Behavioural responses of the moths were scored based on their
135 mere flight towards and actual contact with the surface of the odour source. For all volatile
136 samples, 15 replications were done where each moth was regarded as a replicate. In all cases,
137 surfaces of the glassware platforms where the volatile component and the insects were placed
138 were first cleaned with 70 % ethanol before being heated at 100 °C for 30 minutes prior to
139 assaying any replicate. Surfaces of the wind tunnel were similarly cleaned with 70 % ethanol,
140 and allowed to dry, but without the oven heating, before each assay.

141

142 *Coupled Gas chromatography-mass spectrometry (GC-MS)*

143 A capillary gas chromatography column (HP1, 50m length, 0.25mm i.d., 0.25 µm film
144 thickness) coupled directly to a mass spectrometer (VG Autospec, Fisons Instruments,
145 Machester, UK) was used to analyse aliquots of the headspace samples. Oven temperature
146 was programmed to remain at 50 °C for 3 mins before ramping up to 200 °C at 7 °C min^{-1}
147 and then further heated to 300 °C at 100 °C min^{-1} where it was held constant for 2 min.
148 Ionization was achieved by electron impact (70 Ev, 250 °C). Comparison of retention indices
149 and mass spectra of authentic standards with those of the samples was used to identify
150 compounds.

151 *Chemicals*

152 Commercially sourced synthetic standards; decanal, decane, *R)*-Linalool, (E)-(1R,9S)-
153 caryophyllene, eugenol, α-Pinene, methyl salicylate and (Z)-3-hexenyl acetate were
154 purchased from Avocado Research Chemicals and Sigma Aldrich. Following Leopold
155 (1990), (E)-4,8-Dimethyl-1,3,7-nonatriene (DMNT) (> 98%) and (E,E)-4,8,12-trimethyl-
156 1,3,7,11-tridecatetraene (TMTT) (> 98%) were synthesised through the oxidation of
157 corresponding aldehydes in geraniol and (E,E)-farnesol respectively then followed by a
158 Wittig methylenation. High purity (E)-Ocimene (>95% by GC) was synthesised in a two step
159 protocol. First, 3-methyl-3-sulpholene was functionalised using 1-bromo-3-methylbut-2-ene
160 (Chou et al. 1984) and then followed by extrusion of sulphur dioxide using lithium
161 aluminium hydride (Gaoni 1977).
162

163 *Electroantennogram (EAG) recordings*

164 Coupled GC-electroantennography (GC-EAG) was carried out using antennae of female *C.*
165 *partellus* and the headspace samples of plants. EAG recordings were made using Ag–AgCl
166 glass electrodes filled with saline solution [composition as in Maddrell (1969), but without
167 glucose]. An antenna was excised and suspended between the two electrodes. The tip of the
168 terminal part of the antenna was removed to ensure a good contact. Signals were passed
169 through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and
170 analysed using a customised software package (Syntech). The GC-EAG system, in which the
171 effluent from the GC column is simultaneously directed to the antennal preparation and the
172 GC detector, has been described previously (Wadhams 1990). Separation of the volatiles was
173 achieved on a GC (Agilent Technologies, 6890N) equipped with a cold on-column injector
174 and a FID using a HP-1 column (50 m, 0.32 mm ID, 0.52 lm film thickness). The oven
175 temperature was maintained at 30 °C for 2 mins and then ramped up at 15 °C min^{-1} to 250 °C.
176 The carrier gas was helium. Outputs from the EAG amplifier and the FID were monitored
177 simultaneously and analysed using the Syntech software package. Peaks eluting from the GC
178 column were judged to be active if they elicited EAG activity in three or more of five coupled
179 runs.

180 **Statistical analysis**

181 All statistical analyses were performed in R programme (R Foundation for statistical
182 computing, 2010). A Chi-square test was used to determine the differences in i) the number
183 of flights towards and ii) actual contact with the odour source among control and treatment
184 samples of the same species. Student *t*-tests were done to test significant differences in the
185 means of the quantities of the EAG active compounds that were emitted by drought-stressed
186 and irrigated plants of each species. Total amounts of eluted compounds from the plant

187 samples were analysed as normalized peak area units based on the GC-MS analysis.
188 Differences between the volatiles from drought stressed and irrigated plants of each species
189 were tested using the Mann-Whitney *U*-tests. Thereafter, the sequential Bonferroni method
190 was carried out to correct for any potential family wise error rate (Holm 1979)

191 **Results**

192 *Wind tunnel behavioural responses*

193 Gravid moths were more attracted to odour samples from irrigated Napier grass (χ^2 = 7.033,
194 d.f. = 1, P = 0.008) and signal grass (χ^2 = 4.887, d.f. = 1, P = 0.031) than their respective
195 drought stressed individuals as indicated by the number of moths that contacted the odour
196 source (Table 1). However, there was no immediate discrimination in the initial flight
197 response towards the odour source between control and treatment plants of both Napier grass
198 (χ^2 = 0.682, d.f. = 1, P = 0.408) and signal grass (χ^2 = 1.429, d.f. = 1, P = 0.232). Odour
199 samples from both treatment and control molasses grasses, Mulato II, Silverleaf and
200 Greenleaf *Desmodium* were not attractive as shown by the low responses in both the initial
201 flight behaviour towards odour and actual contact with the odour source for all samples
202 (Table 1).

203 *Identification of electrophysiologically active compounds*

204 Gravid *C. partellus* moths detected a total of 24 different electrophysiologically active
205 compounds from EAG analysis of the samples of drought-stressed and irrigated plants of the
206 6 plant species (Fig. 1). Nineteen of these active compounds were characterised by either
207 GC-MS analyses or coinjection on both polar (HP-1) and non-polar (DB-wax) columns
208 guided by the Kovat indices of the synthetic standards. Traces of GC-EAG responses of
209 gravid *C. partellus* moths to drought-stressed Napier, molasses, signal grasses and Mulato II

210 are shown in Fig. 1. Within each species, most compounds either appeared in both drought-
211 stressed and irrigated plants or were not detected at all in both the 2 sets of plants (Table 2).
212 Some of these compounds were common to all the plant species and treatments. There was a
213 significant increase in the emission of DMNT in both drought stressed Napier and signal
214 grasses (Table 2; Fig 2). However, there was no significant difference in the emission of
215 DMNT between drought-stressed and irrigated molasses grass, Mulato II and the two plants
216 species of the genus *Desmodium*.

217 Drought stress resulted in the increase of (*E*)-ocimene in molasses and signal grasses,
218 and Greenleaf *Desmodium*. However, there was no such significant difference in the emission
219 of (*E*)-ocimene between drought-stressed and irrigated Silverleaf *Desmodium*. No (*E*)-
220 ocimene was detected in Napier grass and Mulato II. There was an increase of the emission
221 of (*R*) linalool in drought-stressed Napier, molasses and signal grasses, Mulato II but not in
222 Silverleaf *Desmodium* where there was no significant difference. In all the plant species, the
223 acute drought stress generally elicited increased emission of particular compounds and
224 overall total volatile emission (Fig. 2). However, in Napier grass, there was a significant
225 reduction in the emission of Z3HA and methyl salicylate following drought stress (Table 2.
226 Fig. 2)

227 **Discussion**

228 Our study suggests that drought stress alters the emission of plant VOCs with
229 subsequent impact on trophic interactions. In wind tunnel bioassays, volatile samples of
230 drought-stressed Napier and signal grasses, wild hosts of *C. partellus*, were not as attractive
231 to the moths when compared with those from the irrigated plants (Table 1). These changes in
232 moth preferences can be explained by the increased emission of monoterpenes such as (*E*)

233 ocimene, (*R*) linalool and the hormotepene DMNT in drought-stressed Napier and signal
234 grasses. These terpenes have already been reported to be of importance in mediating plant-
235 insect interactions in various studies. For example, upon perception of insect herbivore
236 attack, plants signal metabolic events that result in increased emission, among other VOCs, of
237 DMNT, and sesquiterpenes (*E*) caryophyllene and TMTT (Gouinguene et al. 2001; 2003;
238 Degen et al. 2012; Tamiru et al. 2011; 2012). In most cases, such herbivore induced plant
239 volatiles (HIPVs) serve as recruitment cues of egg and larval parasitoids as an indirect
240 defence mechanism (Tumlinson et al. 1993; Schnee et al. 2006; Degenhardt 2009; Dicke and
241 Baldwin, 2010; Tamiru et al. 2011; 2012) and can also be exploited by hyperparasitoids
242 (Poelman et al. 2012)

243 Conversely, HIPVs elicited by feeding larvae have been demonstrated to be repellents
244 of conspecific adult female moths searching for oviposition sites (De Moraes et al. 2001;
245 Allmann et al., 2013; Zakir et al. 2013b). Such ability of plant HIPVs to repel searching
246 gravid moths has been regarded as a way of the plant to prevent further insect colonisation.
247 However, other reports have regarded the oviposition non-preference of HIPVs as a way by
248 which gravid insects can maximise progeny fitness potential through avoidance of
249 intraspecific competition and increased risk of predation (Rodriguez-Saona and Frost 2010).
250 Our present study gives evidence that may inadvertently support both postulations. First, the
251 increased emission of VOCs similar to HIPVs in drought-stressed Napier and signal grasses
252 prevents any further stress due to insect herbivore damage thereby improving the fitness
253 potential of the plant in such circumstances. Second, *C. partellus* moths may have evolved to
254 detect VOCs produced by plants under abiotic stress, which may lead to plant mortality, as
255 cues to avoid poor quality hosts for maximisation of offspring performance. In our previous
256 study, oviposition preference and acceptance was reduced in Napier and signal grasses.

257 Furthermore larval performance was poor in drought stressed Napier and signal grasses
258 (Chidawanyika et al. 2014). Even though the larval survival was also poor in the more
259 attractive irrigated Napier and signal grasses, the non-preference of VOCs from drought
260 stressed plants of these species show how insect herbivores may use cues from plants to
261 select the ideal host. Through acute drought stress these modifies emissions of plant VOCs
262 among natural and managed grassland communities may offer windows of for the plant to
263 escape insect herbivore attack. However, it is not clear how much such an escape of
264 herbivore attack actually contributes to the plants` typical Darwinian fitness upon resumption
265 of watering since these periodic abiotic stressors also affect fitness traits such as reproduction
266 and dispersal, and longevity.

267 Apart from quantitative changes in VOC emissions, drought stress could have
268 influenced the quality of the VOCs resulting in differential responses of the moths in the
269 wind tunnel bioassays. In previous studies, Bruce et al. (2010) showed that an increased ratio
270 of Z3HA to minor component VOCs due to reduction in its emission rates by signal grass
271 upon insect oviposition resulted in reduced oviposition preference by *C. partellus* moths. In
272 the present study, drought-stressed Napier and signal grasses had a dramatic reduction in the
273 emission of Z3HA compared to the corresponding irrigated plants (Table 2; Fig. 2). Even
274 though we did not test for the specific responses of the moths to the synthetic standards at
275 different ratios, relative changes in proportions of Z3HA in the formerly attractive Napier and
276 signal grasses could have influenced moth behavioural responses in our study. The reduction
277 in emission of Z3HA and other green leaf volatiles during drought stress was also present in
278 other species such as Mulato and Greenleaf *Desmodium* (Table 2, Fig. 2) Even though the
279 latter is not known as a host of *C. partellus*, this marked reduction in Z3HA relative to other
280 compounds may have contributed to the non-preference by the moths. Furthermore, other

281 non-hosts such as molasses grass and both *Desmodium* species which constitutively emit the
282 repulsive DMNT had similar emission profiles even under drought stress (Table 2; Fig 2). In
283 some case, drought stress appeared to result in an increase in the emission of DMNT thereby
284 augmenting their repulsive nature against the gravid *C. partellus* moths.

285 The mechanisms and functional ecological role played by plant VOCs that are
286 induced by abiotic stress remains an area of active debate (Holopainen, 2004; 2011;
287 Niinemets, 2010; Blande et al. 2014; Loreto et al. 2014). Terpenoids are one major group of
288 emitted plant VOCs upon the perception of stress due to abiotic stressors (Gershenzon and
289 Dudareva, 2007; Sharkey et al. 2008; Vickers et al. 2009a; Sharkey and Monson 2014). In
290 our study, most of the plant species that we tested were either induced or maintained the
291 emission of various terpernoids followed by a reduction in the emission of other group of
292 compounds such as green leaf volatiles (Table 2; Fig. 2). Even though production of plant
293 volatiles may have metabolic costs for the plant (Sharkey et al. 2008; Neilson et al. 2013), the
294 prioritised emission of terpenes during drought stress in our study indicates that they may
295 therefore be of importance in relieving plants undergoing abiotic stress. Our findings are also
296 similar to those reported by Gouingerne and Turlings (2001). In their study, a decrease in soil
297 humidity resulted in an increase in the emission of the terpenes DMNT and (*E*) β farnesene in
298 corn plants. However, unlike the case in our study, they also reported an increase in the green
299 leaf volatile Z3HA. Therefore even though the majority of the responses to abiotic stress may
300 be generic, some remain species specific.

301 The present study also has direct implications to the push-pull system and may help
302 clarify the mechanistic basis of ecological interactions therein under environmental change.
303 First, the ability of formerly 'pull' or attractive plants of the push-pull system to act as
304 repellents through increased emission of mostly terpenoids under abiotic stress is a form of

305 phenotypic plasticity under environmental variability. The plasticity of Napier grass shown in
306 the present study may therefore allow the species to perform dual roles of attracting and
307 repelling moths under optimal and drought stress conditions respectively. In the latter,
308 however, the increased repellent effect by both the peripheral and intercrop may have been
309 the key to efficacy of the push-pull against stemborers during periodic water deficits. Periodic
310 drought events of between 10 to 15 days within a crop growing season are common in east
311 Africa where the push-pull system is currently practised (Chidawanyika et al. 2014).
312 However, the push-pull has been successful in suppressing crop attack using Napier grass as
313 a 'pull' companion plant under such conditions. Furthermore, collected VOCs of Napier and
314 signal grasses under drought stress were attractive to the larval parasitoid *C. sesamiae*
315 *(unpublished data)*. This attraction is based on the increase in already identified compounds
316 that are constitutively emitted by companion plants such as molasses grass (Khan et al.
317 (1997) and HIPVs in maize landraces Tamiru et al. 2011; 2012) with subsequent increased
318 parasitisation of the pests.

319 Second, the more drought tolerant Mulato II which constitutively emits DMNT also
320 emitted the compound in even larger quantities under drought stress (Table 2; Fig. 3). This
321 induction of increased DMNT emission was not present in with molasses grass, Silverleaf
322 and Greenleaf *Desmodium* as there was no difference in their constitutive emission of DMNT
323 under irrigated and drought stress conditions. In molasses grass and Greenleaf *Desmodium*
324 however, there was a significant increase in the constitutive emission of another terpene (*E*)
325 ocimene under drought stress conditions. This was not the case with Mulato II and Silverleaf
326 *Desmodium*. These variations in the emission profiles of the plant species in response to
327 stress may have been a result of a variety of factors (Mittler 2006; Niinemets 2010). Even
328 though all the plants were exposed to similar periods of water deficit, differences in VOC

329 emission responses, apart from genetic limitations, may also have been due to the relative
330 severity (mild *vs* severe) of the stress to a particular plant species. Furthermore, differences in
331 the perception, transduction and propagation of the stress signals across the different species
332 could have contributed to the differences in the emission profiles (see discussions in
333 Niinemets 2010). However, our observations during the study showed differences in the
334 plants to cope with stress as some already showed signs of wilting. This may have also been
335 due to the high metabolic cost of the emission of the terpenoids itself as they are known to be
336 more expensive to produce per unit weight when compared to other biogenic VOCs
337 (Gershenzon 1994; Paré and Tumlinson 1999). Furthermore, terpenes are also an important
338 source of olefinic compounds which are precursors of phytotoxic products (Monson and Fall
339 1989; Buchbauer et al. 1994). This phytoxicity often occurs when the terpenoids accumulate
340 as they become pollutants comprising of photooxidants and ozone (Paré and Tumlinson
341 1999). However, this phytoxicity has mostly been demonstrated in trees and not grasses (e.g.
342 Monson and fall 1989; Buchbauer et al. 1994). It is therefore not clear if this form of
343 phytotoxicity occurred in our study system for the plants which showed more phenotypic
344 signs of stress coincidentally emitted more induced terpenes.

345 Selection of plants for more resilient plants in the push-pull should therefore
346 incorporate agronomic performance of the companion plants in addition to volatile emission
347 profiles. Such agronomic performance and volatile emission based selection of companion
348 plants are already showing high potential in drier areas of Kenya. Under field conditions the
349 replacement of Napier grass as the perimeter plant with the more drought resilient Mulato in
350 the push-pull system significantly reduces pest damage even in drier environments (Khan et
351 al. 2014). Last, the behavioural responses of the moths to the VOCs of the known repellent
352 plants remained the same under variable emission profiles from irrigated and drought

353 conditions (Table. 1). Therefore, the protection of crops in the push-pull system under
354 drought conditions remains possible through the associational resistance offered by non-host
355 plants and the induced production of pest repellent terpenoids in peripheral companion plants
356 such as Napier and signal grasses. Furthermore, this associational resistance may be further
357 augmented by the increased pest parasitism due to increased attraction of parasitoids by the
358 same terpene compounds (*unpublished data*).

359 In conclusion, the study demonstrates that abiotic stressors can affect plant ecological
360 interactions through alterations in volatile emission profiles. However such responses to
361 abiotic stressors or lack thereof, are species specific. Even though we did not do any
362 transcriptional analysis, there were generic responses of some grasses in production of
363 terpenes in response to abiotic stressors as is the case upon insect herbivory. This shows that
364 there is some form of cross-talk in the signaling pathways of plants under biotic and abiotic
365 stressors to potentially maximise on defense mechanisms when faced with multiple stressors,
366 as is the norm under field conditions (see discussions in Fujita et al., 2006; Atkinson and
367 Urwin 2012).

368

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Conflicts of interest

The authors declare that there is no conflict of interest.

References

Agelopoulos NG, Hooper AM, Maniar SP, Pickett JA, Wadhams LJ (1999) A novel approach for isolation of volatile chemicals released by individual leaves of a plant *in situ*. J Chem Ecol 25: 1411-1425

Allmann S, Späthe A, Bisch-Knaden et al. (2013) Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. eLife 2: e00421. DOI: 10.7554/eLife.00421

Atkinson NJ, Urwin P (2012) The interaction of plant biotic and abiotic stresses: from genes to field. J Exp Bot DOI: 10.1093/jxb/ers100

Birkett MA, Chamberlain K, Khan ZR, Pickett JA, Toshova T, Wadhams LJ, Woodcock CM (2006) Electrophysiological responses of Lepidopterous stemborers *Chilo partellus* and *Busseola fusca* to volatiles from wild and cultivated host plants. J Chem Ecol 32: 2475-2487

392 Blande JD, Holopainen JK, Niinemets Ü (2014) Plant volatiles in polluted atmospheres:
393 stress responses and signal degradation. Plant Cell and Environ 37: 1892-1904

394 Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation.
395 Trends Plant Sci 6: 269-273

396 Bruce TJA, Midega CAO, Birkett MA, Pickett JA, Khan ZR (2010) Is quality more important
397 than quantity ? Insect behavioural responses to changes in volatile blends after
398 stemborer oviposition in African grass. Biol Lett 6: 314-317

399 Bruce TJA, Pickett JA (2011) Perception of volatile blends by herbivorous insects: finding
400 the right mix. Phytochemistry 72: 1605-1611

401 Buchbauer G, Jirovetz L, Wasicky M, Nikiforov A (1994) Comparative investigation of
402 Douglas fir headspace samples, essential oils, and extracts (needles and twigs) using
403 GC-FID and GC-FTIR-MS. J Agric Food Chem 42: 2852–2854

404 Chidawanyika F, Midega CAO, Bruce TJA, Duncan F, Pickett JA, Khan ZR (2014)
405 Oviposition acceptance and larval development of *Chilo partellus* stemborers in
406 drought-stressed wild and cultivated grasses of East Africa. Entomol Exp Appl 151:
407 209-217

408 Chou T, Tso H, Chang L (1984) Stereoselective one-step syntheses of trans ocimene and
409 alpha farnesene. J Chem Soc Chem Commun 20: 1323-1324

410 Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest
411 management strategies. Annu Rev Entomol 52: 375-400

412 Curkovic T, Brunner JF (2006) Evaluation of permethrin for attracticide development against
413 *Choristoneura rosaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae) males.
414 Crop Prot 25: 973-976

415 Curkovic T, Brunner JF, Landolt PJ (2009) Field and laboratory responses of male leaf roller
416 moths, *Choristoneura rosaceana* and *Pandemis pyrusana*, to pheromone
417 concentrations in an attracticide paste formulation. J Insect Sci 9: 1-14

418 Degen T,Bakalovic N, Bergvinson D, Turlings TCJ (2012) Differential performance and
419 parasitism of caterpillars on maize inbred lines with distinctly different herbivore-
420 induced volatile emissions PloS ONE 7: e47589

421 Degenhardt J (2009) Indirect defense responses to herbivory in grasses. Plant Physiol 149:
422 96-102

423 DeLucia EH, Nabity PD, Zavala JA, Berenbaum MR (2012) Climate change: resetting plant-
424 insect interactions. Plant Physiol 160: 1677-1685

425 De Moraes CM, Mescheer MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant
426 volatiles repel nonspecific females. Nature 410: 577-580

427 Dicke M, Baldwin I (2010) The evolutionary context of herbivore-induced plant volatiles:
428 beyond the 'cry for help'. Trends Plant Sci 15: 167-175

429 Fujita M, Fujita Y, Noutoshi Y et al., (2006) Crosstalk between abiotic and biotic stress
430 responses: a current view from the points of convergence in the stress signaling
431 networks. Curr Opin Plant Biol 9: 436-442

Gaoni Y (1977) Lithium aluminium hybride promoted extrusion of SO2 from sulfolenes and sulfolene-adducts. 1, 3-Dienes, 3-chloro- and 3,3 –dichloro-1,4-di-enes and skipped polyenes. Tetrahedron Lett 11: 947-950

Gershenzon J (1994) Metabolic costs of terpenoid accumulation in higher plants. J Chem Ecol 20: 12811328

Gershenzon J, Dudareva N (2007) The function of terpenes in the natural world. Nat Chem Biol 3: 408-414

Gouinguené SP, Turlings TCJ, Degen T (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). Chemoecology 11: 9-16

Gouinguené SP, Turlings TCJ (2002) The effect of abiotic factors on induced volatile emission in corn plants. Plant Physiol 129: 1296-1307

Gouinguené SP, Alborn H, Turlings TCJ (2003) Induction of volatile emissions in maize by different larval instars of *Spodoptera littoralis*. J Chem Ecol 29: 145-162

Holm S (1979) A simple sequential rejective multiple test procedure. Scand J Stat 6: 65-77

Holopainen JK (2004) Multiple functions of induced plant volatiles. Trends Plant Sci 9: 529-533

Holopainen JK, Gershenzon J (2010) Multiple stress factors and the emission of plant VOCs. Trends Plant Sci 15: 176-184

Holopainen JK (2011) Can forest trees compensate for stress-generated growth loses by induced production of volatile compounds. Tree Physiol 31: 1356-1377

453 Jactel H, Birgersson G, Andersson S (2011) Non-host volatiles mediate associational
454 resistance to the pine processionary moth. Oecologia 166: 703-711

455 Kfir R, Overholt WA, Khan ZR, Polaszek (2002) Biology and management of economically
456 important stemborers in Africa. Annu Rev Entomol 47: 701-731

457 Khan ZR, Ampong-Nyarko K, Chiliswa P et al., (1997) Intercropping increases parasitism of
458 pests. Nature 388: 631-632

459 Khan ZR, Pickett JA, van den Berg J, Wadhams LJ, Woodcock CM (2000) Exploiting
460 chemical ecology and species diversity: stem borer and Striga control for maize and
461 sorghum in Africa. Pest Manag Sci 56: 957-962

462 Khan ZR, Midega CAO, Hutter NJ, Wilkins RM, Wadhams LJ (2006) Assessment of the
463 potential of Napier grass (*Pennisetum purpureum*) varieties as trap plants for
464 management of *Chilo partellus*. Entomol Exp Appl 119: 15-22

465 Khan ZR, Midega CAO, Bruce TJA, Hooper AM, Pickett JA (2010) Exploiting
466 phytochemicals for developing 'push-pull' crop protection strategy for cereal farmers
467 in Africa. J Exp Bot 61: 4185-4196

468 Khan ZR, Midega CAO, Pittchar JO (2014) Achieving food security for one-million sub-
469 Saharan African poor through push-pull innovation by 2020. Philos Trans R Soc Lond
470 B Biol Sci 369: 20120284. DOI: 10.1098/rstb.2012.0284

471 Loreto F, Dicke M, Schnitzler J-G, Turlings TSJ (2014) Plant volatiles and the environment.
472 Plant Cell Environ 37: 1905-1908

473 Maddrell SHP (1969) Secretion by the Malphigian tubules of *Rhodnius*. The movement of
474 ions and water. J Exp Biol 51: 71-97

475 Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant
476 Sci 11: 15-19

477 Monson RK, Fall R (1989) Isoprene emission from aspen leaves. Plant Physiol 90: 267-274

478 Neislon EH, Goodger JQD, Woodrow IE, Møller BL (2013) Plant chemical defence: at what
479 cost? Trends Plant Sci 18: 250-258

480 Niinemets Ü (2010) Mild versus severe stress and BVOCs: thresholds, priming and
481 consequences. Trends Plant Sci 15: 145-153

482 Onyango FO, Ochieng Odero JPR (1994) Continuous rearing of the maize stemborer
483 Busceola fusca on an artificial diet. Entomol Exp et Appl 73:139-144

484 Paré PW, Tumlinson JH (1999) Plant volatiles as defense against insect herbivores. Plant
485 Physiol 121: 325-332

486 Plath M, Dorn S, Riedl J, Barrios H, Mody K (2012) Associational resistance and
487 associational susceptibility: specialist herbivores show contrasting responses to tree
488 stand diversification. Oecologia 169: 477-487

489 Poelman EH, Bruinsma M, Zhu F et al., (2012) Hyperparasitoids use herbivore induced plant
490 volatiles to locate their parasitoid host PloS Biol 0(11): e1001435.
491 doi:10.1371/journal.pbio.1001435

492 Reisenman CE, Riffell JA, Duffy K, Pesque A, Mikles D, Goodwin B (2013) Species-
493 specific effects of herbivory on the oviposition behavior of the moth *Manduca sexta*. J
494 Chem Ecol 39: 76-89

495 Rodriguez-Saona CR, Frost CJ (2010) New evidence for a multi-functional role of herbivore-
496 induced plant volatiles in defense against herbivores. Plant Signal Behav 5: 1-3

497 Schnee C, Köllner TG, Held M, Turlings TCJ, Gershenzon J, Degenhardt J (2006) he
498 products of a single maize sesquiterpene synthase form a volatile defense signal that
499 attracts natural enemies of maize herbivores. Proc Natl Acad Sci USA 103: 1129-
500 1134

501 Sharkey TD, Wiberley, Donohue AR (2008) Isoprene emission from plants: why and how.
502 Ann Bot 101: 5-18

503 Sharkey TD, Monson RK (2014) The future of isoprene emission from leaves, canopies and
504 landscapes. Plant Cell Environ 37: 1727-1740

505 Sholes ODV (2008) Effects of associational resistance and host density on woodland insect
506 herbivores. J Anim Ecol 77: 16-23

507 Späthe A, Reinecke A, Olsson SB, Kesavan S, Knaden M, Hansson BS (2013) Plant species-
508 and status-specific odorant blends guide oviposition choice in the moth *Manduca*
509 *sexta*. Chem Senses 38: 147-159

510 Tamiru A, Bruce TJA, Woodcock CM et al. (2011) Maize landraces recruit egg and larval
511 parasitoids in response to egg deposition by a herbivore. Ecol Lett 14: 1075-1083

512 Tamiru A, Bruce TJA, Midega CAO et al. (2012) Oviposition induced volatile emissions
513 from African smallholder farmers` maize varieties. J Chem Ecol 38: 231-234

514 Tumlinson JH, Lewis WJ, Vet LEM (1993) How parasitic wasps find their hosts. Sci Am
515 268: 100-106

516 Vickers CE, Gershenzon J, Ledau MT, Loreto F (2009a) A unified mechanism of action for
517 volatile isoprenoids in plant abiotic stress. Nature Chem Biol 5: 283-291

518 Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J, Ryan A, Mullineaux
519 PM, Hewitt (2009b) Isoprene synthesis protects transgenic tobacco plants from
520 oxidative stress. Plant Cell Environ 32: 520-531

521 Wäschke N, Hardge K, Hancock C, Hilker M, Obermaier E, Meiners T (2014) Habitats as
522 complex odour environments: how does plant diversity affect herbivore and parasitoid
523 orientation? PloS ONE 9(1): e85152. doi:10.1371/journal.pone.0085152

524 Winter TR, Borkowski L, Zeier J, Rostas M (2012) Heavy metal stress can prime for
525 herbivore- induced plant volatile emission. Plant Cell Environ 35: 1287-1298

526 Zakir A, Sadek MM, Bengtsson M, Hansson BS, Witzgall P, Anderson P (2013a) Herbivore-
527 induced plant volatiles provide associational resistance against an ovipositing
528 herbivore. J Ecol 101: 410-417

529 Zakir A, Bengtsson M, Sadek MM et al., (2013b) Specific response to herbivore-induced *de*
530 *novo* synthesized plant volatiles provides reliable information for host plant selection
531 in a moth. J Exp Biol 216: 3257-3263

532

533 **Table 1:** Behavioural responses of *Chilo partellus* to volatile samples from various plant
534 species of the push-pull system that were either optimally irrigated or drought stressed

			Towards odour source		Contact with odour source	
Plant Species	df	N	χ^2	P-value	χ^2	P-value
Napier grass	1	30	0.682	0.408	7.033	0.008
Molasses grass	1	30	0.37	0.53	0.025	0.543
signal grass	1	30	1.429	0.232	4.887	0.031
Mulato II	1	30	2.727	0.098	1.154	0.273
Silverleaf *Desmodium*	1	30	1.132	0.343	0.391	0.71
Greenleaf *Desmodium*	1	30	1.126	0.283	0.411	0.68

535

Table 2 (continued)

	signal grass				Mulato II			
Compound	Drought stressed	irrigated	t	P	Drought stressed	irrigated	t	P
6-methyl-5-hepten-2-one	nd	nd	nd	nd	17.42 (± 4.58)	8.52 (± 3.45)	4.23	0.02
benzaldehyde	2.38 (±0.66)	11.24 (±4.36)	5.34	0.02	nd	nd	nd	nd
(Z)-3-hexenyl acetate	trace	trace	trace	trace	3.1 (± 1.2)	9.12 (± 3.21)	2.1	0.001
(E)-ocimene	46.1 (± 13.14)	16.24 (± 6.87)	8.73	< 0.001	nd	nd	nd	nd
(R)-linalool	64.72 (± 18.1)	28 (± 12.78)	5.22	0.001	35.12 (±11.41)	18.1 (± 5.74)	6.2	< 0.001
DMNT	88.16 (± 22.3)	4.52 (± 1.52)	6.24	< 0.001	12.4 (± 5.1)	10.76 (± 3.8)	2.1	0.35
methyl salicylate	1.34 (± 0.4)	1.74 (± 0.6)	3.21	0.63	2.41 (± 0.56)	53.18 (± 1.2)	3.5	< 0.001
decanal	0.54 (± 0.2)	0.94 (± 0.3)	1.85	0.78	nd	nd	nd	nd
α-pinene	12.1 (± 6.3)	3.1 (± 1.22)	3.34	0.001	nd	nd	nd	nd
eugenol	nd	nd	nd	nd	nd	nd	nd	nd
α-murolene	nd	nd	nd	nd	nd	nd	nd	nd
(E)-caryophyllene	16.1 (± 6.85)	2.46 (± 0.89)	6.7	0.001	nd	nd	nd	nd
TMTT	22.1 (± 7.85)	9.2 (± 0.89)	4.5	< 0.001	nd	nd	nd	nd
Total	253.54 (± 88.4)	77.44 (± 22.3)	9.32	< 0.001	70.45 (± 22.3)	99.68 (± 39.2)	4.52	0.63

Table 2 (continued)

	Silverleaf *Desmodium*				Greenleaf *Desmodium*			
Compound	Drought stressed	irrigated	t	P	Drought stressed	irrigated	t	P
6-methyl-5-hepten-2-one	nd	nd	nd	nd	nd	nd	nd	nd
benzaldehyde	nd	nd	nd	nd	nd	nd	nd	nd
(Z)-3-hexenyl acetate	8.14 (± 4.66)	6.98 (± 3.33)	2.44	0.73	1.3 (± 0.3)	4.33± (1.24)	2.89	0.001
(E)-ocimene	2.54 (± 0.98)	1.78 (± 0.6)	4.28	0.82	45.3 (± 12)	21.3 (± 6.24)	2.64	0.001
(R)-linalool	0.6 (± 0.1)	2.1 (± 0.7)	5.63	0.001	nd	nd	nd	nd
DMNT	16.34 (± 4.24)	11.76 (± 3.2)	1.67	0.48	7.43 (± 2.88)	5.4 (± 2.7)	0.4	0.72
methyl salicylate	22.12 (± 9.21)	16.44 (± 5.2)	8.3	0.02	22.12 (± 9.21)	16.44 (± 5.2)	8.3	0.57
decanal	nd	nd	nd	nd	nd	nd	nd	nd
α-pinene	nd	nd	nd	nd	nd	nd	nd	nd
eugenol	1.6 (± 0.25)	2.45 (± 1.21)	2.33	0.62	3.6 (± 1.22)	2.95 (± 0.96)	3.72	0.48
α-murolene	nd	nd	nd	nd	nd	nd	nd	nd
(E)-caryophyllene	nd	nd	nd	nd	nd	nd	nd	nd
TMTT	nd	nd	nd	nd	4.26 (± 1.6)	1.3 (± 0.4)	4.77	0.02
Total	51.34 (± 19.63)	41.51 (± 11.1)	3.31	0.45	84.01 (± 58.5)	51.72 (± 32.14)	4.21	0.83

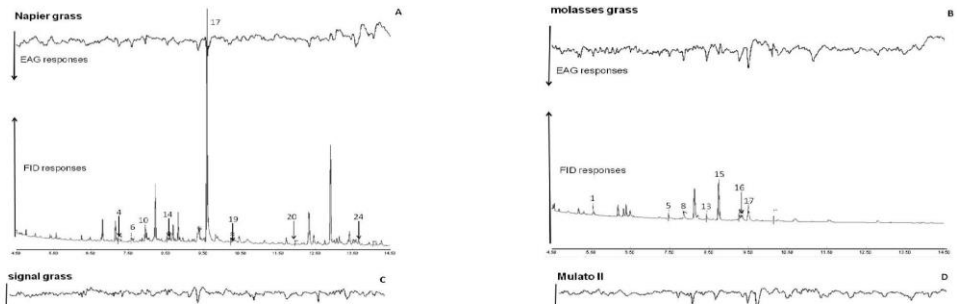
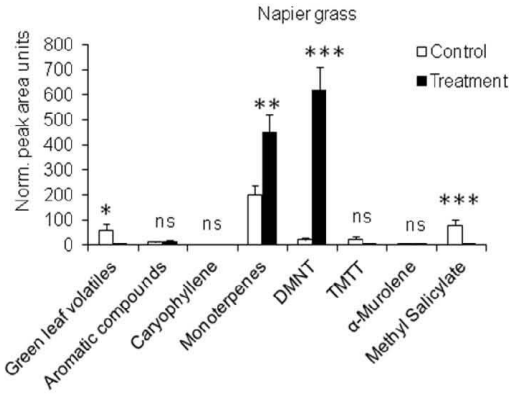
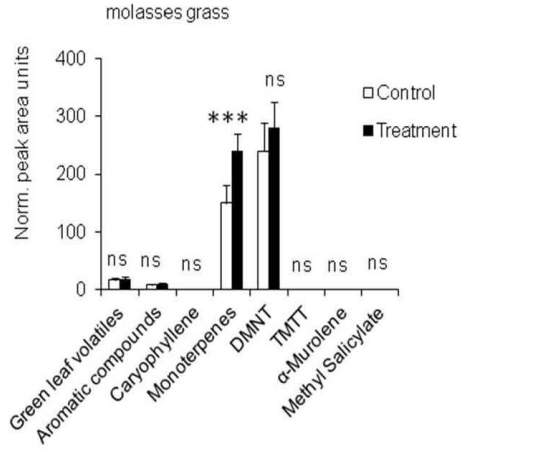
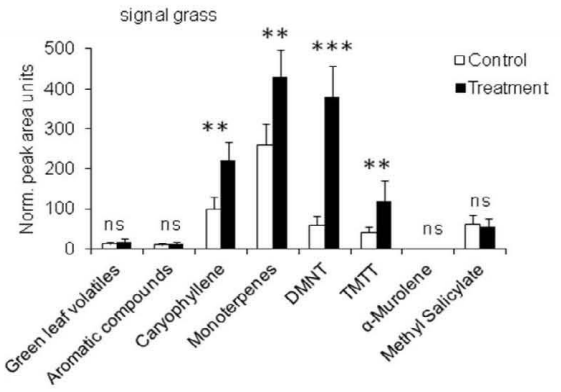
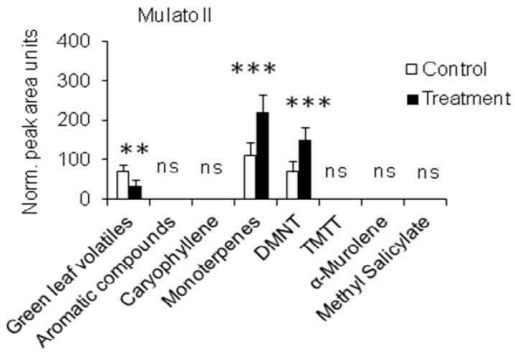


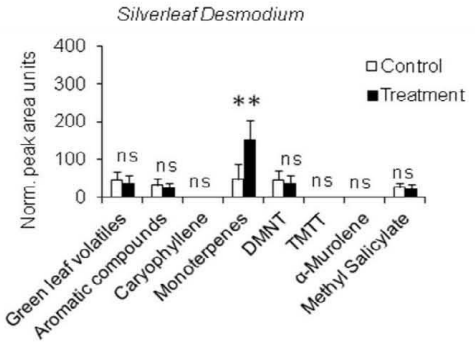
Figure 1: GC-EAG responses of gravid *C. partellus* to volatiles from drought stressed grasses of the push-pull system. Represented compounds are 1, Z-3-hexenal; 2, ethylbenzene; 3, M-xylene; 4, butyl isothioayanate; 5, benzaldehyde; 6, α-pinene; 7, *Z*-2-heptenal; 8, trans2-ethyl-2-hexen-1-ol; 9, 6-methyl-5-hepten-2-one; 10, Z-2-octan-01-ol; 11, Z-3-hexen-1-ol acetate; 12, decane; 13, benzeneacetaldehyde; 14, cyclohexanone; 15, *E*-ocimene; 16, linalool; 17, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT);18, methyl salicylate; 19, 1-nonanol; 20, eugenol; 21, caryophyllene; 22, β-bisbolene; 23, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT); 24, α-muurolene.


Napier grass
Norm. peak area units
Control
Treatment
Green leaf volatiles
Aromatic compounds
Caryophyllene
Monoterpenes
DMNT
TMTT
α-Murolene
Methyl Salicylate


molasses grass
Norm. peak area units
Control
Treatment
Green leaf volatiles
Aromatic compounds
Caryophyllene
Monoterpenes
DMNT
TMTT
α-Murolene
Methyl Salicylate


signal grass
Norm. peak area units
Control
Treatment
Green leaf volatiles
Aromatic compounds
Caryophyllene
Monoterpenes
DMNT
TMTT
α-Murolene
Methyl Salicylate


Mulato II
Norm. peak area units
Control
Treatment
Green leaf volatiles
Aromatic compounds
Caryophyllene
Monoterpenes
DMNT
TMTT
α-Murolene
Methyl Salicylate


Silverleaf Desmodium
Norm. peak area units
Control
Treatment
Green leaf volatiles
Aromatic compounds
Caryophyllene
Monoterpenes
DMNT
TMTT
α-Murolene
Methyl Salicylate

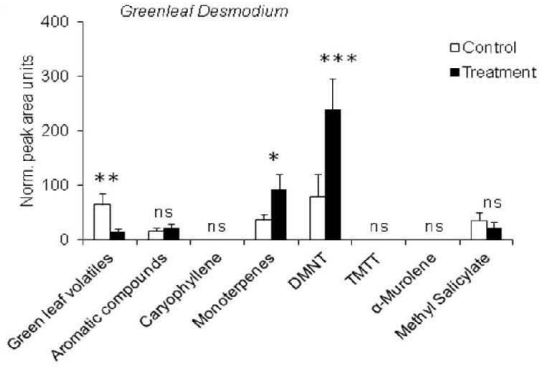

Greenleaf Desmodium
Norm. peak area units
Control
Treatment
Green leaf volatiles
Aromatic compounds
Caryophyllene
Monoterpenes
DMNT
TMTT
α-Murolene
Methyl Salicylate

Figure 2: Quantitative emission rates of volatile compounds by Napier, molasses and signal grasses, Mulato II and, *Silverleaf and Greenleaf Desmodium* under irrigated and drought stress conditions.