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Molasses Grass Induces Direct and Indirect Defense Responses in Neighbouring Maize Plants

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Abstract

Plants have evolved intricate defence strategies against herbivore attack which can include activation of defence in response to stress-related volatile organic compounds (VOCs) emitted by neighbouring plants. VOCs released by intact molasses grass (Melinis minutiflora), have been shown to repel stemborer, Chilo partellus (Swinhoe), from maize and enhance parasitism by Cotesia sesamiae (Cameron). In this study, we tested whether the molasses grass VOCs have a role in plant-plant communication by exposing different maize cultivars to molasses grass for a 3-week induction period and then observing insect responses to the exposed plants. In bioassays, C. partellus preferred non-exposed maize landrace plants for egg deposition to those exposed to molasses grass. Conversely, C. sesamiae parasitoid wasps preferred volatiles from molasses grass exposed maize landraces compared to volatiles from unexposed control plants. Interestingly, the molasses grass induced defence responses were not observed on hybrid maize varieties tested, suggesting that the effect was not simply due to absorption and re-emission of VOCs. Chemical and electrophysiological analyses revealed strong induction of bioactive compounds such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene from maize landraces exposed to molasses grass volatiles. Our results suggest that constitutively emitted molasses grass VOCs can induce direct and indirect defence responses in neighbouring maize landraces. Plants activating defences by VOC exposure alone could realize enhanced levels of resistance and fitness compared to those that launch defence responses upon herbivore attack. Opportunities for exploiting plant-plant signalling to develop ecologically sustainable crop protection strategies against devastating insect pests such as stemborer C. partellus are discussed.

Keywords Chilo partellus · Induced defence · Volatile organic compounds (VOCs) · Maize landraces · Melinis minutiflora

Introduction

Plants have evolved intricate defence strategies to protect themselves, directly and indirectly, against attacking herbi-vores. Directly, plants under attack, produce volatile organic compounds (VOCs), proteins and metabolites that repel phy-tophagous insects (De Moraes et al. 2001; Kessler and Baldwin 2001); whereas, indirectly, they emit VOCs which attract natural enemies of the attacking herbivore such as pred-ators and parasitoid wasps (Heil 2008; Tamiru et al. 2011). Apart from their role in indirect plant defence, herbivore- or wound-induced VOCs may also serve as vital external signals to elicit defence responses in neighbouring plants to warn them of impending attack (Mutyambai et al. 2016; Ton et al. 2006). Generally, VOC-mediated signalling between plants involves four main stages, the signal release and transport by the emitter plant and absorption and perception by the receiver plant (Baldwin et al. 2006). Once the signal has been

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recognized by receiver plant, it may respond with changes in signal transduction, transcriptome, proteome, and metabo-lome which may enhance levels of direct and indirect plant resistance against insect attack (Baldwin et al. 2006; Heil and Karban 2010; Ton et al. 2006). Several studies of plant-plant signalling suggested that exposure to VOCs can induce or prime plants to have faster defence responses upon subsequent herbivore attack (Heil and Silva Bueno 2007; Himanen et al. 2010). For example, lima bean plants exposed to VOCs from beetle-damaged conspecific shoots experienced reduced her-bivore damage and increased the growth rate (Heil and Silva Bueno 2007). Exposure of maize seedlings to VOCs from Spodoptera littoralis infested plants led to stronger induction of defence-related gene expression upon subsequent elicita-tion conferring reduced caterpillar feeding and development as well as attraction of a parasitic Cotesia marginiventris wasps (Ton et al. 2006). Recent studies have shown that VOC-mediated communication between undamaged plants may also lead to changes in the volatile profile of neighbouring plants with subsequent effects on the behaviour of herbivorous insect pests and their natural enemies (Dahlin et al. 2015; Glinwood et al. 2011; Ninkovic et al. 2019).

Our previous studies of maize intercropped with molasses grass, Melinis minutiflora (P. Beauv.), showed a significant reduction in stemborer infestation and increase in larval para-sitism by Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae) (Khan et al. 1997, 2000). This has led to the development of a plant volatile-mediated crop protection strat-egy, known as a ‘Push-Pull’ or ‘stimulo-deterrent diversion-ary’ against cereal stemborers for smallholder farming system in Africa (Khan et al. 2010). The strategy uses knowledge of plant chemistry and insect behaviour to manipulate agro-ecosystems in a manner that is unfavourable to pests, whilst simultaneously promoting crop yield through reduced pest damage (Hassanali et al. 2008; Khan et al. 2010). The ‘Push-Pull’ system involves intercropping maize with repel-lent plants, such as molasses grass or desmodium, while plant-ing attractive trap plants, such as Napier grass or Brachiaria grass, around the borders of the main crop (Cheruiyot et al. 2018; Khan et al. 2010). The intercrop ‘pushes’ away ovipositing stemborer moths by releasing VOCs that indicate low quality of food or presence of competitors; whereas, the trap plants attract the pest and provide a resource for laying their eggs. However, when the eggs hatch on the trap crop, the larvae are unable to survive or their development is constrained, thus reducing pest populations (Khan et al. 2000, 2010). The bioactive compounds responsible for repel-ling the pests and attracting the parasitoids are constitutively released from intact M. minutiflora and include (E)-ocimene, (E)- 4, 8-dime th yl- 1, 3, 7-n on at rie ne (DM NT) , (E)-caryophyllene, humulene and α-terpinolene (Khan et al. 2000; Pickett et al. 2006). Most of these VOCs have also been shown to be produced by maize in response to insect herbivory and/or egg deposition and are implicated in plant’s defence against the herbivore (Dicke and van Loon 2000; Tamiru et al. 2011).

Although the functions of plant derived VOCs from the com-panion M. minutiflora plant have been demonstrated in ecologi-cal pest management (Khan et al. 2010), their role in plant-plant signalling has not yet been established. Here, we investigated plant-plant chemical communication between undamaged M. minutiflora and different maize cultivars and its subsequent effects on direct and indirect defence responses of the crop against the stemborer Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) pest. Specifically, we determined effects of constitu-tive M. minutiflora VOCs exposure on volatile emission profiles of maize cultivars from different origins, i.e. maize landraces and commercial hybrids. Moreover, we examined effects of induced VOCs from the maize cultivars exposed to M. minutiflora on oviposition behaviour of stemborer, C. partellus and its natural enemy, the parasitic C. sesamiae wasp and identified bioactive compounds mediating the observed behavioural responses. VOCs emitted by M. minutiflora exposed maize plants could enhance levels of resistance in the crop if they deter stemborer pest and/or attract herbivore’s natural enemies. As far as we know, this is the first study to consider role of constitutive VOCs from undamaged M. minutiflora on volatile emission pro-files of neighbouring maize cultivars from contrasting origin and determine subsequent effects of the induced VOCs at three tro-phic levels. Earlier studies on maize focused on effects of plant-plant communication from herbivore damaged plants and tritrophic interactions therein.

We hypothesise that VOCs from intact molasses grass can induce changes in volatile profiles of maize cultivars leading to subsequent effects on the next trophic level and plant– stemborer-parasitoid interactions. Plants activating direct and indirect defences by VOC exposure alone, without actual her-bivore attack, could realize improved levels of fitness compared to plants that launch defence responses after herbivore attack. The stemborer C. partellus is one of the most injurious pests of maize, Zea mays L. (Poaceae), in sub-Saharan Africa, causing yield losses ranging between 20 and 80% depending on age of the crop at time of infestation and pest pressure (Kfir et al. 2002). It is furthermore predicted that the relative importance of C. partellus in the Lepidoptera pest complex of maize will increase in future due to the changing climate (Ntiri et al. 2016, 2019; Tamiru et al. 2007). Plant-plant signalling could be exploited to develop novel and ecologically sustainable crop protection strategies against the devastating stemborer pest and hence enhance smallholder maize production in the region.

Methods and Materials

Study Site Studies were carried out at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and

Ecology (icipe), Mbita point (0°25’S, 34°12′E, 1200 m a.s.l. and around 900 mm annual rainfall). The site is situated on the shores of Lake Victoria in western Kenya, where C. partellus is the key insect pest of maize (Khan et al. 2006).

Insects and Plants Chilo partellus were obtained from the insect mass rearing unit of icipe. The C. partellus colony was established from field-collected larvae and reared on a semi-synthetic diet as described by Ochieng et al. (1985). The mass-reared C. partellus culture was infused with a field-collected insect population every 3 months to avoid ge-netic decay and maintain the original behavioural characteris-tics of the species. Adults were sexed and gravid females selected for oviposition experiments and electrophysiology. Maize seeds of local varieties (landraces) ‘Jowi-red’ and ‘Nyamula’ were obtained from farmers in western Kenya; whereas, hybrid maize varieties ‘WS505’ and ‘PH4’ were obtained from commercial seed suppliers (Western Seed Company Ltd. and Kenya Seed Company Ltd., respectively). Seeds of molasses grass were obtained from push-pull field plots at icipe. Seeds of the maize varieties and molasses grass, M. minutiflora, were planted individually in pots filled with fertilized soil. The experimental plants were maintained inside insect proof screen houses under natural conditions (25 °C, 65% RH, 12 L:12D).

Plant-Plant Communication The plant-plant communication experiment was conducted by placing pots of newly planted maize varieties in between rows of 5–6 weeks old potted molasses grass until the exposed maize plants were 3 weeks old. The experimental plants were placed with a spacing of 75 cm between plants (within rows) and 100 cm between rows to ensure no direct plant to plant physical contact. After 3 weeks induction period, the plants exposed to molasses grass were removed and kept in a separate screen house until they were used in oviposition experiments and headspace volatile collection. Each maize variety was subjected to six different treat-ments, based on the time interval since removal from exposure to molasses grass VOCs prior to use in experiments, i.e. 0 h (immediately used), 24 h, 48 h, 72 h, 96 h and 1 week after removal. Ten replicates were done for each treatment.

Volatile Collection Volatile organic compounds (VOCs) from experimental plants, i.e. molasses grass, maize plants exposed to molasses grass and unexposed control plants, were collect-ed using headspace sampling (Tamiru et al. 2011). The leaves of experimental plants were placed gently inside polyethyleneterephtalate (PET) bags (volume 3.2 l, ~12.5 mm thickness) through an open end of the bag. The PET bags were sterilized at 150 °C before use. Purified char-coal filtered air was pumped through the bottom of the bag at a rate of 600 ml/min. The adsorbent, Porapak Q (0.05 g, 60 / 80 mesh; Supelco) were placed at the outlet valve where air was drawn at 400 ml/min. A low flow rate in the outlet valve allows adequate time and pressure for the Porapak Q to effectively adsorb the plant derived VOCs and minimize influx of air from outside through an open end of the bag. Prior to headspace sampling of M. minutiflora exposed maize varieties, the plants were removed from in-between rows of molasses grass after 3 weeks of growing together (induction period). Then, volatile samples were collected based on different lapse of time since removal from molasses grass intercrop, i.e. 0 h (immediately used), 24 h, 48 h, 72 h, 96 h and 1 week after removal. Control plants of corresponding maize varieties were planted in a different screen house under similar natural conditions but without exposure to the molasses grass. After 48 h of entrainment, volatile samples were eluted from Porapak Q with 0.5 ml of dichloromethane in 2 ml sample vials and kept in a freezer at −20 °C until they were used in bioassay and chemical analysis.

Oviposition Bioassay Two-choice tests were carried out using a modified methodology of Khan et al. (2007) in oviposition cages (90 × 60 × 60 cm) covered by a fine wire mesh netting. To determine C. partellus oviposition preference, molasses grass-exposed and non-exposed maize plants were placed in-side the oviposition cages adjacent to each other and five gravid female C. partellus moths were introduced into each cage and allowed to oviposit overnight. The following morn-ing, plants were removed from the cage and the number of eggs laid on each plant were counted under a light microscope at 6.5X magnification. A total of 10 replicates were done for each treatment.

Four-Arm Olfactometer Bioassay Responses of parasitic wasp, C. sesamiae, to VOCs collected from treatment and control plants were tested in a Perspex four-arm olfactometer (Tamiru et al. 2011). Aliquots of headspace samples (10 μl) from ex-posed and non-exposed maize plants were applied on to a piece of filter paper (4 × 25 mm) using micropipette and were placed at the two opposite ends of the olfactometer arms, while the remaining arms were solvent controls. Air was drawn through the four arms towards the centre of the olfactometer at a rate of 260 ml min^{-1}. Mated female C. sesamiae parasitoids, without any previous exposure to plants or hosts, were transferred individually into the central chamber of the olfactometer using a custom-made piece of glass tubing. Time spent and number of entries into each arm was recorded using ‘Olfa’ software (F. Nazzi, Udine, Italy) for 12 min. The olfactometer was rotated every 3 min to avoid any bias due to directional effect. The experiments were replicated 12 times for each treatment sample.

Gas Chromatography (GC) Analysis Collected VOC samples were analysed using an Agilent 7890 GC instrument (Agilent Technologies) equipped with a cool on-column injector, a non-polar HP-1 capillary column (50 m, 0.32 mm internal diameter, 0.52 μm film thickness) and a flame ionization de-tector (FID). Aliquots (2 μl) of headspace samples were injected into the injector port of the GC instrument for analy-sis. The oven temperature was maintained at 30 °C for 2 min and then programmed at 5 °C min^{-1} to 250 °C. The carrier gas was hydrogen. The GC data were analysed using HP Chemstation software. The stereochemistry of linalool was determined by co-injections on GC fitted with a β-cyclodextrin chiral capillary column (Supelco, 30 m × 0.25 mm i.d., 0.25 μm film thickness) (Tamiru et al. 2011). The GC oven was maintained at 40 °C for 1 min and then raised by 5 °C min^{-1} to 150 °C, where it was held for 30 min. After confirming successful separation of synthetic enantio-mers, peak enhancement confirmed the presence of R-enan-tiomer in the headspace sample.

Coupled GC-Electroantennography (GC-EAG) Analysis

Coupled GC-EAG analysis was carried out using antennae of gravid female C. partellus and VOC samples collected from M. minutiflora exposed maize varieties and molasses grass. Five insects were used per selected representative head-space samples. The glass Ag-AgCl electrodes were filled with saline solution (composition as in Maddrell (1969) but with-out glucose). Female moths were chilled for 1 min before the antennae were excised. The tips of the antennae were removed to ensure a good contact when suspended between the two saline solution filled electrodes. 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 efflu-ent from the GC column is simultaneously delivered to the antennal preparation and the GC detector was as described previously (Tamiru et al. 2011, 2015). Separation of the VOCs was achieved on a GC (Agilent Technologies, 6890 N) equipped with a cold on-column injector and a FID using a HP-1 column (50 m, 0.32 mm ID, 0.52 μm film thick-ness). The oven temperature was maintained at 30 °C for 2 min and then programmed at 15 °C min^{-1} to 250 °C. The carrier gas was hydrogen. Outputs from the EAG amplifier and the FID were analysed using the Syntech software pack-age. Peaks eluting from the GC column were judged to be bioactive only when they elicited consistent EAG activity in each of the five coupled runs (to avoid mistaking background noise for an electrophysiological response).

Coupled GC-Mass Spectrometry (GC-MS) Analysis

Aliquots of VOC samples were analysed on a capillary GC column (HP-1, 50 m, 0.32 mm i.d., 0.52 μm) directly coupled to a mass spectrom eter (VG Autospec, Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionisation was performed by electron impact (70 eV, 250 °C). The oven temperature was maintained at 30 °C for 5 min and then programmed at 5 °C min^{-1} to 250 °C. Tentative identification of compounds was made by compar-ison of spectra with mass spectral databases (NIST 2005) and confirmed through co-injection with authentic standards.

Statistical Analysis

Two-sample (unpaired) Student's t test was used to compare significant differences in the number of C. partellus eggs between M. minutiflora exposed and non-exposed maize plants. Behavioural responses (attraction) of C. sesamiae to headspace volatiles from maize exposed to molasses grass and unexposed control were compared in a four-way olfactometer choice test. Bioassay data were gener-ated using OLFA software, which provided a summary of time spent by C. sesamiae in each of the four olfactometer arms. The time spent (preference) data were then converted into proportions to address dependence of visiting time by C. sesamiae within the olfactometer fields, and then a log-ratio transformation (log10) was applied to account for the compositional nature of the proportions (Aitchison 1986; Tamiru et al. 2011). The transformed data were checked for normality and then subjected to analysis of variance to deter-mine significant differences between treatments and controls. The data analysis was implemented in R statistical software, version 3.2.3 (R Development Core Team 2015).

Results

Oviposition Preference (Two-Choice Test)

A significant reduc-tion was observed ($P < 0.05$, Fig. 1) in proportions of C. partellus eggs laid on maize plants of the landraces 'Nyamula' and 'Jowi-red' that were previously exposed to M. minutiflora VOCs, compared to non-exposed plants, for all the treatment durations after removal from molasses grass exposure (0 h, 24 h, 48 h, 72 h, 96 h and 1 week after remov-al). In contrast, the proportions of eggs laid on M. minutiflora VOC-exposed and non-exposed hybrid maize varieties (WS505 and PH4) did not differ significantly ($P > 0.05$, Fig. 2).

Behavioral Responses of Parasitoids to Maize Headspace Samples

In a four-arm olfactometer bioassay, gravid female C. sesamiae parasitoids were significantly attracted ($P < 0.05$; Fig. 3) to headspace samples of plant volatiles collected from M. minutiflora VOC-exposed maize landraces, Nyamula and Jowi-red, compared to non-exposed and solvent controls, for all the treatments except for Jowi-red maize plants after 1 week of removal from exposure to M. minutiflora. In contrast, there was no significant difference in C. sesamiae preference to volatiles collected from the two hybrid varieties, WS505 and PH4, exposed to M. minutiflora VOCs and non-exposed ($P > 0.05$; Fig. 4).

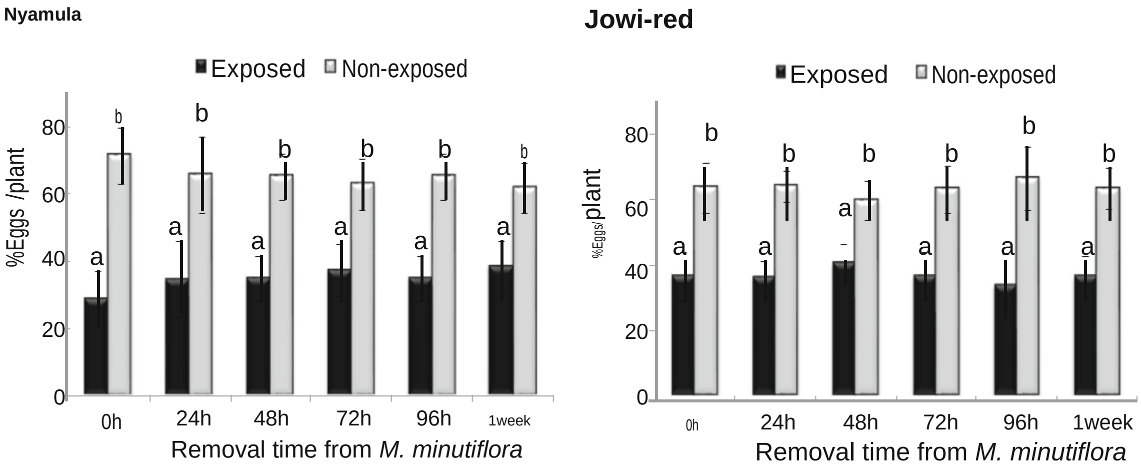

Fig. 1 Mean (± s.e.) percentage of Chilo partellus eggs laid on molasses grass-exposed and non-exposed maize landrace varieties, Nyamula and Jowi-red, in two-choice tests, at different removal time (N = 10). Means with different letters above the bars are significantly different using Student's t test (P < 0.05)

Identification of Induced Volatile Compounds GC analysis of the VOCs collected from M. minutiflora VOC-exposed and non-exposed maize landrace cultivars, 'Jowi-red' and 'Nayamula', revealed differences in volatile profile (Figs. 5 and 6). There was strong induction of bioactive volatile compounds such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) in M. minutiflora VOC-exposed maize landraces (Figs. 5 and 6). In contrast, volatile emission profiles of M. minutiflora VOC-exposed and non-exposed hybrid maize plants were very similar (Fig. 7). Coupled GC-EAG recordings using the VOCs collected from M. minutiflora VOC-exposed maize landrace plants showed consistent responses from antennae of female C. partellus to induced VOCs such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). GC linked mass spectrometry analysis of volatiles from intact M. minutiflora revealed a variety of bioactive VOCs associated with direct and indirect plant de-fences comprising monoterpenes (ocimene, nonanal), homoterpenes ((E)-4,8-dimethyl-1,3,7-nonatriene) and sesquiterpenes (α-cedrene, (E)-caryophyllene, α-bergamotene, (E)-β-farnesene) (Fig. 8).

Discussion

Our findings demonstrate that exposure of local maize landrace cultivars, 'Nyamula' and 'Jowi-red', to M. minutiflora VOCs elicits enhanced direct and indirect defence responses against C. partellus stemborers. As the experimental plants were in pots and there was no connection through the rhizosphere, VOCs rather than root exudates and microbiome are implicated in the plant-plant communication observed here. In oviposition bioassays, fewer C. partellus eggs were laid on M. minutiflora exposed maize plants compared to non-

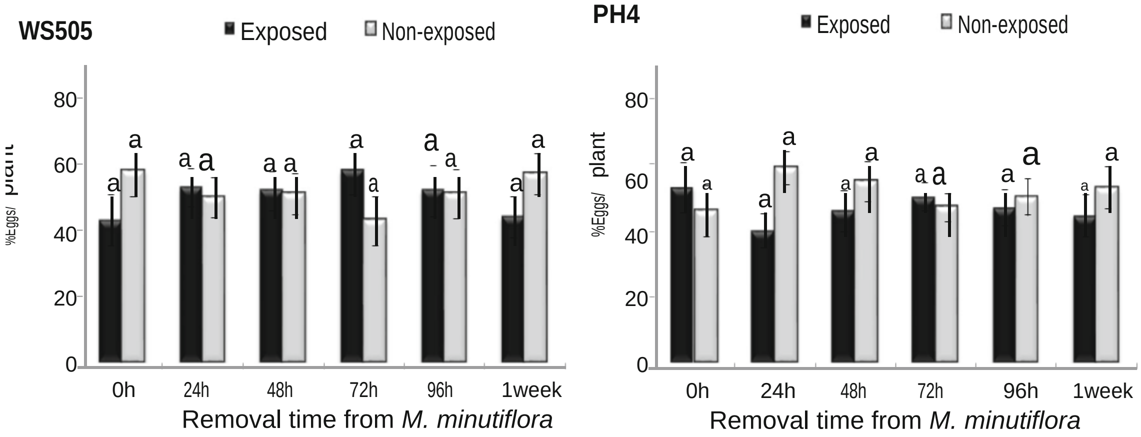

Fig. 2 Mean (± s.e.) percentage of Chilo partellus eggs laid on molasses grass-exposed and non-exposed hybrid maize varieties, WS505 and PH4, in two-choice tests, at different removal time (N = 10). Means with same letters above the bars are not significantly different using Student's t test (P < 0.05)

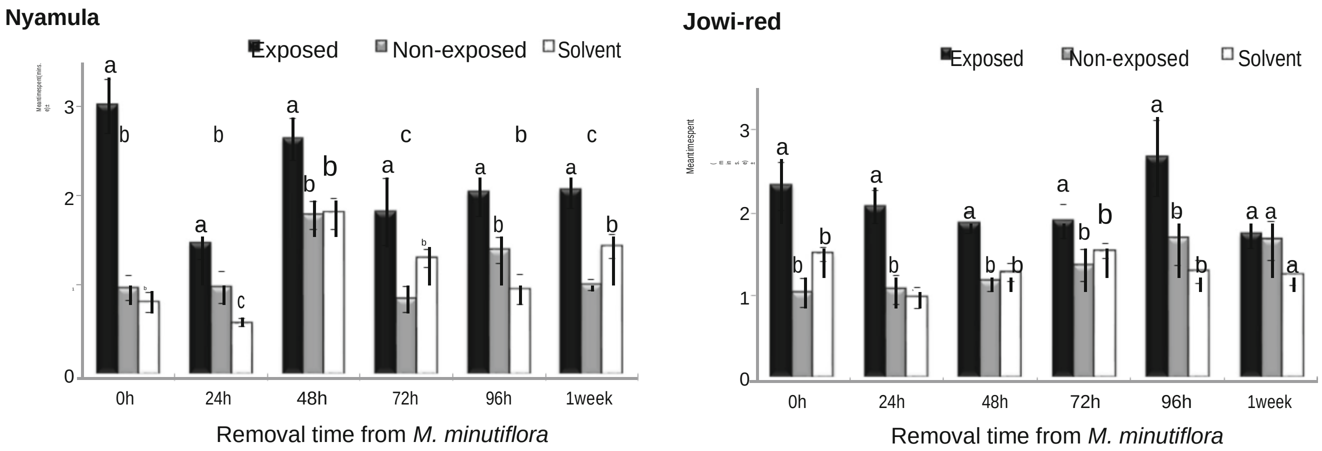

Fig. 3 Behavioural responses of female Cotesia sesamiae parasitoid in a four-arm olfactometer bioassay to volatiles collected from maize landraces 'Nyamula' and 'Jowi-red' exposed to M. minutiflora volatiles and unexposed control. Each parasitoid was observed for 12 min (N = 12). Time spent (min; mean ± s.e.) by female C. sesamiae in the treatment and control regions of the olfactometer is shown. Error bars indicate standard error and different letters above bars show statistically significant differ-ences based on the SNK test (P < 0.05)

exposed plants and the effect lasted at least for a week after exposure. The reduced oviposition preference for the exposed plants could be linked to the observed changes in volatile emission profiles of M. minutiflora exposed maize plants, however, changes in contact cues cannot be ruled out as moths were in contact with the plants in the oviposition bioassay. Plant preference by ovipositing moths is influenced by intrinsic properties of plants, particularly plant volatile chemistry, and central processing of olfactory signals by the insect (Renwick and Chew 1994; Nylin and Janz 1996; Konstantopoulou et al. 2002; Bruce et al. 2005). Volatiles collected from M. minutiflora exposed maize plants and analysed by GC-MS were different from VOCs of non-exposed plants. Furthermore, C. sesamiae parasitoid wasps preferred headspace samples of volatiles collected from maize plants exposed to M. minutiflora VOCs than those from non-exposed control plants in olfactometer bioassays.

To compensate for their sessile nature, plants have evolved intricate mechanisms of detecting VOCs from neighbouring plants as one of the ways to sense their environment and respond accordingly (Ninkovic et al. 2019). Most previous research on plant-plant chemical communication has focused on VOCs mediated signalling by neighbouring herbivore- or pathogen-attacked plants (Ton et al. 2006; Baldwin et al. 2006; Karban et al. 2014). However, recent studies have demonstrated that exposure to volatiles from unattacked plants can also induce neighbouring plants to change their volatile emission profiles (Dahlin et al. 2015; Glinwood et al. 2011;

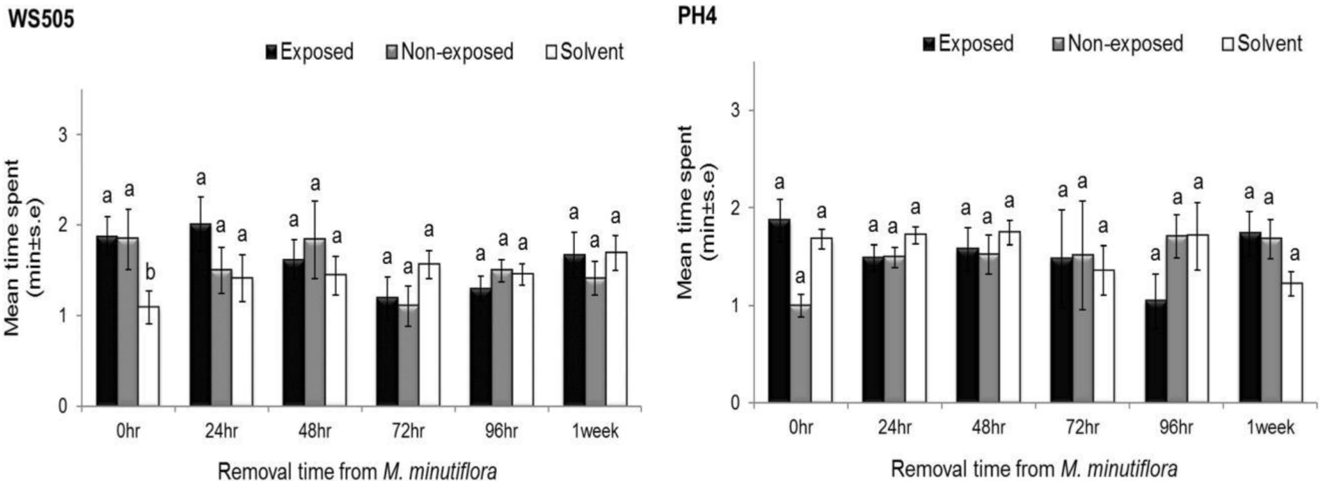

Fig. 4 Behavioural responses of female Cotesia sesamiae in a four-arm olfactometer bioassay to volatiles collected from maize hybrids 'WS505' and 'PH4' exposed to M. minutiflora volatiles and unexposed control. Each parasitoid was observed for 12 min (N = 12). Time spent (min; mean ± s.e.) by female C. sesamiae in the treatment and control regions of the olfactometer is shown. Error bars indicate standard error and dif-ferent letters above bars show statistically significant differences based on the SNK test (P < 0.05)

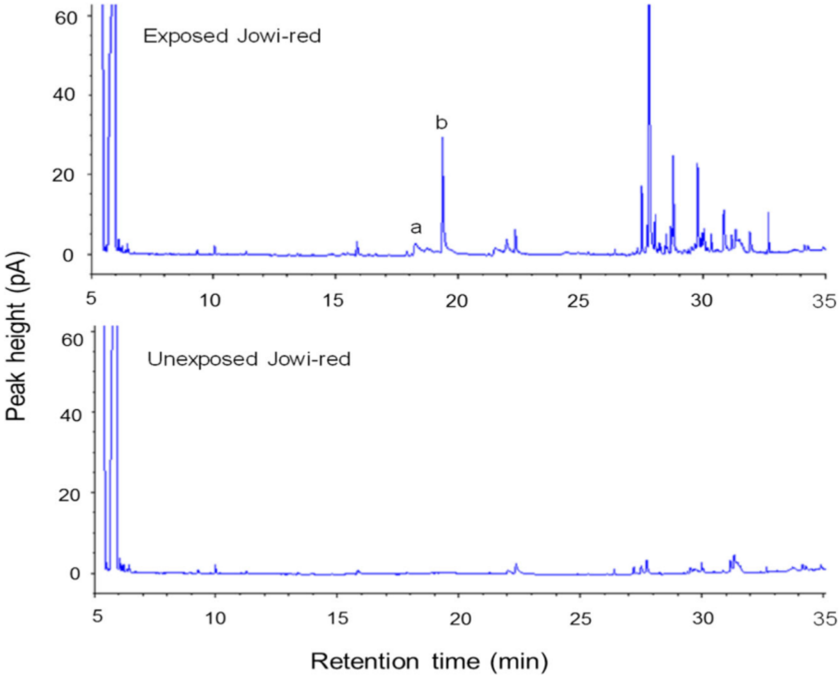

Fig. 5 Representative GC profiles of volatile organic compounds (VOCs) collected from maize landrace 'Jowi-red' exposed to molasses grass and non-exposed controls (N = 6). Represented are the bioactive compounds: a (R)-linalool, b (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT)

Ninkovic et al. 2013). These neighbour-induced VOCs have been involved in insect-plant interactions such as repelling herbivores and attracting their natural enemies. For example, VOCs from onion plants induced neighbouring potato plants to emit greater quantities of two terpenoids, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and nerolidol, which repelled green peach aphid, Myzus persicae Sulzer (Hemiptera: Aphididae) (Dahlin et al. 2015) and attracted 7-spot ladybird Coccinella septempunctata, an important natural enemy of aphids (Vucetic et al. 2014). In related study, expo-sure of barley crop to volatiles from Chenopodium album L. (Amaranthaceae) and Solanum nigrum L. (Solanaceae) result-ed in significant reduction in aphid acceptance rate when com-pared with unexposed plants in laboratory and field experi-ments (Ninkovic et al. 2009). Our current findings from the M. minutiflora -maize cropping system add to the limited number of studies where volatiles from an intact nonhost plant activate defence response in neighbouring host plants by

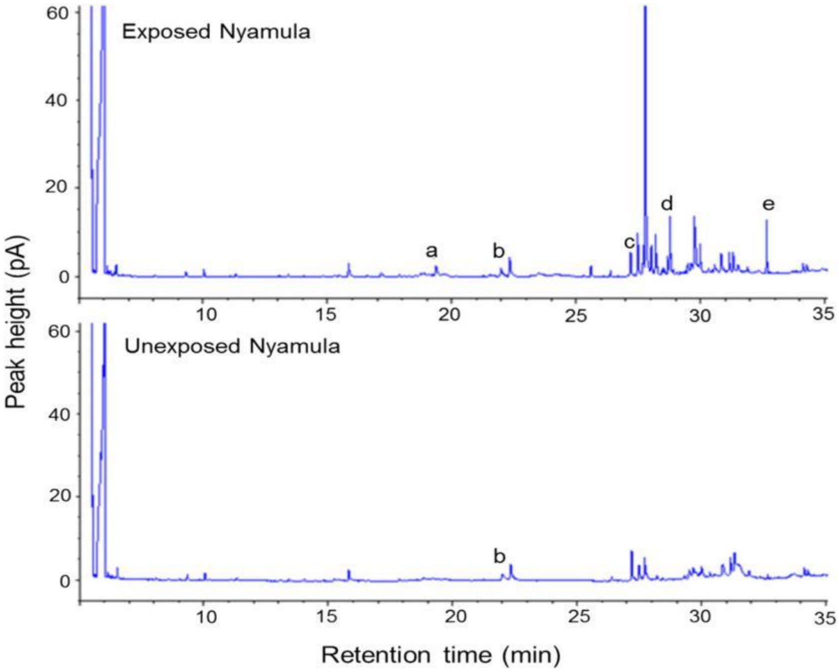

Fig. 6 Representative GC profiles of volatile organic compounds (VOCs) collected from maize landrace 'Nyamula' exposed to molasses grass and non-exposed controls (N = 6). Represented are the bioactive compounds: a (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), b decanal, c (Z)-jasmone, d (E)-β-farnesene, e (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT)

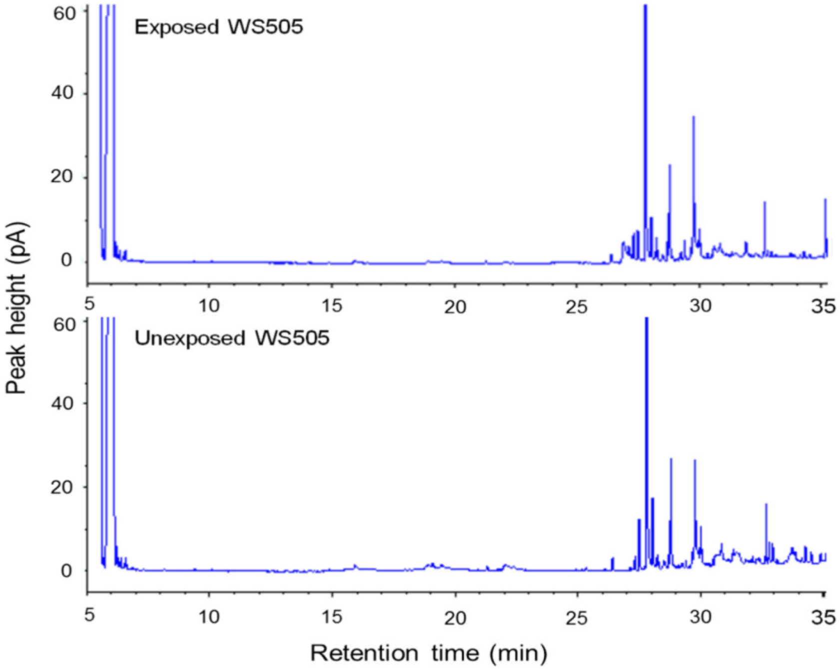

Fig. 7 Representative GC profiles of volatile organic compounds (VOCs) collected from the hybrid maize variety WS505 exposed to molasses grass and non-exposed controls (N = 6). Very little difference in volatile emission profile was found between M. minutiflora exposed and unexposed WS505 maize plants

changing their volatile profiles as well as influencing their interaction with organism on other trophic level (herbivore). It is likely that plant responses to VOCs have evolved because they were associated with neighbouring insect attacked plants, or even damaged parts of the same plant, but here the neighbouring plant releases them constitutively.

Our study also demonstrated that VOCs from intact M. minutiflora induce changes in volatile profiles that have a role in indirect defence responses of neighbouring maize land-race cultivars. In the olfactometer bioassay, female C. sesamiae wasps were significantly attracted to volatiles from maize landraces exposed M. minutiflora compared to non-exposed control plants. This suggests the VOCs mediated communication between the plants could enhance the foraging efficiency of natural enemies and thus improve their ecological fitness on M. minutiflora-maize intercropped fields infested with host herbivore. Comparison of volatile profiles revealed strong induction of bioactive compounds such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) in maize landraces exposed to M. minutiflora compared to non-exposed plants. Previous behavioural and electrophysiological studies established responses of C. sesamiae to these compounds (Bruce et al. 2010; Tamiru et al. 2011, 2012, 2015).

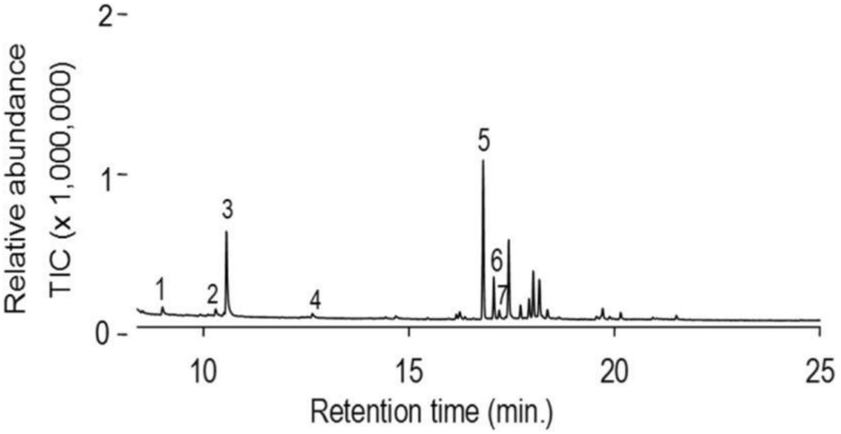

Fig. 8 GC-MS profiles of headspace volatiles from an intact molasses grass (Melinis minutiflora Beauv.). The identity of represented bioactive compounds is as follows: (1) ocimene (2) nonanal (3) (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (4) α-cedrene (5) (E)-caryophyllene (6) α-bergamotene (7) (E)-β-farnesene. Our previous studies established these VOCs elicit behavioural and electrophysiological responses in the para-sitic C. sesamiae wasp (Tamiru et al. 2011, 2012, 2015)

Melinis minutiflora constitutively emits bioactive compounds including DMNT (Fig. 8) implicated as a cue for recruiting predators and parasitoids. Attractiveness of odours from M. minutiflora exposed maize landraces implies that M. minutiflora can trigger similar indirect defence responses in heterospecific neighbouring maize plants making them bet-ter prepared for upcoming insect attack.

The gas chromatography coupled electroantennographic (GC–EAG) recording confirmed that the VOCs induced after maize landrace exposure to M. minutiflora (e.g. (R)-linalool and DMNT) elicited consistent electrophysiological responses from antennae of female C. partellus. Our earlier studies have shown that the induced VOCs such as (R)-linalool, DMNT and TMTT are attractive to C. sesamiae in bioassays (Tamiru et al. 2011, 2012, 2015). This provides an explanation for the attraction of C. sesamiae wasps to M. minutiflora ex-posed maize plants and deterrence of C. partellus from egg deposition on the exposed plants. Insect responses to odour sources are mainly determined by the qualitative and quantitative composition of volatiles emitted by plants (Bruce and Pickett 2011; Tamiru et al. 2015). Neighbour-emitted VOCs could trigger changes in volatile profile of adjacent plants that are ecologically beneficial to the receiving plant when the induced production of volatiles deter herbivores and/or attract their natural enemies (Himanen et al. 2010). Our study demonstrates changes in maize volatile profiles, induced by neighbouring M. minutiflora VOCs, which inhibits oviposition by stemborer pest while attracting parasitic C. sesamiae wasps, a key natural enemy of the pest. Such VOC mediated defence activation among plants in proximity could have practical value in pest management especially under smallholder African agricultural systems as intercropping accounts for the majority of region’s crop production practice.

Interestingly, the hybrid maize varieties used in this study (WS505 and PH4) appear to lack the ability to perceive and respond to molasses grass VOCs unlike the maize landraces. The absence of statistically significant difference in the number of C. partellus eggs laid between M. minutiflora exposed and non-exposed maize hybrids suggests lack of preference by ovipositing moths. Moreover, chemical analysis revealed very little difference in the volatile profiles of maize hybrids exposed to molasses grass and unexposed control, explaining the reason for the lack of behavioural response from the herbivore and its natural enemy to odours from hybrids exposed to M. minutiflora (Tamiru et al. 2011). Although the mechanisms by which plants accurately detect volatile cues from neighbouring plants are not well known, previous studies have indicated that plant-plant chemical interactions may occur in specific cultivar combinations of emitter and receiver within and between species (Ninkovic et al. 2019). For example, a study conducted to investigate effects of airborne interaction between different barley cultivars demonstrated that odour differences between barley cultivars could affect natural enemy behaviour (Glinwood et al. 2009). Crucially, the difference in response between maize cultivars we observed demonstrates that the effect was not simply due to absorption and re-emission of VOCs because there would not be the marked differences between the hybrids and landraces as observed here. Moreover, some VOCs induced in maize landrace (e.g. TMTT in Nyamula) after exposure to M. minutiflora are not inherently emitted by molasses grass.

In summary, our findings provide evidence that VOCs inherently produced by adjacent M. minutiflora prepare the neighbouring maize landrace plants to better respond to insect attack by triggering their induced defences. Maize plants exposed to M. minutiflora VOCs were more attractive to C. sesamiae wasps and repellent to moths of the damaging herbivore, C. partellus. Recently a small but increasing number of studies have demonstrated volatile interaction between unattacked plants can elicit changes in volatile emission profiles of the receiving plants that reduce their attractiveness to insect herbivores and lead to attraction of herbivores’ natural enemies (Dahlin et al. 2015; Glinwood et al. 2011; Ninkovic et al. 2019; Vucetic et al. 2014). Activating plant defences by VOC expo-sure alone, without actual herbivore attack, could enable plants to realize improved levels of fitness compared to plants that launch defence responses upon pest attack because defences are already deployed before arrival of the herbivore. This im-portant ecological interaction can be exploited for better protec-tion the crop from pest damage. For example, the ‘push-pull’ pest management strategy has been developed by exploiting inherent abilities of companion crops that produced VOCs that repel herbivorous insects from main crop while recruiting nat-ural enemies of the pest (Khan et al. 2000). Induction of defence VOCs in the main maize crop itself, to deter pest oviposition while simultaneously attracting natural enemies through plant-plant communication could enhance their competitive ability in situations where there is a high risk of herbivory. These findings from the current study not only deepen our understanding on the chemical ecology of plant-plant interactions but also pave a way for development of novel and ecologically sustainable crop protection strategies against the devastating stemborer pests. Further studies are required to determine the mechanism of induction and whether the inducible defence trait is available in other maize genotypes including mainstream commercial hybrids. There is also scope for introgressing inducible defence traits into elite hybrid maize varieties which lack the trait, espe-cially for resource constrained smallholder maize farmers in sub-Saharan Africa who cannot afford pesticides for crop pro-tection. The prospects of exploiting VOCs' mediated plant sig-nalling to improve crop resistance against insect pests has been demonstrated (Khan et al. 2010; Pickett and Khan 2016).

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References

Aitchison J (1986) The statistical analysis of compositional data. Chapman and Hall, London XII 416pp

Baldwin IT, Halitschke R, Paschold A, Von Dahl CC, Preston CA (2006) Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. Science 311:812–815

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10(6):269–274

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

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

Cheruiyot D, Midega CAO, Bruce TJA, Van den Berg J, Pickett JA, Khan ZR (2018) Suitability of Brachiaria grass as a trap crop for management of Chilo partellus. Entomol Exp Appl 166:139–149

Dahlin I, Vucetic A, Ninkovic V (2015) Changed host plant volatile emissions induced by chemical interaction between unattacked plants reduce aphid plant acceptance with intermorph variation. J Pest Sci 88:249–257

De Moraes CM, Mescher MC, Tumlinson JH (2001) Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410:577– 580

Dicke M, van Loon JJA (2000) Multitrophic effects of herbivore induced plant volatiles in an evolutionary context. Entomol Exp Appl 97: 237–249

Glinwood R, Ahmed E, Qvarfordt E, Ninkovic V, Pettersson J (2009) Airborne interactions between undamaged plants of different culti-vars affect insect herbivores and natural enemies. Arthropod-Plant Interact 3:215–224

Glinwood R, Ninkovic V, Pettersson J (2011) Chemical interaction be-tween undamaged plants-effects on herbivores and natural enemies. Phytochemistry 72(13):1683–1689

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 agricul-tural systems including animal husbandry. Philos Trans Royal Soc B 363:611–621

Heil M, Silva Bueno JC (2007) Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. Proc Natl Acad Sci U S A 104:5467–5472

Heil M (2008) Indirect defence via tritrophic interactions. New Phytol 178:41–61

Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends Ecol Evol 25:137–144

Himanen SJ, Blande JD, Klemola T, Pulkkinen J, Heijari J, Holopainen JK (2010) Birch (Betula spp.) leaves adsorb and re-release volatiles specific to neighbouring plants - A mechanism for associational herbivore resistance? New Phytologist 186(3):722–732

Karban R, Yang LH, Edwards KF (2014) Volatile communication be-tween plants that affects herbivory: a meta-analysis. Ecol Lett 17: 44–52

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Sci 291:2141–2144

Kfir R, Overholt WA, Khan ZR, Polaszek A (2002) Biology and man-agement of economically important lepidopteran cereal stem borers in Africa. Annu Rev Entomol 47:701–731

Khan ZR, Ampong-Nyarko K, Chiliswa P, Hassanali A, Kimani S, Lwande W, Overholt WA (1997) Intercropping increases parasitism of pests. Nature 388:631–632

Khan ZR, Pickett JA, Van den Berg J, Wadhams LJ, Woodcock CM (2000) Exploiting chemical ecology and species diversity. Stemborer and Striga control for maize and sorghum in Africa. Pest Manag Sci 56:957–962

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. Entomol Exp Appl 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. Entomol Exp Appl 124: 201–211

Khan ZR, Midega CAO, Bruce TJA, Hooper AM, Pickett JA (2010) Exploiting phytochemicals for developing the push-pull crop pro-tection strategy for cereal farmers in Africa. J Exp Bot 61:4185– 4196

Konstantopoulou MA, Krokos FD, Mazomenos BE (2002) Chemical stimuli from corn plants affect host selection and oviposition behav-iour of Sesamia nonagrioides (Lepidoptera: Noctuidae). J Econ Entomol 95:1289–1293

Maddrell SHP (1969) Secretion by the Malphigian tubules of Rhodnius. The movement of ions and water. J Exp Biol 51:71–97

Mutyambai DM, Bruce TJA, van den Berg J, Midega CAO, Pickett JA, Khan ZR (2016) An indirect defence trait mediated through egg-induced maize volatiles from neighbouring plants. PLoS One 11(7): e0158744. https://doi.org/10.1371/journal.pone.0158744

Ninkovic V, Dahlin I, Vucetic A, Petrovic-Obradovic O, Glinwood R, Webster B (2013) Volatile exchange between undamaged plants - a new mechanism affecting insect orientation in intercropping. PLoS One 8:e69431. https://doi.org/10.1371/journal.pone.0069431

Ninkovic V, Glinwood R, Dahlin I (2009) Weed-barley interactions affect plant acceptance by aphids in laboratory and field experiments. Entomol Exp Appl 133:38–45

Ninkovic V, Rensing M, Dahlin I, Markovic D (2019) Who is my neigh-bor? Volatile cues in plant interactions. Plant Signal Behav 3:1–6. https://doi.org/10.1080/15592324.2019.1634993

NIST (2005) NIST mass spectral search for the NIST/EPA/NIH mass spectral library version 2.0. Office of the standard reference data base. National Institute of Standards and Technology, Gaithersburg, Maryland

Ntiri ES, Calatayud PA, Van den Berg J, Schulthess F, Le Ru B (2016) Influence of temperature on intra- and interspecific resource utilisation within a community of lepidopteran maize stemborers. PLOS ONE 11(2):e0148735. https://doi.org/10.1371/journal.pone. 0148735

Ntiri ES, Calatayud PA, Musyoka B, Van den Berg J, Le Ru BP (2019) Spatio-temporal interactions between maize lepidopteran stemborer communities and possible implications from the recent invasion of Spodoptera frugiperda (Lepidoptera: Noctuidae) in sub-Saharan Africa. Env Entomol 48:573–582

Nylin S, Janz N (1996) Host plant preference in the comma butterfly (Polygonia c-album): do parents and offspring agree? Ecoscience 3:285–289

Ochieng RS, Onyango FO, Bungu MDO (1985) Improvement of tech-niques for mass-culture of Chilo partellus (Swinhoe). Insect Sci Appl 6:425–428

Pickett JA, Khan ZR (2016) Plant volatile-mediated signalling and its application in agriculture: successes and challenges. New Phytol 212(4):856–870

Pickett JA, Bruce TJA, Chamberlain K, Hassanali A, Khan ZR, Matthes MC, Napier JA, Smart LE, Wadhams LJ, Woodcock CM (2006) Plant volatiles yielding new ways to exploit plant defence. In: Dicke M, Takken W (eds) Chemical ecology from gene to ecosys-tem. Springer, The Netherlands, pp 161–173

Renwick JAA, Chew FS (1994) Oviposition behaviour in lepidoptera. Annu Rev Entomol 39:377–400

R Core Team (2015) R: a language and environment for statistical com-puting. R Foundation for Statistical Computing, Vienna

Tamiru A, Getu E, Jembere B (2007) Role of some ecological factors for an altitudinal expansion of Chilo partellus (Sinhoe) (Lepidoptera: Crambidae). SINET: Ethiop J Sci 30:71–76

Tamiru A, Bruce TJA, Woodcock CM, Caulfield JC, Midega CAO, Ogol CKPO, Mayon P, Birkett MA, Pickett JA, Khan ZR (2011) Maize landraces recruit egg and larval parasitoids in response to egg depo-sition by a herbivore. Ecol Lett 14:1075–1083

Tamiru A, Bruce TJA, Midega CAO, Woodcock CM, Birkett MA, Pickett JA, Khan ZR (2012) Oviposition induced volatile emissions from African smallholder farmers' maize varieties. J Chem Ecol 38: 231–234

Tamiru A, Bruce TJA, Woodcock CM, Birkett MA, Midega CAO, Pickett JA, Khan ZR (2015) Chemical cues modulating electrophys-iological and behavioural responses in the parasiticwasp. Cotesia sesamiae. Can J Zool 93:281–287

Ton J, D'Alessandro M, Jourdie V, Jakab G, Karlen D, Held M, Mauch-Mani B, Turlings TCJ (2006) Priming by airborne signals boosts direct and indirect resistance in maize. Plant J 49:16–26

Vucetic A, Dahlin I, Petrovic-Obradovic O, Glinwood R, Webster B, Ninkovic V (2014) Volatile interaction between undamaged plants affects tritrophic interactions through changed plant volatile emis-sion. Plant Signal Behav 9(8):e29517. https://doi.org/10.4161/psb. 29517