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**Signal Grass (*Brachiaria brizantha*) Oviposited by
Stemborer (*Chilo partellus*) Emits Herbivore-Induced
Plant Volatiles That Induce Neighbouring Local Maize
(*Zea mays*) Varieties to Recruit Cereal Stemborer Larval
Parasitoid *Cotessia sesamiae***

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Abstract

Plants respond to attack by herbivores with the release of herbivore-induced plant volatiles (HIPVs). In return, natural enemies (predators and parasitoids) respond to these emitted herbivore-induced plant volatiles while foraging for their hosts. Neighboring plants of the same family may be induced by the emitted HIPVs. This is a tritrophic interaction that leads to an 'indirect' plant defense characteristic within species that recruit the natural enemies to ward off pests. The objective of this study was to evaluate the effect of HIPVs of *B. brizantha* on neighbouring maize plants on the management of *Chilo partellus*. This was done by entrainment of volatiles

from five maize varieties: Nyamula (local maize), jowi (local maize), Cuba 91 (a landrace from Cuba), Western hybrid 505 (WH505) and Pwani hybrid 4 (PH4). In the first set up we exposed the five maize varieties to *B. brizantha* with *C. partellus* eggs for 48 hours and removed them from the grass and then entrained them. In the second set up we exposed these five maize varieties to *B. brizantha* without *C. partellus* eggs for 48 hours and removed them from the grass and then entrained them. Lastly in the third set up we entrained non-exposed maize plants. Olfactory responses of the cereal stemborer parasitoid *Cotesia sesamiae* to volatiles emitted by these maize plant varieties were studied in a 4-way olfactometer. Gas chromatography and coupled Gas chromatography-Mass spectrometry was done to identify physiologically active compounds of the collected volatiles. The findings indicated that egg ovi position by the gravid *Chilo partellus* on signal grass *Brachiaria brizantha* caused production of HIPVs that further induced smallholder farmers' own maize varieties (Nyamula and Jowi) and a landrace maize from Latin America (Cuba 91) to attract *Cotesia sesamiae*, a parasitoid of *Chilo partellus*. Gravid females of *Cotesia sesamiae* were significantly more attracted to the volatiles of the smallholder farmers' own maize varieties and Latin America landrace maize exposed to *B. brizantha* with *Chilo partellus* eggs. In contrast, olfactometer bioassays showed that female *C. sesamiae* were significantly less attracted to HIPVs from plants exposed to *B. brizantha* without *C. partellus* eggs and to volatiles from non-exposed maize plants to either grass with eggs or without eggs. Hybrids were not induced hence had no significant result. This holds a promise for development of a sophisticated defense strategy whereby parasitoids are recruited in advance, awaiting the eggs to hatch. When the eggs hatch, the larvae are attacked, stopping them from damaging the maize crop. Our findings indicate that *B. brizantha* can be used to induce defense in a neighboring maize crop to attract *C. sesamiae* parasitoids and as a trap crop to ward off stemborers from the maize crop. This trait in *B. brizantha* should be tested further in other cereal crops to access their potential of being induced. The possible role of signal grass in pest management is discussed

Keywords: *Brachiaria brizantha*; *Chilo partellus*; *Cotesia sesamiae*; Herbivore induced plant volatiles; inducing defense; Insect oviposition; trap crop; Tritrophic interaction; *Zea mays*.

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1. Introduction

The Lepidopteran stemborer *Chilo partellus* (Swinhoe) is one of the serious insect pests that damage maize (*Zea mays* L.) in the field, the most important staple food crop in Africa [27]. Its larvae bore into the stems and leaves and feed on the inside contents rendering the stems being weak and reduce the surface area for photosynthesis in leaves [35, 26, 38]. Yield losses of up to 80% in some places have been recorded on maize [22, 37, 27]. In addition to maize, stemborers have been noted in sorghum and wild grasses that act as reservoirs during non-cropping periods [23, 24]. The management of this insect pest mainly depends on the use of expensive synthetic insecticides by the resource poor farmers. In recent years, consumers' awareness of health hazards from residual toxicity and the rising problem of insect resistance to conventional insecticide and upsurge of secondary pests

have led to researchers to look for alternative methods for field crop protection [7, 5]. With ever increasing emphasis on reduction of environmental pollution, Herbivore-Induced Plant Volatiles (HIPVs) will play an important role in replacing conventional insecticides as field crop protectants. The HIPVs are considered to be an alternative to conventional insecticides [42, 46, 18, 27]. The HIPVs have a natural origin, cheap, nontoxic and are environmentally friendly [25, 17, 8]. Studies have shown that like any other plant, signal grass, *B. brizantha* leaves emit natural green-leaf volatiles which serve as cues to attract female stemborer to the plant to deposit eggs [4]. When this grass is damaged it gives out stress chemicals called herbivore induced plant volatiles (HIPVs). These HIPVs attract natural enemies such as *C. sesamiae* of the stemborer larvae while other HIPVs repel and inhibit further colonization of the plant by stemborer female moths [40, 10, 5]. Furthermore, some HIPVs are taken up by neighboring undamaged *B. brizantha* hence tailoring them as a part of the defense against attack from stemborers [2, 43, 21, 44, 5, 13]. No studies on the effect of herbivore-induced plant volatiles of signal grass *Brachiaria brizantha* on neighbouring maize for *Chilo partellus* management have been reported. This would provide information on whether signal grass has potential for increasing parasitism of stemborers in cereal-based farming systems if grown at the border of the cereals. This paper provides an overview of Signal grass and presents data on the inducement of smallholder farmers' maize varieties by Signal grass with *C. partellus* eggs exhibited by foraging *C. sesamiae*. This was done by exposing various smallholder farmers' varieties and Latin America landrace and hybrid maize seedlings to *B. brizantha* with *C. partellus* oviposition, some maize seedlings to non-oviposited *B. brizantha* and some non-exposed maize seedlings. The volatiles from these maize seedlings were collected by entrainment. Then there was analysis of the emitted volatile chemical compounds in these seedlings with a view of determining whether *B. brizantha* could be employed to induce the maize plants in the field to produce HIPVs that mask the maize from *C. partellus* and attract parasitoids to protect them from stemborer infestation and damage. If *C. sesamiae* preference for maize plants volatiles exposed to signal grass with eggs is high, which seems to be suggested by these observations, the possibility exists that this plant could be used as an inducing agent plant around crops on which stemborers are a problem. This technique of using wild grasses as trap crops for stem borers is used effectively in Africa in a push-pull strategy where Napier grass (*Pennisetum purpureum*) is used to concentrate oviposition away from maize crops and to reduce subsequent population development [23, 24, 45, 8].

2. Materials and Methods

2.1 Insects and Plants

A culture of *Chilo partellus* was started field-collected stemborers and reared on a semi-synthetic diet described by [30]. The larval parasitoid *C. sesamiae* were reared on larvae using protocols described previously by [31]. Experimental insects were maintained at the insect mass rearing unit of *icipe*-Thomas Odhiambo campus (24 ± 3 °C, $70 \pm 5\%$ RH, 12L: 12D). The mass-reared culture was mixed with field-collected insect population every 3 months to avoid genetic decay and maintain the original behavioural characteristics of the species. Naive mated female parasitoids obtained from the fourth to fifth generation were used in the experiments.

Smallholders farmers' own maize (*Z. mays*) varieties were obtained from local farmers; the Latin America landrace maize, from the International Maize and Wheat Improvement Center (CIMMYT) while hybrid varieties were obtained from commercial seed suppliers (Western Seed Company Ltd. and Kenya Seed Company Ltd.). Signal grass, *B. brizantha* splits were obtained from *icipe*. The plants were grown individually in pots filled with fertilized soil in an insect-proof screen house at *icipe*'s Thomas Odhiambo campus, Mbita Point (0°25'S, 34°12'E; 1200 m above sea level), Western Kenya. All plants were grown under natural conditions (25 °C, 65% RH; 12L: 12D) and used in the experiments when 4 weeks old.

2.2 Collection of volatiles

Volatiles from intact potted maize seedlings, exposed to *B. brizantha* with and without stemborer eggs and non-exposed maize plants (to any kind of *B. brizantha*) were collected by headspace sampling [1, 3] for use in subsequent bioassays and electrophysiological and chemical analyses. Care was taken not to damage the plants while setting up the entrainment to avoid possible release of volatiles in response to mechanical damage of the plant. Prior to volatile collection, Signal grass, *B. brizantha* seedlings (4 weeks old) were placed inside oviposition cages (80 × 40 × 40 cm) into which 20 gravid female stemborer moths were introduced and kept overnight for oviposition. Each cage was standing in lids containing clean water to prevent predator arthropods such as brown ants and spiders from killing the stemborer moths and also from attacking the eggs deposited by these moths on the grass. Concurrently, control plants were kept inside similar cages, but without stemborer moths. The following day, the oviposited *B. brizantha* grasses were removed from the cages and taken back into the screen house whereby they were arranged into two rows 1m apart. One row of maize comprising of five potted maize plants was placed in between the two rows of Signal grass for three days. The above set up was repeated for another five maize plants but with *B. brizantha* not exposed to gravid stemborer moths and another set up comprised of non-exposed (clean) maize to either *B. brizantha* with eggs or without eggs. Volatiles were collected after the third day, starting at the last two hour of photo-phase, for 48 hours. Leaves of maize plants (45 grams) exposed to *B. brizantha* with or without eggs and non-exposed (clean) ones were enclosed in polyethyleneterephthalate (PET) bag (Volume 3.2L, approximately 12.5mm thickness) heated at 150°C before use and fitted with Swagelock inlet and outlet ports. Charcoal-filtered air was pumped (600 mL min⁻¹) through the inlet port. Volatiles were collected on Porapak Q (0.05 g, 60/80 mesh; Supelco) filters inserted in the outlet port through which air was drawn at 450 mL min⁻¹. Pumping rates were controlled using flow meters on entrainment kits to make sure more purified air is pumped in than drawn out to avoid influx of unfiltered air from outside. After entrainment, volatiles were eluted with 0.5 mL dichloromethane and used immediately in bioassays or stored at -20°C for later use. Each experiment was replicated 12 times. Control plants were kept under similar conditions, but without exposure to *C. partellus*.

2.3 Four-arm olfactometer bioassay

Responses of gravid female larval parasitoid, *C. sesamiae* to volatiles emitted by maize seedlings exposed to *B. brizantha* with stemborer eggs, *B. brizantha* without stemborer eggs and non-exposed maize seedlings.

Responses of parasitoids to volatiles were tested in a Perspex four-arm olfactometer [33]. Air was drawn through the four arms towards the centre at 260 mL min^{-1} . Headspace samples (10 μL aliquots) from various maize plants exposed to Signal grass, *B. brizantha* with eggs and control plants (non-exposed ones exposed to the grass without eggs or non-exposed maize to any kind of grass) were applied, using a micropipette (Drummond 'microcap', Drummond Scientific Co., Broomall, PA, USA), to a piece of filter paper ($4 \times 25 \text{ mm}$) subsequently placed in an inlet port at the end of each olfactometer arm. Gravid female parasitoids, without 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 in each olfactometer arm was recorded with 'Olfa' software (F. Nazzi, Udine, Italy) for 12 min. The experiments were replicated 12 times. A choice test was carried out to compare insect responses to headspace samples from oviposition-induced and control plants. The two opposite arms held the test stimuli (10 μL aliquots of headspace sample). This dose was approximately equal to that emitted by 12 plants over 12 minutes. The remaining two arms were solvent controls.

2.4 Coupled Gas chromatography-Mass spectrometry (GC-MS) analyses

Aliquots of attractive headspace samples were analyzed on a capillary GC column (HP-1, 50 m, 0.32 mm i.d., 0.52 μm) directly coupled to a mass spectrometer (VG Autospec; Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionization was performed by electron impact (70 eV, 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 GC-MS identifications were confirmed by peak enhancement with authentic standards on two GC columns of different polarities (non-polar, HP-1 column, 50 m, 0.32 mm i.d., 0.52 μm film thickness; polar DB-wax column, 30 m, 0.32 mm i.d., 0.5 μm film thickness) [47, 48].

2.5 Statistical analysis

Bioassay data from the four-arm olfactometer, i.e. time spent in each arm, were compared by analysis of variance (ANOVA) after conversion of the data into proportions and a log ratio transformation. Means were separated using Fisher's LSD test with α set at 0.05 (SAS version 10, VSN International, Hemel Hempstead, UK). All data was checked for normality before analyses. The physiologically active compounds in selected farmers own maize varieties and Cuba landrace were analyzed using a GC and GC-MS coupled machine by comparing the GC profiles and then identifying the active compounds.

3. Results

3.1 Behavioural responses of parasitoids to headspace samples of volatiles from maize exposed to Signal grass *Brachiaria brizantha* with and without eggs and non-exposed maize

Olfactometer bioassays showed that female *C. sesamiae* wasps were significantly attracted to HIPVs from the smallholder farmers' own varieties Nyamula, and Jowi and the Latin America landrace (Cuba 91), exposed to *B. brizantha* with *C. partellus* eggs (Nyamula $F_{2,33} = 8.32$, $P = 0.0012$; Jowi $F_{2,33} = 17.21$, $P < 0.0001$; Cuba 91 $F_{2,33} = 12.68$, $P < 0.0001$) Volatiles from non-exposed plants and blank controls were less attractive (Figure 1(a), 1(b) and 1(c)). A similar behavioural response was seen in volatiles from plants exposed to *B. brizantha*

with *C. partellus* eggs compared to those from plants exposed to *B. brizantha* without *C. partellus* eggs and the blank controls (Nyamula $F_{2,33}=23.53, P<0.0001$; Jowi $F_{2,33}=17.21, P<0.0001$; Cuba 91 $F_{2,33}=12.68, P<0.0001$) (Figures 2(a), 2(b), and 2(c)). In contrast, olfactometer bioassays showed that female *C. sesamiae* were not significantly attracted to HIPVs from plants exposed to *B. brizantha* without *C. partellus* eggs, compared to volatiles from non-exposed plants and the blank controls (Nyamula $F_{2,33}=2.25, P=0.1214$; Jowi $F_{2,33}=2.8, P=0.0705$; Cuba 91 $F_{2,33}=2.63, P=0.0868$) (Figures 3(a), 3(b), and 3(c)). Notably, volatiles collected from two standard commercial hybrid varieties [Western Seed Company Ltd (WH505) and Kenya Seed Company Ltd (PH4)] after exposure to *B. brizantha* with *C. partellus* eggs were less attractive to *C. sesamiae* (WH505: $F_{2,33}=4.63, P=0.0168$; PH4: $F_{2,34}=4.04, P=0.269$) when compared to volatiles from non-exposed WH505 and PH4 and the blank control arms (Figure 1 (d) and 1 (e)).

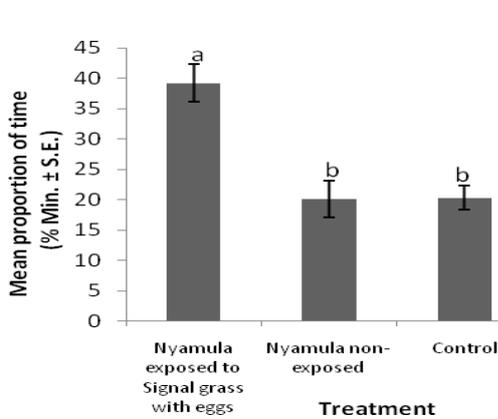


Fig. 1 (a) Nyamula

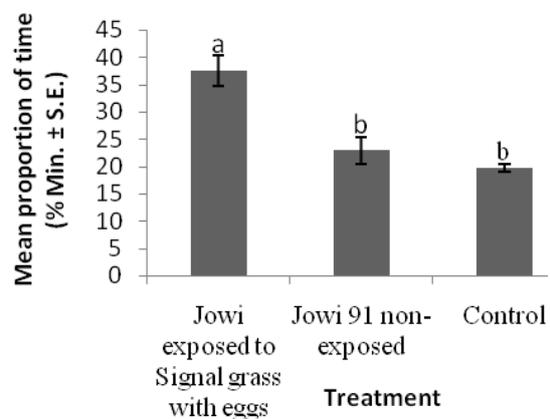


Fig. 1 (b) Jowi

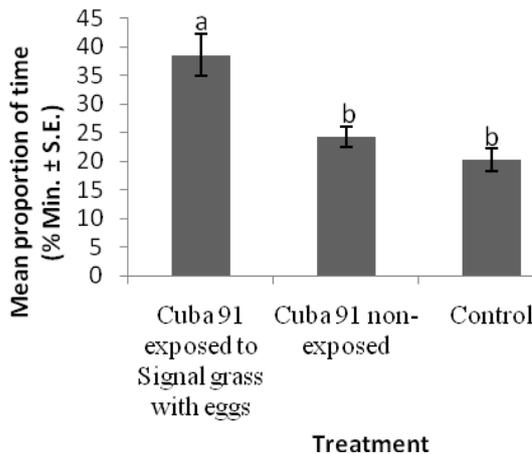


Fig. 1 (c) Cuba 91

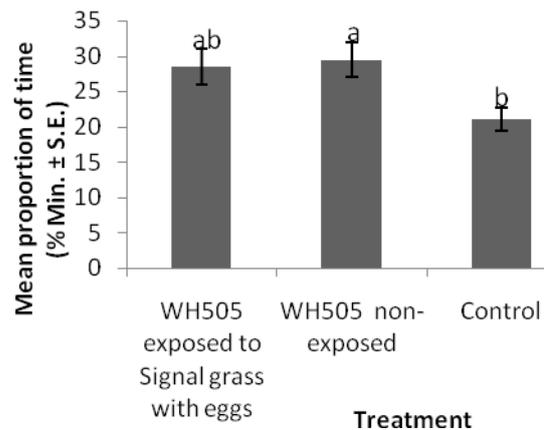


Fig. 1 (d) WH505

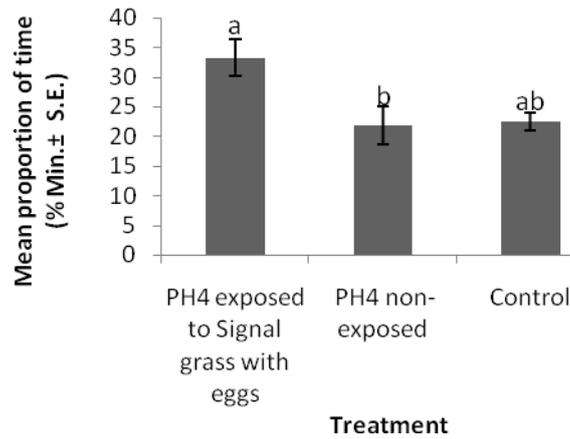


Fig. 1 (e) PH4

Figure 1. Responses of gravid *C. sesamiae* (parasitoid) to volatiles collected from maize exposed to *B. brizantha* with *C. partellus* eggs and non-exposed maize in a four-arm olfactometer bioassay. Each female parasitoid was observed for 12 min ($n = 12$). Mean % (\pm SE) for time spent (min) in each part of the olfactometer is shown. Parasitoid responses were compared by anova after conversion of the data into proportions and log-ratio transformation. Different letters indicate a significant difference while same letters indicate no significant difference using Fisher's LSD test ($P < 0.05$).

The same trend was observed when volatiles collected from these two hybrid varieties were exposed to *B. brizantha* with *C. partellus* eggs (WH505: $F_{2, 33} = 3.96$, $P = 0.288$; PH4: $F_{2, 33} = 3.08$, $P = 0.0593$) when compared to volatiles from WH505 and PH4 exposed to *B. brizantha* without *C. partellus* eggs and the blank control arms (Figure 2(d) and 2(e)). Lastly, when volatiles collected from these two hybrid varieties after exposure to *B. brizantha* without *C. partellus* eggs (WH505: $F_{2, 33} = 2.86$, $P = 0.0718$; PH4: $F_{2, 33} = 2.59$, $P = 0.0900$) were compared to volatiles from unexposed WH505 and unexposed PH4 and the blank control arms, no significant differences were observed (Figure 3(d) and 3 (e)). Responses of adult *C. sesamiae* to plant volatile cues emitted even before their prey hatched were particularly interesting and we decided to focus our study on this species.

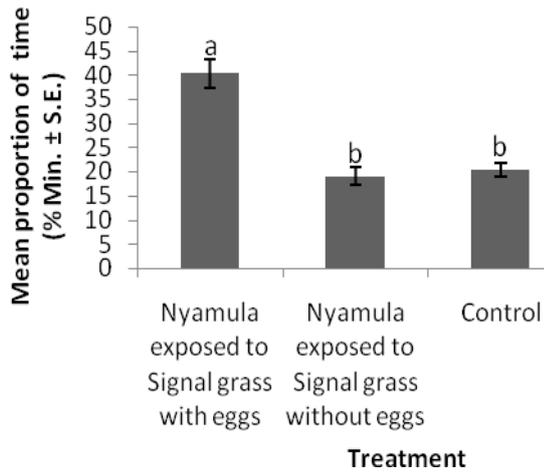


Fig. 2 (a) Nyamula

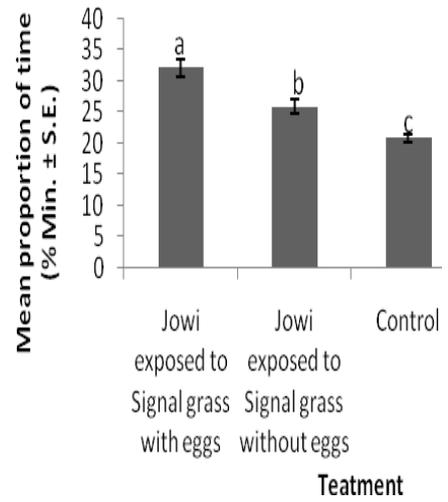


Fig. 2 (b) Jowi

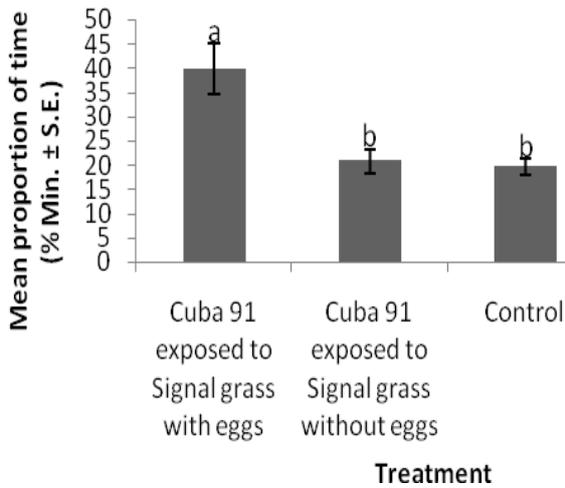


Fig. 2(c) Cuba 91

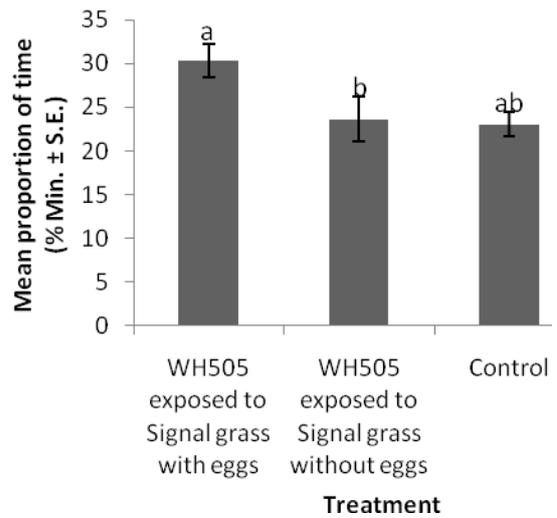


Fig. 2 (d) WH505

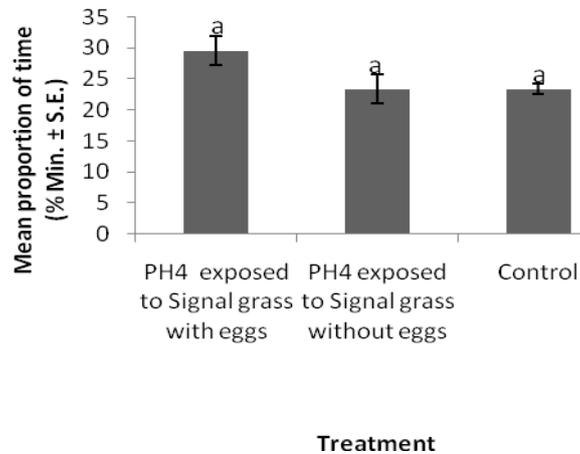


Fig. 2 (e) PH4

Figure 2. Responses of gravid *C. sesamiae* (parasitoid) to volatiles collected from maize exposed to *B. brizantha* with *C. partellus* eggs and maize exposed to *B. brizantha* without *C. partellus* eggs in a four-arm olfactometer bioassay. Each female parasitoid was observed for 12 min ($n = 12$). Mean % (\pm SE) for time spent (min) in each part of the olfactometer is shown. Parasitoid responses were compared by anova after conversion of the data into proportions and log-ratio transformation. Different letters indicate a significant difference while same letters indicate no significant difference using Fisher's LSD test ($P < 0.05$).

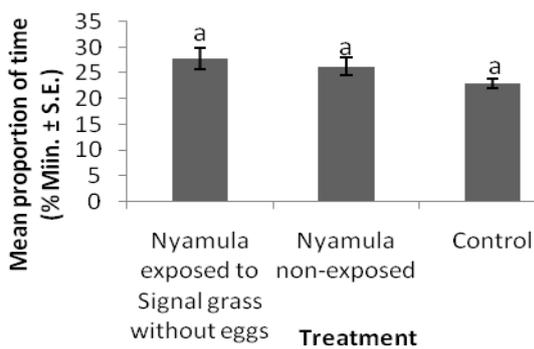


Fig. 3 (a) Nyamula

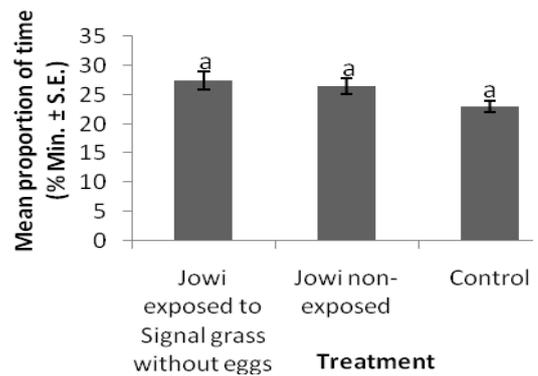


Fig. 3 (b) Jowi

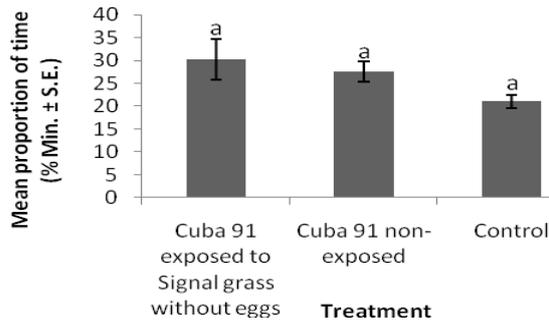


Fig. 3 (b) Cuba 91

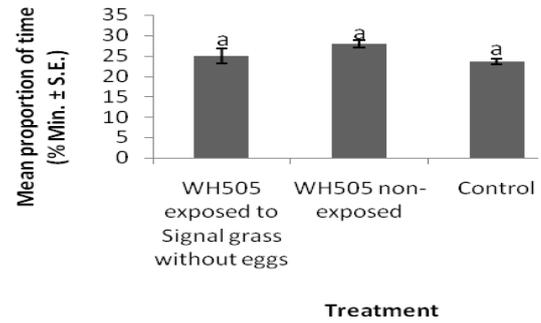


Fig. 3 (d) WH505

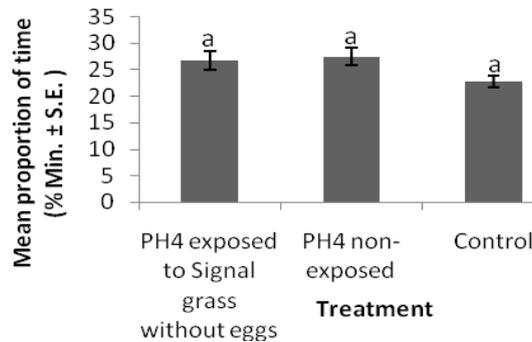


Fig. 3 (e) PH4

Figure 3. Responses of gravid *C. sesamiae* (parasitoid) to volatiles collected from maize exposed to *B. brizantha* without *C. partellus* eggs and non-exposed maize in a four-arm olfactometer bioassay. Each female parasitoid was observed for 12 min ($n = 12$). Mean % (\pm SE) for time spent (min) in each part of the olfactometer is shown. Parasitoid responses were compared by anova after conversion of the data into proportions and log-ratio transformation. Different letters indicate a significant difference while same letters indicate no significant difference using Fisher's LSD test ($P < 0.05$).

3.2 Identification of physiologically active compounds in the Herbivore Induced Plant volatiles (HIPVs)

Comparison of GC profiles of volatiles emitted from maize seedlings exposed to *B. brizantha* with *C. partellus* eggs revealed that the smallholder farmers' own seed varieties (Nyamula and Jowi) and a Latin America landrace (Cuba 91) emitted more HIPVs than the non-exposed ones or those exposed to *B. brizantha* without *C. partellus* eggs (Figures 4a, 4b and 4c).

The GC-MS analyses of the volatiles from three representative maize lines (Nyamula, Jowi and Cuba 91) exposed to *B. brizantha* with *C. partellus* eggs revealed the presence of seven key compounds. The most abundant components from Nyamula were identified as: (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), Decanal and (*E*)-Caryophyllene. The most abundant components from Jowi were identified as Linalool, Nananal, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), Methyl Salicylate and (*3E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), while the most abundant components from Cuba 91 were identified as: Linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E*)- β -farnesene. The above compounds were induced in the maize exposed to *B. brizantha* with *C. partellus* eggs but were insignificant in the maize exposed to *B. brizantha* without *C. partellus* eggs and the non-exposed ones as expressed in the farmers maize varieties and Latin America landrace maize line.

The GC and GC-MS recordings with the attractive HIPV samples from representative smallholder farmer's own varieties Nyamula and Jowi and a landrace from Latin America line Cuba 91 revealed that *C. sesamiae* were responsive to (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT), decanal and (*E*)-caryophyllene (for Nyamula); Linalool, (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT) (*E*)- β -farnesene (for Cuba 91) and Linalool + nonanal, DMNT, methyl salicylate, and (*3E,7E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) (for Jowi ja muomo). (Fig. 4a, 4b and 4c). Mean abundance of most GC-MS active compounds was significantly higher in Nyamula, Jowi and Cuba 91 maize plants exposed to *B. brizantha* with *C. partellus* eggs compared to Nyamula, Jowi and Cuba 91 maize plants exposed to *B. brizantha* without *C. partellus* eggs and non-exposed maize volatile. Egg deposition by *C. partellus* on the *B. brizantha* studied was thus found to induce local maize to emit HIPVs which attract *C. sasemiae*, a key natural enemy of the *C. partellus*.

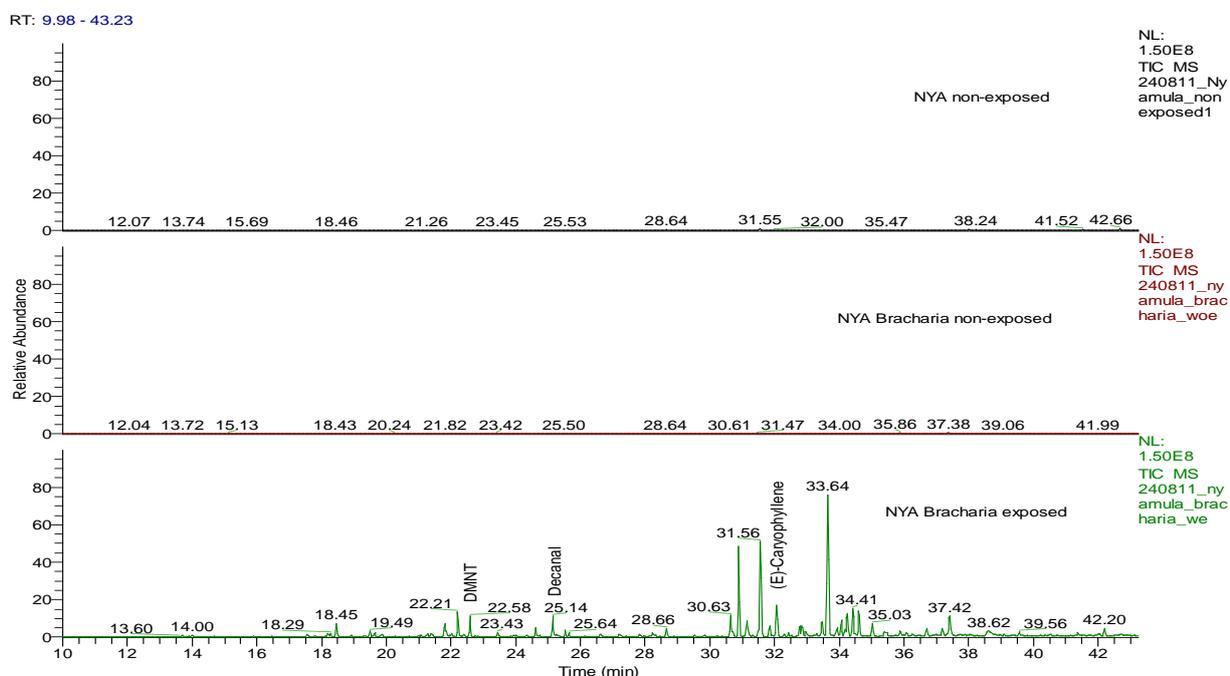


Fig. 4a. GC profiles of headspace volatiles from Nyamula exposed to *B. brizantha* with and without *C. partellus* eggs and non-exposed maize to either kind of the *B. brizantha*. The identities of GC-MS active compounds in Nyamula are: (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT), decanal and (*E*)-caryophyllene.

RT: 9.98 - 43.01

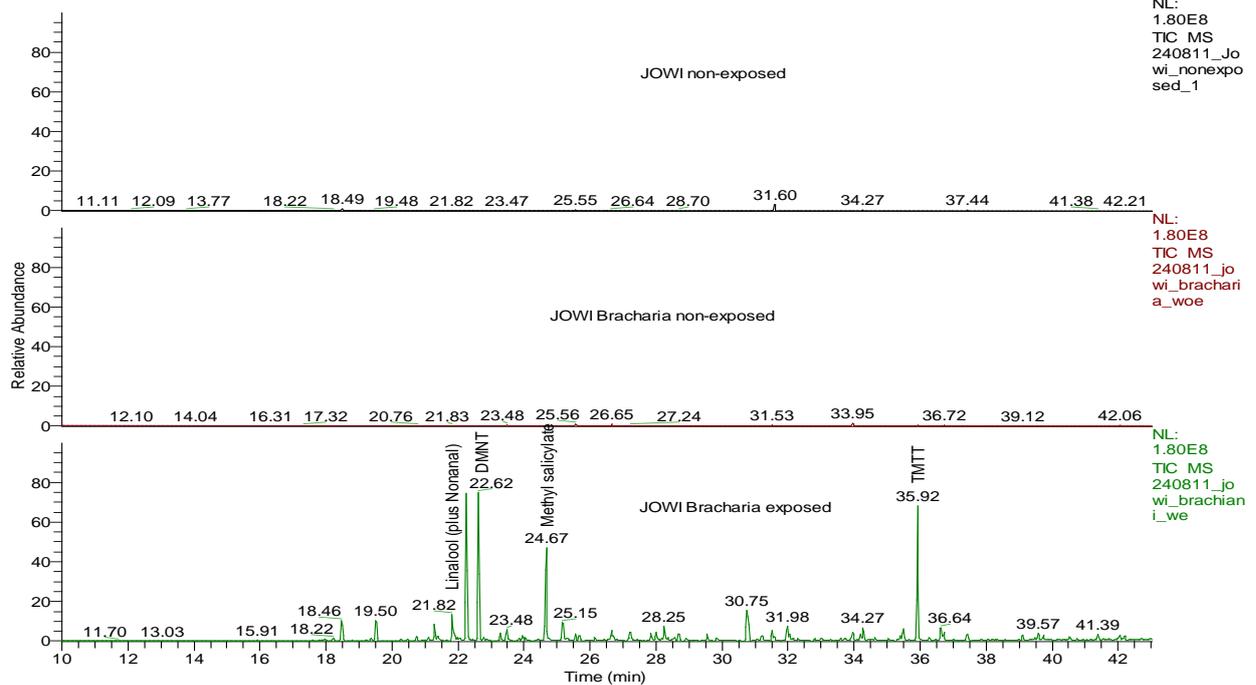


Fig. 4b. GC profiles of headspace volatiles from Jowi exposed to *B. brizantha* with and without *C. partellus* eggs and non-exposed maize to either kind of the *B. brizantha*. The identities of GC-MS active compounds in Jowi are: linalool + nonanal, DMNT, methyl salicylate and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

RT: 9.98 - 42.93

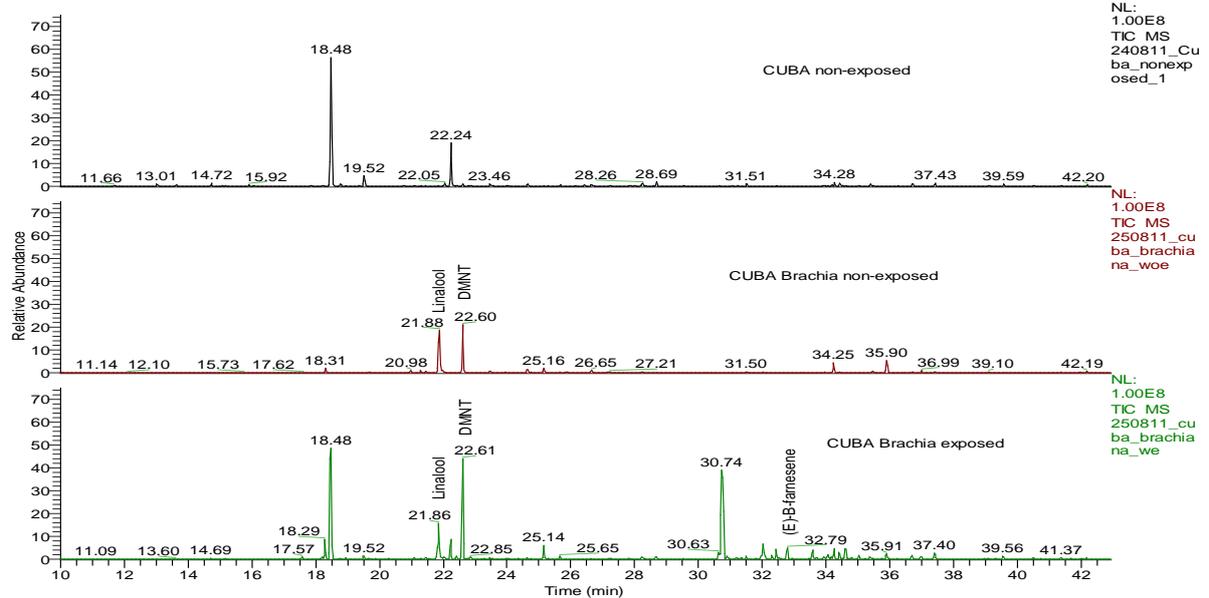


Fig. 4c. GC profiles of headspace volatiles from Cuba 91 exposed to *B. brizantha* with and without *C. partellus* eggs and non-exposed maize to either kind of the *B. brizantha*. The identities of GC-MS active compounds in Cuba 91 are linalool, (*E*)-4,8-dimethyl-1,3,7, nonatriene (DMNT) (*E*)-β-farnesene.

4. Discussion

Oviposition by phytophagous insects can induce indirect plant defence responses whereby volatiles that attract larval parasitoids are emitted [19, 39]. These same volatiles can also deter the gravid phytophagous insect from further ovipositing eggs and feeding on the crop hence protecting the crop [5, 32]. HIPVs provide parasitoids with early alert cues from plants colonized by their host and thus enhance their foraging efficacy [6, 19, 5, 38]. Plants that are induced by HIPVs in response to egg deposition on other host plants have the advantage of defending themselves early on, before larvae can damage the plant. Our study is the first to demonstrate that the HIPVs emitted from *B. brizantha* have effects on neighbouring maize, an economically important crop plant. However, the effect only occurred in tested local smallholder farmers' own varieties and the landrace maize plants and not in the tested hybrid varieties. Two tested local maize varieties Nyamula (Kenya) and Jowi jamuomo (Kenya) and one Latin America landrace maize variety, Cuba 91 (Cuba) exposed to *B. brizantha* with *C. partellus* eggs, were found to produce HIPVs that were attractive to *C. sesamiae*.

The HIPV emission following oviposition enables larval parasitoids to differentiate odours of plants colonized by hosts. Moreover, the attraction of larval parasitoids in response to oviposition indicates that their recruitment occurs in anticipation of larval hatching and before they damage the plant. Eggs and larvae are both present in fields where maize is attacked by *C. partellus*, a herbivore which has a short life cycle under tropical conditions, with eggs hatching 4 days after laying [16].

It is therefore highly likely that presence of eggs also implies larval presence, explaining the observed attraction from the perspective of the larval parasitoid. Being an annual plant with a short life cycle, maize will benefit from HIPVs from *B. brizantha* with *C. partellus* eggs in recruiting parasitoids even more than perennial plants investigated previously [19, 15]. Moreover, the maize landrace varieties identified in the current study were capable of being induced by HIPVs from *B. brizantha* following egg deposition, hence attracting larval parasitoids of *C. partellus*. While it is of adaptive value to the plant to emit HIPVs, there is also selection pressure on the parasitoids to respond to such signals, as it enhances their foraging efficiency and thus improves their ecological fitness [20, 38].

These induced defensive responses were not shown by the commercial hybrid maize varieties tested, implying that the ability to be induced by HIPVs from *B. brizantha* with oviposition at this early stage of herbivory may have been lost during the breeding process. Previous reports have indicated possible loss of direct defenses [36, 9] and below-ground indirect defenses [34, 28, 29, 38] during breeding and domestication processes. As far as our research is concerned, this is the first demonstration of an above-ground indirect defense characteristic, caused by insect eggs, that is present in smallholder farmers' own maize varieties and Latin America landrace, but absent in commercial hybrid maize varieties. The variation in induction could be due to genetic variability amongst the various farmers maize varieties and Latin America landrace line maize varieties as reflected by the representative peaks.

5. Conclusion

In conclusion, our study shows that oviposition by *C. partellus* on *B. brizantha* induces smallholder farmers' own maize varieties and Latin America landrace maize to release volatiles that attract larval parasitoids (*C. sesamiae*) and repel *C. partellus*. These HIPVs demonstrate that oviposition-associated HIPVs can be taken up by unattacked neighbouring maize plants for their protection from damage. This gives the neighbouring maize crop an added advantage as it increases the total amount of induced volatiles by the maize plant like the *B. brizantha* with oviposited eggs and hence the strength of the defence signals [42, 49, 12, 11, 20, 14, 15]. This proposed indirect defense strategy, however, appears to have been lost in modern maize hybrids. The present study will contribute to the development of management tactics that rely on exploitation of plant to plant communication through semiochemicals to manipulate oviposition behaviour of the maize stemborers. Therefore, the use of stimulo-deterrent diversional strategy that takes advantage of naturally-occurring semiochemicals in small scale maize farms in Kenya appears to be feasible in the short term.

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