

Responses of the fall armyworm (*Spodoptera frugiperda*) to different host plants: Implications for its management strategy

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Abstract

Background: The selection of suitable host plants for oviposition is critical for herbivorous insects to maximise survival of their offspring. Olfaction plays an important role in this process. However, little is known about how olfaction shapes the interaction between the fall armyworm (*Spodoptera frugiperda*) and host plants. In this study, we tested the hypothesis that olfaction guides the host selection process in the fall armyworm using oviposition and wind tunnel bioassays.

Results: In no-choice and dual-choice assays, female moths oviposited on all seven host plants that were tested (maize, sorghum, wheat, bean, cowpea, tomato and cabbage). However, in multiple-choice assays, no eggs were deposited on cowpea and cabbage. We found that maize, sorghum and wheat were most preferred for oviposition, whereas cowpea was least preferred. Wind tunnel assays confirmed these divergent oviposition preferences, with maize, sorghum and wheat odours being the most attractive. Gas chromatography–mass spectrometry analysis followed by random forest classification identified terpenes as the potential host-plant attractants.

Conclusion: Our results improve our understanding of the chemical ecology of the fall armyworm and suggest that some of these host plants could offer potential for use in an intercropping strategy to manage *S. frugiperda*.

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Supporting information may be found in the online version of this article.

Keywords: fall armyworm; oviposition; olfaction; host plant; terpenes; intercropping

1 INTRODUCTION

Plant volatiles play an important role in the host-plant selection process of herbivorous insects.¹ Insects have chemoreceptors to detect both short- and long-range volatile organic chemicals (VOCs).² They select host plants on which to oviposit considering the nutritional requirements of their offspring and to reduce the risks of higher predation, parasitism and competition.³ Some insect species express high specificity in the host-plant selection process, whereas polyphagous insects may oviposit on any available hosts when the preferred hosts are absent or scarce.⁴ Furthermore, plant volatile compounds have other functions such as mediating tritrophic (plant–herbivore–natural enemy) interactions typically determined by the quality and quantity of volatiles released by the plant.^{5–8}

Plant-derived volatile semiochemicals can be exploited to manipulate insect pest behaviour in an integrated pest management strategy.⁹ Previous studies demonstrated the attractiveness of plant volatiles to different noctuid moths. For example, volatile compounds released from pigeon pea (*Cajanus cajan*) and chickpea (*Cicer arietinum*) were shown to be attractive to *Helicoverpa armigera* moths in the laboratory and field.^{10–12} Field observations conducted by Zhu *et al.*¹³ indicated that volatiles released from flowers of early-blooming and late-blooming plants were highly attractive to several noctuid species, including armyworm, black cutworm and corn earworm. A better understanding of the biological and ecological

functions of plant volatiles could enable effective utilisation of volatile semiochemicals for insect pest management.⁷

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), an important noctuid pest native to tropical and subtropical regions of the Americas, has become a new invasive pest to the African, Asian and Australian continents.^{14–20} Currently, the pest has rapidly spread across 100 countries worldwide.¹⁸ The fall armyworm is a highly polyphagous pest, attacking more than 353 host plants, including maize (*Zea mays*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), cabbage (*Brassica oleracea*), tomato (*Solanum lycopersicum*), beans (*Phaseolus vulgaris*)

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and cowpea (*Vigna unguiculata*).²¹ In Africa, *S. frugiperda* has been primarily reported to infest maize followed by sorghum.^{15,21} According to estimates from 12 African countries, *S. frugiperda* causes an annual loss of 4.1 to 17.7 million tons of maize.²² The pest has caused estimated yield losses of 11.5% in Ethiopia,²³ 11.6% in Zimbabwe,²⁴ 45% in Ghana and 40% in Zambia.²⁵ In Kenya, *S. frugiperda* causes yield losses of about a third of annual maize production, estimated at about 1 million tons.²⁶

Female *S. frugiperda* moths discriminate between host plants to lay eggs.²⁷ It has been reported that *S. frugiperda* infestation can be significantly reduced when maize is intercropped with other plants such as legumes, soybean, beans and groundnut.^{28–30} The mechanisms underlying these observations are not fully understood. Thus, a better understanding of *S. frugiperda* interaction with host plants and the chemical basis for their preferences is essential to know how the pest responds to a mix of preferred and less preferred host plant volatiles in an intercropping system. Behavioural studies can fill this knowledge gap and guide the selection of potential intercrops to manage *S. frugiperda*.

In this study, we tested the hypothesis that olfaction guides the host selection process in *S. frugiperda*. To achieve this, we first compared the oviposition responses of *S. frugiperda* to seven host plants: maize, sorghum, wheat, bean, cowpea, tomato and cabbage. Second, we used wind tunnel assays to investigate the role of olfaction in *S. frugiperda* oviposition responses. Third, we used coupled gas chromatography–mass spectrometry followed by random forest analysis to identify the most discriminant volatiles from the headspace of the seven host plants. Our results reveal which host plants can potentially be used in an intercropping system to reduce fall armyworm infestation.

2 MATERIALS AND METHODS

2.1 Host plants

All experiments in this study were conducted at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya (1.22170° S, 36.89648° E and 1600 m above sea level [masl]). Cereal crops (maize variety 'SC Duma 43', sorghum variety 'Seredo' and wheat variety 'Chui'), pulses (kidney bean variety 'Nyayo' and cowpea variety 'Kenya Kunde 1') and vegetables (tomato variety 'Money-maker' and cabbage variety 'Gloria') were purchased from the Kenya Seed Company and used in the experiment. The plants were grown in plastic pots (3 L) in an insect-proof greenhouse under natural conditions [25 ± 3 °C, 50 ± 5% relative humidity (RH), 12 h light:12 h dark (12 L:12D) photoperiod]. The pots were filled with soil at a ratio of 2:1:1 topsoil, compost and sand soil, respectively. Watering and other agronomic practices were applied based on crop requirements. The experimental plants

were used in the experiment when they were 3–4 weeks old because they are known to release higher levels of volatiles to attract ovipositing moths at this stage.³¹

2.2 *S. frugiperda* colony

The *S. frugiperda* culture was established from larvae collected from unsprayed maize farms at Rugakuru, Embu County (0.72150° S, 37.48889° E and 1123 masl), and Nyataro, Kisii County, (0.850111° S, 39.47439° E and 1485 masl) in March and August 2020. Field-collected *S. frugiperda* were reared in the laboratory at 25 ± 3 °C, 60 ± 5% RH and 12 L:12D photoperiod. The larvae were placed in vials (30 mL) and fed on an artificial diet as described by Prasanna *et al.*³¹ To ensure adequate mating, pupae were sexed by examining key distinguishing features of the terminal segments using a Leica EZ4 HD stereo microscope (Leica Microsystems, Wetzlar, Germany) at 10× magnification. In female pupae, the gap between the genital and anal openings is wider than in male pupae.³² The sexed pupae were then placed in a Petri dish inside an oviposition cage at a ratio of 1:1. No more than three pairs of pupae were placed in each cage to avoid overcrowding. The emerged adults were fed from a cotton ball soaked with 10% honey-water solution. Wax paper was placed inside the oviposition cage as an egg-laying substrate. Gravid female *S. frugiperda* moths (2 days old) from the second to fourth laboratory generations were used in the assays. The colony was infused periodically with field-collected populations to ensure colony vigour.

2.3 Oviposition assays

The oviposition preference of *S. frugiperda* moths to different plant species was studied using circular wire mesh cages in multiple-choice, dual-choice and no-choice situations. A density of two plants per pot was used for each plant species in the experiments. For each test, gravid females (2 days old) were released at the beginning of the scotophase and left to oviposit for 3 days. Egg masses were then collected, and the number of eggs found per plant was counted using a Leica EZ4 HD stereo microscope (Leica Microsystems) at 16× magnification.

2.3.1 Multiple-choice oviposition assays

All the seven host plants were placed inside a circular wire mesh cage (diameter 1.9 m, height 1.5 m). The experimental plants were randomly arranged in a circle inside the cage at similar distances (87 cm) from each other. Ten pairs of *S. frugiperda* moths were released per cage. The insect release was made at the centre of the cage at equal distance from each of the plant species. The experiment was replicated 10 times, to give a total of 20 plants in 10 pots.

Table 1. Dual-choice combinations during oviposition and wind tunnel bioassays

No.	Treatment combination	No.	Treatment combination	No.	Treatment combination
1.	Maize vs bean	8.	Sorghum vs tomato	15.	Wheat vs cowpea
2.	Maize vs cabbage	9.	Sorghum vs cowpea	16.	Bean vs tomato
3.	Maize vs sorghum	10.	Sorghum vs wheat	17.	Bean vs cabbage
4.	Maize vs wheat	11.	Sorghum vs cabbage	18.	Bean vs cowpea
5.	Maize vs tomato	12.	Wheat vs bean	19.	Tomato vs cabbage
6.	Maize vs cowpea	13.	Wheat vs cabbage	20.	Tomato vs cowpea
7.	Sorghum vs bean	14.	Wheat vs tomato	21.	Cabbage vs cowpea

2.3.2 Dual-choice oviposition assays

Each host plant was tested in a dual-choice setup with another plant species inside a wire mesh cage ($1 \times 1 \times 1$ m) (Table 1). Five pairs of *S. frugiperda* moths were released per cage. The experiment was replicated six times. Each replicate consisted of two pots with two similar plants per pot. A total of 12 plants from each plant species and 30 pairs of moths was used.

2.3.3 No-choice oviposition assays

Each host plant species was placed alone in the oviposition cage ($1 \times 1 \times 1$ m). The experiment was replicated six times. Each replicate had four pots with two of the same plant species in each. A total of 48 plants from each plant species was used. Ten pairs of *S. frugiperda* moths were released per cage. A total of 60 pairs of moths was released per each plant species.

2.3.4 Oviposition deterrent effect of host plants

We evaluated whether some nonpreferred plants deter oviposition in female *S. frugiperda* by combining them with the most preferred plants from the above experiments (maize and sorghum). Each plant species was placed with maize and sorghum plants inside a wire mesh cage ($1 \times 1 \times 1$ m). Maize and sorghum plants were kept individually in a separate cage of similar size for comparison. Five pairs of *S. frugiperda* moths were released per cage and left to oviposit for 3 days. The experiment was replicated six times. Each replicate consisted of two pots with two plants per pot. A total of 12 plants from each plant species and 30 pairs of moths was used.

2.4 Wind tunnel bioassays

The behavioural response of gravid *S. frugiperda* moths to different host-plant odours was studied using an aluminium-framed wind tunnel (100 cm long \times 30 cm wide \times 30 cm high). Odour sources placed outside both ends of the wind tunnel were connected by Teflon tubes to transport headspace odours from the plant into the wind tunnel. Air from the chamber was continuously vented out through an exhaust tube connected at the middle top of the wind tunnel to prevent recirculation of used odour (Supporting Information Figure S9). The gravid moths were released at the middle centre of the wind tunnel with equal distance (50 cm) from the odour source of the experimental plants. Plant odours were delivered to the wind tunnel chamber by pumping charcoal-filtered air into the headspace of intact potted plants enclosed in multipurpose cooking bags (dimension 250×380 mm, Sainsbury's). A light source provided by a bulb (40 W, fitted with a red filter) positioned at 50 cm above the tunnel was used to observe insect movement. The experiments were conducted at 25 ± 2 °C and $60 \pm 10\%$ RH, and the air speed inside the wind tunnel was 20 cm/s. Insects were acclimatised to the wind tunnel room conditions 1 h before testing. A single gravid *S. frugiperda* moth was released at the centre of the wind tunnel and its flight towards different plant odours located at both ends of the wind tunnel was observed for 5 min. Twenty-one pairs of choice tests were carried out since odour sources from seven plant species were used in a dual-choice setup (Table 1). Each moth was used only once and a total of 30 moths were tested for each plant pair. Behavioural responses such as the number of approaches (20 cm from the odour source) and landing distance were recorded visually for 5 min.

2.5 Collection of volatiles

Volatiles were collected by headspace sampling from 3–4-week-old plants (bean, cowpea, maize, sorghum, wheat, cabbage and tomato) using a portable volatile collection kit (B.J. Pye, Hertfordshire, UK) according to Tamiru *et al.*³³ Each plant was carefully covered with multipurpose cooking bags (250×380 mm, Sainsbury's) that had been precleaned by heating to 150 °C for 1 h and tied to the bottom of the stem. The plant leaves were carefully placed inside the cooking bags to avoid possible release of stress-related volatiles. An adsorbent tube (Porapak Q, 50 mg, 60/80 mesh; Supelco, Bellefonte, Pennsylvania, US) was inserted into a hole at the top corner of the bag. The Porapak Q tube was conditioned by rinsing 10 times with dichloromethane (99.9% purity; Merck, Germany) and kept at 50 °C for 1 h before using it for volatile collection. Charcoal-filtered air was pumped into the entrainment at 500 mL min^{-1} and volatiles were collected on Porapak Q filters inserted in the outlet port through which air was drawn at 300 mL min^{-1} . Collection of volatiles was done for 24 h with six replications. Air entrainment in a similar setup but from empty cooking bags, i.e. without any plant species, were used as controls. Headspace volatile samples were eluted with 0.5 mL of dichloromethane and stored in a freezer at -20 °C in 1.5-mL glass vials (Agilent) until required for chemical analysis.

2.6 Analysis of volatiles

Volatiles were analysed using a gas chromatograph coupled to a mass spectrometer (GC–MS; 7890A GC and MSD 5975C triple-axis; Agilent Technologies, Palo Alto, CA, USA) set to an electron impact ionisation mode of 70 eV. The mass spectrometer was equipped with a HP-5MSI low-bleed capillary column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ }\mu\text{m}$). Helium was used as the carrier gas at 1.2 mL min^{-1} . The oven temperature was maintained at 35 °C for 5 min and then raised by $10 \text{ }^\circ\text{C min}^{-1}$ to a final temperature of 280 °C. Aliquots of headspace samples (2 μL) were injected into the GC with the help of an autosampler. The compounds were tentatively identified by comparing their mass spectra data with library data in the GC–MS library (Adams) and National Institute of Standards and Technology databases. Further confirmation was done by comparison of retention time, retention index and mass spectra of authentic standards run under the same conditions. All compounds detected in the control were considered to be contaminants and therefore ignored during identification. The Kovats retention indices of each chemical were calculated according to Adams³⁴ and Hérent *et al.*³⁵ with reference to *n*-alkane (C8–C23) standards.

2.7 Data analyses

The normality and homogeneity of all data were checked using the Shapiro–Wilk and Bartlett tests, respectively. Egg count data in the multiple-choice and no-choice oviposition assays were analysed using a generalised linear model (GLM) with negative binomial distribution. A GLM with Poisson distribution was used to analyse egg count data in the dual-choice oviposition assays. The numbers of egg masses and eggs per mass were taken as the response variables. The moth approaches and landing responses in the wind tunnel were analysed using the Wilcoxon test. The amounts of volatile compounds between the plant species were compared using analysis of variance (ANOVA). Tukey's range test ($P \leq 0.05$) was used to compare the mean values of all data. We used random forest (RF) analysis³⁶ to select the most discriminant volatiles between plant species, as used in previous studies.^{37,38} The mean decrease in accuracy (MDA) of the most

predictive variables was calculated using the RF 'importance' function. Higher MDA values indicate which volatiles are the most important in the classification.³⁸ A sparse partial least squares discriminant analysis (sPLS-DA) biplot was created using concentrations of the most discriminant volatile compounds to show differences in compound emission between plant species.³⁹ The mixOmics package's 'perf' function was used to validate the sPLS-DA.⁴⁰ A heatmap clustering analysis was performed to depict the data matrix using a colour gradient. Lower concentrations of the compounds were represented by stronger intensities of one colour, whereas higher concentrations of the compound were represented by increasing intensities of a different colour. Before performing heatmap clustering analysis, all data were converted to percentages.⁴¹ R statistical software version 4.0.4 was used for all statistical analyses.⁴²

3 RESULTS

3.1 Multiple-choice oviposition assays

The number of egg masses deposited by *S. frugiperda* females was highest on maize as compared to other plant species tested (bean, tomato, sorghum, wheat, cabbage and cowpea) ($df = 6$, $\chi^2 = 164.4$, $P < 0.001$) (Fig. 1(a)). Sorghum and wheat ranked second and third after maize in the number of egg masses deposited, but Tukey's test did not detect a significant difference between them. The numbers of egg masses laid on bean and tomato were not significantly different, while no eggs were laid on cabbage and cowpea (Fig. 1(a)). Likewise, the mean number of eggs laid

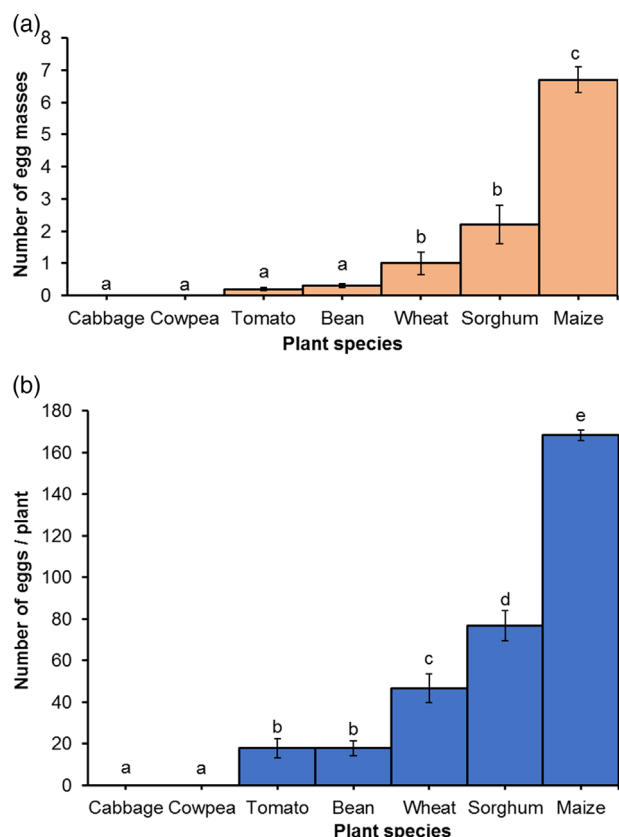


Figure 1. Oviposition by gravid *S. frugiperda* on different host plants in multiple-choice assays. Mean numbers (\pm SE) of *S. frugiperda* egg masses per plant (a) and eggs per plant (b) are shown. Bars capped with different letters are significantly different at $P \leq 0.05$ using Tukey's range test.

per plant was significantly different among the host plants ($df = 6$, $\chi^2 = 4382.5$, $P < 0.001$) (Fig. 1(b)). Gravid *S. frugiperda* moths laid the highest number of eggs on maize followed by sorghum and wheat. The numbers of eggs laid in tomato and bean were not significantly different ($P > 0.05$).

3.2 Dual-choice oviposition assays

The total numbers of egg masses and eggs laid per plant varied between plant species (Fig. 2). Significantly more egg masses were laid on maize than on cabbage ($\chi^2 = 24.9$, $P < 0.001$), cowpea ($\chi^2 = 27.7$, $P < 0.001$), bean ($\chi^2 = 27.6$, $P < 0.001$), wheat ($\chi^2 = 10.6$, $P < 0.001$) and sorghum ($\chi^2 = 54.2$, $P < 0.001$) (Fig. 2(a)). Similarly, significantly more egg masses were laid on sorghum than on cowpea ($\chi^2 = 27.7$, $P < 0.001$), cabbage ($\chi^2 = 12.2$, $P < 0.001$), tomato ($\chi^2 = 14.1$, $P < 0.001$) and bean ($\chi^2 = 13.7$, $P < 0.001$). Furthermore, significantly more egg masses were deposited on wheat than on bean ($\chi^2 = 13.6$, $P < 0.001$), cabbage ($\chi^2 = 19.8$, $P < 0.001$) and cowpea ($\chi^2 = 20.8$, $P < 0.001$).

Significantly more eggs were also laid on bean ($\chi^2 = 6.7$, $P < 0.01$) and tomato ($\chi^2 = 9.8$, $P < 0.01$) than on cowpea (Fig. 2(a)). On the other hand, the numbers of eggs laid were not statistically different when tomato was compared with bean ($\chi^2 = 1.9$, $P > 0.05$). Likewise, the numbers of egg masses laid on cowpea and cabbage were not statistically different ($\chi^2 = 1.9$, $P > 0.05$) (Fig. 2(a)). Significantly higher numbers of eggs were laid on maize than on cabbage ($\chi^2 = 524$, $P < 0.001$), cowpea ($\chi^2 = 1205.9$, $P < 0.001$), bean ($\chi^2 = 681.2$, $P < 0.001$), wheat ($\chi^2 = 129.4$, $P < 0.001$), tomato ($\chi^2 = 146.8$, $P < 0.001$) and sorghum ($\chi^2 = 4.6$, $P < 0.05$) (Fig. 2(b)). Significantly more eggs were laid on sorghum than on cowpea ($\chi^2 = 1391.3$, $P < 0.001$), cabbage ($\chi^2 = 767.8$, $P < 0.001$), tomato ($\chi^2 = 479.7$, $P < 0.001$), bean ($\chi^2 = 335.3$, $P < 0.001$) and wheat ($\chi^2 = 51.2$, $P < 0.001$) (Fig. 2(b)). Significantly higher numbers of eggs were laid on wheat than on bean ($\chi^2 = 560.1$, $P < 0.001$), cabbage ($\chi^2 = 180.5$, $P < 0.001$), cowpea ($\chi^2 = 83.2$, $P < 0.001$) and tomato ($\chi^2 = 5.7$, $P < 0.05$). The difference in the numbers of eggs laid between tomato and bean was not statistically significant ($\chi^2 = 1.7$, $P > 0.05$) (Fig. 2(b)).

3.3 No-choice oviposition assays

The total numbers of egg masses and eggs laid on each plant species differed in the no-choice assay (Fig. 3). Significantly higher numbers of egg masses were laid on maize followed by sorghum and wheat ($\chi^2 = 72.9$, $P < 0.001$) (Fig. 3(a)). The numbers of egg masses laid on maize and sorghum were not significantly different. Likewise, the numbers of egg masses laid on bean, tomato and cabbage were not statistically different ($P > 0.05$) (Fig. 3(a)). Correspondingly, the highest number of eggs was laid on maize followed by sorghum and wheat ($\chi^2 = 352.9$, $P < 0.001$) (Fig. 3(b)). The numbers of eggs laid on tomato and bean were significantly higher than those laid on cabbage and cowpea ($P < 0.05$). The fewest egg masses and eggs were found on cowpea (Fig. 3). In contrast, the highest numbers of egg masses laid on the cage walls were recorded when the moths were exposed to cowpea or cabbage ($\chi^2 = 73.9$, $P < 0.001$). The fewest egg masses were found on the cage walls when *S. frugiperda* moths were allowed to oviposit on maize, sorghum or wheat (Fig. 3(a)).

3.4 Oviposition deterrent effect of host plants

There were significant differences in the oviposition responses of *S. frugiperda* females among plant combinations (Fig. 4). *S. frugiperda* females deposited significantly higher numbers of eggs when maize was placed alone ($\chi^2 = 93$, $P < 0.05$) compared with other plant combinations. This was followed by a

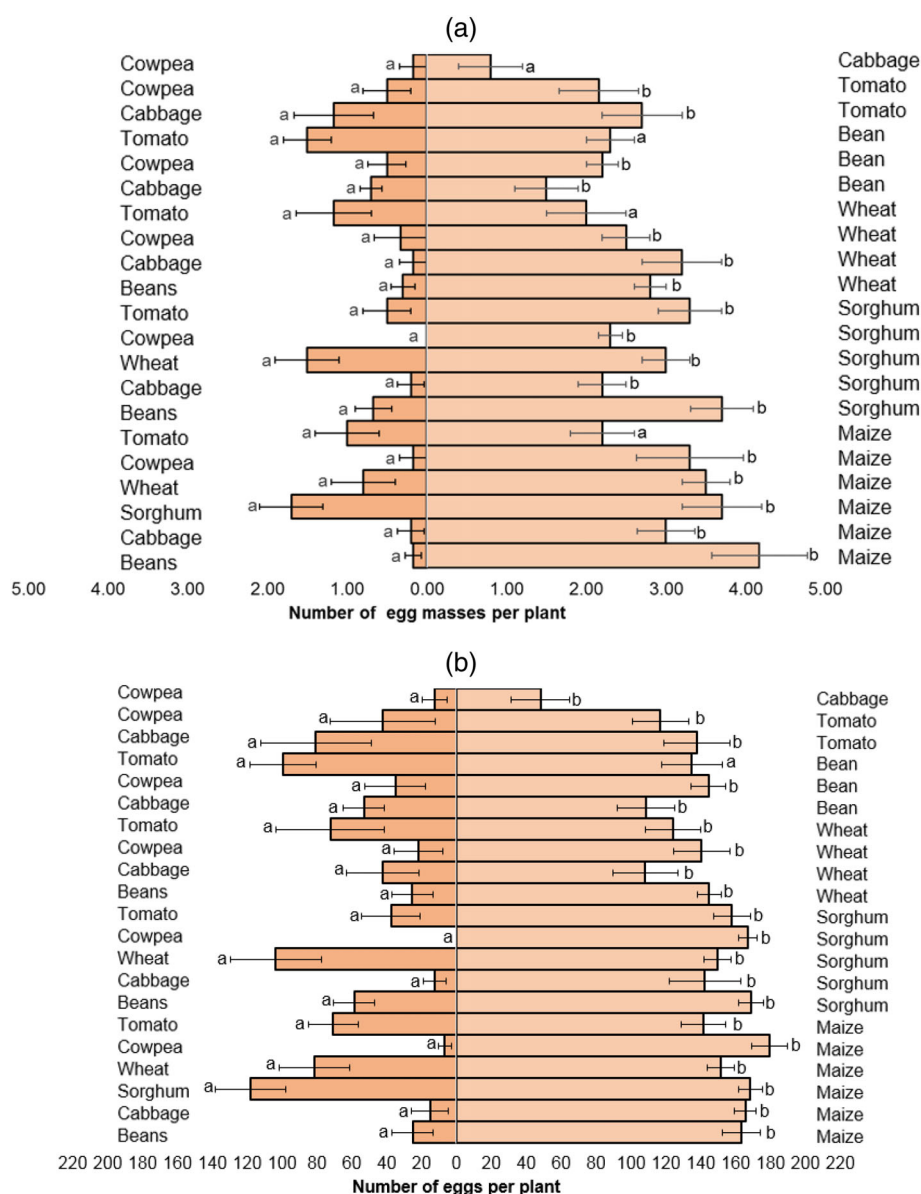


Figure 2. Oviposition by gravid *S. frugiperda* on different host plants in a dual-choice assay. Mean numbers (\pm SE) of egg masses per plant (a) and eggs per plant (b) are shown. Bars capped with different letters are significantly different at $P \leq 0.05$ using Tukey's range test.

combination of maize and sorghum for the number of eggs laid. There was no significant difference in the number of eggs laid on maize when combined with wheat and tomato. The lowest number of eggs was laid on maize when combined with cabbage, bean, cowpea and tomato (Fig. 4(a)). A significantly higher number of eggs was laid on sorghum placed alone compared to in combinations ($\chi^2 = 78$, $P < 0.05$) (Fig. 4(b)). The combination of sorghum with wheat had the second highest number of eggs, which was not significantly different from number of eggs laid when sorghum was combined with bean and tomato. The lowest number of eggs was laid on a combination of sorghum and cabbage, which was not significantly different from the number of eggs laid in combinations with cowpea or tomato (Fig. 4(b)).

3.5 Behavioural responses in a dual-choice wind tunnel

The upwind flight behaviour of *S. frugiperda* moths towards the odour sources of different host plants varied significantly (Fig. 5).

Female moths flew significantly closer to odour source odours from maize than towards those from tomato ($W = 747.5$, $P < 0.001$), cabbage ($W = 95.5$, $P < 0.001$), bean ($W = 158$, $P < 0.0001$), cowpea ($W = 161.5$, $P < 0.001$) and wheat ($W = 747.5$, $P < 0.001$). Similarly, there were more advances towards sorghum odours than towards tomato ($W = 716$, $P < 0.001$), cabbage ($W = 160$, $P < 0.001$), cowpea ($W = 241.5$, $P < 0.01$) and bean ($W = 317$, $P < 0.05$) odours. Upwind approaches to wheat odours were higher than cowpea ($W = 229.5$, $P < 0.001$) and tomato ($W = 281.5$, $P < 0.05$). However, more approaches towards bean odours were observed than towards cowpea ($W = 661$, $P < 0.01$). On the other hand, the female *S. frugiperda* responses to maize and sorghum odours were not statistically different ($P > 0.05$). The approaches towards the remaining treatments were also not statistically different ($P > 0.05$) (Fig. 5).

There were statistically significant differences in the landing responses of female *S. frugiperda* moths towards different odour sources in the wind tunnel (Fig. 6). The moths landed significantly

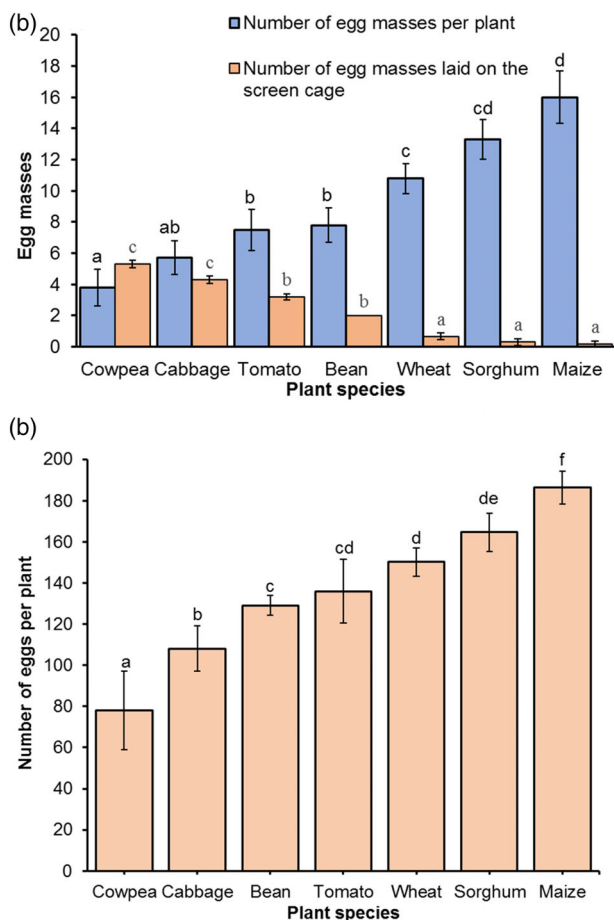


Figure 3. Oviposition by gravid *S. frugiperda* on different host plants in a no-choice assay. Mean numbers (\pm SE) of egg masses (a) and eggs per plant (b) are shown. Bars capped with different letters are significantly different at $P \leq 0.05$ using Tukey's range test.

closer to maize odours than to tomato ($W = 782$, $P < 0.001$), cabbage ($W = 120.5$, $P < 0.001$), bean ($W = 35.5$, $P < 0.001$), cowpea ($W = 193.5$, $P < 0.001$), sorghum ($W = 627.5$, $P < 0.01$) and wheat ($W = 780.5$, $P < 0.001$) odours. Similarly, significantly higher landing responses were recorded towards odours from sorghum than from tomato ($W = 789.5$, $P < 0.001$), cabbage ($W = 101$, $P < 0.001$), bean ($W = 159.5$, $P < 0.001$), cowpea ($W = 91$, $P < 0.0001$) and wheat ($W = 587.5$, $P < 0.05$) odours. The moths also landed closer to wheat odours than to bean ($W = 297$, $P < 0.05$), cowpea ($W = 219.5$, $P < 0.001$), tomato ($W = 137$, $P < 0.001$) and cabbage ($W = 165$, $P < 0.001$) odours. The moths landed closer to tomato odours compared to cowpea odours ($W = 266$, $P < 0.01$), and closer to cabbage odours when compared with cowpea odours ($W = 594$, $P < 0.05$). The landing response of moths towards tomato odours was not significantly different from that recorded for bean and cabbage odours ($P > 0.05$) (Fig. 6).

3.6 Analysis of volatiles

There were qualitative and quantitative variations in the volatile profiles of the different host plants (Table 2 and Figs 7 and 8). A total of 34 volatile organic compounds from four major chemical classes were identified, namely aldehydes, ester, alcohols and

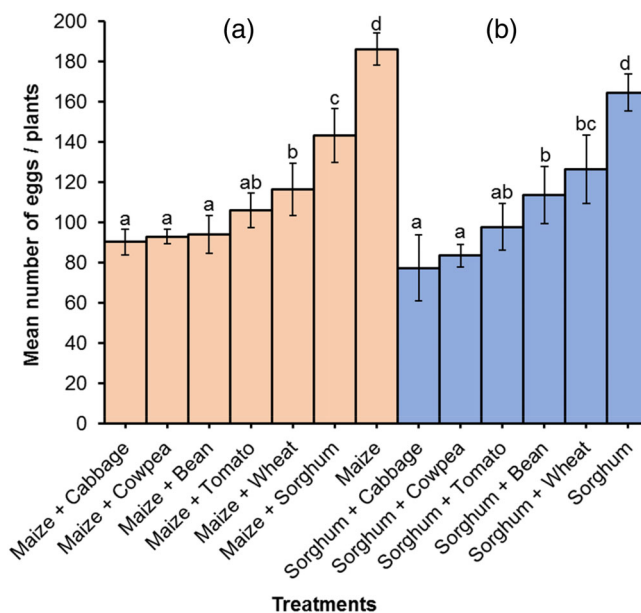


Figure 4. Oviposition by gravid *S. frugiperda* on different host-plant combinations in an oviposition cage. Combination of maize (a) and sorghum (b) with other host plants. The mean number (\pm SE) of *S. frugiperda* eggs laid is shown. Bars capped with different letters are significantly different at $P \leq 0.05$ using Tukey's range test.

terpenes (Table 2). Terpenes dominated the volatile profiles, comprising 17 monoterpenes [α -thujene, α -pinene, sabinene, β -pinene, β -myrcene, 2-carene, α -phellandrene, 3-carene, α -terpinene, p -cymene, limonene, β -phellandrene, (*E*)- β -ocimene, γ -terpinene, terpinolene, linalool and camphor], homoterpene [(*Z*)-4,8-dimethyl-1,3,7-nonatriene (DMNT)] and 10 sesquiterpenes [δ -elemene, cyclosativene, α -yalangene, α -copaene, (*E*)- β -caryophyllene, (*E*)- α -bergamotene, (*E*)- β -farnesene, α -humulene, β -selinene and α -muurolene]. The remaining compounds comprised aldehydes [(*Z*)-3-hexenal and decanal, ester [(*Z*)-3-hexen-1-ol acetate] and alcohols [2-hexanol, (*Z*)-3-hexen-1-ol and 1-octen-3-ol].

Significantly greater amounts of β -myrcene and (*E*)- β -ocimene were released from maize and sorghum than from the other test plants. The amounts of these two compounds were 2.4–3.4-fold higher in maize than in sorghum plant. The amount of (*E*)- β -ocimene in cereals was significantly higher compared to the amount in pulses and vegetables except in tomato ($P < 0.001$) (Table 2). The compounds α -yalangene and (*E*)- β -farnesene were detected only in maize, sorghum and wheat plants. The amount of α -yalangene was significantly higher, i.e., 2.6–9-fold, in sorghum compared to maize and wheat, while (*E*)- β -farnesene was significantly higher, i.e., 3–5.3-fold, in maize compared to sorghum and wheat. Nearly equal amounts of (*E*)- α -bergamotene were released by maize, sorghum and wheat. However, the amount of (*E*)- α -bergamotene was 9.6–10.6-fold higher in maize, sorghum and wheat than bean ($P < 0.01$) while it was not detected in other plant species. The compound α -pinene was detected in all tested plants, but in greater amounts in maize and tomato ($P < 0.01$) (Table 2).

The RF analysis highlighted 16 volatile compounds as discriminating between plant species (Fig. 8(a)). The compounds in rank order are β -myrcene, α -copaene, (*E*)- β -caryophyllene, α -terpinene, α -pinene, α -phellandrene, (*E*)- β -farnesene, β -pinene, β -phellandrene, limonene, (*E*)- α -bergamotene, sabinene, decanal, (*E*)-

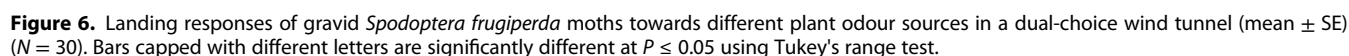
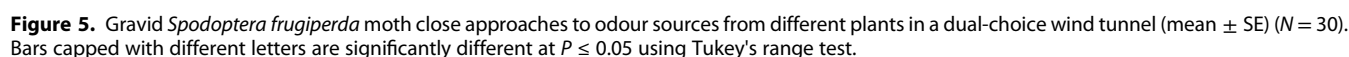


Table 2. Mean amount (ng/plant/h) of volatile compounds identified in the headspace samples of healthy plants ($n = 4$)

Peak no.	RT	RI _{alk}	RI _L	Compound	Plant species							P value
					Maize	Cabbage	Bean	Sorghum	Wheat	Cowpea	Tomato	
1	6.74	811	811	2-hexanol	0.02 ± 0.00 ^a	nd	0.03 ± 0.01 ^a	0.04 ± 0.02 ^a	0.01 ± 0.01 ^a	0.02 ± 0.00 ^a	nd	0.1
2	8.02	859	nc	(Z)-3-hexenal	0.1 ± 0.01 ^a	nd	0.01 ± 0.00 ^a	0.02 ± 0.02 ^a	0.01 ± 0.01 ^a	nd	nd	0.06
3	8.08	862	859	(Z)-3-hexen-1-ol	0.05 ± 0.01 ^a	nd	0.06 ± 0.01 ^a	0.2 ± 0.02 ^b	0.04 ± 0.02 ^a	nd	nd	0.03
4	9.69	929	927	α -thujene	nd	nd	0.1 ± 0.01 ^a	nd	nd	nd	0.7 ± 0.00 ^b	0.02
5	9.83	935	934	α -pinene ^a	6.1 ± 1.0 ^d	0.03 ± 0.01 ^a	0.06 ± 0.02 ^{ab}	0.3 ± 0.1 ^c	0.2 ± 0.07 ^c	0.08 ± 0.01 ^b	4.6 ± 0.5 ^d	0.01
6	10.68	975	975	Sabinene	nd	0.02 ± 0.01 ^a	nd	0.1 ± 0.03 ^a	nd	nd	6.8 ± 2.2 ^b	0.01
7	10.73	978	981	β -pinene	0.1 ± 0.02 ^a	0.05 ± 0.03 ^a	nd	nd	0.1 ± 0.03 ^a	nd	0.7 ± 0.2 ^a	0.1
8	10.83	982	983	1-octen-3-ol ^a	0.07 ± 0.00 ^a	nd	nd	nd	0.2 ± 0.00 ^b	0.06 ± 0.00 ^a	nd	0.04
9	11.04	992	992	β -myrcene	60.5 ± 7.6 ^d	0.7 ± 0.05 ^a	2.7 ± 0.8 ^b	25.08 ± 1.5 ^c	0.8 ± 0.02 ^a	nd	0.2 ± 0.06 ^a	0.001
10	11.2	1001	1001	2-carene	nd	nd	nd	nd	nd	nd	0.5 ± 0.08	–
11	11.3	1005	1005	α -phellandrene ^a	nd	0.03 ± 0.01 ^a	0.04 ± 0.02 ^a	nd	nd	0.1 ± 0.03 ^b	1.7 ± 0.1 ^c	0.03
12	11.36	1008	1007	(Z)-3-hexen-1-ol acetate ^a	0.3 ± 0.04 ^a	nd	0.2 ± 0.06 ^a	0.3 ± 0.05 ^a	0.2 ± 0.04 ^a	nd	nd	0.5
13	11.4	1009	1007	3-carene	nd	nd	nd	nd	nd	nd	0.4 ± 0.06	–
14	11.5	1015	1018	α -terpinene	3.6 ± 1.3 ^b	nd	nd	0.5 ± 0.1 ^a	nd	0.3 ± 0.1 ^a	5.4 ± 2.1 ^b	0.007
15	11.67	1026	1026	<i>p</i> -cymene	nd	nd	nd	nd	nd	nd	0.5 ± 0.09	–
16	11.75	1030	1030	Limonene	0.06 ± 0.02 ^a	0.9 ± 0.01 ^b	0.05 ± 0.01 ^a	nd	0.1 ± 0.04 ^a	nd	nd	0.02
17	11.77	1032	1035	β -phellandrene	nd	0.3 ± 0.2 ^a	0.3 ± 0.1 ^a	nd	nd	0.2 ± 0.01 ^a	1.5 ± 0.2 ^b	0.02
18	12.09	1050	1050	(E)- β -ocimene	57.8 ± 7.9 ^d	0.2 ± 0.05 ^a	8.0 ± 2.3 ^b	17 ± 6.9 ^c	13.7 ± 5.8 ^{bc}	nd	22.5 ± 10.2 ^c	0.001
19	12.3	1060	1060	γ -terpinene	nd	nd	nd	nd	nd	nd	0.3 ± 0.02	–
20	12.8	1090	1091	Terpinolene	nd	nd	nd	nd	nd	nd	0.2 ± 0.01	–
21	12.99	1100	1101	Linalool	0.3 ± 0.01 ^a	nd	0.02 ± 0.01 ^a	nd	0.02 ± 0.00 ^a	nd	nd	0.06
22	13.29	1118	1117	DMNT	0.5 ± 0.1	nd	nd	nd	nd	nd	nd	–
23	13.79	1150	1151	Camphor	0.03 ± 0.01 ^a	nd	nd	0.03 ± 0.01 ^a	nd	nd	nd	0.06
24	14.79	1214	1212	Decanal ^a	0.2 ± 0.01 ^a	0.2 ± 0.001 ^a	0.3 ± 0.1 ^a	2.6 ± 0.4 ^b	0.2 ± 0.01 ^a	nd	nd	0.02
25	16.65	1343	1342	δ -elemene	nd	nd	nd	nd	nd	nd	0.3 ± 0.06	–
26	17.1	1360	1368	Cyclosativene	0.2 ± 0.01	nd	nd	nd	nd	nd	nd	–
27	17.14	1362	1370	α -yalangene	2.8 ± 0.5 ^b	nd	nd	7.4 ± 1.8 ^c	0.8 ± 0.07 ^a	nd	nd	0.008
28	17.2	1365	1375	α -copaene	1.9 ± 0.7 ^b	nd	0.7 ± 0.2 ^a	0.9 ± 0.1 ^a	1.1 ± 0.4 ^a	0.5 ± 0.2 ^a	0.5 ± 0.03 ^a	0.03
29	17.8	1397	1396	(E)- β -caryophyllene ^a	2.8 ± 0.9 ^a	nd	nd	18.1 ± 3.1 ^c	nd	nd	10.0 ± 2.5 ^b	0.01
30	17.94	1414	1413	(E)- α -bergamotene	8.5 ± 1.1 ^b	nd	0.8 ± 0.4 ^a	7.9 ± 0.8 ^b	7.7 ± 1.5 ^b	nd	nd	0.01
31	18.2	1444	1444	(E)- β -farnesene	5.9 ± 1.2 ^b	nd	nd	2.0 ± 0.9 ^a	1.1 ± 0.1 ^a	nd	nd	0.02
32	18.3	1450	1449	α -humulene	0.02 ± 0.01 ^a	0.7 ± 0.1 ^b	nd	nd	nd	nd	0.8 ± 0.04 ^b	0.03
33	18.5	1488	1488	β -selinene	0.3 ± 0.1 ^a	0.2 ± 0.1 ^a	nd	0.2 ± 0.1 ^a	nd	nd	4.3 ± 0.3 ^b	0.01
34	18.56	1492	1491	α -muurolene	0.2 ± 0.01 ^a	nd	nd	nd	nd	nd	0.3 ± 0.01 ^a	0.1

Means with different superscript letters within a row are significantly different at $P \leq 0.05$ using Tukey's range test. Significant values are highlighted in bold.^a Compounds identified based on the comparison of retention time and mass spectra data with an authentic standard.DMNT, (Z)-4,8-dimethyl-1,3,7-nonatriene; nc, not calculated; nd, not detected; RI_{alk}, retention index relative to C8–C23 *n*-alkanes; RI_L, retention index obtained from the literature.^{43–46}

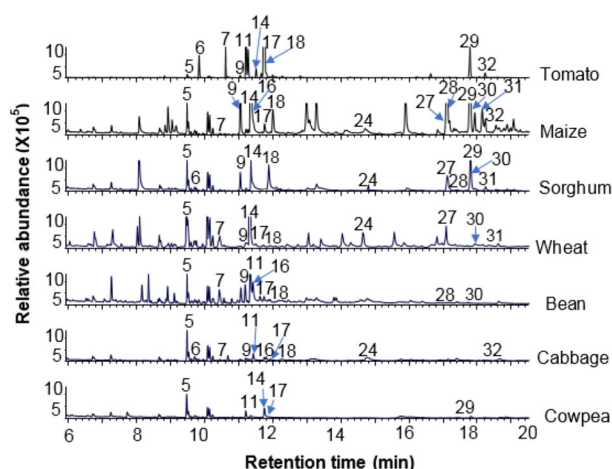


Figure 7. Gas chromatography-mass spectrometry (GC-MS) chromatograms of plant volatiles. The peaks of the most discriminant plant compounds are indicated by numbers. The numbers corresponding to volatile compounds are indicated in Table 2.

β -ocimene, α -yalangene and α -humulene (Fig. 8(a)). The sPLS-DA grouped plant species into clusters and displayed the correlation between the discriminant VOCs and the plant species (Fig. 8(b)). Dimension 1 was primarily linked with β -myrcene, α -copaene, (*E*)- β -farnesene, (*E*)- α -bergamotene and α -yalangene, and accounted for 42% of the total variation. Dimension 2 was highly linked to α -terpinene, (*E*)- β -caryophyllene, α -humulene and sabinene, and accounted for 28% of the total variation. The two dimensions of the sPLS-DA biplot explained 70% of the total variation (Fig. 8(b)). Dimension 1 was most associated with the increasing preference of *S. frugiperda* towards the cereal crops. This was largely confirmed by heatmap clustering. The compounds β -myrcene, (*E*)- β -farnesene and α -yalangene were highly associated with maize and sorghum. Similarly, (*E*)- α -bergamotene was strongly related with maize, sorghum and wheat. (*E*)- β -caryophyllene was associated with tomato, sorghum and maize, whereas sabinene was found mainly in tomato. On the other hand, (*E*)- β -ocimene was present in all tested plants except cowpea (Fig. 8(c)).

4 DISCUSSION

Our study examined the oviposition preference of *S. frugiperda* to different host plants in choice and no-choice setups. The results revealed distinct ovipositional preferences of *S. frugiperda* to certain host plants. The gravid *S. frugiperda* moths oviposited considerably more eggs on maize plants, followed by sorghum and wheat in multiple-choice, dual-choice and no-choice tests. Fewest eggs were laid on tomato and bean plants in multiple-choice tests while no eggs were deposited on cowpea and cabbage. Our results confirm earlier reports which showed the oviposition preference of *S. frugiperda* moths to maize over other host plants.⁴⁷ Similarly, recent studies^{48–50} have also reported a higher amount of *S. frugiperda* egg deposition on maize compared to other host plants, including sorghum, peanut, wheat, cotton and cabbage. We also observed differences in preferences within cereals. Similarly, Keerthi *et al.*⁵⁰ found that fodder maize was preferred by *S. frugiperda* moths over blue panicum grass and teosinte plants. They also found that no eggs were deposited on cowpea plants, which concurs with our findings.

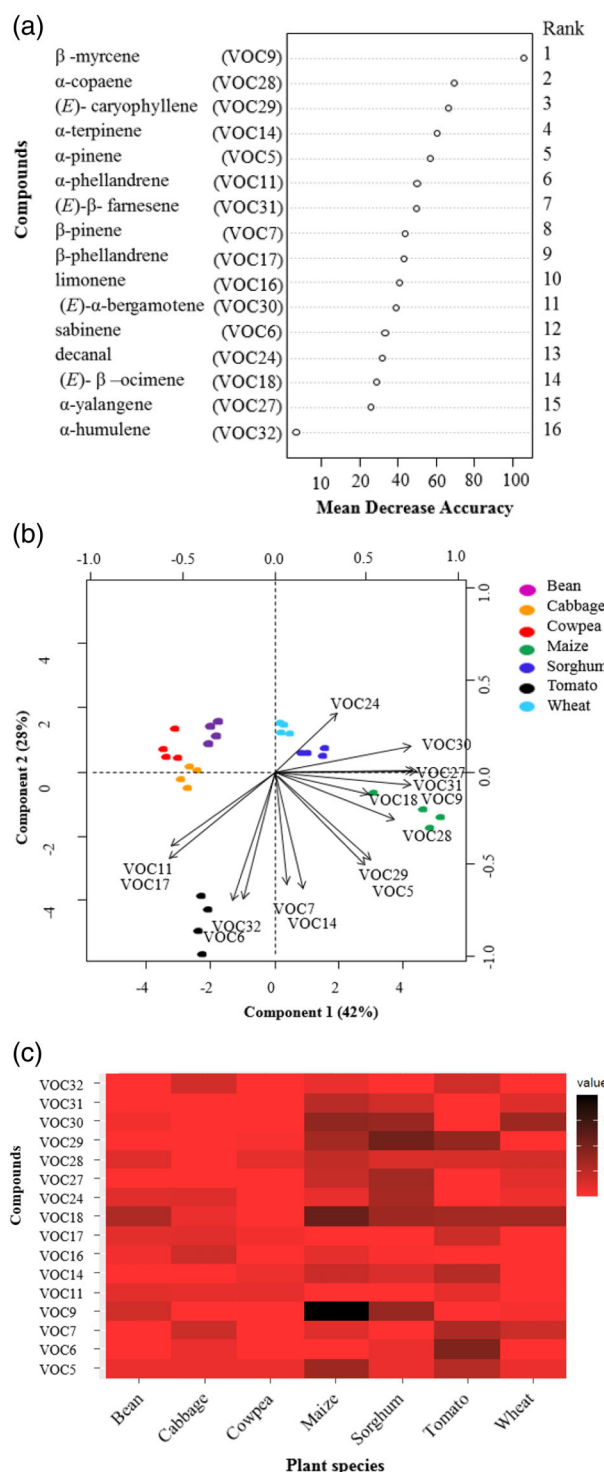


Figure 8. Random forest (RF) analysis of volatile profiles from different host plants. (a) The most discriminant plant volatiles are listed based on mean decrease in accuracy (MDA) following RF analysis. (b) sPLS-DA biplot showing the correlation of the most discriminant volatiles in the plant species. (d) Heatmap clustering showing the mean abundance of the most discriminant volatile compounds from crop species.

The strong preference of the female *S. frugiperda* moths to lay eggs on maize may also explain the heavy fall armyworm damage reported on maize fields despite the presence of other host plants nearby.^{15,22} Insect pests have evolved intricate behavioural and sensory mechanisms to locate and accept preferred hosts for

egg laying and reject unsuitable plants.^{51,52} Judicious choice of suitable host plants for egg laying by the adult lepidopteran female is crucial because the hatching larvae are often immobile with little opportunity to change their development location to feed, grow and survive.^{1,4,51,53} The adaptive nature of female oviposition preference in lepidopteran insects has been well documented.⁵¹ Interestingly, during no-choice oviposition bioassays, *S. frugiperda* moths laid their eggs on plants that were not preferred for oviposition under multiple-choice conditions, such as cowpea and cabbage. This indicates the potential of female *S. frugiperda* moths to lay eggs on various plant species in the absence of preferred host plants. However, more egg masses were laid on the cage wall during no-choice test conditions with less preferred host plants, as compared to preferred host plants. In contrast to our results, a recent study by Sotelo-Cardona *et al.*⁴⁸ reported equal numbers of eggs laid on maize and cage walls. These differences could be due to the different oviposition cage sizes used in the study, which in this case was smaller (0.6 × 0.6 × 0.6 m), as well as the host rearing conditions. On the other hand, more eggs were laid on the cage wall during the no-choice test with tomato plants, which supports our findings. Moths' preference to lay more eggs on oviposition cage walls away from nearby plants inside the cage could indicate repellent effects of plant-derived volatiles.

Physical characteristics such as texture (smooth vs grooved), shape, colour, veins and leaf orientation of the host plants have been reported to influence the *S. frugiperda* oviposition site selection.^{26,50} However, our results from wind tunnel bioassays also contribute to understanding the chemical cues employed by *S. frugiperda* to discriminate between preferred and nonpreferred host plants as the moths do not have direct contact with the test plant except for their odours. According to our findings, the odours of different plant species had varied levels of attractiveness for the gravid *S. frugiperda* female moth. In the dual-choice wind tunnel bioassay, gravid *S. frugiperda* moths showed more approaches towards maize and sorghum odour sources than to odours from other host plants. In contrast, moths made significantly fewer approaches and landed further away from cabbage and cowpea odour sources. The observed differential responses towards odours from various host plants, in the absence of visual stimuli and physical contact, corroborate the role of plant-derived volatiles in the host-finding behaviour of adult *S. frugiperda*.

Several studies have demonstrated the importance of plant volatiles in the host-selection process of the pest.^{6,28,54,56,57} Signorotti *et al.*⁵⁴ conducted bioassays to determine the olfactory responses of *S. frugiperda* gravid female moths to damaged and undamaged maize plant odours. The moth prefers undamaged maize plants to avoid competitors and natural enemies for their offspring. Furthermore, a recent study conducted by Sobhy *et al.*²⁸ found that female *S. frugiperda* moths were more attracted to the maize odours than the companion crop desmodium volatiles in a wind tunnel bioassay.

Qualitative and quantitative variations in the volatile profiles of host plants allow odour discrimination by ovipositing insects. In our study, a total of 34 volatile organic compounds in intact bean, maize, wheat, cabbage, sorghum, cowpea and tomato were identified. The result of the RF analysis revealed 16 compounds that were ranked as most important in discriminating between the plant species. The terpene compounds, β -myrcene, (*E*)- β -ocimene, α -yalangene, (*E*)- β -farnesene and (*E*)- α -bergamotene were released in greater amounts from maize and sorghum plants, whereas they were only present in trace amounts or completely

absent in wheat, bean, tomato, cabbage and cowpea plants. The discriminant compounds (*E*)- β -farnesene, α -yalangene and (*E*)- α -bergamotene, which were found in maize and sorghum in higher amounts and in wheat in small amounts, could explain the oviposition preference of *S. frugiperda* towards these plants. This finding is supported by an earlier study by Pinto-Zevallos *et al.*,⁵⁵ who observed higher antennal responses of *S. frugiperda* female moths to (*E*)- β -farnesene and α -yalangene. Moreover, Yac-tayo *et al.*⁵⁶ reported (*E*)- α -bergamotene as oviposition attractants for *S. frugiperda* adult moths. A recent study conducted by Sobhy *et al.*²⁸ also showed that the compound (*E*)- β -farnesene elicited antennal responses in *S. frugiperda* females. Some of the other discriminant compounds in this study, such as (*E*)- β -caryophyllene, β -copaene and (*E*)- β -ocimene, also elicited antennae responses in *S. frugiperda* females.²⁷ Similarly, linalool, cyclosativene, (*Z*)-3-hexenyl acetate, 2-hexenol, (*Z*)-3-hexenol, DMNT and β -selinene, which were present in trace amounts or completely absent in some host plants, elicited antennal responses in *S. frugiperda* females.^{28,55,57}

Our findings demonstrated that plants lacking key discriminate compounds and not preferred by gravid moths may have an oviposition deterrent effect on female *S. frugiperda* moths. The number of eggs laid by the moths was reduced when maize and sorghum plants were combined with cowpea, cabbage and bean in the oviposition bioassay. On the other hand, when maize plants were combined with other cereals, such as sorghum and wheat, the number of eggs laid increased. This indicates that intercropping with nonpreferred plants in the field could reduce *S. frugiperda* oviposition on the preferred plant. In a related study, Baudron *et al.*²⁴ found that female *S. frugiperda* are attracted to both graminaceous plants and weeds for oviposition. As a result, it may be prudent to avoid mixing graminaceous plants with maize in the field. However, graminaceous plants could be planted as a trap crop around the field. Several studies have also shown that infestation by *S. frugiperda* is suppressed in maize intercrops compared to monocrops in the field.^{29,30,58} For example, Hailu *et al.*²⁹ reported maize intercropped with soybean, beans and groundnuts had a lower *S. frugiperda* infestation than mono-cropped maize. Tanyi *et al.*³⁰ conducted a similar study and discovered that maize intercropped with beans had lower fall armyworm infestation than mono-crop plots. Additionally, Altieri *et al.*⁵⁸ reported low *S. frugiperda* infestation in Colombia when maize was intercropped with beans. Similarly, climate-adapted push-pull intercropping technology, initially developed for stem-borers and striga weed management, significantly reduced *S. frugiperda* infestation in East African smallholder farming systems.^{28,59}

5 CONCLUSIONS

S. frugiperda shows divergent ovipositional preferences for selected host plants. The gravid *S. frugiperda* moth preferred maize plants, followed by sorghum and wheat in both oviposition and wind tunnel bioassays. On the other hand, cowpea and cabbage were the least preferred plants for oviposition by *S. frugiperda* moths. Chemical differences in host plant volatiles may account for the differential ovipositional preferences. Volatile compounds, such as (*E*)- β -farnesene, α -yalangene and (*E*)- α -bergamotene, could be responsible for the moth's attraction. The plants that are not preferred by gravid moths could be exploited in an intercropping strategy to manage *S. frugiperda*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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