Desmodium Volatiles in "Push-Pull" Agriculture and Protection Against the Fall Armyworm, *Spodoptera frugiperda*

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Abstract Push-pull systems for sustainable pest management of crop plants employ repellent stimuli from intercrops ("push") to repel herbivores and attract their predators and parasitoids, and attracting stimuli from border plants ("pull") to lead herbivorous insects out of the crop. The most widespread implementation, intercropping with the legume *Desmodium*, reduces herbivory damage from the invasive fall armyworm (FAW) *Spodoptera frugiperda*. However, the three publications to date investigating underlying mechanisms disagree whether the *Desmodium* intercrop emits bioactive volatiles that repel FAW. We aimed to resolve this controversy by measuring volatile emission from *Desmodium intortum* (greenleaf *Desmodium*) and *D. incanum* intercrops in push-pull fields, and assaying their effects on the behavior of the FAW in oviposition and wind tunnel choice bioassays. We detected many volatile substances in the headspaces of both *Desmodium* species, which exhibited similar profiles, including substances previously reported to repel lepidopteran herbivores. FAW moths preferred to oviposit on maize over *Desmodium*, but not on maize further from versus closer to *Desmodium* plants that were inaccessible to the moths, but sharing the air. In a wind tunnel bioassay, the moths tended to prefer maize volatiles over a combination of volatiles from maize and *D. intortum*, but not *D. incanum*. In conclusion, we found *D. intortum* and *D. incanum* to emit volatiles that have been shown to be bioactive, and FAW moths to prefer maize over either *Desmodium* species. Moreover, additional mechanisms are likely important for reducing FAW damage to maize under push-pull cultivation.

Introduction

The sustainable intensification of agriculture is essential to achieve Sustainable Development Goal (SDG) 2, zero hunger, and reduce hidden costs of meeting requirements of other SDGs such as 13, climate action (***FAO et al., 2020***). This is particularly important in the developing Global South

where larger yield gaps are observed compared to the developed countries with more financial resources (**David Tilman et al., 2011**). Intercropping may offer sustainable solutions with potential for strong positive effects on pest and disease control as well as associated biodiversity (**Beillouin et al., 2021**).

The concept of "push-pull" mixed cropping was first reported in 1987 as a "stimulo-deterrence" strategy for the reduction of pest damage in cotton, combining a pest repellent intercrop ("push") interspersed with the main crop, and an attractant border crop ("pull") to trap pests at the field perimeter (**Bruce et al., 1987**). As most widely practiced, current push-pull systems benefit from the attractive and repellent stimuli of perennial inter- and border crops (referred to as companion crops), thereby reducing or eliminating the need for pesticides (**Pickett et al., 2014**). In Kenya, the first push-pull system, introduced in 1997, was designed to reduce yield losses caused by stem-borer species and comprised a combination of the main crop maize (*Zea mays* L), the intercrop molasses grass (*Melinis minutiflora*), and the border crop Napier grass (*Pennisetum purpureum*) or Sudan grass (*Sorghum vulgare sudanense*) (**Khan et al., 1997b**).

Several volatile substances emitted from molasses grass repelled female stem borers and simultaneously attracted their parasitoid *Cotesia sesamiae* (**Khan et al., 1997a**). Following the initial success of this system, there was interest in replacing molasses grass with a leguminous repellent intercrop that could improve soil quality by fixing nitrogen, and provide high-quality fodder. The legumes silverleaf *Desmodium* (*Desmodium uncinatum*) and its congeneric (*Desmodium intortum*) were shown to repel ovipositing stem borers and furthermore to suppress parasitic *Striga* weeds. (**Khan et al., 2000, 2002**). The first *Desmodium*-intercropped version of push-pull used *D. uncinatum* in place of *M. minutiflora* together with *P. purpureum*. As a result of screening for more drought-tolerant companion plants, a version using *D. intortum* combined with the border crop *Brachiaria* cv Mulato II was developed as "climate-adapted push-pull", which has been demonstrated to increase maize yield by a factor of 2.5 (**Midega et al., 2015**). Recently, a "third generation" push-pull system was evaluated using the intercrop *D. incanum* to replace *D. intortum* – which does not flower and set seed in many parts of tropical Africa and thus limits adoption and spread of push-pull by limiting seed supply for the intercrop – with *Brachiaria brizantha* cv Xaraes as border crop, which is more resistant to herbivorous mites that attack the mulato cultivar (**Cheruiyot et al., 2021**).

The invasive fall armyworm (FAW), *Spodoptera frugiperda*, originating from a maize-specialized strain from northern and central America, has become a major threat to African maize crops since 2016 (**Goergen et al., 2016**; **Day et al., 2017**; **Hailu et al., 2021**; **Zhang et al., 2023**). It spread rapidly through East Africa, with most of the farmers in Ethiopia and Kenya encountering the FAW after the long rains in the first half of 2017 (**Kumela et al., 2018**). The invasiveness of the moth is fueled by its relatively short life cycle of about four weeks and the capability of the adult females to lay hundreds of eggs (**Sparks, 1979**). Other traits favoring the FAW's spread in Africa and beyond are its strong flight capacity, lack of diapause, reported survival in diverse habitats, rapid development of resistance to insecticides / viruses, and the polyphagous nature (**Wan et al., 2021**). Although its wide host range of at least 353 host species across 76 plant families (**Montezano et al., 2018**), the high financial and yield losses on the main crop maize due to the pest are particularly devastating (**Eschen et al., 2021**). The FAW is reported to outcompete resident stemborers (**Hailu et al., 2021**; **Sokame et al., 2021**; **Mutyambai et al., 2022**). The use of pesticides is a popular approach to control the FAW, posing risks to environmental and human health, which includes acute pesticide-related illnesses as many smallholder farmers do not use personal protective equipment while spraying (**Tambo et al., 2020**). Therefore, sole reliance on pesticides is not sufficient to manage FAW sustainably, and Integrated Pest Management (IPM) strategies, such as the promotion of natural enemies, are desirable (**Nyamutukwa et al., 2022**; **Van den Berg and du Plessis, 2022**). Climate-adapted and third-generation push-pull systems have been reported to reduce plant damage and yield loss caused by the FAW (**Midega et al., 2018**; **Hailu et al., 2018**; **Cheruiyot et al., 2021**; **Yeboah et al., 2021**; **Mutyambai et al., 2022**).

A systematic review on the chemical ecology of push-pull systems by **Lang et al.** (**2022**) found

only thirty publications (seven primary sources, and 23 publications reporting on results from these primary sources) on the chemistry of push-pull and related mixed-cropping systems, from which 206 compounds were reported to be potentially associated with push and pull effects. Of these, 101 were categorized as plant volatiles and reported by studies with a focus on plant-insect interactions (as opposed to studies with a focus on *Striga* control). However, none of these publications reported volatiles sampled under field conditions. Two recent papers, both published after the literature review conducted by ***Lang et al.*** (***2022***), reported additional potentially bioactive compounds from companion plants in maize-*Desmodium*-grass push-pull systems, and both publications supported the hypothesis that volatiles from the companion crop *D. intortum* repel the FAW and attract parasitic wasps (***Sobhy et al., 2022***; ***Peter et al., 2023***). In contrast, a third recent publication by ***Erdei et al.*** (***2024***) detected low levels of volatiles from *D. intortum* plants and found no consistent preference of FAW for maize over either *D. intortum* or maize together with *D. intortum*, and concluded that the protective effects of the intercrop were more likely to result from physical trapping of FAW larvae.

Thus, further investigations are needed to understand the mechanisms by which the maize-*Desmodium*-grass push-pull system can protect maize from FAW. A summary of the studies to date on push-pull effects on the FAW can be found in ***Figure 1***. To our knowledge, no data on plant volatiles from the more drought-resistant *D. incanum* have been published so far. Therefore, this paper focuses on the volatiles of *D. intortum* and *D. incanum* and the effects of exposure to the headspaces of these plants, versus direct exposure to the plants, on the invasive pest FAW. We collected volatiles from plants in farmers' fields as well as in bioassay setups under semi-field conditions, and conducted oviposition and flight tunnel bioassays to assess FAW moth preferences.

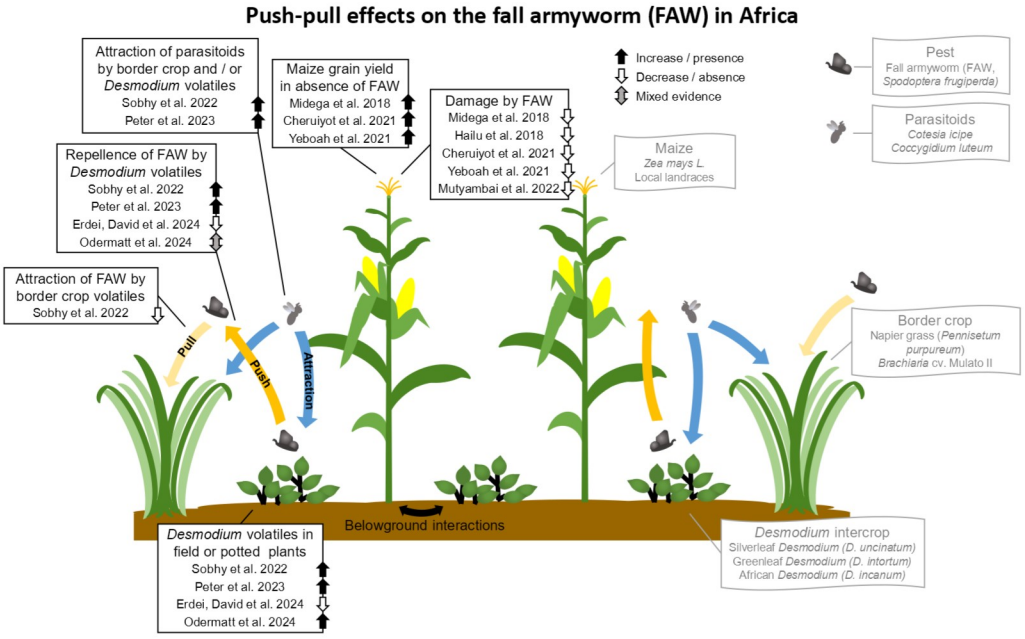

Figure 1. Studies to date on push-pull effects on the fall armyworm (FAW) embedded in the mechanisms according to the current state of knowledge of the system. Volatiles (and potentially other traits) of the intercrop repel the herbivorous insect and additionally attract its parasitoids, while volatiles (and potentially other traits) of the border crop attract herbivores away from the main crop (***Pickett et al., 2014***; ***Khan et al., 2018***; ***Eigenbrode et al., 2016***). The pull effect of the border crop Napier grass, *Pennisetum purpureum* was observed in earlier systems, as it attracted stemborers (***Khan et al., 1997b***), but could not be confirmed for the FAW with the border crop *Brachiaria* cv Mulato II (***Sobhy et al., 2022***). For more detail on all experiments, see supplementary file 1.

Results

Desmodium Volatile Profiles

A total of 25 substances were measured in at least 2/3 of field-collected samples from each of the *Desmodium* species, of which 11 occurred in both species. These substances include (*E*)-*β*-ocimene, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, acetate, 1-octen-3-ol, 3-octanone, caryophyllene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), germacrene d, (3*E*,7*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene (TMTT), and five other peaks that were not fully identifiable. The unidentified peaks were categorized based on mass spectra and relative retention times as a benzene derivative, a naphthalene derivative, a monoterpene, and two sequiterpenes (for feature information, see ***Table 1***. The sesquiterpene (*E*)-*α*-bergamotene only occurred in *D. incanum*, while another unidentified sesquiterpene only occurred in *D. intortum* (see ***Figure 2*** and for a per-sample heatmap ***Figure 2—figure Supplement 1***). Of these substances, (*Z*)-1,5-octadien-3-ol, 1,2,3-trimethylbenzene, 3-pentanol, and hexyl acetate are here for the first time reported in connection with the chemical ecology of push-pull cropping systems (***Lang et al., 2022***; ***Sobhy et al., 2022***; ***Peter et al., 2023***). Germacrene D and 1-hexanol were not yet reported from *Desmodium* species, but were reported in relation to mixed cropping for pest control. Germacrene D was detected in elevated levels in maize plants grown in soil from push-pull fields in comparison with those grown in soil from maize monocultures (***Mutyambai et al., 2019***), while germacrene D and 1-hexanol both were reported to be emitted from bean plants, *Vicia faba*, and used by the black bean aphid, *Aphis fabae*, for host detection (***Pickett and Khan, 2016***; ***Webster et al., 2008***).

Oviposition Bioassays

Oviposition choice bioassays were conducted to determine the influence of direct or indirect contact with *Desmodium* plants on the oviposition behavior of FAW moths. A fraction of the potted *Desmodium* plants used in the oviposition assays was sampled for volatiles the night before the start of trials, and the composition of these samples was compared with the substances and features detected from *Desmodium* plants sampled in farmers' fields. In the comparison between field *Desmodium* plants, and potted bioassay plants that were sampled in a smaller number, the majority of substances showed a higher relative abundance in potted *D. incanum* (bioassay conditions), whereas in *D. intortum*, some substances occurred in higher concentrations in the potted plants, while others were not detected. Sixteen of the volatiles detected in *Desmodium* plants in farmers' fields were also present in either uninfested or infested maize plants, with 14 substances overlapping (see ***Figure 3***).

The count of eggs and egg batches showed that fewer eggs were laid directly on *Desmodium* plants, with the egg count on maize plants being 7.9 or 6.8 times higher than on *D. incanum* or *D. intortum*, respectively (see ***Figure 4*** and ***Figure 4—figure Supplement 1***). A mixed model with egg counts as the observed variable and accounting for plant position detected a significant difference between the control and all other treatments ($F(1) = 9.21$, $p = 0.003$), as well as for the comparison of the indirect and the direct treatments ($F(1) = 9.32$, $p = 0.003$). However, no significant difference was found between the two *Desmodium* species ($F(1) = 1.01$, $p = 0.32$) (see ***Table 3***).

Wind Tunnel Bioassays

Wind tunnel bioassays were conducted to determine short-term effects of plant volatiles on the flight behavior of FAW moths.

Two-thirds of the moths tested showed ten or fewer segment changes during the five minutes of each experiment. Across all treatments, at least 77% of the moths showed no movement in the last 2 min of the experiment or only little activity, with a maximum of two segment changes in the last 3 min of the experiment, which was interpreted to mean that they had made a decision within the five-minute timeframe of the experiment. Overall, moths showed a reduction in segment changes over time, particularly for the comparison of maize vs. maize + *D. intortum* (see ***Figure 5***).

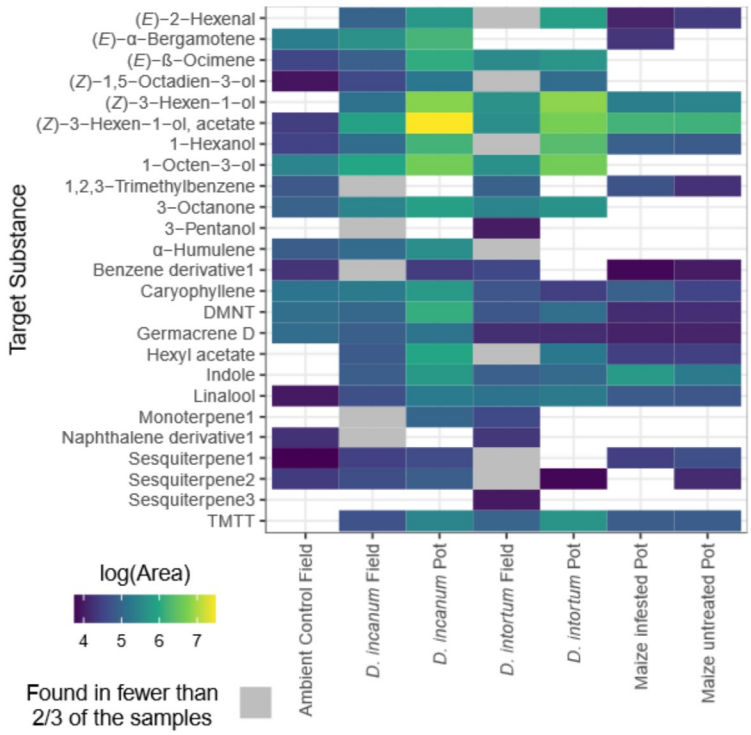

Figure 2. Heatmap comparing the log10-transformed peak area of the non-zero hits of substances present in at least 2/3 of the samples of at least one of the *Desmodium* species in the field, showing relative peak areas detected in field- or pot-grown (bioassay conditions) *D. intortum* and *D. incanum*, with pot-grown maize for comparison. For the field *Desmodium* samples all substances occurring in less then 2/3 of all samples were removed and here displayed in grey, while lower frequency was accepted for the potted samples and the controls that showed a smaller sampling size. The x-axis displays the species and their growth conditions (field or pot) and the y-axis shows the target substances in alphabetical order. The color indicates mean of the log-10 transformed peak area of all samples with the substance present. The grey color indicates that a substance occurs in more than one, but less than 2/3 of the samples from the field. "Maize Infested" refers to maize plants that were exposed to FAW eggs, as moths were allowed to oviposit on the plants two nights prior to volatile sampling. Sample sizes: Ambient Control Field = 4, *D. incanum* Field = 14, *D. incanum* Pot = 3, *D. intortum* Field = 11, *D. intortum* Pot = 5, Maize infested Pot = 4, Maize Pot = 4.

Figure 2—figure supplement 1. Per-sample heatmap

Across all treatments, a few individuals displayed high flight activity of up to 76 segment changes with no indication of decline over time.

No significant effects were detected when comparing the stays in the maize segments versus the *Desmodium* segments across the treatments using a mixed model (see ***Table 4***). However, a preference for the side with maize is apparent for the comparison of maize vs. maize + *D.intortum*, especially when comparing the mean stay duration as well as the final settling location of the moth, whereas moths showed a slight preference for maize + *D. incanum* instead of maize alone (see ***Figure 5***). Fourteen of nineteen moths exposed to airflow from maize vs. maize + *D.intortum* settled in a segment of the wind tunnel within the first three minutes of the experiment, a larger proportion than in the maize vs. maize control or the maize vs. maize + *D. incanum* treatment.

Discussion

The fall armyworm (FAW) *Spodoptera frugiperda* invaded sub-Saharan Africa in 2016 and is responsible for major yield losses in maize crops (***Day et al., 2017***; ***Goergen et al., 2016***). Push-pull cropping of maize with *Desmodium* and border grasses is reported to reduce FAW damage (***Midega et al., 2015***; ***Cheruiyot et al., 2021***; ***Mutyambai et al., 2022***; ***Yeboah et al., 2021***; ***Hailu et al., 2018***) and

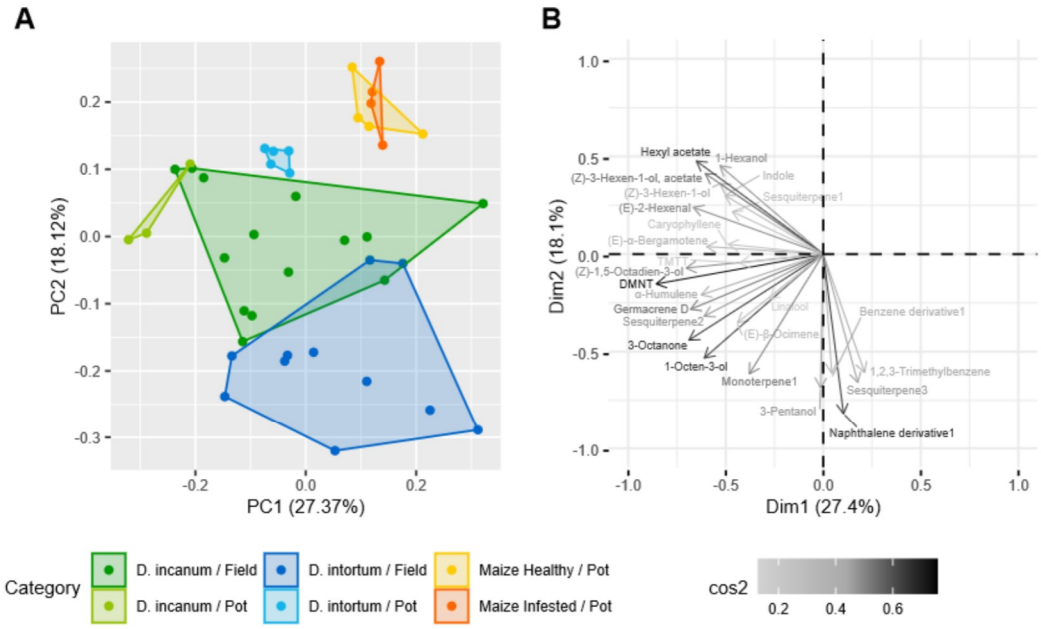

Figure 3. A: Principal component analysis plot based on the normalized peak areas of the 25 target substances. Sample sizes: Ambient Control Field = 4, *D. incanum* Field = 14, *D. incanum* Pot = 3, *D. intortum* Field = 11, *D. intortum* Pot = 5, Maize infested Pot = 4, Maize Pot = 4 **B:** Loading plot of the projection of the variables on the first two dimensions. The x-axis displays PC1 (Dimension 1) and the y-axis shows PC2 (Dimension 2). The color indicates the cos2, whereby dark and long arrows indicate a better representation of a loading on these first two dimensions.

to increase maize yield (***Midega et al., 2018***; ***Cheruiyot et al., 2021***; ***Yeboah et al., 2021***). Push-pull cropping systems benefit from the attractive and repellent stimuli of perennial companion crops (***Pickett et al., 2014***), with more than one hundred plant volatiles from different classes, especially terpenoids and fatty acid-derived "green leaf" volatiles, reported to be associated with push-pull effects (***Lang et al., 2022***). Nevertheless, the repellent role of volatiles from greenleaf *Desmodium* (*D. intortum*) has been investigated in only three studies and almost exclusively in potted plants, with conflicting reports of whether these volatiles repel FAW moths, and the only study reporting field measurements recovering few volatiles in relatively low abundance (***Sobhy et al., 2022***; ***Peter et al., 2023***; ***Erdei et al., 2024***). Here, we characterized volatiles sampled from both *D. intortum* (greenleaf *Desmodium*) and *D. incanum* growing in farmer fields or in pots, and conducted bioassays with FAW moths to determine whether the presence of *Desmodium* plants or their volatiles would alter moth preferences for maize. We identified up to 17 - 19 substances in headspace samples from *Desmodium* plants, often in abundance similar to or greater than volatiles measured from maize plants under comparable conditions, and we observed that FAW moths preferred to oviposit on maize rather than on *Desmodium* plants of either species. However, proximity to a *Desmodium* plant sharing the airspace did not influence FAW oviposition preferences for maize in a greenhouse cage choice assay, nor did FAW moths show a significant preference for maize volatiles over volatiles from a combination of maize and *Desmodium* plants in a wind tunnel choice assay. We note that these assays were performed in a risk area for malaria and other mosquito-borne illnesses, and so experimenters were protected with DEET, which has low volatility but is active in the vapor phase, where it can cause host avoidance by binding to insect olfactory receptors (although this has been mostly studied in the dipterans *Aedes aegypti* and *Drosophila melanogaster*) (***DeGennaro, 2015***). We reduced any potential influence on the lepidopteran in our experiments by using minimal amounts of DEET, avoiding direct contact of any DEET with experimental materials, and conducting behav-

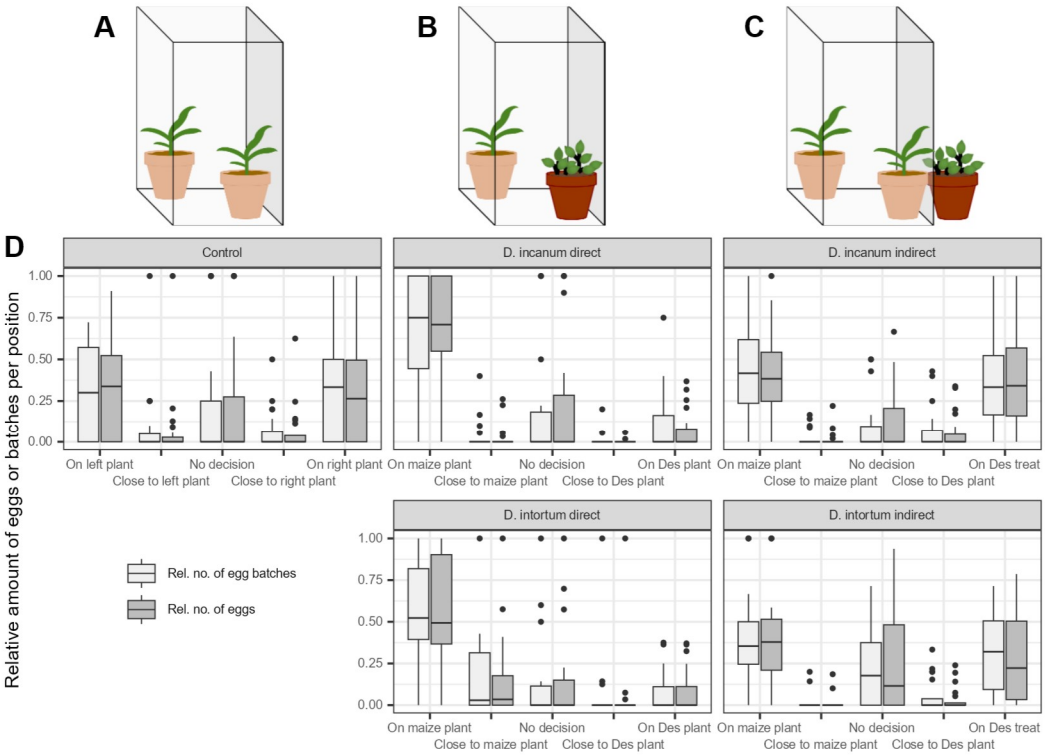

Figure 4. A: Setup of the control with two maize plants inside the cage. **B:** Setup of the direct treatments with one maize and one *Desmodium* plant inside the cage. **C:** Setup of the indirect treatments with two maize plants placed inside the cage and one *Desmodium* plant placed in proximity to one of the maize plants, but unreachable for the moths. **D:** The boxplots display the relative number of eggs (light grey boxes) or egg batches (dark grey boxes) per position (x axis) and treatment (superordinate boxes). The lower and upper hinges correspond to the 25th and 75th percentiles, while the whiskers extend to the largest and smallest non-outlier values, respectively. Outliers are values outside a window of 1.5 x the interquartile range and are plotted individually. *Desmodium* is abbreviated with 'Des' and treatment is abbreviated with 'treat'. See ***Figure 4—figure Supplement 1*** for a breakdown of eggs per repetition and batch. Sample sizes (n, replicate units of a given treatment): Control = 21, *D. incanum* direct = 19, *D. intortum* direct = 20, *D. incanum* indirect = 19, *D. intortum* indirect = 20.

Figure 4—figure supplement 1. Total egg count per repetition and per batch during oviposition bioassays

Figure 4—figure supplement 2. Schemes and photographs of the experimental setup of the choice oviposition bioassays

Figure 4—figure supplement 3. Scheme of the cage setup in greenhouse 1B

Figure 4—figure supplement 4. Scheme of the cage setup in greenhouse 3B

Figure 4—figure supplement 5. Photographs of the egg collection process

ioral assays without the insects being exposed to volatiles from the experimenter (experimenters were generally not present during oviposition assays, and the insects were in an isolated airspace during wind tunnel bioassays). We conclude that *Desmodium* plants in push-pull cultivation emit known bioactive volatiles, but cannot confirm that these volatiles alone repel FAW moths based on our bioassays, and therefore suggest that other features of the maize-*Desmodium*-border grass push-pull system, including the influence of border grasses as well as belowground interactions and other repellent traits of *Desmodium*, may be important for protecting maize from FAW moths and their larvae.

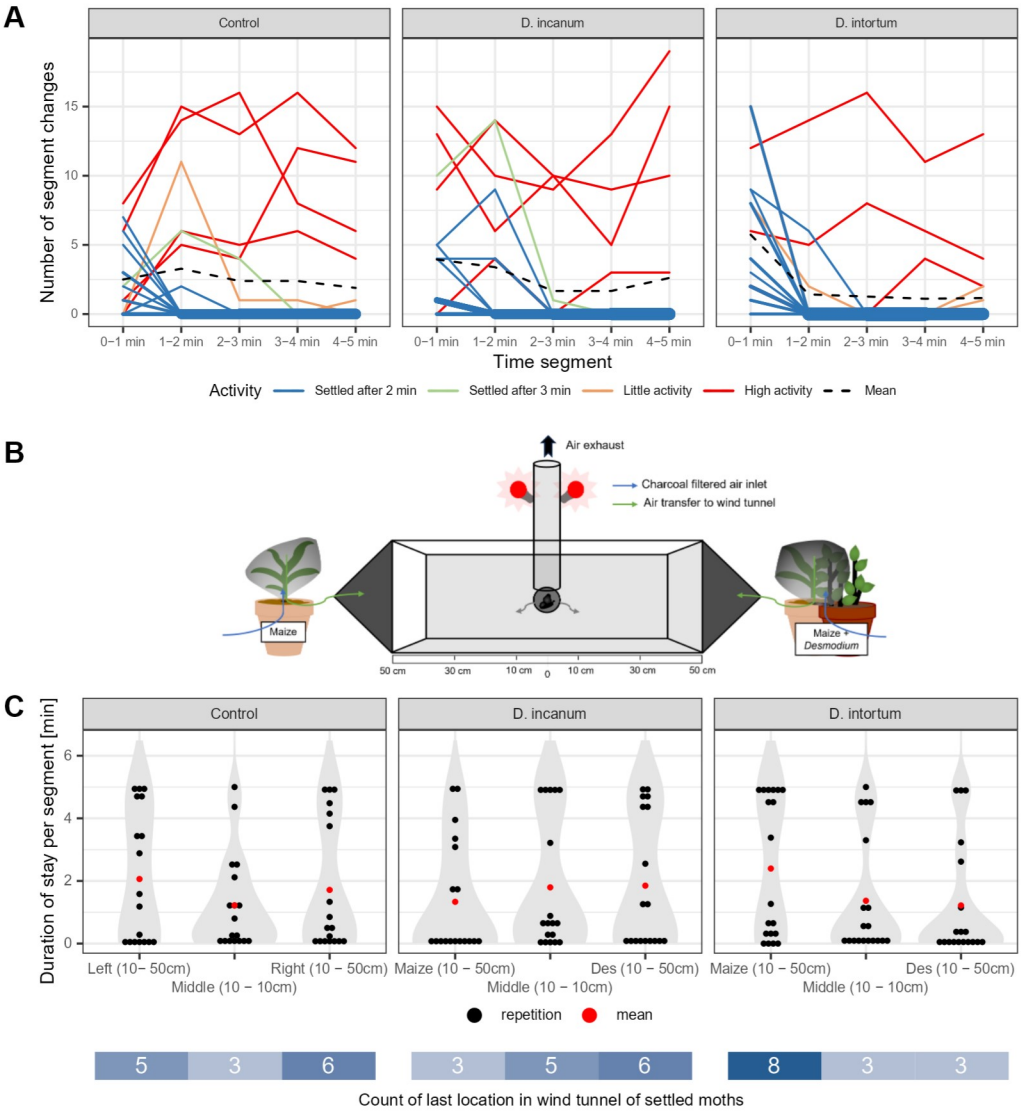

Figure 5. A: Line plot displaying the number of segment changes per minute until the experiment ended after 5 min. The line width indicates the number of overlaying lines and the colors indicate the moth activity level: 'High Activity' = Moths showed higher activity with at least 6 segment changes in the last 3 min of the experiment. 'Little Activity' = Moths showed max. 2 segment changes in the last 3 min of the experiment. 'Settled after 3 min' = No segment changes after 3 min. 'Settled after 2 min' = No segment changes after 2 min. The mean of all repetitions is represented by the black dashed line. **B:** Setup of the wind tunnel for a *Desmodium* treatment comparing a maize plant (left) vs. a maize and a *Desmodium* plant (right). The moths are released through an opening in the center (at 0 cm) and observed for 5 min. **C:** Violin plot displaying the duration of stays in each segment in treatments with *D. incanum* and *D. intortum* (comparison of maize and maize + *Desmodium*) with the inclusion of the individual data points per repetition (black) and their mean (red): The x-axis displays the location in the wind tunnel and the y-axis the total duration that each moth stayed in each segment. Below the x-axis, the last position of the moths that were considered settled (settled after <3min or little activity) is displayed. See ***Figure 5—figure Supplement 1*** for the set-up and segmentation of the two-choice wind tunnel setup. Sample sizes (n, replicate units of a given treatment: Control = 18, *D. incanum* = 18, *D. intortum* = 19.)

Figure 5—figure supplement 1. Photograph of the experimental setup of the two-choice wind tunnel bioassay

Emission of Potentially Bioactive Volatiles by *Desmodium* Intercrops

The headspace of *Desmodium* plants was dynamically sampled on Tenax TA adsorbent directly in push-pull fields, as well as from potted plants used for oviposition bioassays, overnight.

All substances occurring in fewer than 2/3 of the field samples were excluded from further analysis, which represents an arbitrary limit. Quantitative comparisons within single substances are possible, but must be handled with care, as normalization based on the biomass and leaf area of the plants was not possible. The chosen adsorbent, Tenax TA, is known to be effective for lipophilic to medium-polarity organic compounds of intermediate molecular weight (ca. C7-C26) (***Dettmer and Engewald, 2002***; ***Tholl et al., 2020***). It is possible that headspace samples changed during storage (up to 3 months tightly closed at room temperature) as degradation or thermal rearrangements on Tenax TA material have been reported before (***Dettmer and Engewald, 2002***; ***Alborn et al., 2021***). We extracted headspace samples using thermal desorption, which is simple and sensitive, but not appropriate for some compounds, such as the sesquiterpene germacrene A, which are susceptible to thermal rearrangement or degradation (***Tholl et al., 2006***).

Despite these caveats, our samples yielded a variety of volatiles with high signal:noise ratios, of which 13 had been previously reported from sampling the headspace of potted *D. intortum* plants onto Porapak Q for sampling durations of 24 h or 48 h followed by solvent extraction and GC-MS analysis (***Sobhy et al., 2022***; ***Peter et al., 2023***). In contrast, ***Erdei et al.*** (***2024***) detected fewer volatiles in lower relative abundance from *D. intortum* using solid-phase microextraction (SPME) and a saturation time of 18 - 24h to sample intact, mechanically damaged or herbivory-induced potted plants or intact plants in Tanzanian and Ugandan fields. Our study is the only one of these that limited the window of sampling strictly to the nighttime hours corresponding to the reported activity window for the FAW(***Sparks, 1979***). In pretests no shorter activity window could be determined as the moths still showed mating behaviour and potential oviposition at around midnight (see Appendix 1). Differences might be explained due to the different use of sampling techniques, as the sensitivity of SPME is lower in comparison to dynamic headspace collection (***Tholl et al., 2006***). To our knowledge, the volatile profile of *D. incanum* was not previously reported. In conclusion, the hypothesis that *D. intortum* and *D. incanum* emit potentially bioactive volatiles is supported.

Oviposition Choice Bioassay

FAW oviposition behaviour was observed over three nights in two treatments per *Desmodium* species. In one treatment, FAW moths had direct contact with *Desmodium* plants and in another, they had only indirect contact through aerial exposure to plants outside of, but directly next to a mesh cage. Moths always had direct contact with maize plants.

Critical consideration must be given to differences in the volatile profiles between the potted plants and those measured in farmers' fields, which led to a separation of potted and field *D. intortum* in the second dimension of a PCA, and a near-separation of *D. incanum* in the first dimension. As the untargeted analysis was conducted based on the field *Desmodium* samples, any volatile substances that only occur in the potted plants were not identified or analyzed further (full results of GC-MS analysis are available with the source data for this publication). Oviposition on maize can alter the maize volatile profile and affect volatile emission in the following days, which is why the maize samples are divided into plants before and after (infested) oviposition exposure. Of the substances occurring in field *Desmodium* plants, approximately half were abundant in healthy and infested potted maize plants, with only one substance, (*E*)-α-Bergamotene, being present solely in infested maize plants. The sampling of potted plants was conducted using the same procedure as in the field, but in a greenhouse with netted walls on two sides that allowed air circulation as for the oviposition bioassays. Overall, this highlights the importance of studying oviposition cues and moth preferences directly in push-pull fields in the future.

In all treatments, the number of eggs laid per batch showed no difference depending on the position, which indicates that FAW moths did not adjust their behavior once they started laying

eggs. This differs slightly from the outcomes reported by **Peter et al.** (**2023**), where a reduction in egg number per batch was observed and the number of eggs, but not the number of egg batches, differed between maize vs. maize + *D. intortum*. However, a clear preference for maize compared to the two *Desmodium* species was observed in the treatments where FAW moths had direct contact with both plant species, with a large effect size of 7 – 8 times more eggs on maize than on *Desmodium*. The preference of maize over *D. intortum* in oviposition assays with direct contact to both plants is consistent with various publications using similarly controlled setups (**Sobhy et al., 2022**; **Peter et al., 2023**; **Erdei et al., 2024**). There was little oviposition on the cage in our bioassays, which indicates that moths primarily chose between plants in our setup, but differs from the strong preference found by **Sobhy et al.** (**2022**) of FAW moths to oviposit directly on the mesh of a bioassay cage rather than on the plants when offered maize co-planted with *D. intortum*. *Desmodium* and maize plants were planted in the same pots by **Sobhy et al.** (**2022**), and therefore maize volatiles may have been affected by belowground interactions or through priming by *Desmodium* volatiles prior to or during oviposition bioassays. This is in fact closer to the configuration in push-pull fields, but does not isolate the influence of the *Desmodium* headspace, as we sought to do here.

In our bioassays, no significant effect could be seen in the treatments where moths had only indirect contact with *Desmodium* volatiles coming from a *Desmodium* plant proximate to one maize plant, but outside of the cage. This is not consistent with the hypothesis that *Desmodium* volatiles repel FAW moths as proposed (**Pickett et al., 2014**; **Sobhy et al., 2022**). It is possible that the distance of about 60 cm between the plants in the cages was not suitable to create a gradient of volatiles sufficient to allow moths to choose between maize plants closer to versus further from *Desmodium*, in which case neighboring treatments with distances of 50 – 100 cm could have also affected each other. To our knowledge, the only comparable setup was reported by **Erdei et al.** (**2024**), in which two maize plants were exposed to volatiles of either *D. intortum* or an artificial plant in a modified wind tunnel, and moths did not show a significant preference for either maize plant.

In conclusion, we found a preference of FAW for maize over *D. intortum* and *D. incanum*, but this preference could not be solely attributed to *Desmodium* volatiles in our experiments. It is not to be expected that herbivores use all potential host plants equally, and preferences among host plants or for host over non-host plants can be based on different plant traits. FAW moths may also prefer maize over *Desmodium* due to other traits such as the surface texture, as proposed in the findings from **Erdei et al.** (**2024**) that silicate trichomes on *D. intortum* pose a danger to FAW larvae; or chemical repellents directly on the leaf surface. Belowground interactions or priming between *Desmodium* and maize plants co-planted in push-pull fields may also change moths' preference for maize, an effect which could not have been observed with our experimental design, but might explain inconsistencies between our findings and those from **Erdei et al.** (**2024**) versus those from **Sobhy et al.** (**2022**).

Wind Tunnel Bioassay

The short-term flight behavior of FAW moths was tested in a two-choice wind tunnel setup where volatiles of a maize plant vs. maize + *Desmodium* were compared against each other. Flight behavior showed large variation among the individual moths, but three-quarters of the moths had settled after at least three minutes or showed fewer than three segment changes in the last two minutes of the experiment. This experimental setup has limited space and duration, and moths may behave differently over longer times or when given more space to maneuver, as under normal field conditions. Furthermore, the quarter of the moths that showed high activity indicate the difficulty of quantifying the responses of such active fliers. Interestingly, a larger proportion of moths settled after 2 minutes when presented with maize versus maize + *D. intortum*, rather than continuing to fly. The moths showed a weak preference for the volatiles of the single maize plant over the volatiles of maize + *D. intortum*, whereby they slightly preferred volatiles from *D. incanum* + maize over maize alone but did not distinguish between volatiles from two maize plants (negative con-

trol). However, none of the treatments showed any significant preference. In contrast, repulsion of moths by *D. intortum* volatiles with or without the addition of maize was observed in no-choice assays when the total distance flown upwind at the end of the experiment was used as a metric (***Sobhy et al., 2022***; ***Peter et al., 2023***). Based on these wind tunnel results, it cannot be excluded that the exchange of *D. intortum* with *D. incanum* in third-generation push-pull fields may reduce protection against FAW moths. However, in direct oviposition choice assays (in which moths were enclosed in a cage with one maize and one *Desmodium* plant), maize was preferred equally over either *Desmodium* species.

Conclusion

Several field studies have described positive effects of push-pull systems with the companion crops *D. intortum* or *D. incanum* on maize yield (***Midega et al., 2015***, ***2018***; ***Cheruiyot et al., 2021***). Here, we show that 17 - 19 volatile substances that have been previously shown to affect pest host choice were found in the headspaces of both companion crops *D. intortum* and *D. incanum* within the activity window of FAW moths. However, we did not observe a clear repellent effect of *Desmodium* volatiles on the FAW in our bioassays. FAW moths clearly preferred to oviposit on maize over *D. intortum* or *D. incanum* plants. Our results thus indicate that moths prefer maize over *Desmodium* and that this may be influenced by short-range mechanisms such as an unfavorable leaf surface of *Desmodium*. However, the main interest in the context of push-pull is how *Desmodium* contributes to protecting co-planted maize. A reduction of FAW damage on maize under push-pull cultivation has been demonstrated in multiple studies (***Midega et al., 2018***; ***Hailu et al., 2018***; ***Cheruiyot et al., 2021***; ***Yeboah et al., 2021***; ***Mutyambai et al., 2022***). Based on the literature, the pull effect of border crop volatiles may be important for reducing FAW damage in push-pull fields, in combination with the attraction of parasitoids by *Desmodium* volatiles and unfavorable host qualities of *Desmodium* plants for FAW (***Figure 1***). Furthermore, the *Desmodium* intercrop may be equally or more important for weed suppression, soil fertility, and possibly improving maize plant health and rigor as for volatile-mediated repellence of FAW in the push-pull system.

Methods and Materials

All volatile sampling and bioassays were performed with two *Desmodium* species, *Desmodium intortum* (greenleaf *Desmodium*) and *Desmodium incanum* (often referred to as African *Desmodium* by the push-pull farmers) and in the method section referred to as *Desmodium* for simplicity. All raw data and code used for the statistical analysis can be found on Zenodo (CERN, Geneva, Switzerland, https://doi.org/10.5281/zenodo.11633890) and Github (github.com/ DariaMOdermatt/DesmodiumVolatilesinPush-Pull).

Pretest Moth Activity Window

The determination of the activity window of the FAW was relevant for the choice of the duration of the volatile sampling. The FAW is reported to be nocturnal (***Sparks, 1979***), without report of a more precise activity window. For the first cycle, two single maize leaves of the landrace 'Jowi white' were placed in wooden cages with meshed side walls (100 x 60 x 60 cm) in high cylindrical glasses filled with water, but sealed with a piece of cotton to prevent moths of entering the glasses. Moths of the age of 3 - 4 days were released in pairs of one female and one male in 10 different cages (resulting in 20 moths in total) and observed from 7pm - 11.30pm in two consecutive nights. The moths were recollected in falcon tubes in the morning after the first night, sealed with a piece of cotton to allow air circulation and stored at a temperature of approx. 25 °C until they were released in the same combinations on the following evening. For the second night fresh maize leaves were provided for the moths to oviposit. For the second cycle, potted maize plants with multiple leaves of the landrace 'Jowi white' were placed inside the wooden cages with a piece of cotton soaked with water. Three female and two male moths of different ages were placed each in three cages

(resulting in 15 moths in total) and observed between 6pm - 1am in two consecutive nights. During day time the moths and the plant were left unaltered in the cage.

Volatile Sampling

Volatiles of *Desmodium* were collected from plants in push-pull fields of local smallholder farmers near Mbita in the counties Homa bay and Siaya (coordinates determined with WGS84 coordinate system (latitude, longitude): *D. incanum* = (-0.382096, 34.175487), (-0.4298279, 34.207891), (0.189957, 34.36072), *D. intortum* = (-0.382096, 34.175487), (-0.6302736, 34.494595), (-0.551479, 34.314673)). *Desmodium* volatiles were collected on three different push-pull fields in May and June 2023 during the long rains, selecting four representative plants per field. As it is becoming more common, some of the push-pull fields were mixed with vegetables such as kale or cowpeas. Volatile collections were also performed from potted *Desmodium* plants, as well as from healthy and infested potted maize plants on the research campus. Five three-week-old maize plants, later labeled as"infested", were exposed in two cages, each with each three female and two male moths, for two nights prior to the volatile sampling, which resulted in 1 to 10 batches per plant. Potted plants were placed in a greenhouse with a glass roof and two glass walls, in which air was able to circulate through netted walls on the opposing longer walls of the greenhouse.
As an adsorbent, commercially available Tenax TA glass tubes (containing 150 mg adsorbent, 35 – 60 mesh, Markes International Ltd, England or 186 mg adsorbent, 60 – 80 mesh, Merck, Germany) were used. A handful of leaves were enclosed in a preheated roasting bag (Sainsbury's, London, UK). The inlet tubing pushed 500 mL/min charcoal-filtered air into the bags from the lower bag rim, while the Tenax TA tube was connected to an air outlet tubing through a small hole at the top of the bag. Air flows were regulated by PYE Volatile Collection Kits (B. J. Pye, Kings Walden, UK) as described in***Steen et al.*** (***2019***). Volatiles were accumulated on the adsorbent by aspirating the air at 200 mL/min (+/- 40 mL/min) for 11.75 h (+/- 30 min) overnight from 7 pm until 6.45 am of the following day. Under the same conditions, four ambient controls were sampled with an empty roasting bag on two fields with each *Desmodium* species. Additionally, a Tenax TA storage control was stored with all tubes, but never taken to the fields.

TD-GC-MS Measurement

All samples were measured on a TD-GC-MS instrument (Thermal Desorption – Gas Chromatography – Mass Spectrometer) from Shimadzu (TD30-QP2020NX Kyoto, Japan). The Tenax TA tubes were desorbed for 15 min at 220 °C under 80 mL/min nitrogen flow. Volatiles were trapped on a Tenax cooling trap at -20 °C and after desorption was completed, the cooling trap was heated rapidly to 230 °C. The sample was injected with a split ratio of 1:5 and separated on a Rtx-Wax column (30 m, 0.25 mmID, 0.25 μm, Restek Corporation, USA) with the following 35 min oven program: holding at 40 °C for 2 min, heating to 150 °C with 10 °C/min, holding at 150 °C for 2 min, heating to 190 °C with 3 °C/min, heating to 230 °C with 10 °C/min and holding at 230 °C for 3 min. Mass fragments from 33 m/z to 400 m/z resulting from electron ionization at 70 eV were recorded with a scan rate of 5 Hz at an ion source temperature of 230 °C from 3 – 30 min.

Feature Detection

A feature list was generated in MZmine (Version 3.9.0, (***Schmid et al., 2023***)) from all compounds that occurred in at least one-third of the field samples of either *Desmodium* species and in less than 4/5 of the combined control samples (ambient control and storage control). Features were numbered and considered as unidentified if there was no corresponding reference compound. After removal of contaminants, the features were added to an existing target list that was composed with commercially available plant volatiles that can be mainly classified as terpenoids and benzenoids, which as a whole contain a large variety of functional groups such as alcohols, aldehydes, ketones and esters. Additionally, the existing list included four green leaf volatiles ((*E*)-2-hexen-1-ol acetate, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol acetate) and a selection of more specific

substances reported in push-pull fields such as (*E*)-4,8–dimethyl–1,3,7- nonatriene (DMNT) and (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). Integration of all substances and features was manually checked for the all control samples (ambient and storage control) using the LabSolutions Insight GCMS software (Shimadzu corporation, Kyoto, Japan). Peaks for all targets that occurred in at least 1/2 of the *Desmodium* field samples and in no more than 3/5 of controls were manually checked for all samples. Eventually, all targets that occured in at least 2/3 of the field samples of *D. intortum* or *D. incanum* and in no more than 3/5 of the controls were considered as present. In ***Table 1*** the electron impact ionization (EI)-MS data of the unidentified features are displayed, and in ***Table 2*** the origin of all reference standards used to identify the target hits can be found.

Table 1. Mass fragments of the unknown features detected by EI-MS in field *D. intortum* and / or *D. incanum*

Name	Retention index	Peaks with relative intensity >5% [m/z (intensity in %)]
Monoterpene	1229	93 (100.0), 121 (29.4), 77 (28.2), 120 (11.6), 105 (10.0), 80 (10.0), 136 (8.8), 107 (8.0), 91 (6.7), 92 (5.7)
Benzene derrivative	1356	119 (100.0), 117 (54.6), 134 (30.8), 91 (18.9), 118 (18.7), 115 (17.9), 102 (12.4), 120 (8.6), 77 (7.2), 65 (6.0), 51 (5.5), 63 (5.1)
Sesquiterpene1	1477	119 (100.0), 105 (94.6), 161 (77.1), 93 (45.7), 91 (39.5), 81 (26.8), 92 (26.3), 120 (23.7), 41 (19.1), 77 (17.7), 79 (15.8), 133 (14.3), 121 (14.0), 204 (13.6), 106 (13.5), 107 (13.3), 55 (12.3), 117 (12.3), 162 (11.1), 43 (10.0), 136 (8.4), 69 (7.3), 115 (7.2), 65 (6.6), 53 (6.4), 94 (6.4), 131 (6.0), 39 (5.8), 67 (5.7)
Sesquiterpene2	1716	105 (100.0), 93 (77.0), 119 (49.5), 91 (39.9), 41 (39.1), 107 (32.9), 79 (32.5), 161 (30.8), 94 (30.6), 81 (29.3), 69 (28.4), 55 (24.7), 204 (24.2), 133 (23.2), 77 (22.8), 189 (18.1), 106 (17.4), 121 (17.4), 147 (13.1), 53 (13.1), 92 (12.7), 95 (11.3), 109 (10.5), 39 (10.1), 120 (8.9), 162 (8.5), 67 (8.5), 134 (8.3), 108 (8.3), 82 (8.1), 43 (7.8), 122 (7.6), 65 (7.1), 148 (6.9), 175 (6.6), 123 (6.6), 54 (6.3), 135 (6.0), 80 (6.0), 117 (5.6), 110 (5.3)
Sesquiterpene3	1762	81 (100.0), 93 (99.0), 107 (84.5), 91 (74.1), 79 (70.5), 80 (68.5), 55 (56.8), 105 (54.9), 43 (54.3), 95 (53.8), 119 (51.7), 108 (50.6), 41 (50.0), 94 (48.6), 109 (42.8), 67 (41.7), 133 (35.1), 77 (32.9), 70 (28.9), 121 (28.7), 147 (23.9), 189 (22.9), 53 (20.4), 106 (19.3), 59 (18.6), 122 (17.9), 39 (17.8), 123 (17.4), 92 (16.9), 57 (16.6), 134 (16.4), 65 (16.2), 71 (15.3), 161 (15.0), 120 (13.7), 56 (13.5), 175 (13.0), 82 (12.0), 113 (10.9), 78 (10.2), 204 (9.2), 148 (9.0), 117 (8.7), 42 (8.2), 135 (8.1), 149 (7.8), 83 (7.7), 66 (7.5), 68 (7.2), 85 (7.2), 132 (7.2), 126 (6.9), 124 (6.7), 162 (6.5), 96 (6.4), 69 (6.3), 131 (6.1), 97 (5.9), 63 (5.6), 110 (5.3), 111 (5.0), 136 (5.0)
Naphthalene derivative	1834	142 (100.0), 141 (81.3), 115 (34.7), 91 (20.4), 143 (11.3), 43 (11.3), 139 (10.9), 105 (8.8), 135 (7.7), 71 (7.3), 63 (5.6), 147 (5.1)

Table 2. Origin of the reference standards of all identified target substances present in field *D. intortum* and / or *D. incanum*. TMTT = (3*E*,7*E*)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene, DMNT = (*E*)-4,8-Dimethylnona-1,3,7-triene

Compound Name	Distributor	Article No	CAS No	Quantifier [m/z]	Qualifiers [m/z (intensity in %)]	RI
(*E*)-2-Hexenal	Aldrich	132659-25g	5728-26-3	41	55.00 (82.67) 69.00 (72.87)	1215
(*E*)-α-Bergamotene	isobionics	-	-	119	93.00 (95.07) 91.00 (38.14)	1594
(*E*)-β-Ocimene	Sigma-Aldrich	W353901-SAMPLE	13877-91-3	93	91.00 (47.39) 79.00 (45.35)	1248
(*Z*)-1,5-octadien-3-ol	Givaudan	-	-	57	70.00 (45.40) 55.00 (34.19)	1473
(*Z*)-3-Hexen-1-ol	Phyto Technology Laborato-ries	H4000	928-96-1	67	82.00 (46.66) 55.00 (42.27)	1378
(*Z*)- 3-Hexen-1-ol, acetate	Sigma-Aldrich	74597-1ml	3681-71-8	43	67.00 (98.54) 82.00 (52.15)	1317
1-Hexanol	Fluka	52830	111-27-3	56	55.00 (47.00) 69.00 (28.00)	1346
1-Octen-3-ol	Sigma-Aldrich	05284-25g	3391-86-4	57	43.00 (24.99) 72.00 (16.88)	1446
1,2,3-Trimethylbenzene	Aldrich	T73202-5mL	526-73-8	105	120.00 (50.09) 77.00 (11.00)	1333
3-Octanone	Sigma-Aldrich	136913-25g	106-68-3	57	71.00 (70.00) 99.00 (67.00)	1255
3-Pentanol	Aldrich	P8025	584-02-1	59	41.00 (19.00) 58.00 (9.00)	1102
α-Humulene	PhytoLab	83351-100mg	6753-98-6	93	80.00 (32.44) 121.00 (26.27)	1679
Caryophyllene	Sigma-Aldrich	22075-5ml-F	87-44-5	93	133.00 (91.17) 69.00 (87.31)	1606
DMNT	trc	TRC-D475810	19945-61-0	69	79.00 (14.49) 81.00 (13.76)	1310
Germacrene D	Cayman Chemical Company	26539		161	105.00 (78.00) 119.00 (52.00)	1710
Hexyl acetate	Sigma-Aldrich	25539-1mL	142-92-7	43	56.00 (77.00) 61.00 (34.00)	1270
Linalool	Aldrich	L2602-5g	78-70-6	71	93.00 (82.13) 121.00 (24.00)	1544
Indole	Sigma-Aldrich	442619	120-72-9	117	90.00 (45.80) 89.00 (31.87)	2483
TMTT	trc	TRC-T797630	62235-06-7	69	81.00 (43.14) 79.00 (11.34)	1814

Oviposition Bioassays

Plants

All plants were planted in plastic pots in black cotton soil in Mbita, Kenya. Maize plants (SC Duma 43, Seed Co Limited, Nairobi, Kenya) were grown from seeds with the addition of fertilizer (3 g DAP 18-46-0, Yara East Africa LTD, Nairobi, Kenya) and used at the age of 2 – 4 weeks with five to seven fully-grown leaves. *Desmodium* plants were obtained from a push-pull field on the icipe Mbita campus and kept in pots without fertilizer.

Fall Armyworm Moths

To form the FAW colony, wild individuals were collected in the counties Siaya, Kisumu, Migori, and Vihiga in Western Kenya. Larvae were fed on artificial diet based on soy flour, wheat germ, raw linseed oil, mineral mix, sugar, aureomycin, vitamins, agar, methyl parabene, sorbic acid, and calcium propionate (Article No 870-265-3747, Southland Products Inc, Lake Village AR, USA). Moths were fed on water and in rare cases after emergence on 10% honey solution. The colony was occasionally restocked with wild FAW moths. Each day after hatching, moths of both sexes were transferred to a cage to allow mating until the start of the experiment.

Experimental Procedure

Cages (100 x 60 x 60 cm) lined with wooden floors and ceilings and netted walls were placed in greenhouses that allowed air circulation through mesh side walls (for pictures of the real setup, see ***Figure 4—figure Supplement 2***, for exact positioning in the greenhouses, see ***Figure 4—figure Supplement 3*** and ***Figure 4—figure Supplement 4***). Two plants were placed within the cage at the greatest distance possible (approx. 60 cm) in two opposite corners. For the control treatment, two maize plants were placed in the cage, while the direct *Desmodium* treatment consisted of one maize plant and one *Desmodium* plant in the cage. In two additional treatments with indirect contact, two maize plants were placed in the cage, while one *Desmodium* plant was placed outside the cage unreachable for the FAW moths, but in proximity to one maize plant. Three female and two male moths at the age of 4 – 5 days were released approx. 1 h before dusk and allowed to oviposit under natural conditions of L12:D12 for three consecutive nights. Eggs were collected in five different groups depending on the position in which they were laid, either on one of the two plants, close to one of the plants within a maximum of 20 cm distance, or on the cage further than 20 cm from any plant (labeled as “No Decision”). Each treatment was repeated 19 – 21 times in total over five cycles, with up to 5 replicates per treatment per cycle.

Egg Count

Each batch of FAW eggs of was collected with sticky tape to separate the layers and spread all eggs out to one dimension. Each egg batch was taped to white paper and photographed in a UV imaging system (Syngene, Cambridge, England) against UV light coming from underneath the paper. A script for semiautomatic counts in ImageJ (Version 1.54f, National Institutes of Health, USA) was developed and used for counting the exact number of eggs per picture (see ***Figure 4—figure Supplement 5***). The code is available in the data depsitory.

Wind tunnel Bioassays

Plants

All plants were planted from seeds without the addition of fertilizer in plastic pots in standard red soil mixed with manure (ratio of 2:1) at the icipe campus in Nairobi, Kenya. Maize plants (SC Duma 43, Seed Co Limited, Nairobi, Kenya) were used at the age of 4 – 7 weeks with five to eight fully-grown leaves. It was observed that maize plants seemed to grow more slowly in Nairobi than in Mbita, which could be explained by the generally lower temperatures in Nairobi. *D. intortum* and *D. incanum* seeds were obtained from a push-pull field on the icipe campus Mbita and were 6 – 8 weeks and 7 months old when used, respectively.

Fall Armyworm Moths

To form the FAW colony, wild individuals were collected in Central Kenya such as in the counties Kiambu, Muranga, and Embu and restocked with wild individuals every three months. Larvae were reared on maize leaves, while moths were only fed with water. The colony was occasionally restocked with wild FAW moths. Each day after hatching, moths of both sexes were transferred to a cage to allow mating until the start of the experiment. At least 2 h prior to the experiment, female moths were separated from males and and placed in the darkened experimental room for adjustment to temperature and humidity.

Experimental Procedure

The five upper leaves of a maize plant were wrapped in a preheated roasting bag (Sainsbury's, London, UK) with the addition of approximately the same biomass of *Desmodium* on one side of a two-choice wind tunnel (100 x 30 x 30 cm) and compared to a single maize plant wrapped in the same manner on the other side. Control treatments were conducted with one maize plant wrapped in this way on each side. Air was pushed through activated charcoal filters and into the roasting bag from the lower rim of the bag at a rate of 740 – 820 mL/min and a Teflon tube conveyed the plant volatiles from the top of the roasting bag to one side of the wind tunnel. Inside the wind tunnel, the air was pumped out from the center at the 0 cm mark resulting in an air stream at the air transfer tubing of 480 – 570 mL/min with no greater difference than 4 mL/min between the two sides. One female moth at an age of 4 – 5 days was released through a hole in the center at 0 cm and its flight behavior was observed for 5 minutes. The wind tunnel was separated into 5 sectors of 20 cm each, and a time stamp was set every time the moth changed the segment (See ***Figure 5—figure Supplement 1***). All experiments were conducted between 7 pm and 1:30 am under red light. Each trial was recorded on video using a mobile phone camera (Fairphone 4, 48 MP), which captured the wind tunnel from a front view. The videos were rewatched for the data acquisition and in cases where moths were barely visible, complemented with the notes from the live observation. To simplify the data analysis, the two left sectors (50 – 30 cm left & 30 – 10 cm left) were combined, as were the two right sectors (10 – 30 cm right, 30 – 50 cm right).

Statistical analysis

All statistical analysis was performed in RStudio (R version 4.3.2, RStudio version 2024.04.2). Mixed models were performed to determine significant effects of the positions of FAW eggs in the oviposition bioassays or for the duration of stay in the different sectors in the wind tunnel bioassays. In both bioassays a control with two maize plants was performed. Therefore, the control replications were included twice in the dataset, once with the egg count data or the duration of stay of both sides interchanged. In addition, the replication was included as a random effect into the model equations. For the oviposition bioassay, the egg counts on the maize or the *Desmodium* plant were compared amongst all treatments, with the inclusion of the greenhouse, the cage (combination of the cage group and the cage number), the start date and the replication number as random effects. For the wind tunnel bioassay, the duration of stay closer to the maize or the *Desmodium* plants were compared amongst all treatments, with the inclusion of the start date and the replication number as random effects.

Table 3. Mixed model applied for Oviposition Bioassays
Maize: Comparison of the laid eggs on the maize plants for all treatments with the following formula: RelEggNoMaize ~ ContrvsTreat + IndvsDir + IncvsInt + Treatment + (1| Greenhouse) + (1| Group:CageNo) + (1| Start.Date) + (1| Rep)
***Desmodium*:** Comparison of the laid eggs on the *Desmodium* plants for all treatments with the following formula: RelEggNoDesmodium ~ ContrvsTreat + IndvsDir + IncvsInt + Treatment + (1| Greenhouse) + (1| Group:CageNo) + (1| Start.Date)+ (1| Rep)
Descriptions of the terms: RelEggNoMaize & RelEggNoDesmodium = The dependent variable was determined by number of eggs laid on maize or *Desmodium* relative to the total number of eggs laid in one repetition. ContrvsTreat = All *Desmodium* treatments are compared with the control treatment IndvsDir = The indirect *Desmodium* treatments (*D. incanum* and *D. intortum*) are compared with the indirect treatments of the two species. IncvsInt = *D. incanum* and *D. intortum* are compared against each other. Treatment = All treatments are compared against each other to detect effects which are not related to terms used before. Greenhouse = Treatments were carried out equally in two different greenhouses. Group:CageNo = Twentyfive cages were repeatedly used, whereby each cage could be unambiguous identified with the group and cage number. Start.date = Treatments were carried out at five different start dates. Rep = All repetitions of the control were inserted twice with inversion of the values of the left and right maize plants. Any effects of the single repetitions are displayed in this term.

On Maize plant

Term	Sum Sq	Mean Sq	Num DF	DenDF	F value	PR (>F)
ContrvsTreat	1.01914	1.01914	1	111.09	11.1142	0.001165
IndvsDir	0.87220	0.87220	1	111.09	9.5117	0.002576
IncvsInt	0.09894	0.09894	1	112.10	1.0789	0.301171
Treatment	0.06341	0.06341	1	111.40	0.6915	0.407421

On *Desmodium* plant

Term	Sum Sq	Mean Sq	Num DF	DenDF	F value	PR (>F)
ContrvsTreat	0.32383	0.32383	1	111.27	5.3626	0.02241
IndvsDir	1.36041	1.36041	1	111.17	22.5287	6.207e-06
IncvsInt	0.03747	0.03747	1	112.61	0.6204	0.43254
Treatment	0.05994	0.05994	1	111.61	0.9927	0.32125

Table 4. Mixed model applied for Wind tunnel Bioassays:
Maize: Comparison of the duration of stay in the compartment of the maize plants for all treatments with the following formula: (y_logitMaize ~ (MaizevsDes + Treatment) + PosMaize + (1|Date)+(1|Rep)
***Desmodium*:** Comparison of the duration of stay in the compartment of the *Desmodium* treatment for all treatments with the following formula: (y_logitDesmodium ~ (MaizevsDes + Treatment) + PosMaize + (1|Date)+(1|Rep)
Descriptions of the terms: y_logitMaize and y_logitDesmodium = The dependent variable was determined by the logit-transformed accumulated duration of the stays in minutes in the two compartments (10 cm - 50 cm) closer to the maize plant or a maize plant combined with a *Desmodium* plant. ContrvsTreat = The *D. incanum* and *D. intortum*treatments are both compared with the control treatment. Treatment = The two *Desmodium* species are compared with each other. PosMaize = The position of the maize plant and the maize + *Desmodium* treatment were equally placed on the left or right side of the wind tunnel. Rep = All repetitions of the control were inserted twice with inversion of the values of the left and right maize plants.

		Side of Maize				
Term	Sum Sq	Mean Sq	Num DF	DenDF	F value	PR (>F)
ContrvsTreat	3.2055	3.2055	1	67	0.2410	0.6251
Treatment	16.8770	16.8770	1	67	1.2687	0.2640
PosMaize	0.4577	0.4577	1	67	0.0344	0.8534

		Side of *Desmodium*				
Term	Sum Sq	Mean Sq	Num DF	DenDF	F value	PR (>F)
ContrvsTreat	0.8454	0.8454	1	66.777	0.0618	0.8044
Treatment	13.2919	13.2919	1	64.002	0.9719	0.3279
PosMaize	6.5060	6.5060	1	48.989	0.4757	0.4936

Author Contribution

Conceptualization: D.M.O., F.C., and M.C.S.; Data curation: D.M.O.; Formal analysis: D.M.O., B.S.; Funding acquisition: M.C.S., F.C.; Investigation: D.M.O.; Methodology: D.M.O., M.C.S., F.C.; Project administration: M.C.S., F.C.; Resources: M.C.S., F.C., A.T.; Supervision: M.C.S., F.C., D.M.M., A.T., L.A.D.; Validation: D.M.O.; Visualization: D.M.O., L.A.D.; Writing – original draft: D.M.O., M.C.S; Writing – review & editing: M.C.S., F.C., B.S., D.M.M., L.A.D., A.T.

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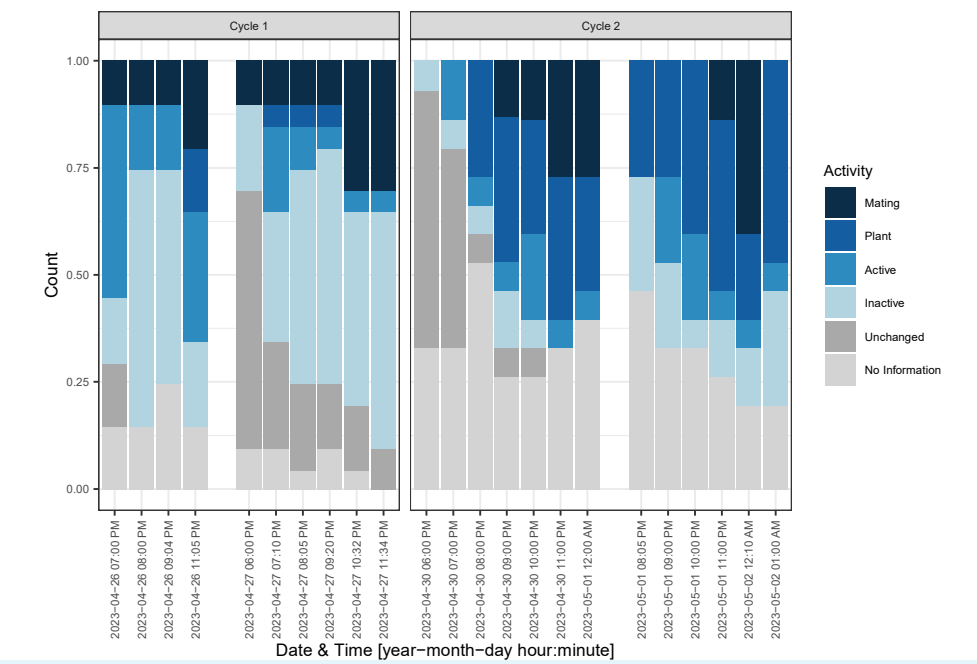
Appendix 1

Pretests: Moth Activity Window

Pretests were carried out to determine the activity window of the FAW moths, as it was important for deciding the time window of the headspace sampling of volatiles that are relevant for the FAW moths. The FAW moth is reported to be nocturnal (***Sparks, 1979***) and was checked regularly from dusk until around midnight. In the first hours only little activity was observed, while toward midnight more flight and mating activity was observed. As a result, the moth activity could not be limited to the few hours of the evening and volatile sampling was proceeded from 7pm - 6.45am covering all dark hours.



Appendix 1—figure 1. Stacked Barplot displaying the moth activity between 6pm and 1am. Several pairs of moths were observed mating for several hours up until 1pm. Explanation color code: Mating = Two moths mate, Plant = Moth sits on the plant and therefore might oviposits, Active = Moth actively move or flies, Inactive = Moth does not move and sits on the frame or the net, Unchanged = Moth does not move from the starting point, No Information = Moth could not found and therefore no information on the activity were possible.

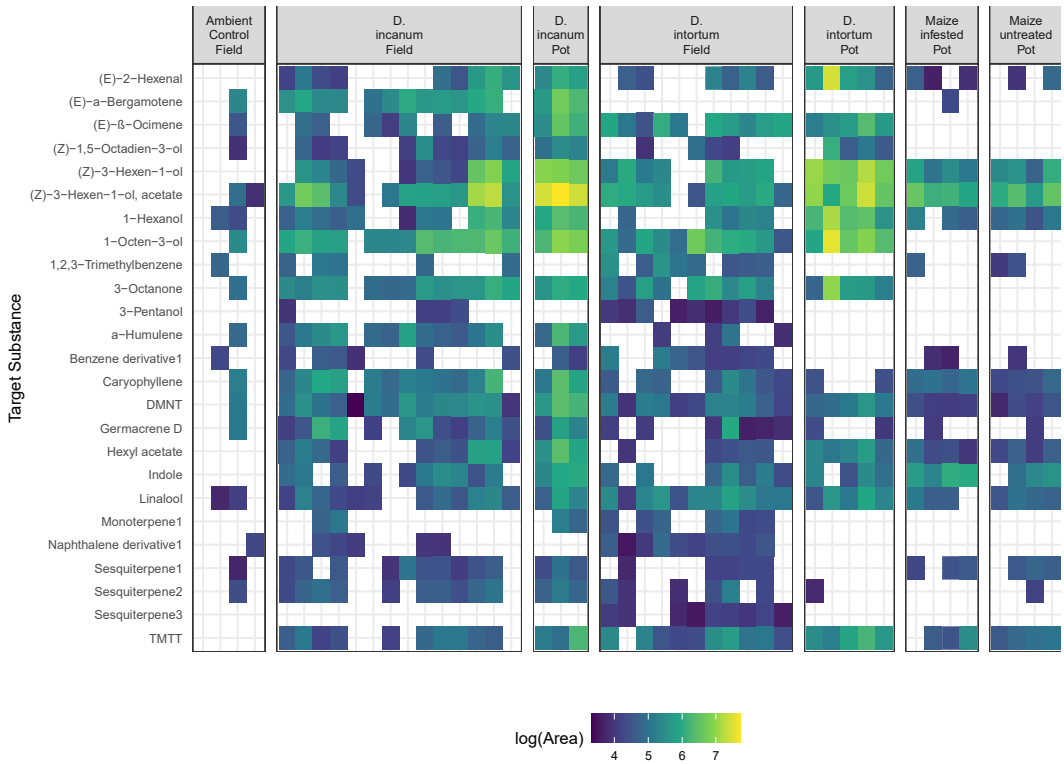


Figure 2—figure supplement 1. Heatmap comparing the log10-transformed peak area of all samples. The x-axis displays the species and their growth conditions (field or pot) and the y-axis shows the target substances in alphabetical order. The color indicates mean of the log-10 transformed peak area, whereby transparency indicates that no peak was found.

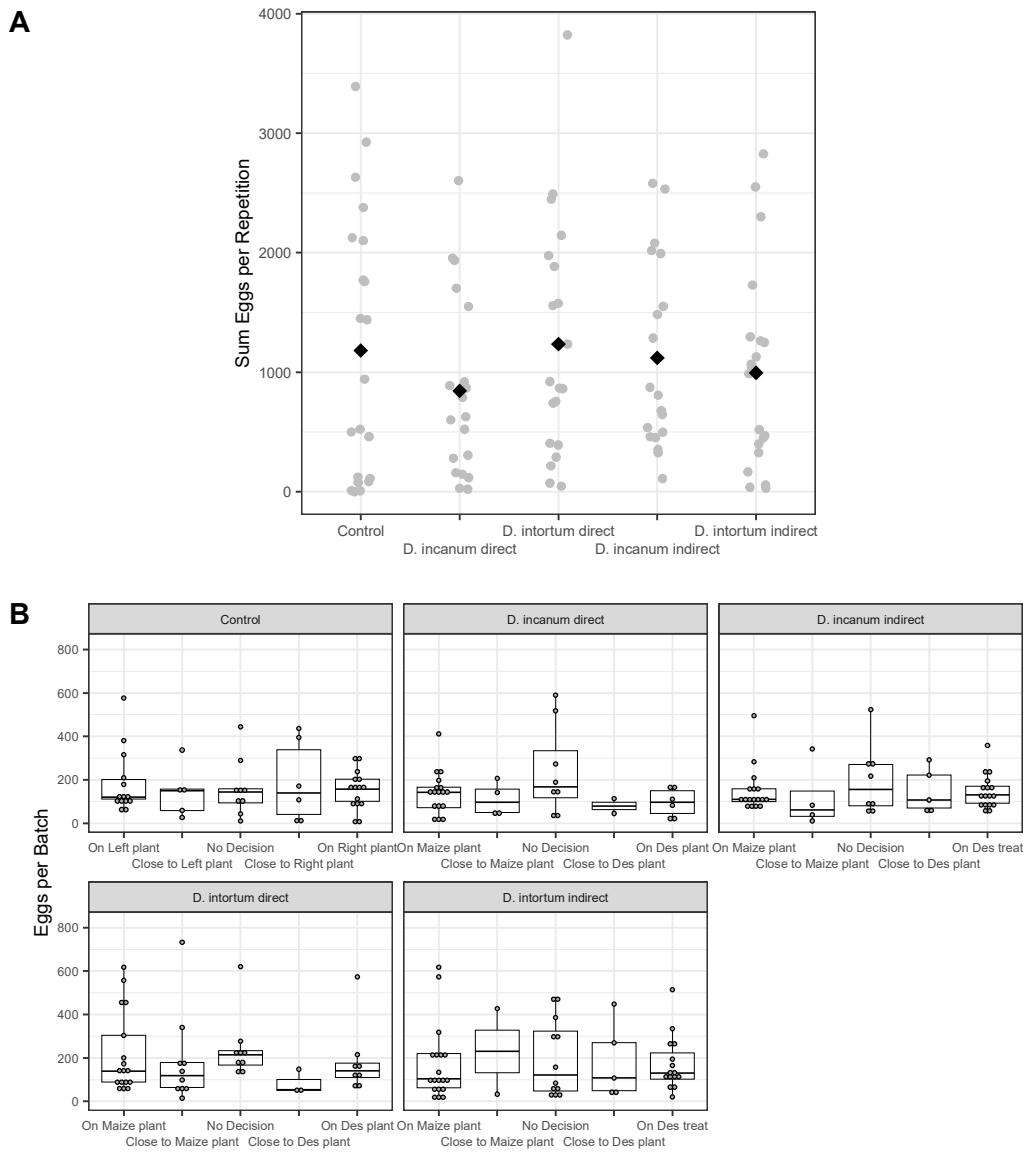


Figure 4—figure supplement 1. A: Variance of total egg count per repetition. The x-axis shows the sum of all egg found in all position within one repetition. The grey points display the total egg count per repetition and the black diamonds show the mean value over all data points per treatment. **B:** Boxplot including individual data points of the egg batch size (egg number per batch) per location (x-axis) and per treatment (superordinate boxes). *Desmodium* is abbreviated with 'Des' and treatments is abbreviated with 'treat'. Samples sizes: Control = 21, *D. incanum* direct = 19, *D. intortum* direct = 20, *D. incanum* indirect = 19, *D. intortum* indirect = 20.

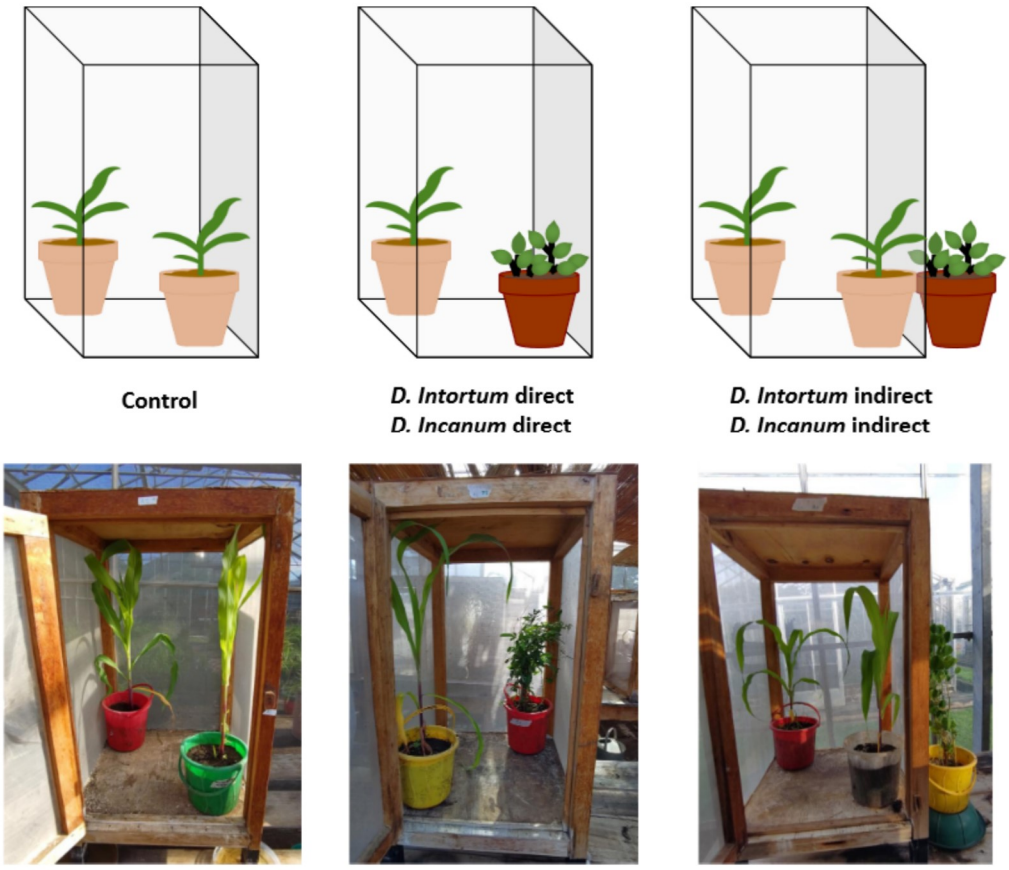


Figure 4—figure supplement 2. Upper Line: Schematic drawing of the placement of the plants in the cages according to the treatments. The rectangle represents the cage, the plant in the beige pot represents maize and the plant in the red pot *Desmodium*. **Lower Line:** Photos of the experimental setup of three treatments control (left), *D. incanum* direct (center) and *D. intortum* indirect (right)

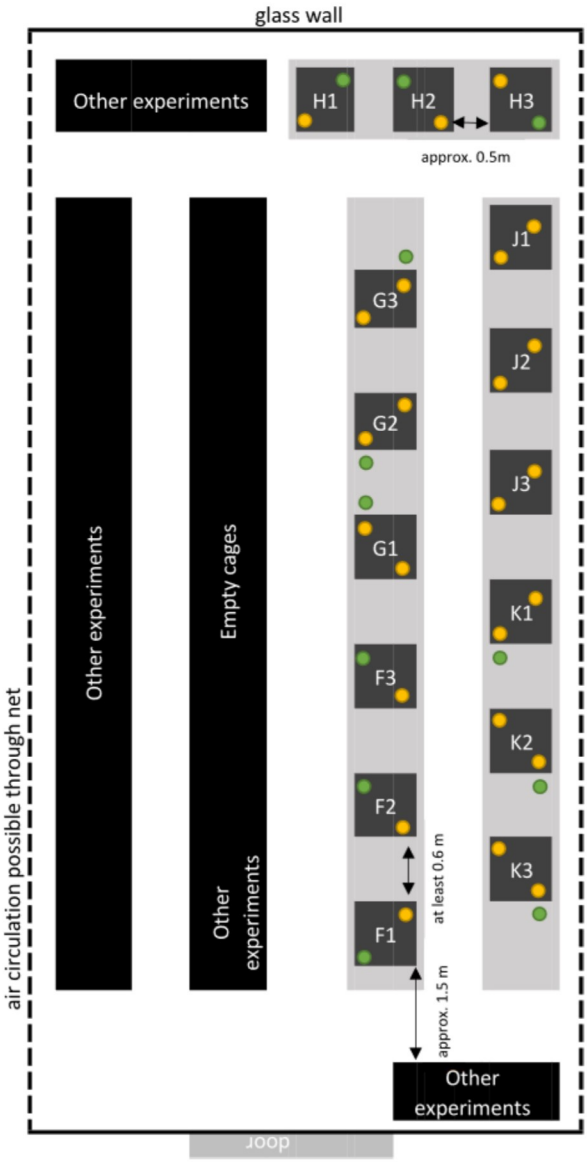


Figure 4—figure supplement 3. To minimize volatile interference of neighboring setups, the treatments were placed in groups next to each other (represented by letters) with the distance of at least one cage length in between, which corresponded to 60 – 100 cm. Due to space limitations on two tables the cages of group H were placed slightly closer with an approximate distance of 50 cm. The positions of the treatments were swapped between each cycle so that treatment was conducted at each position once. In the treatments where *Desmodium* plants were placed outside the cages, the *Desmodium* plants were normally placed between the cages and in greenhouse 1B additionally on the outside of the outermost cage of a group (as can be seen on the cages of G). However, as all treatments were photographed, in retrospect several misplaced plants were noticed (see cage K2). Therefore, it must be considered that only conclusions about the influence of *Desmodium* volatiles in proximity versus those in further distance of >0.5 m can be made and any volatiles reaching longer distances might have affected neighboring replications. **Greenhouse 1B:** Schematic graph of the cage setup in the greenhouse 1B (above) and a photograph taken of the cages H2, J2, J3, K1 & K2 (right). This greenhouse provided space for 15 cages in total, whereby three replicates of each treatment could be repeated per cycle. The plants display the placement of the plants in the replicates of the second cycle from 23. – 26.05.2023. Lightgrey squares = tables, darkgrey squares = cages, yellow circle = maize plants, green circle = *Desmodium* plants; The red circle indicated the situation where a plant was misplaced and therefore was in the closest distance of the neighboring repetition.

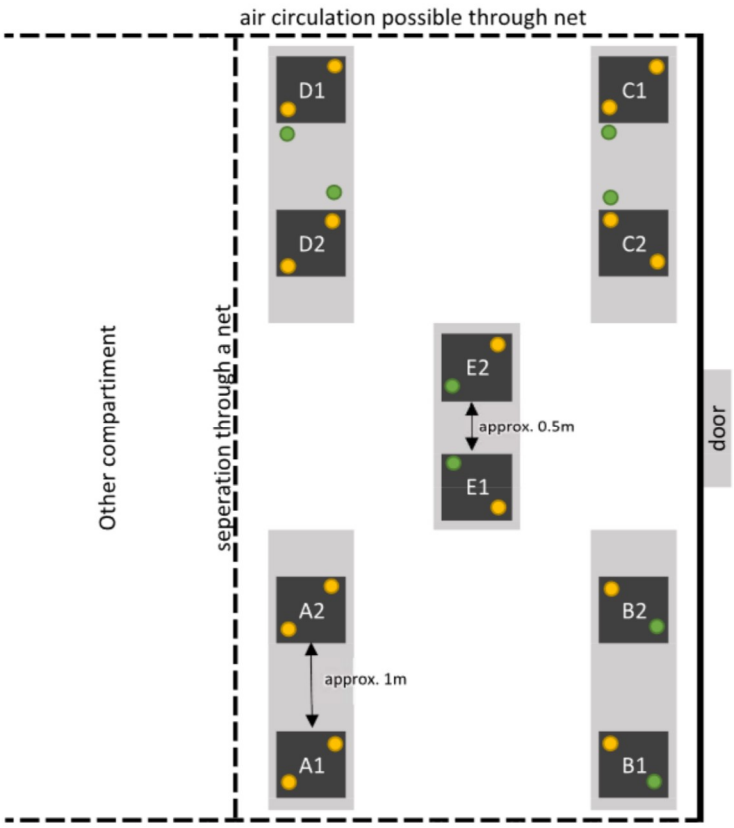


Figure 4—figure supplement 4. To minimize volatile interference of neighboring setups, the treatments were placed in groups next to each other (represented by letters) with the distance of at least one cage length in between, which corresponded to 60 – 100 cm. Due to space limitations on two tables the cages of group E were placed slightly closer with an approximate distance of 50 cm. The positions of the treatments were swapped between each cycle, with all treatments being conducted in 4/5 positions. In the treatments where *Desmodium* plants were placed outside the cages, the *Desmodium* plants were normally placed between the cages and in greenhouse 1B additionally on the outside of the outermost cage of a group (as can be seen on the cages of G). However, as all treatments were photographed, in retrospect several misplaced plants were noticed (see ***Figure 4—figure Supplement 3*** Figure of Greenhouse 1B, cage K2). Therefore, it must be considered that only conclusions about the influence of *Desmodium* volatiles in proximity versus those in further distance of >0.5 m can be made and any volatiles reaching longer distances might have affected neighboring replications. **Greenhouse 3B:** Schematic graph of the cage setup in the greenhouse 3B (left) and a photograph taken from the door toward the cages A1 & A2 (right). This greenhouse consisted of 10 cages in total, whereby two replicates of each treatment could be repeated per cycle. The plants display the placement of the plants in the cycle of 23. – 26.05.2023. lightgrey squares = tables, darkgrey squares = cages, yellow circle = maize plants; green circle = *Desmodium* plants.

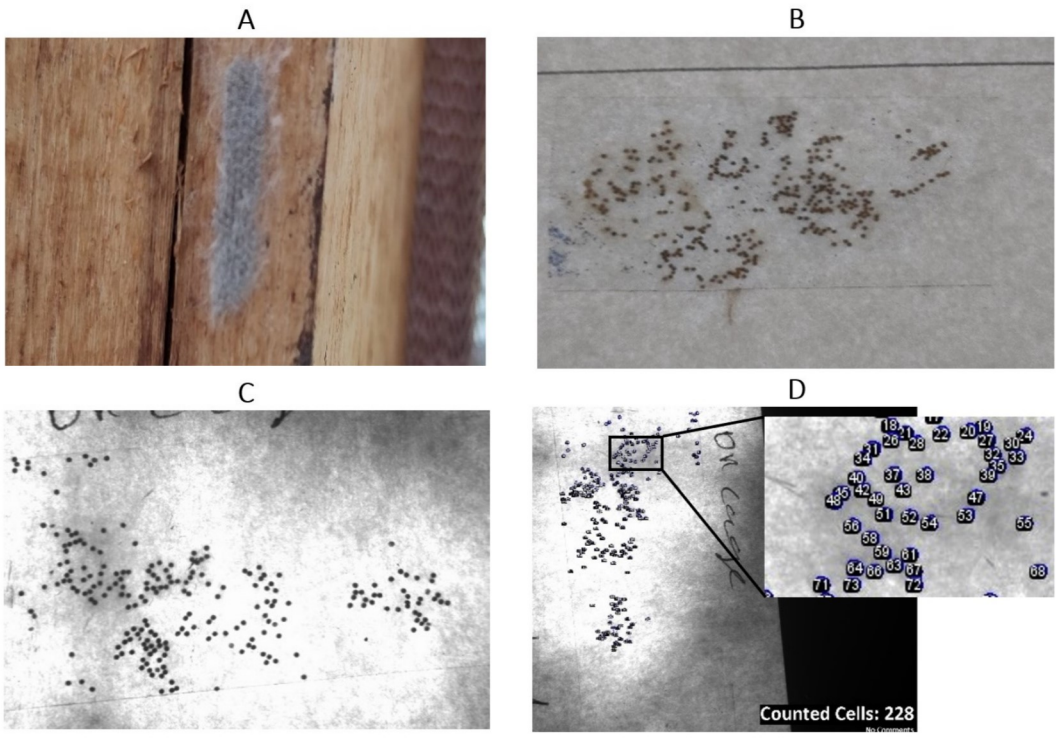


Figure 4—figure supplement 5. Fall armyworm eggs are normally laid staggered in clusters and with hair-like scales on the surface texture which complicates accurate egg counting (see picture **A**). Therefore, eggs were collected with sticky tape to separate the layers and spread all eggs out to one dimension (**B**). The egg batches were taped to white paper, taking care not to squash eggs and photographed in a UV imaging system (Syngene, Cambridge, England) against UV light coming from underneath the paper (**C**). A script for semiautomatic counts in ImageJ (Version 1.54f, National Institutes of Health, USA) was developed and used for counting the exact number of eggs per picture (**D**).

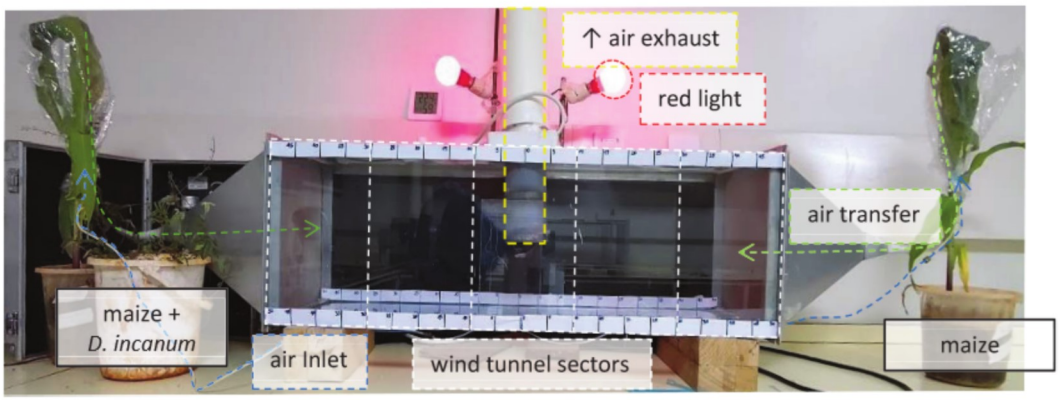


Figure 5—figure supplement 1. Experimental setup of the two-choice wind tunnel with a *D. incanum* treatment on the left and a maize on the right. The photograph was taken in the light, while the experiments were conducted in the dark only with red light. Yellow marks: Air exhaust at the centre of the wind tunnel covered with a fine-meshed net. Red marks: Two red light bulbs placed symmetrical approx. 20 cm above the upper rim of the wind tunnel. White marks: Wind tunnel body (30 x 30 x 100 cm) and its imaginary separation of the length in five sectors of 20 cm. Green marks: Air transfer from the top of the plant bags via teflon tubing to the wind tunnel. Blue marks: Air inlet for charcoal-filtered air provided by Volatile Collection Kits via teflon tubing.