

Determination of the Functional Role of *Desmodium* Volatiles on the Fall Armyworm in Push-Pull Systems

Daria M. Odermatt, Master of Quantitative Environmental Sciences, University of Zurich

Impact and Achievement of the SDGs The sustainable intensification of agriculture is essential to achieve SDG 2, zero hunger, and reduce hidden costs of meeting requirements of other SDGs such as 13, climate action (1). This is particularly important in the developing Global South where larger yield gaps are observed compared to the developed countries with more financial resources (2). Intercropping may offer sustainable solutions with potential for strong positive effects on pest and disease control as well as associated biodiversity (3). This master thesis addresses knowledge gaps in the fundamental functioning of the push-pull intercropping systems and therefore contributes to the goals of the UPSCALE project, funded by the European Commission, to upscale the benefits of push-pull technologies for sustainable agricultural intensification in East Africa.

A. Introduction. Achieving food security for millions of African smallholder farmers requires sustainable intensification (4). “Push-pull” cropping systems promise sustainable crop protection, including against fall armyworm, an invasive moth responsible for most maize yield losses to insect pests in East Africa (5–7). 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 (8). In Kenya, the first push-pull system was introduced in 1997 and designed to reduce yield losses in the main crop maize caused by stemborer species (9). Following the initial success, the system has been further developed by introducing the leguminous repellent intercrop *Desmodium* that could improve soil quality by fixing nitrogen, and provide high-quality fodder and *Brachiaria* species as border crops (10, 11).

The invasive fall armyworm (FAW), *Spodoptera frugiperda* originated from northern and central America and has become a major threat to African maize crops since 2016 (12–14). It spread rapidly through East Africa, with most of the farmers in Ethiopia and Kenya already encountering the FAW after the long rains in the first half of 2017 (15). 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 (16), as well as other traits such as its strong flight capacity, lack of diapause, reported survival in diverse habitats, rapid development of resistance to insecticides / viruses, and the polyphagous nature (17). Although its wide host range of at least 353 host species across 76 plant families (18), the high financial and yield losses on the main crop maize due to the pest are particularly devastating (19). However, the three publications to date investigating underlying mechanisms of *Desmodium* in push-pull systems disagree whether the *Desmodium* intercrop emits bioactive volatiles

that repel FAW. Two publications supported the hypothesis that volatiles from the companion crop *D. intortum* repel the FAW and attract parasitic wasps (20, 21). In contrast, a third recent publication by Erdei, David, and colleagues (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 (22). Thus, further investigations are needed to understand the mechanisms by which the maize-*Desmodium*-grass push-pull system can protect maize from FAW.

B. Research objectives and questions. This master thesis aimed to resolve the controversy over the role of *Desmodium* volatiles in push-pull systems by measuring volatile emission of the two most relevant current *Desmodium* intercrops in push-pull fields, and assaying their effects on the behavior of the FAW in oviposition and wind tunnel choice bioassays. The combination of these methods aims to answer the research questions of whether *Desmodium* intercrops release potentially bioactive volatiles and if these can explain a repellent effect in oviposition and flight behaviour of FAW.

C. Approach and methods. The field work was conducted in collaboration with the International Centre of Insect Physiology and Ecology (icipe) in Kenya, while gas chromatography measurements were carried out in the laboratory of the Spatial Genetics research group at the University of Zurich. All volatile samplings and bioassays were performed with two *Desmodium* species, *D. intortum* (greenleaf *Desmodium*) and *D. incanum*, and are here referred to as *Desmodium* for simplicity.

The emission of volatiles was tested by sampling volatiles of *Desmodium* plants directly on push-pull fields, as well as in potted plants on campus of icipe by trapping volatiles on

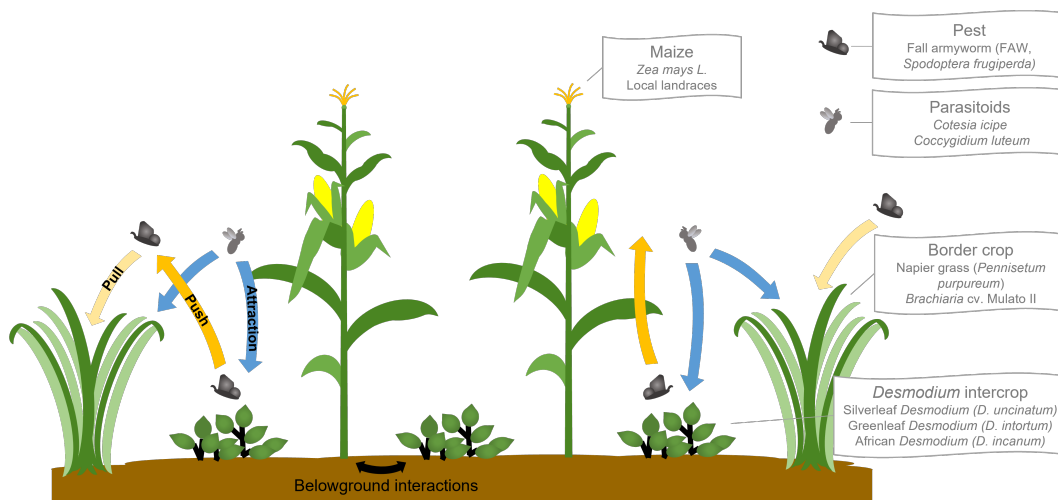


Fig. 1. Scheme displaying the mechanisms of push-pull according to the current state of knowledge of the system. Volatiles (and potentially other traits) of the intercrop repel the herbivorous insect and additionally attract its parasitoids, while volatiles (and potentially other traits) of the border crop attract herbivores away from the main crop (5, 23, 24).

Tenax TA adsorbents during the activity window of the FAW, the night hours. The samples were desorbed and measured by a thermal desorption - gas chromatograph - mass spectrometer (TD-GC-MS) and compared to a target list. The 135 target volatiles were composed of commercially available general known plant volatiles that can be mainly classified as terpenoids and benzenoids with different functional groups, few greenleaf volatiles 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).

The oviposition bioassays were carried out by placing two maize plants or a maize plant and a *Desmodium* plant within a wooden cage with netted walls with the greatest distance possible (approx. 60 cm) in two opposite corners. In two additional indirect treatments, two maize plants were placed in the cage, while one *Desmodium* plant was placed outside the cage and unreachable for the FAW moths but in proximity to one maize plant. Three female and two male moths were allowed to oviposit for three consecutive nights. Eggs located on the two plants and close to the two plants (in distance of maximum 20 cm) were collected and counted.

To test the influence of *Desmodium* volatiles on the short-term flight behaviour of female fall armyworm moths in a wind tunnel, leaves of a single maize plant and a combination of maize and *Desmodium* were wrapped in a preheated roasting bag. This allowed to test the preference of the FAW moths for maize vs. maize + *Desmodium* volatiles. At the start of each experiment one moth was released through a hole in the center of the wind tunnel and had the choice to fly upwind in both directions, whereby its flight behaviour was observed for 5 minutes.

D. Key Results. Dozens of volatile substances were detected 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

the 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, the hypothesis that *D. intortum* and *D. incanum* emit potentially bioactive volatiles can be confirmed, and a clear preference of FAW moths to oviposit directly on maize over either *Desmodium* species was visible. However, a repelling effect of the volatiles alone could not be shown in the scope of this master thesis as the short-term flight behaviour in the wind tunnel showed only a weak effect of repellence when combining maize with greenleaf *Desmodium* (*D. intortum*) volatiles and no effects for the *D. incanum* volatiles. These results therefore indicate that the preference of moths for maize over *Desmodium* is mediated more strongly by a short-range mechanism such as oviposition deterrents or an unfavourable leaf surface than by volatiles. Nevertheless, the measured *Desmodium* volatiles might be useful as attractants of natural enemies of the FAW and by itself could already represent an important effect in reduction of yield losses in maize.

1. FAO, IFAD, UNICEF, WFP, WHO. , pp. Rome, FAO (2020).
2. David Tilman, C Balzer, J Hill, BL Befort. *Proceedings of the National Academy of Sciences of the United States of America* **108** (2011).
3. D Beillouin, T Ben-Ari, E Malézieux, V Seufert, D Makowski. *Global Change Biology* **27**, 4697–4710 (2021).
4. MK van Ittersum, et al. *Proceedings of the National Academy of Sciences* **113**, 14964–14969 (2016).
5. JA Pickett, CM Woodcock, CA Midega, ZR Khan. *Current Opinion in Biotechnology* **26**, 125–132 (2014).
6. CAO Midega, JO Pittchar, JA Pickett, GW Hailu, ZR Khan. *Crop Protection* **105**, 10–15 (2018).
7. ZR Khan, et al. *Journal of Chemical Ecology* **28**, 1871–1885 (2002).
8. P Bruce, M Rice, S Bruce, M Zalucki. *The Australian Cotton Grower* **4**, 7–9 (1987).
9. ZR Khan, et al. *International Journal of Tropical Insect Science* **17**, 143–150 (1997).
10. CAO Midega, et al. *Field Crops Research* **180**, 118–125 (2015).
11. D Cheruiyot, et al. *Experimental Agriculture* **57**, 301–315 (2021).
12. G Goergen, PL Kumar, SB Sankung, A Togola, M Tamò. *PLoS ONE* **11**, e0165632 (2016).
13. R Day, et al. *Outlooks on Pest Management* **28**, 196–201 (2017).
14. G Hailu, et al. *International Journal of Tropical Insect Science* **41**, 1753–1762 (2021).
15. T Kumela, et al. *International Journal of Pest Management* **65**, 1–9 (2018).
16. AN Sparks. *The Florida Entomologist* **62**, 82–87 (1979).
17. J Wan, et al. *Journal of Integrative Agriculture* **20**, 646–663 (2021).
18. DG Montezano, et al. *African Entomology* **26**, 286–300 (2018).
19. R Eschen, et al. *CABI Agriculture and Bioscience* **2**, 1–18 (2021).
20. IS Sobhy, et al. *Frontiers in Ecology and Evolution* **10**, 883020 (2022).
21. E Peter, et al. *Chemical and Biological Technologies in Agriculture* **10**, 61 (2023).
22. AL Erdei, et al. *eLife* **0**, e88695 (2024).
23. ZR Khan, JO Pittchar, CAO Midega, JA Pickett. *Outlooks on Pest Management* **29**, 220–224 (2018).
24. SD Eigenbrode, ANE Birch, S Lindzey, R Meadow, WE Snyder. *Journal of Applied Ecology* **53**, 202–212 (2016).