

Associational effects of Desmodium intercropping on maize resistance and secondary metabolism

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Research Article

Keywords: Associational resistance, Associational susceptibility, Associational effects, Push-pull, Intercropping

Posted Date: November 25th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3641018/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Journal of Chemical Ecology on February 2nd, 2024. See the published version at <https://doi.org/10.1007/s10886-024-01470-5>.

Abstract

Intercropping is drawing increasing attention as a strategy to increase crop yields and manage pest pressure; however the mechanisms of associational resistance remain controversial. We conducted a controlled experiment to assess the impact of co-planting with silverleaf Desmodium (*Desmodium uncinatum*) on maize secondary metabolism and resistance to herbivory by the spotted stemborer (*Chilo partellus*). Two maize varieties were grown either with *Desmodium* or adjacent to it in separate pots. Our findings indicate that co-planting with *Desmodium* influences secondary metabolism and herbivore resistance through both above and below-ground mechanisms. Stronger effects were generally observed when maize and *Desmodium* shared the same pot, implying that direct root contact or soil-borne cues play an important role in mediating associational effects on plant resistance in this system.

INTRODUCTION

Agricultural production in the 21st century is increasingly dominated by monocultural cropping systems with low biodiversity, which can have a number of adverse impacts on ecosystem functioning, such as reduced soil quality and increased pressure from pests and diseases, which in turn decrease crop yield (Horwith 1985). One strategy to mitigate the ecological costs of intensive monocultural production is intercropping, which refers to agricultural production systems where multiple crop species are simultaneously grown together in the same field. There is growing evidence that increasing the biodiversity of agroecosystems through intercropping can increase overall agricultural yields (Chai et al. 2021; Li et al. 2021b; Li et al. 2023) by increasing nutrient acquisition (Li et al. 2014) and reducing pest pressure (Trenbath 1993; Poveda et al. 2008). Diversification of agroecosystems through intercropping generally reduces herbivore and pathogen density, however the size and direction of these effects varies substantially between studies and systems (Letourneau et al. 2011; Chadfield et al. 2022; Hahn and Cammarano 2023). Intercropping could influence resistance to pests and pathogens through at least several hypothesized mechanisms, such as numerical dilution of host plants (and host plant cues) (Tahvanainen and Root 1972; Root 1973), disruption of insect host-finding by non-host cues, increased accumulation of natural enemies like predators and parasitoids, or resistance-enhancing changes in the metabolism of the focal plant species (Poveda et al. 2008; Barbosa et al. 2009). While field-level mechanisms are likely important in driving associational resistance (Finch and Collier 2000; Cook et al. 2007), there is growing recognition that direct effects on the metabolism of crop plants may also be an important component of associational resistance (Zhu and Morel 2019). In order to guide the development and successful implementation of functional intercropping systems, it is necessary to understand the underlying mechanisms that contribute to associational resistance in intercropping systems. While the concept of associational resistance was originally developed in connection with agroecosystems (Tahvanainen and Root 1972) and is often applied in this context (Barbosa et al. 2009), associational resistance can also affect herbivore pressure and community composition in natural plant communities (Massad et al. 2017; Salazar and Marquis 2022; Wang et al. 2023).

One of the most successful applications of intercropping for pest control is the “push-pull” technology developed at the International Centre of Insect Physiology and Ecology (*icipe*) in Kenya. Since its initial development in the 1990s, push-pull intercropping has been widely adopted due to its remarkable success at reducing pest pressure and increasing cereal yields on smallholder farms in Kenya and the surrounding countries (Khan et al. 2014; Ndayisaba et al. 2020). The push-pull system is thought to work by altering the chemical information space in maize fields in order to disrupt the host-finding ability of herbivores, especially stem-boring moths which are among the most damaging pests of maize and sorghum in East Africa (Khan et al. 2000; Khan et al. 2016). Specifically, cereal fields are interspersed with a repellent plant species (typically *Desmodium spp.*) to “push” pest insects away from the focal crop. At the same time, an attractive plant species, (typically *Brachiaria spp.* or Napier grass, *Cenchrus purpureus*, synonym *Pennisetum purpureum*) is planted around the edges of the field to “pull” the pests toward the periphery of the field. While there’s strong evidence from field studies for the efficacy of the push-pull intercropping technique (Khan et al. 2014; Kassie et al. 2018; Luttermoser et al. 2023), the precise mechanisms underlying these effects remains somewhat ambiguous. This is, at least in part, due to the difficulty of replicating the complex dynamics that contribute to the success of push-pull at an experimentally tractable scale. Thus, it has increasingly been called into question to what extent “push” and “pull” are the main mechanisms underlying enhanced resistance due to *Desmodium* intercropping (Erdei et al. 2022). Beyond the direct effects of intercropping on insect host-finding (i.e. the canonical push-pull mechanism (Khan et al. 2000)), intercropping could also contribute to associational resistance by directly altering the metabolism of crop plants in ways that either disrupt insect host-finding or directly enhance the resistance of crop plants (Barbosa et al. 2009; Chadfield et al. 2022). Recent work suggests that intercropping can affect leaf traits such as specific leaf area, leaf toughness, nitrogen content, as well as the concentration of secondary metabolites (Felix et al. 2023). Such neighbor-induced changes in plant secondary metabolism could, in turn, be mediated through the perception of either a) above-ground cues, b) below-ground cues, or c) a mixture of both perceptual modalities by the receiver plant.

In support of a below-ground mechanism of chemical information transduction, soil derived from push-pull fields that used *Desmodium* intercrops has previously been shown to increase the resistance of maize plants to herbivory by the larvae of the spotted stemborer, *Chilo partellus* (Mutiyambai et al. 2019). Moreover, fungal communities were found to differ markedly in the soil of push-pull fields, presumably due to soil conditioning by the intercropped *Desmodium* plants (Owuor et al. 2017; Mwakilili et al. 2021) and/or reduced soil disturbance. However, it is unclear what time scale is necessary for these soil-mediated effects to develop. At the same time, there is growing evidence that mere proximity to some of the intercrops used in push-pull intercropping can reduce stemborer oviposition by directly altering the metabolic phenotype of maize plants (Tolosa et al. 2019; Magara et al. 2020). For example, maize plants growing next to signal grass (*Brachiaria brizantha*) were found to emit more volatiles, apparently resulting in reduced oviposition by *C. partellus* (Magara et al. 2020). However, the direct effects of *Desmodium spp.* on the metabolism of nearby maize plants has not previously been investigated, even though it is the most common “push” plant used in the Kenyan push-pull system.

To address this gap, we conducted a controlled experiment to address the role of direct associational effects of *Desmodium* co-planting on maize secondary metabolism and resistance to herbivory by the common stem-boring moth *C. partellus*. Secondly, we sought to answer whether these direct effects are primarily mediated by below-ground cues (e.g. root exudates) or above-ground cues (e.g. volatile or spectral cues). We hypothesized that maize growing in the same pot with *Desmodium* would have increased herbivore resistance and defensive metabolism, in line with previous research suggesting that push-pull intercropping with *Desmodium* may condition soil to the benefit of maize (Mutiyambai et al. 2019). Alternatively, above-ground cues could be more important in determining maize metabolic response to intercropping. We also hypothesized that the ability of a plant to respond either to above- or below-ground cues could be a function of a plant's genetic background (phenotypic plasticity). Thus, we used two hybrid maize varieties (SC Duma 43 and HB 505) that are commonly used by farmers in Kenya but vary in their ability to respond to environmental stresses. For example, SC Duma 43 was previously shown to exhibit induced responses to oviposition by *C. partellus*, including increased VOC emission and herbivore resistance, whereas HB 505 was insensitive to these oviposition cues (Mutiyambai et al. 2014; Tamiru et al. 2015b).

Our results suggest that both above and below-ground neighbor cues contribute to metabolic reprogramming and herbivore resistance in maize, but stronger effects were generally observed in the co-planting treatment where maize was grown in the same pot with *Desmodium*.

METHODS

The study was conducted in 2018 at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and Ecology (*icipe*), located at Mbita Point (0° 25'S, 34° 12'E; 1,200 m above sea level) on the shore of Lake Victoria in western Kenya.

Experimental Design

We investigated the resistance of maize plants grown a) alone, b) neighboring a silverleaf *Desmodium* (*Desmodium uncinatum*) plant growing in a separate pot, or c) neighboring a silverleaf *Desmodium* plant planted in the same pot. Soil was obtained from an open field within the *icipe* Mbita campus and filled into five-liter plastic planting pots (Kenpoly, Nairobi). Two maize seeds of the same variety were planted in each pot and the pots were placed in a greenhouse (25 ± 2°C, 72 ± 5% RH; 12L: 12D). The pots were watered daily with clear water (no fertilization). Three days after seed germination, the weakest seedling was thinned out. We used two maize varieties commonly grown by farmers in western Kenya: "SC Duma 43" and "HB 505" (Almekinders et al. 2021). SC Duma 43 has been described as a "smart" maize variety, because it responds more strongly than other hybrid maize varieties to oviposition by *Chilo partellus* through induction of chemical defenses. Maize seeds were obtained from Agri-Seedco Ltd (Nairobi, Kenya). The use of just two varieties may limit our ability to draw generalizable conclusions about genotypic effects on inducibility but provides sufficient preliminary data to motivate a more detailed analysis into the mechanisms underlying neighbor-associated inducibility and signal perception. We used

spotted stemborers (*Chilo partellus* C. Swinhoe, Lepidoptera:Crambidae) for plant resistance assays, since they are a major insect pest of maize in East Africa. The insects were obtained from the insect rearing facility at *icipe*, Thomas Odhiambo campus, Mbita point (Mutiyambai et al. 2019). In the oviposition experiments, newly eclosed *C. partellus* moths were allowed to mate for 24 hours before being used in choice experiments. In the larval experiments, newly hatched neonates were used less than 24 hours after emergence.

Plant Growth Rate Measurement

Plant height was measured at weekly intervals throughout the course of the experiment by extending a tape measure from the base of each plant to the apex of the stem.

Oviposition Preference Assay

Oviposition choice assays were conducted in 165 x 60 x 60 cm cages containing three X-week old maize plants arranged in a row. Each cage contained one plant from each of the previously described *Desmodium* exposure treatments (n = 16 cages). Five freshly-eclosed female moths were introduced to each cage at dusk and left overnight to oviposit, after being allowed to mate for 24 hours. The following morning, egg clutches were counted and removed from all plants and plastic pots. The eggs were then individually counted under a stereo microscope. Since almost half of the eggs were laid on the pots rather than the plants themselves, eggs laid on the pots were included in the count for each plant under the assumption that cues from nearby plants would influence oviposition on the pot. Prior work suggests that volatile organic compounds can influence oviposition on artificial substrates by *C. partellus* (Varshney et al. 2003; Singh et al. 2011; Koul et al. 2013) as well as other stem-boring moths (Binder and Robbins 1997; Konstantopoulou et al. 2004). The number of egg clutches as well as the total number of eggs were analyzed using generalized linear mixed models with a Poisson distribution in the lme4 package (Bates et al. 2015) and observation-level random effects (OLRE) to account for over-dispersion in the data (Harrison 2014).

Larval Resistance Assays

Larval resistance was measured in both choice and no-choice feeding assays using 15 mm leaf discs cut from the second youngest fully expanded leaf. No-choice larval performance experiments were conducted in small plastic deli cups containing a layer of agar to maintain plant turgor. Slits were cut into the lids with a razor blade to allow air exchange. Ten larvae were then placed into each cup and allowed to feed for 24 hours. We assayed 8 plants from each neighborhood treatment (four plants for each neighborhood treatment x genotype combination) and the consumption of each plant was measured in two technical replicates. Choice assays were conducted in polystyrene petri dishes containing two leaf disks from different neighborhood treatments. Twenty assays were conducted for each combination of maize genotype and neighborhood treatments, such that there were forty total replicates for each combination of neighborhood treatments (coplant vs control, coplant versus exposure, and exposure vs control). Ten larvae were placed in the center of each plate and allowed to feed for 24 hours. At the end of

the feeding period, the leaf disks were photographed and the area consumed was measured in ImageJ (Schneider et al. 2012).

No-choice larval feeding assays were analyzed using linear mixed models in R (Douma and Weedon 2019) as implemented in the glmmTMB package (Brooks et al. 2017). Leaf consumption was modeled as a function of neighbor treatment and maize genotype, with the number of surviving larvae in each enclosure as a co-variate. Plant number was included as a random factor to account for individual variation in resistance. For the choice assay, the Wilcoxon signed-rank test was used to compare the feeding damage in each combination of neighbor treatments. Separate tests were conducted for each maize genotype (HB 505 and SC Duma 43), as well as an overall test for the total effect of neighborhood treatment (ignoring differences between maize lines).

Volatile Organic Compound Analysis

Volatile organic compounds (VOCs) were collected by open-flow dynamic headspace sampling, according to previously described methods (Mutyambai et al. 2019). Briefly, plants were enclosed in PET oven bags and VOCs were collected by pulling air through charcoal traps (ORBO-32, Supelco, Bellefonte, PA, USA) using a vacuum pump (GAST) powered by a 12V car battery. VOCs were collected from all plants during two 12 hour collection windows – a photophase collection window beginning at dawn (06:00–18:00) and a scotophase collection window beginning at dusk (18:00 to 6:00). VOCs were also collected from an empty bag and an empty pot containing bare soil as ambient controls. Traps were transported to the laboratory on ice and stored at -80°C until they could be extracted and analyzed.

VOCs adsorbed to the traps were eluted with 300 μ l dichloromethane into glass vials using a vacuum manifold. Samples were then analyzed on a Varian Saturn 2200 GC/MS, equipped with a CP-8400 autosampler and a PEG column (Agilent J&W DB-WAX, 30m, 0.25 mm ID, DF = 0.25). The injection temperature was held constant at 225°C and the column temperature was adjusted according to the following program: 45°C to 130°C at 10°C/minute; 130°C to 180 at 5°C/min; 180°C to 250°C at 20°C/minute; hold 250°C for 5 min. The raw data were converted to ANDI-MS CDF files with OpenChrom (Wenig and Odermatt 2010) and analyzed in MS-DIAL (version 4.9.221218) with default parameters except for the minimum peak height which was set to 400 (Tsugawa et al. 2015). VOCs from the two collection windows (photophase and scotophase) were analyzed separately. The resulting peak tables were imported into R for further statistical analysis using mzinspectr (Bass 2023a). Peak areas were then standardized by subtracting the median area of each feature appearing in the ambient soil control from the area of the same feature in each sample. Features identified as likely contaminants (e.g. siloxanes) or appearing in less than 60% of our samples were excluded from the data set. Finally, data were normalized using probabilistic quotient normalization (Dieterle et al. 2006). Features were tentatively identified by matching the retention indices and mass spectra to an internal database derived from commercial standards run in the lab and the NIST 11 Mass Spectral Database.

Leaf and Root Secondary Metabolite Analysis

To investigate the effects of neighborhood on maize metabolism, leaves and roots were harvested at the end of the experiment, submerged in 50% methanol (1 ml/sample) and stored at -20°C. Leaf and root samples were later homogenized by adding 0.9 g Zircon grinding beads (BioSpec, Zirconia/Silica 2.3mm) and beating them in a FastPrep tissue homogenizer (MP Biomedicals, Solon, Ohio, USA). All tissues were beaten twice at 6 m/s for 45 s. The homogenized tissue was then doubly centrifuged at 4°C (6 min at 14,000 rpm/20,800 × g), drawing off the supernatant after each round of centrifugation. Finally, the supernatant was analyzed by high performance liquid chromatography (Agilent 1100 HPLC coupled to a G1315A diode-array detector) with a Gemini C18 reverse-phase column (3 µm, 150 × 4.6 mm, Phenomenex, Torrance, CA, USA). The elution system consisted of aqueous 0.1% phosphoric acid (H_3PO_4) and methanol (MeOH) which were pumped through the column at a rate of 0.7 ml/min with increasing concentrations of methanol: 0–10 min, 20% MeOH; 10–35 min, 20–35% MeOH; 35–50 min, 35–95% MeOH; 50–60 min, 95% MeOH. Raw HPLC data were then imported into R using the chromConverter package v0.4.2 (Bass 2023b) and entab v0.3.1 (Bovee et al. 2022) and processed in chromatographR v0.5.3 (Bass 2022). All chromatograms were normalized by the fresh weight of the plant tissue used in each extraction. Where possible, compounds were identified by matching their retention times and UV spectra to commercial standards.

Statistical Analysis

All data was analyzed in R 4.2.1 (R Core Team 2023). Chemical data were analyzed by permutational analysis of variance to look for overall compositional differences between maize lines and intercropping treatments (*adonis2* in the vegan package using bray-curtis distances and 999 permutations) (Anderson 2001; Oksanen et al. 2022). These tests were then followed up with pairwise PERMANOVAs (*pairwise.adonis2* in the pairwiseAdonis package (Martinez Arbizu 2017)) to determine which of the neighbor treatments were driving differences in plant secondary metabolism. Feature selection was performed with the Boruta algorithm to identify the individual features contributing most strongly to the compositional differences observed among treatment groups (Liaw and Wiener 2002; Kursu and Rudnicki 2010). All figures were generated in R with ggplot2 (Wickham 2016).

RESULTS

Plant Height

Maize plants in the co-cultivation treatment grew less rapidly (-0.64 (95% CI -0.97, -0.31; $p < 0.001$)) than the other two treatments, however there was no evidence that maize growth was affected by above-ground neighbor cues (-0.15 (95% CI -0.49, 0.18; $p = 0.4$)) (Supplementary Table 1).

Oviposition Preference

A total of 232 *C. partellus* egg clutches were collected, of which 141 were laid on maize plants, 86 were laid on pots and 5 were laid on *Desmodium* plants. Because of the large number of eggs laid on pots (37% of the total), these were included in the count for the respective plant. Intercropping with

Desmodium reduced oviposition by spotted stemborers resulting in fewer total eggs (-1.3 (95% CI -2.5, -0.18; $p = 0.024$)) and fewer egg clutches (-0.68 (95% CI -1.5, 0.14; $p = 0.10$)) (Fig. 1, Supplementary Table 2). However, maize plants merely grown in proximity to a *Desmodium* plant attracted the oviposition of more eggs compared to control plants (1.2 (95% CI 0.12, 2.3; $p = 0.029$)) as well as more eggs per clutch (0.74 (95% CI 0.14, 1.3; $p = 0.016$)). No evidence was found that above-ground exposure to a *Desmodium* plant altered the number of egg clutches received (0.37 (95% CI -0.33, 1.1; $p = 0.3$)), indicating that the moths laid larger clutches on maize plants neighboring a *Desmodium*. Of the three plants that received no eggs, all of them were Duma co-planted with *Desmodium*.

More egg clutches were laid overall on HB 505 than SC Duma 43 (0.73 (95% CI 0.01, 1.5; $p = 0.048$)). There was limited evidence that the effect of above-ground neighbor cues on oviposition preference was weaker in HB 505 compared to SC Duma 43 for total eggs (-1.6 (95% CI -3.1, 0.02; $p = 0.053$)) as well as the number of egg clutches (-0.86 (95% CI -1.8, 0.09; $p = 0.077$)). There was no clear evidence that the effect of *Desmodium* co-planting was weaker in HB 505 compared to SC Duma 43 (1.2 (95% CI -0.38, 2.8; $p = 0.14$)).

Larval Resistance

Co-planting with *Desmodium* did not affect larval consumption (-0.24 (95% CI -0.68, 0.20; $p = 0.3$)) in the no-choice assay. However, the above-ground exposure treatment increased the leaf area eaten by the *C. partellus* caterpillars (0.48 (95% CI 0.07, 0.88; $p = 0.021$)) (Fig. 2, Supplementary Table 4). Larval consumption was also very strongly correlated with the number of larvae surviving in each cup at the end of our experiment (0.21 (95% CI 0.09, 0.33; $p < 0.001$)), however there was no evidence that larval mortality differed between treatments (Supplementary Table 5).

In the larval choice assay, there was no evidence that neighborhood affected the number of *C. partellus* larvae attracted to leaf disks (Supplementary Table 6) and little evidence that neighborhood affected the proportion of leaf area eaten by *C. partellus* larvae (Supplementary Table 7). However, larvae ate a slightly greater proportion of leaf area in control plants (0.18 ± 0.02) compared to plants exposed to above-ground *Desmodium* cues (0.13 ± 0.01) ($p = 0.047$). There was weak evidence that more larvae were also attracted to leaf disks from control plants (6 ± 0.5) compared to plants exposed to above-ground *Desmodium* cues (4 ± 0.5) ($p = 0.1$).

Maize Secondary Metabolism

Volatile Organic Compounds

VOC emissions were analyzed to assess changes in the general metabolic state of maize plants induced by exposure to above- and below-ground *Desmodium* cues. VOC bouquets in both collection windows (day and night) differed by treatment ($F_{day} = 2.26$, $p_{day} = 0.0054$; $F_{night} = 1.88$, $p_{night} = 8e-04$) as well as plant genotype ($F_{day} = 2.06$, $p_{day} = 0.034$; $F_{night} = 2.23$, $p_{night} = 4e-04$) (Supplementary Table 8 & Supplementary Table 9, Fig. 3C-D). Pairwise tests suggest that during the photophase collection period,

the composition of maize headspace VOCs was only significantly altered by *Desmodium* co-planting in comparison to the control ($F_{day} = 3.19$, $p_{day} = 0.005$). However, during the scotophase collection period, all pairwise comparisons showed significant differences between treatments: above-ground exposure versus control ($F_{night} = 1.76$, $p_{night} = 0.02$), *Desmodium* co-planting versus the control ($F_{night} = 1.83$, $p_{night} = 0.011$), and *Desmodium* co-planting versus exposure ($F_{night} = 2.09$, $p_{night} = 0.0015$). Total volatile organic compound production did not differ between neighbor treatments ($F_{day} = 2.61$, $p_{day} = 0.11$; $F_{night} = 1.67$, $p_{night} = 0.22$) or genotypes ($F_{day} = 1.17$, $p_{day} = 0.3$; $F_{night} = 0.19$, $p_{night} = 0.67$) during either collection window.

A number of VOCs were also identified in both collection windows through Boruta feature selection (Kursa and Rudnicki 2010) as important features that explain differences in the VOC composition between treatments. The Boruta algorithm selected 11 features during the photophase collection window and 14 in the scotophase collection window as important predictors of neighborhood treatment. Maize co-planted with *Desmodium* showed elevated levels of short-chain aldehydes like octanal and decanal during both collection windows (Fig. 4B-C). During the photophase collection window, a number of terpenoids, such as 3,7-Dimethylocta-1,3,7-triene (ocimene), 3,7-Dimethylocta-1,6-dien-3-ol (linalool) and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) were suppressed to varying degrees (Fig. 4D). At night however, when adult stemborers are active, linalool and TMTT were strongly induced by above-ground *Desmodium* exposure, while ocimene emissions were equal across treatments (Fig. 4E).

Differences between treatments were also visualized by principal components analysis. During the photophase collection window, VOC bouquets from maize plants co-planted with *Desmodium* were shifted in chemical space along PC1 (Fig. 3C). PC1 was strongly influenced by alkylbenzenes such as 2-Ethyltoluene (1-ethyl-2-methyl-benzene), psuedocumene (1,2,4-trimethyl benzene), durene (1,2,4,5-tetramethyl-benzene), and mesitylene (1,3,5-trimethylbenzene), which were induced by co-planting with *Desmodium* and to a lesser degree by above-ground exposure (Fig. 4A). Linalool and TMTT were also associated with PC1 but had negative loadings, indicating that they are negatively correlated with the alkylbenzenes (Fig. 4D)).

Leaf and Root Metabolites:

Leaf and root chemistry were both altered in maize co-planted with *Desmodium* in the same pot, however there was no effect of above-ground exposure. Benzoxazinoids were strongly suppressed by co-planting with *Desmodium* in root tissue and to a lesser extent in leaves (Fig. 6).

We conducted permutational analysis of variance on peak tables generated from leaf and root chromatograms. Both leaf and root metabolites were strongly affected by neighborhood ($F_{leaf} = 2.77$, $p_{leaf} = 0.001$; $F_{root} = 6.8$, $p_{root} = 0.001$) as well as plant genotype ($F_{leaf} = 1.19$, $p_{leaf} = 0.29$; $F_{root} = 2.69$, $p_{root} = 0.054$), but there was no evidence of an interaction between neighborhood and genotype ($F_{leaf} = 0.83$, $p_{leaf} = 0.66$; $F_{root} = 0.96$, $p_{root} = 0.45$) (Supplementary Table 11, Supplementary Table 10).

In pairwise PERMANOVA tests, leaf metabolites were altered by *Desmodium* co-planting compared with the control ($F_{leaf} = 4.3$, $p_{leaf} = 0.001$) and above-ground exposure ($F_{leaf} = 3.01$, $p_{leaf} = 0.003$) treatments, but no evidence was found of a difference between the above ground exposure treatment and the control ($F_{leaf} = 0.67$, $p_{leaf} = 0.79$). Similarly, root metabolites differed between the co-planted maize and the control treatments ($F_{root} = 4.3$, $p_{root} = 0.001$) as well as the above-ground exposure treatment ($F_{root} = 3.01$, $p_{root} = 0.003$), but not between the exposure treatment and the control ($F_{root} = 0.67$, $p_{root} = 0.77$).

The Boruta algorithm selected 9 peaks in leaves and 15 peaks in roots as important predictors of neighborhood treatment. In the root dataset, this included many of the features with the highest loadings along PC1 including the top six features, which were mainly benzoxazinoids. Co-planting with *Desmodium* resulted in major suppression of root benzoxazinoids including 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (-26 (95% CI -41, -11; $p = 0.002$; $q = 0.004$)) and 6-methoxy-2-benzoxazolinone (MBOA) (-11 (95% CI -23, 0.05; $p = 0.051$; $q = 0.2$)), as well as several unidentified benzoxazinoid glycosides (Fig. 6B). Suppression of leaf benzoxazinoids was also observed, though to a much lesser degree (Fig. 6A).

Figure 6 Metabolites in leaves (panel A) and roots (panel B) of maize plants exposed to different *Desmodium* neighborhood treatments. Boxes sharing no letters in common are significantly different by Tukey's range test at a 5% significance level in univariate comparisons. Plots are also annotated with p-values and FDR-corrected q-values from a two-way ANOVA testing the effects of neighborhood treatment (crossed with genotype) on each metabolite. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), MBOA (6-methoxy-2-benzoxazolinone) and three unidentified hydroxycinnamic acid glycosides (Bx-GLC) were strongly suppressed in the roots of maize plants that were co-planted with *Desmodium uncinatum*. In the leaves, DIMBOA, MBOA and an unidentified hydroxycinnamic acid (HC1) were moderately suppressed in maize co-planted with *D. uncinatum*. Metabolites marked with a dagger (†) were selected by Boruta random feature selection as important predictors of neighborhood treatment.

DISCUSSION

While there's substantial evidence from the field that intercropping with *Desmodium* effectively reduces herbivory by cereal stemborers (Khan et al. 2008; Ndayisaba et al. 2020), this is one of the first published datasets to show directly that interplanting with *Desmodium* can reduce oviposition by stemborers at a microcosmic scale. Most importantly, we demonstrate that maize secondary metabolism can be directly altered through association with a neighboring *Desmodium* plant. The effects of co-planting with *Desmodium* on maize secondary metabolism was much greater in magnitude than the effect of maize genotype (Supplementary Table 8, Supplementary Table 9, Supplementary Table 10, Supplementary Table 11). While both above and below-ground neighbor cues influenced maize metabolism, the effects were stronger when maize plants shared the same pot with *Desmodium*, suggesting that direct root contact or soil-borne chemical cues are an important factor in the perception of neighborhood by maize. These neighbor-associated metabolic changes could influence resistance to herbivores and/or

pathogens, promoting either associational resistance or susceptibility. Thus, these results have major implications for the study and application for other intercropping systems.

Contrary to our expectations, some defensive leaf and root metabolites such as benzoxazinoids were strongly suppressed by *Desmodium* co-planting. However emissions of some VOCs such as short-chain aldehydes and alkylbenzenes were induced, especially during the photophase collection window. We also found evidence that the modality of the interaction between *Desmodium* and maize can affect attractiveness to herbivores. *Chilo partellus* adults were less likely to oviposit on plants co-planted with *Desmodium* in the oviposition choice assay (Supplementary Table 2). However, plants exposed only to above-ground neighbor cues showed increased susceptibility to stemborer herbivory in the oviposition choice assay (Fig. 1, Supplementary Table 2, and in a no-choice larval resistance assay (Fig. 2).

The experimental approach used here cannot determine whether and to what extent the avoidance of intercropped plants by *C. partellus* is driven primarily by neighbor-induced changes in maize metabolism or by cues associated with the *Desmodium* plant itself. However, the concordance between stemborer preference in the larval feeding assay and the oviposition choice assay suggests that the avoidance of intercropped plants may accurately reflect differences in the palatability of maize between treatments. Thus, the data suggest that neighbor-associated metabolic changes may be an important mediator of resistance in push-pull intercropping. In consequence, our data raise important new hypotheses on the chemical ecology of interspecific plant-plant interactions in general and intercropping applications in particular.

Functional and ecological implications of neighbor-associated metabolic changes

Several recent studies have shown that neighbor effects associated with various intercrops used in push-pull intercropping can reduce oviposition by stemborers (Tolosa et al. 2019; Magara et al. 2020). For example, maize plants growing next to molasses grass (*Melinis minutiflora*) or herbivore-induced signal grass (*Brachiaria brizantha*) showed increased emissions of volatiles, apparently resulting in reduced oviposition by *C. partellus* (Tolosa et al. 2019; Magara et al. 2020). In both cases, short-chain aldehydes like nonanal and decanal as well as E- β -farnesene were induced by the presence of a neighbor in at least some maize varieties. Our study adds to this body of work suggesting that associational effects on maize secondary metabolism could contribute to the benefits of push-pull intercropping. Like these prior studies, we found that neighbor perception had a strong effect on VOC emissions as well as effects on oviposition and larval feeding by *C. partellus*. Unlike these prior studies however, we did not find evidence of major differences in plasticity between SC Duma 43 and HB 505.

SC Duma 43 maize plants exposed to above-ground cues from a neighboring *Desmodium* plant received more eggs (Fig. 1, Supplementary Table 2) and were consumed at a higher rate in the no-choice larval feeding assay (Fig. 2, Supplementary Table 4). However, this was slightly contradicted by the results of our choice assay where plants exposed to above-ground *Desmodium* cues were slightly preferred by *C.*

partellus larvae (Supplementary Table 6, Supplementary Table 7). The increased consumption of neighbor-exposed SC Duma 43 plants suggests that the response of SC Duma 43 to above-ground neighbor cues may actually suppress direct resistance to larval feeding. This slightly contradicts the results of other recent studies, which have generally shown reduced oviposition by *C. partellus* and increased attraction of parasitoid wasps to maize exposed to intercrops such as *Brachiaria brizantha* (signal grass) and *Melinis minutiflora* (molasses grass) (Tolosa et al. 2019; Magara et al. 2020). However, these other studies have not investigated the effect of neighborhood on larval feeding. It also cannot be ruled out that our results instead reflect compensatory feeding by stemborer larvae due to reduced digestibility of maize leaves (Browne 1975). Thus, more work is needed to assess how neighbor-associated changes in crop metabolism affect herbivore feeding and performance.

Implications of Neighborhood-Associated Shifts in VOC Composition

A number of VOCs were identified during both collection windows that are influenced by *Desmodium* neighborhood and could impact plant resistance to herbivores and pathogens. For example, short-chain aldehydes like octanal and decanal were upregulated at both day and night. These compounds elicit very strong electrophysiological responses in a variety of stemborer species, including *Chilo partellus* and *Busseola fusca* (Waladde et al. 1990; Birkett et al. 2006; Huang et al. 2009; Molnár et al. 2015), suggesting that they may play a role in host recognition. However the literature contains conflicting evidence about the specific effects of these compounds on stemborer behavior, as they have alternatively been reported as stemborer oviposition stimulants (Khan et al. 2000; Solé et al. 2010; Molnár et al. 2015) and repellents (Konstantopoulou et al. 2004; Huang et al. 2009; Jiang et al. 2015; Magara et al. 2020; Yu et al. 2020) in different studies. This variability could be related to variation in the relative or absolute concentrations of these compounds (Molnár et al. 2015) or differences in behavior between different stemborer species. Decanal is induced by stemborer oviposition (Tamiru et al. 2011; Mutyambai et al. 2016) in maize and sorghum (Shwetha and Padmaja 2023), so it may be perceived by stemborers as a cue of unpalatability or increased potential for competition. Short-chain aldehydes have also been reported to deter oviposition by other maize pests like the maize weevil (*Commiphora rostrata*) (Lwande et al. 1992). Such compounds may also play a role in the attraction of predators (Yu et al. 2008) and parasitoids to infested maize plants (Yu et al. 2010; QingXing et al. 2015; Yang et al. 2016).

Co-planting with *Desmodium* also led to the suppression of some volatile terpenoids during the daytime collection window, such as (E)- β -ocimene and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). At night however, TMTT and linalool were strongly induced by exposure to above-ground *Desmodium* cues. Biosynthesis of linalool and TMTT are dependent on the same enzyme (TPS2) (Richter et al. 2016) and are systemically induced in herbivore damaged plants (Turlings and Tumlinson 1992; Escobar-Bravo et al. 2022). Both of these compounds have previously been proposed to play a role in the attraction of parasitoids (Turlings et al. 1991; Bruce et al. 2009; Tamiru et al. 2011; Tamiru et al. 2015a) and/or the repulsion of stemborers from push-pull fields (Khan et al. 2000). Linalool is induced in maize plants by herbivory (Turlings and Tumlinson 1992; Tamiru et al. 2012; Pinto-Zevallos et al. 2016; Sokame et al.

2019) and elicits a strong electrophysiological response from stemborers (Birkett et al. 2006) and *Spodoptera frugiperda* moths (Malo et al. 2004; Pinto-Zevallos et al. 2016). Linalool is generally considered to be an oviposition stimulant for gravid stemborers (Khan et al. 2000; Magara et al. 2020) and also functions as an attractant of sixth-instar fall armyworm caterpillars (*S. frugiperda*) (Carroll et al. 2006), however it's unclear how it influences oviposition behavior in fall armyworm. Increased emissions of linalool could explain the increased attraction of *C. partellus* to maize plants conditioned by above-ground *Desmodium* cues in our study. TMTT is also induced by stemborer oviposition (Tamiru et al. 2011) and may repel spotted stemborers (Magara et al. 2020) as well as attracting parasitoids (Turlings and Tumlinson 1992; Bruce et al. 2009; Tamiru et al. 2011; Tamiru et al. 2015a).

Many of the volatile secondary metabolites altered by *Desmodium* neighborhood also play a role in pathogen resistance, suggesting that metabolic changes associated with push-pull intercropping could contribute to the suppression of mycotoxigenic corn ear-rots observed in several recent studies (Owuor et al. 2017; Njeru et al. 2020). Several of the VOCs upregulated by *Desmodium* co-planting have direct antifungal activity or have been shown to induce resistance to pathogen infection via plant-plant communication. For example, octanal and decanal have been shown to increase pathogen resistance by directly inhibiting the growth of plant pathogens and mycotoxigenic molds (Zeringue and McCormick 1990; Hamilton-Kemp et al. 1992; Wright et al. 2000; Fauguel et al. 2017; Quintana-Rodriguez et al. 2018; Li et al. 2021a). These short-chain aldehydes are also emitted by maize kernels and silks, where they have been proposed as a key factor affecting resistance to *Aspergillus* ear rots and aflatoxin contamination (Zeringue and McCormick 1990; Zeringue et al. 1996; Zeringue 2000; Wright et al. 2000). Genetic variation in the production of these aliphatic aldehydes has been associated with reduced susceptibility to aflatoxin contamination in the field (Zeringue et al. 1996). Nonanal has also been implicated as a mediator of induced resistance against pathogens via plant-plant communication. For example, exposure to nonanal increased resistance to pathogen infection in barley (*Hordeum vulgare* L.) (Brambilla et al. 2022) and lima bean (*Phaseolus lunatus* L.) by inducing pathogenesis-related protein expression (Yi et al. 2009; Girón-Calva et al. 2012). Linalool also inhibits germination of *Aspergillus* and *Fusarium* spores (Li et al. 2022; Qi et al. 2023).

Thus, while the suppression of mycotoxigenic fungi in push-pull fields has generally been attributed to suppression of insects that vector the disease, our results suggest that intercropping with *Desmodium* could also contribute to the suppression of pathogens through the direct induction of defensive metabolism. The reduction of benzoxazinoids in maize roots could also contribute to the suppression of mycotoxigenic fungi that may gain a competitive advantage through resistance to these compounds (Saunders and Kohn 2009). Such a mechanism could also contribute to the large effects of soil type on the efficacy of push-pull intercropping observed in some previous studies (Mutyambai et al. 2019). Future work should investigate if push-pull intercropping alters the chemistry of maize kernels or silks in a way that increases resistance to fungal pathogens.

Implications of Neighborhood-Associated Benzoxazinoid Suppression

The other major effect of *Desmodium* co-planting in our study was the suppression of benzoxazinoid production in roots and (to a lesser extent) in leaves. Benzoxazinoids play a major role in structuring soil microbial communities and are generally considered to improve herbivore and pathogen resistance but can have variable effects on plant growth and yield (Hu et al. 2018; Cotton et al. 2019; Cadot et al. 2021). Exposure to benzoxazinoids in soil has generally been shown to reduce maize growth while increasing herbivore and pathogen resistance (Hu et al. 2018; Cadot et al. 2021). However, in some cases, benzoxazinoids can increase maize yields by mitigating heterospecific negative plant soil-feedbacks (Gfeller et al. 2023). MBOA and 2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one (HDMBOA) were recently identified as inhibitors of biological nitrification inhibition (BNI) (Otaka et al. 2022, 2023), suggesting that benzoxazinoid exudation may be part of a strategy to increase nitrogen use efficiency. Thus, it is possible that the presence of *Desmodium* obviates the need to engage in BNI by increasing nitrogen availability and uptake. Alternatively, competition with *Desmodium* may drive down soil nitrogen levels low enough that investment in biological nitrification inhibition no longer provides a net benefit. While intercropping with *Desmodium* increases nitrogen availability in the field (Drinkwater et al. 2021), it's unclear how the presence of *Desmodium* might affect nitrogen availability in a pot environment. Previous studies have found that ammonium induced the biosynthesis of biological nitrification inhibitors in *Brachiaria humidicola* and rice (*Oryza sativa* L.) (Subbarao et al. 2007; Zhang et al. 2019). Thus, very low levels of nitrogen availability may suppress BNI activity.

Benzoxazinoids (and their degradation products) also have in vitro antifungal activity against many plant pathogens (Martyniuk et al. 2006; Song et al. 2011; Duan et al. 2022) and it is generally assumed that they function to suppress pathogens in the rhizosphere. However, many fungal pathogens of cereals are able to detoxify benzoxazinoids which may limit the efficacy of these compounds as antifungal defenses (Glenn et al. 2001; Robert and Mateo 2022). In fact, several lines of evidence suggest that benzoxazinoids may increase pathogenesis in maize. For example, colonization of maize by mycotoxigenic *Fusarium* ear rots was reduced in benzoxazinoid knockout lines (Saunders and Kohn 2009) and benzoxazinoids have even been used in a semi-selective medium for the isolation of mycotoxigenic *Fusarium* species like *F. verticilloides* (Glenn et al. 2001). Conflicting evidence on the role of benzoxazinoids in pathogen suppression has also been reported based on amplicon sequencing data. Whereas benzoxazinoids have sometimes been found to suppress the abundance of OTUs associated with plant pathogens in rhizosphere soil (Kudjardjie et al. 2019; Cotton et al. 2019), other studies have found that benzoxazinoids were associated with enrichment of pathogen-associated OTUs (Cadot et al. 2021). In leaves, benzoxazinoids like DIM²BOA-Glc (2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3-one) may also be associated with increased susceptibility to Northern Corn Leaf Blight (*Exserohilum turcicum*) (Yang et al. 2019).

Neighbor perception is dependent on interaction modality but not plant genotype

Maize secondary metabolism was affected differently by the presence of a neighboring *Desmodium* plant according to the modality of the interaction. For example, leaf and root secondary metabolism were

both suppressed in maize plants growing in the same pot with *Desmodium*, but not in plants exposed only to above-ground *Desmodium* cues. Meanwhile, VOC emissions were affected by both *Desmodium* treatments (but not always in the same way). We found little evidence for our prediction that SC Duma 43 would be more responsive to neighborhood as compared with HB 505. There was no significant interaction between treatment and genotype in our multivariate analyses, suggesting that the overall metabolic response to neighborhood treatment was not heavily influenced by plant genetic background under our experimental conditions. Interactions with genotype were observed for some metabolites in univariate analyses but no consistent pattern was observed. Nevertheless, there was weak evidence that the effects of *Desmodium* neighborhood on oviposition were weaker in the HB 505 genetic background compared to SC Duma 43.

Mechanisms of neighbor perception

While it's known that plants can respond to the perception of a neighbor through multiple sensory modalities, such as nutrient limitation (Bechtold and Field 2018), shading (Ballaré 2014), and detection of root exudates (Wang et al. 2020), it remains poorly understood how these multiple stimuli are integrated by the plant to produce an adaptive response (Bilas et al. 2021; Chautá and Kessler 2022). Our experiment suggests that associational effects on plant resistance and metabolism may be dependent on the integration of above- and below-ground neighbor cues.

Aboveground Mechanisms of Associational Effects

While associational resistance in the push-pull intercropping system has generally been attributed to volatile-mediated plant-plant communication, it cannot be ruled out that the observed effects could also be influenced by other perceptual modalities. For example, light-based cues are known to have complex effects on plant immunity and the allocation of resources to plant defense (Ballaré et al. 2012; Ballaré 2014). Moreover, growing evidence suggests that spectral neighbor cues may be integrated with volatile cues to produce complex responses in receiver plants (Chautá and Kessler 2022; Escobar-Bravo et al. 2022). Nevertheless, prior experiments on associational effects mediated by push-pull intercropping have generally assumed that above-ground communication between plants is mediated primarily by VOCs. Accordingly, these experiments have generally not attempted to control for alternative modalities of neighbor-perception such as spectral reflectance cues. Our experiment is no exception in this regard, since maize plants were simultaneously exposed to VOC and light cues in both *Desmodium* exposure treatments. Thus, more mechanistic work is needed to understand different modalities of neighbor perception by plants and how they may interact to shape associational effects of intercropping on plant performance and resistance.

The neighborhood-induced changes in the VOC profiles of our plants were broadly consistent with prior studies conducted with molasses grass and *Brachiaria spp.* grass, suggesting that these neighborhood effects could represent a non-specific response to increased planting density. Recent studies have found that exposure to far red light can increase the sensitivity of plants to induction by HIPVs from a neighbor (Chautá and Kessler 2022; Escobar-Bravo et al. 2022). Thus, multiple neighbor cues may interact to

determine a plant's response. While increased exposure to far-red light generally reduces the resource allocation to defense (Ballaré 2014), it has been suggested that, in some cases, plants may compensate for these changes through increased investment in indirect defenses (Cortés et al. 2016; Chautá and Kessler 2022; Escobar-Bravo et al. 2022). Thus, phytochrome-mediated changes in plant metabolism could, in principle, explain the pattern of increased VOC emissions associated with proximity to intercrops observed in several recent studies. Our results are broadly consistent with the predicted trade-off between direct and indirect defenses under far-red light exposure (Ballaré 2014), insofar as plants exposed to above-ground neighbor cues showed increased VOC emissions but reduced resistance to larval feeding. Unfortunately, most studies on direct associational effects of push-pull intercropping have not reported data on direct resistance to larval feeding, so it is unclear how common this pattern may be.

Belowground Mechanisms of Associational Effects

Co-cultivation with *Desmodium* resulted in major suppression of secondary metabolites (primarily, benzoxazinoids) in maize roots and, to a lesser extent, in leaves, while also changing the emissions profile of VOCs. This pattern somewhat contradicts the evidence from several other studies, where competition has generally been shown to increase benzoxazinoid content in maize (Ding et al. 2015) as well as other cereals like wheat and rye (Zhang et al. 2016; Kong et al. 2018; Rakoczy-Trojanowska et al. 2020). The mechanisms and functional significance of this suppression of defensive metabolism aren't entirely clear. Several factors could contribute to the pattern we observed, such as changes in nutrient availability, microbial community composition, light quality, or direct chemical interactions between the plants.

Since maize plants grown in the intercropping treatment showed reduced height, the suppression of benzoxazinoids could simply be the result of resource limitation. In line with this hypothesis, nutrient limitation has been shown to suppress benzoxazinoid content in some prior work (Walker et al. 2012), however high fertility has also been shown to reduce benzoxazinoid content in rye (Mwaja et al. 1995) and maize (Nie et al. 2005). There could also be differential impacts on benzoxazinoid production depending on which nutrient is limited. For example, there is some evidence that benzoxazinoids may be suppressed by nitrogen limitation but promoted by phosphorus limitation (Schlüter et al. 2013; Ma et al. 2020).

Alternatively, microbes promoted by *Desmodium* could contribute to reduced investment in benzoxazinoids by maize. There is mixed evidence on the role of microbial interactions on benzoxazinoid production. However, current evidence suggests that colonization by AMF or other beneficial microbes generally increases investment in benzoxazinoid production in maize (Song et al. 2011; Walker et al. 2012). In wheat however, there is some evidence that colonization by arbuscular mycorrhizal fungi may reduce benzoxazinoid content in roots (Frew et al. 2018).

Alternatively, a strong shade avoidance response could underlie the observed disinvestment in root defenses (Ballaré and Austin 2019). Shading is generally understood to reduce investment in root growth in favor of increasing investment in above-ground biomass. This reduced allocation to root growth could

also translate into reduced defensive investment. Several recent studies have found evidence of increased VOC emissions in response to shading (Cortés et al. 2016; Chautá and Kessler 2022; Escobar-Bravo et al. 2022), suggesting that phytochrome-mediated suppression of direct defenses may sometimes be compensated by increased investment into VOC production. However, the insensitivity of root metabolism to the above-ground exposure treatment suggests that shading is insufficient on its own to completely explain the observed effect.

Finally, maize plants may be responding directly to chemical cues emitted from the roots of the neighbor. A study in rye found that competition with various species of legumes reduced the content of aglycone benzoxazinoids in roots but increased the content of several benzoxazinoid glycosides (Hazrati et al. 2020, 2021). These shifts in root benzoxazinoid composition were interpreted as an adaptive strategy, since benzoxazinoid glycosides are more mobile in soil and may thus be more toxic to neighboring plants. Competition also reduced the content of 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) in rye shoots, however other benzoxazinoids were not affected by competition (Hazrati et al. 2021).

Thus, our results suggest that below-ground maize metabolism is highly responsive to the presence of a neighbor and these changes apparently depend on the perception of below-ground neighbor cues. In contrast, above-ground neighbor perception had a substantial effect on foliar VOC emissions, but did not perceptibly alter root or leaf metabolism. Some above-ground volatile emissions were attenuated when plants were simultaneously exposed to above- and below-ground cues (4).

It also cannot be ruled out that interactions with *Desmodium* in pots are not fully reflective of what happens in the field. For example, competition between potted plants may be stronger compared to intercropped plants in field settings. The observed effects of *Desmodium* co-planting on maize secondary metabolism could be specific to the pot environment, since we controlled for a number of factors that could affect the plant response in field settings. Thus, more work is needed to understand the mechanisms and adaptive significance of multimodal integration of information about neighborhood.

CONCLUSION

Our results provide additional evidence that *Desmodium* intercropping mediates associational resistance to herbivory in maize plants and expand our understanding of its underlying mechanisms beyond the initially hypothesized push-pull mechanism. In addition to the resistance-mediating effects of *Desmodium* intercropping on soil quality described in a previous study (Mutyambai et al. 2019), we now add direct signal transduction between plants as an additional mechanism through which intercropping could enhance maize resistance. This suggests that the resistance-mediating effects of push-pull intercropping may depend on multiple mechanisms, including direct host disruption by intercrop cues (i.e. the canonical push-pull mechanism), soil-mediated resistance, as well as direct signal transduction between plants, both above and below ground. To further advance our understanding, future research should focus on quantifying the relative contributions of these mechanisms to associational resistance in intercropping systems. Our findings also emphasize the potential for improving intercropping

strategies by developing a more detailed understanding of the specific chemical cues and signal transduction pathways that shape resistance-mediating changes in crop-plant metabolism. Enhanced knowledge of these mechanisms could prove invaluable for the further development and refinement of functional intercropping strategies.

Declarations

Statements and Declarations

Funding

Funding for this study was provided by an Academic Venture Fund and a Postdoctoral Fellowship Award from the Atkinson Center for Sustainable Future at Cornell University. The following organizations and agencies provide financial assistance to the Push-Pull Programme of icipe through various projects: European Union, UK's Foreign, Commonwealth & Development Office (FCDO); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Norwegian Agency for Development Cooperation (NORAD); Biovision Foundation (Switzerland) the Federal Democratic Republic of Ethiopia and the Government of the Republic of Kenya.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design of the study. Experiments were conducted by DM. EB analyzed the data and wrote the first draft of the manuscript. All authors contributed to and approved the final version of the manuscript.

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Figures

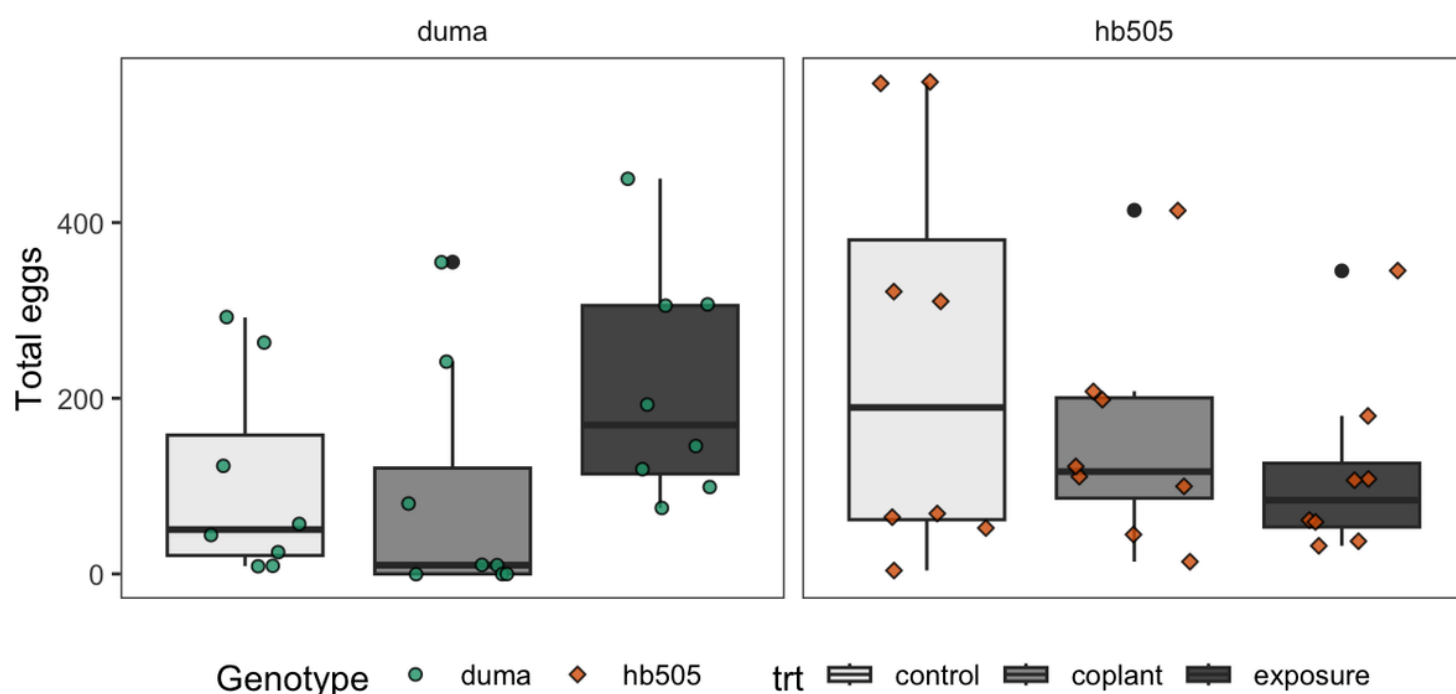


Figure 1

Box plot showing the total number of eggs oviposited by gravid *C. partellus* moths on maize plants by *Desmodium* neighborhood and plant genotype in a three-way choice test ($n = 8$ cages per genotype). *C. partellus* moths were offered a choice between an isolated (control) maize plant, a maize plant occupying the same pot with a *Desmodium unincatum* neighbor (coplant) or a maize plant that was previously exposed to visual and volatile cues from a neighboring *D. unincatum* plant in a separate pot (exposure).

Data are plotted by maize variety – either SC Duma 43 (left panel, teal circles) or HB 505 (right panel, red diamonds).

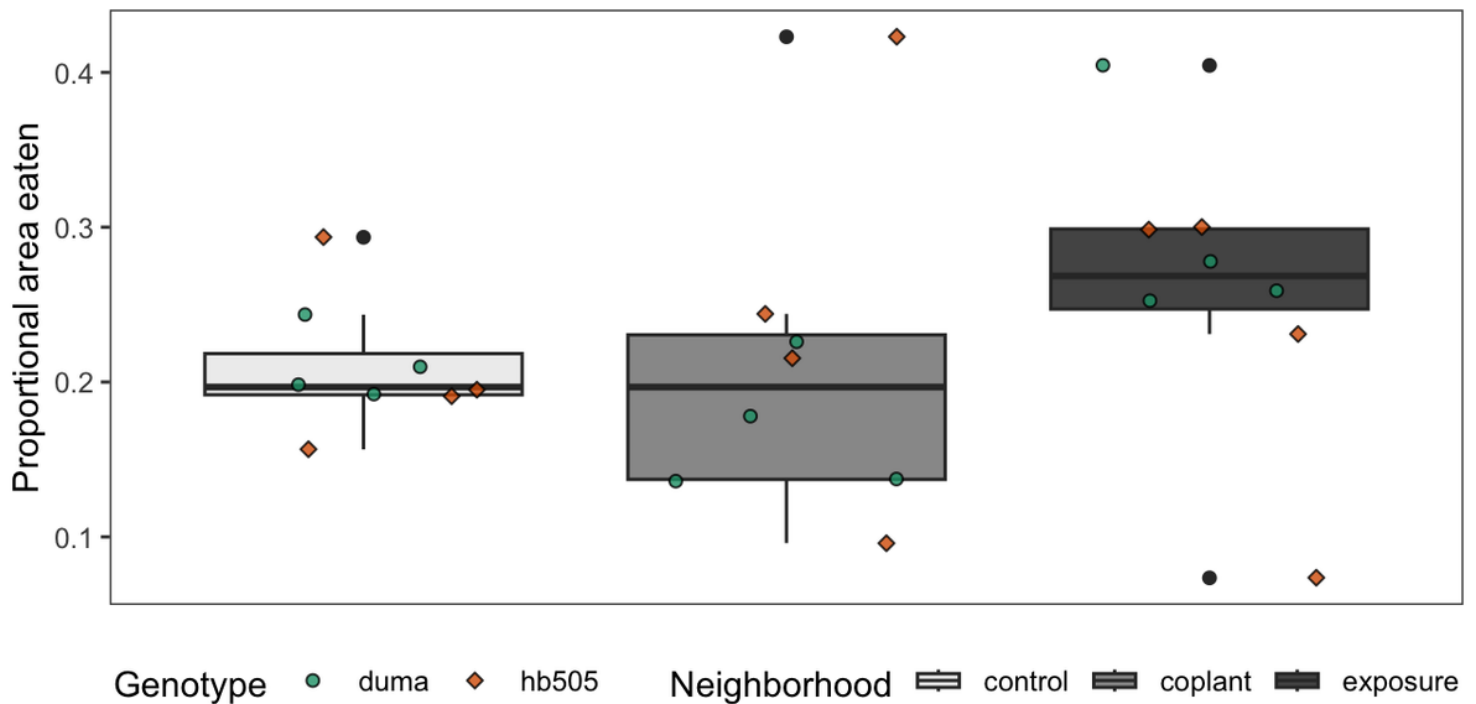


Figure 2

Box plot showing the proportion of leaf area eaten by *C. partellus* neonates on isolated (control) maize plants, maize plants grown in the same pot with a *Desmodium unincatum* neighbor (coplant), or maize plants that were previously exposed to visual and volatile cues from a neighboring *D. unincatum* plant growing in a separate pot (exposure). Each point is the mean of two technical replicates. Points are styled by maize variety (SC Duma 43 = teal circles; HB505 = red diamonds).

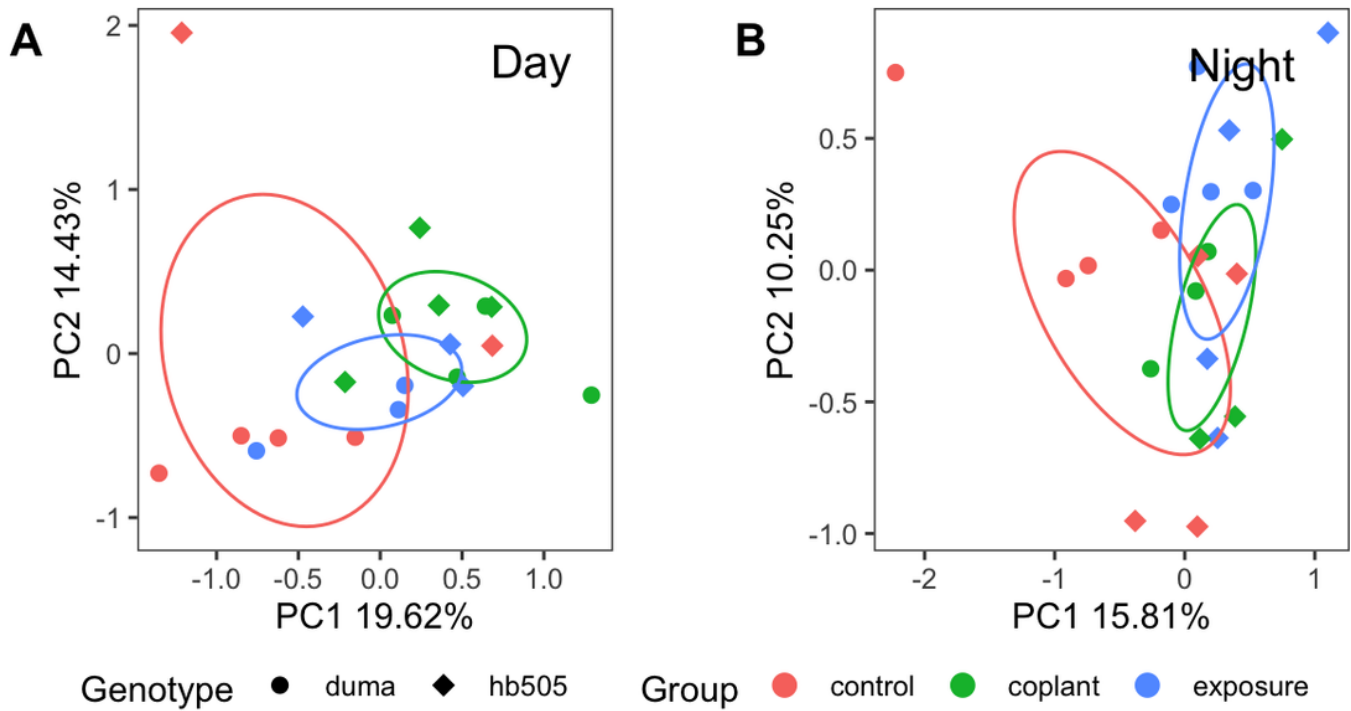


Figure 3

Ordination plots showing the distribution of photophase and scotophase VOCs from maize (Panels A and B) on the first two principal components in chemical space. Samples are colored by neighbor treatment (red = control, green = co-planting with *Desmodium*, blue = neighboring *Desmodium*). In panel A, maize plants sharing a pot with *Desmodium* are shifted to the right along PC1 (representing 11.48% and 16.64% of the variance respectively in leaves and roots). No separation is seen between maize plants exposed to above-ground *Desmodium* cues from a neighboring pot and the control. Standard deviation ellipses represent the distribution of each group in chemical space.

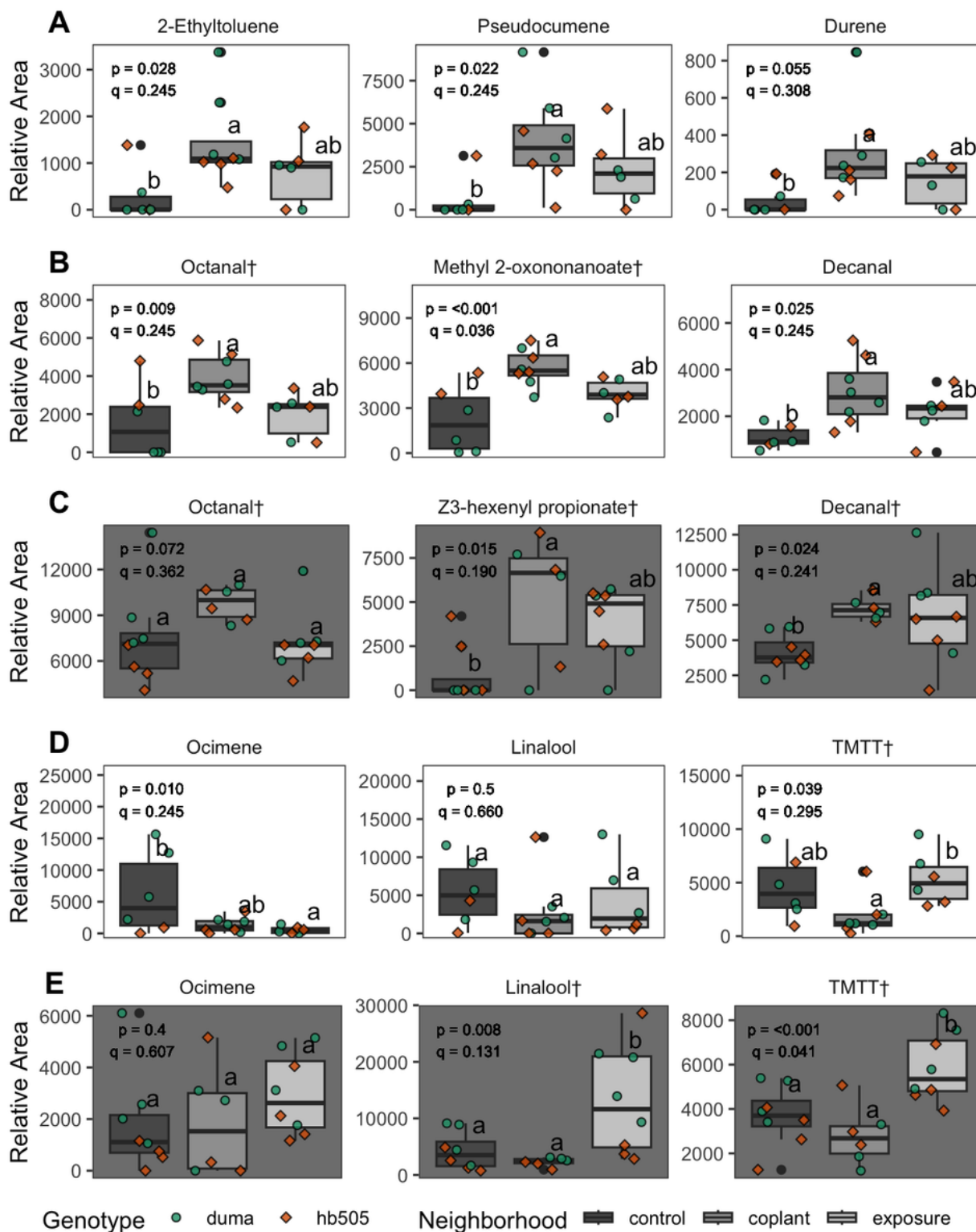


Figure 4

VOCs affected by neighborhood treatment during the photophase (panels A, B, and D) and scotophase (panels C and E) collection windows. Boxes sharing no letters in common are significantly different by Tukey's range test in univariate comparisons at a 5% significance level. Plots are also annotated with p-values and FDR-corrected q-values from two-way ANOVAs testing the effects of neighborhood treatment (crossed with genotype) on each metabolite. Alkylbenzenes and short-chain aldehydes (panels A & B)

were generally upregulated by co-planting with *Desmodium* during the photophase collection window, while mono- and homoterpenes like ocimene, linalool (3,7-Dimethylocta-1,6-dien-3-ol), and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) were suppressed (panel D). During the scotophase collection window, short-chain aldehydes were similarly induced by co-planting with *Desmodium* (panel C). In addition, some terpenoids, such as linalool and TMTT were induced in maize plants exposed to above-ground *Desmodium* cues (panel E). The alkylbenzenes in panel A were selected from the five photophase VOCs with the highest loadings along PC1, while compounds marked with a dagger (†) were identified by Boruta feature selection as important predictors of neighbor treatment.

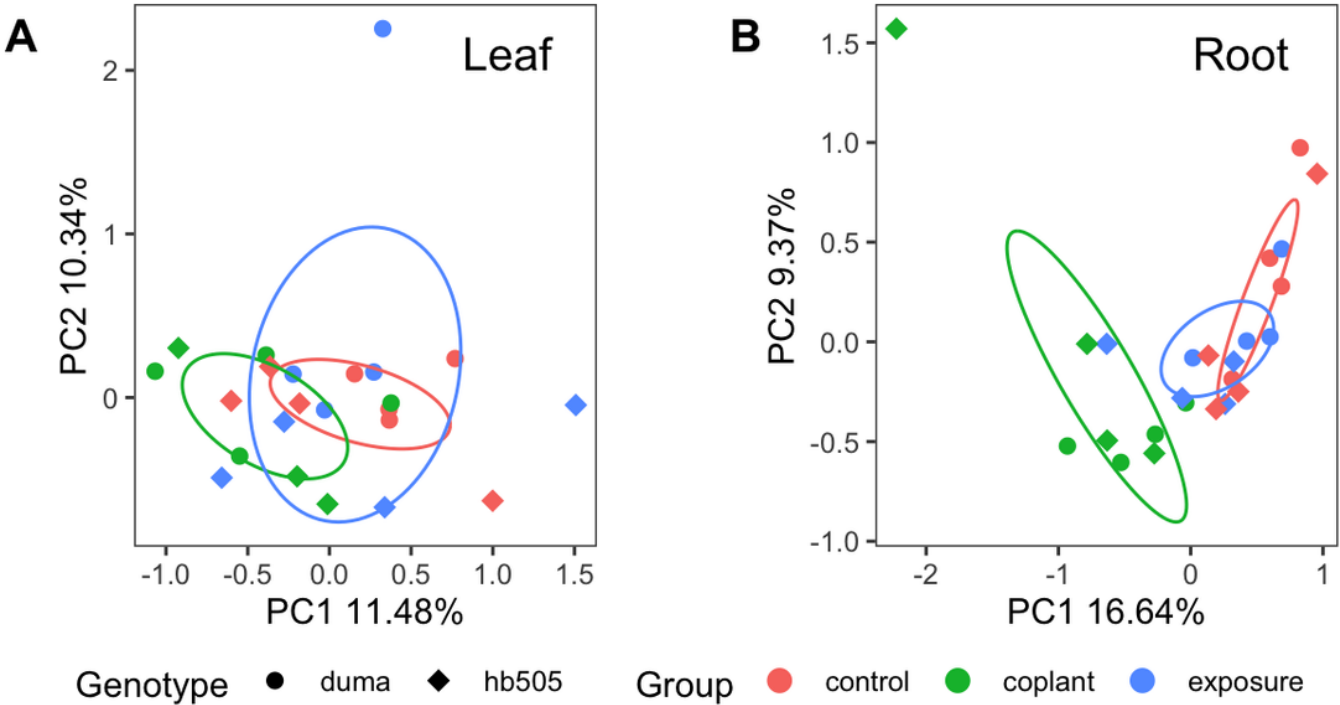


Figure 5

Principal components analysis showing the distribution of samples in leaves (Panel A) and roots (Panel B) along the first two principal components in chemical space. Samples are colored by neighbor treatment (red = control, green = co-planting with *Desmodium*, blue = neighboring *Desmodium*). In both plots, the maize plants sharing a pot with *Desmodium* are shifted to the left along PC1 (representing 11.48% and 16.64% of the variance respectively in leaves and roots). No separation is seen between

maize plants exposed to above-ground *Desmodium* cues from a neighboring pot and the control. Standard deviation ellipses represent the distribution of each group in chemical space.

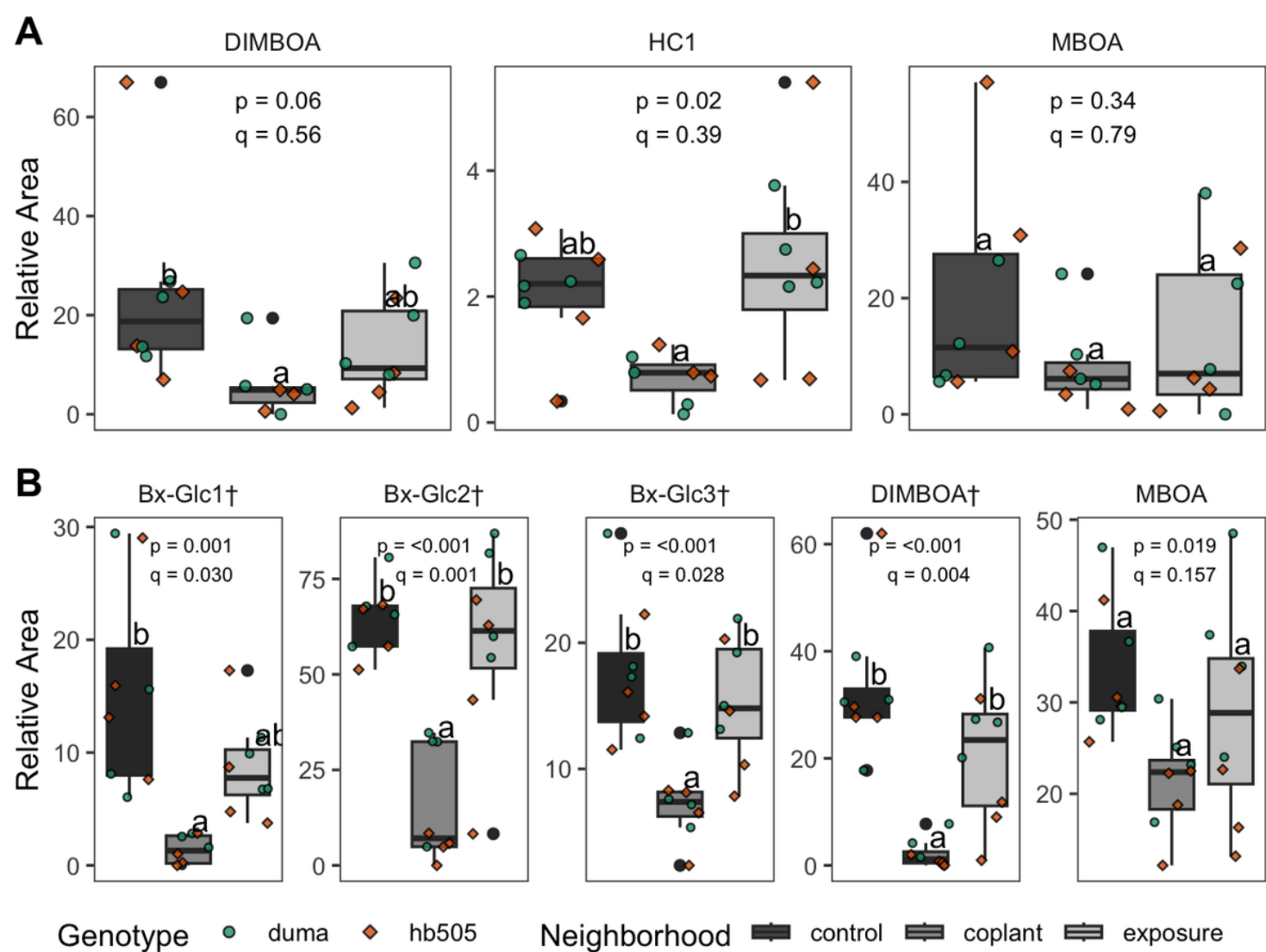


Figure 6

Metabolites in leaves (panel A) and roots (panel B) of maize plants exposed to different *Desmodium* neighborhood treatments. Boxes sharing no letters in common are significantly different by Tukey’s range test at a 5% significance level in univariate comparisons. Plots are also annotated with p-values and FDR-corrected q-values from a two-way ANOVA testing the effects of neighborhood treatment (crossed with genotype) on each metabolite. DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), MBOA (6-methoxy-2-benzoxazolinone) and three unidentified hydroxycinnamic acid glycosides (Bx-GLC) were strongly suppressed in the roots of maize plants that were co-planted with *Desmodium uncinatum*. In the leaves, DIMBOA, MBOA and an unidentified hydroxycinnamic acid (HC1) were moderately suppressed in maize co-planted with *D. uncinatum*. Metabolites marked with a dagger (†) were selected by Boruta random feature selection as important predictors of neighborhood treatment.

Supplementary Files

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- [SupplementaryMaterials.docx](#)