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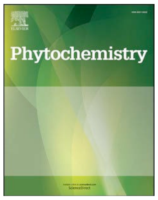

# Maize terpene synthase 1 impacts insect behavior via the production of monoterpene volatiles β-myrcene and linalool

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ABSTRACT

Plant-derived volatiles are important mediators of plant-insect interactions as they can provide cues for host location and quality, or act as direct or indirect defense molecules. The volatiles produced by *Zea mays* (maize) include a range of terpenes, likely produced by several of the terpene synthases (TPS) present in maize. Determining the roles of specific terpene volatiles and individual TPSs in maize-insect interactions is challenging due to the promiscuous nature of TPSs *in vitro* and their potential for functional redundancy. In this study, we used metabolite GWAS of a sweetcorn diversity panel infested with *Spodoptera frugiperda* (fall armyworm) to identify genetic correlations between TPSs and individual volatiles. This analysis revealed a correlation between maize terpene synthase 1 (*Zm*TPS1) and emission of the monoterpene volatiles linalool and β-myrcene. Electroantennogram assays showed gravid *S. frugiperda* could detect both linalool and β-myrcene. Quantification of headspace volatiles in a maize *tps1* loss-of-function mutant confirmed that *Zm*TPS1 is an important contributor to linalool and β-myrcene emission in maize. Furthermore, pairwise choice assays between *tps1* mutant and wild-type plants showed that *Zm*TPS1, and by extension its volatile products, aid host location in the chewing insect *S. frugiperda,* yet repel the sap-sucking pest, *Rhopalosiphum maidis* (corn leaf aphid). On the other hand, *Zm*TPS1 had no impact on indirect defense via the recruitment of the parasitoid *Cotesia marginiventris*. *Zm*TPS1 is therefore an important mediator of the interactions between maize and its insect pests.

## 1. Introduction

Volatile organic compounds are low molecular weight compounds that can mediate the interactions of plants with their environment. Many volatile organic compounds are induced in response to biotic or abiotic stress. They function in plant-plant communication, pollinator recruitment and the interaction of plants with other organisms. Different plant species produce different blends of volatiles. For instance, most of the volatiles produced by maize (*Zea mays*) are terpenes, which originate from the conjugation of the five-carbon compound dimethylallyl diphosphate (DMAPP) and its isomer isopentenyl diphosphate (IPP). The prenyl diphosphates produced from two, three, and four of these C5-units are geranyl diphosphate (C10), farnesyl diphosphate (C15) and geranylgeranyl diphosphate (C20), respectively (Tholl, 2015). Subsequent rearrangements of these molecules via terpene synthases (TPSs) leads to the production of monoterpenes, sesquiterpenes and diterpenes (Davis and Croteau, 2000). Maize has approximately 30 TPS genes (Chen et al., 2011; Ding et al., 2017), of which about half have been characterized to some extent (Block et al., 2019). Despite these characterization efforts, the apparent redundancy of TPS genes, coupled with the promiscuity of their *in vitro* activities, has meant that the relative contribution of many of these enzymes to the production of specific volatile terpenes in maize remains elusive.

The folivorous insect fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a major pest of maize throughout the Western Hemisphere, and a significant rising threat in sub-Saharan Africa and Asia (Nagoshi et al., 2018; Rane et al., 2023). Gravid *S. frugiperda* moths are attracted to maize volatiles, and differing volatile

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profiles can influence their choice of host plant (Signoretti et al., 2012; Block et al., 2020; Yactayo-Chang et al., 2021). *Spodoptera frugiperda* moths can detect a range of plant volatiles, including terpenes, methyl salicylate, and several short chain alcohols such as n-hexanol (Malo et al., 2004). In contrast to their role in host location, plant volatiles can also serve as direct defense compounds by repelling insect pests, or as indirect defense compounds by recruiting parasitoids and predators to infested plants. Maize for instance, releases large amounts of terpene volatiles when infested with *Spodoptera exigua* (beet armyworm). A blend of these volatiles attracts the *S. exigua* and *S. frugiperda* parasitoid *Cotesia marginiventris* and thus act as an indirect defense mechanism, as parasitized caterpillars cause less damage to the host plant (Turlings et al., 1990, 1991).

The identification of specific maize volatiles that impact *S. frugiperda* host location, and the genes responsible for their biosynthesis, could provide important molecular breeding targets for the development of maize lines that are less attractive to this pest. To tackle this problem and determine which TPS genes are important for the production of maize monoterpene volatiles, we leveraged the fact that different maize inbred lines vary in the quantity and composition of the volatile blends they produce (Degen et al., 2004; Block et al., 2018) and performed metabolite Genome Wide Association Studies (mGWAS) on volatiles $\beta$-myrcene and linalool in a sweetcorn diversity panel. This analysis led to the identification of an association between these monoterpene volatiles and the maize gene terpene synthase 1 (*Zm*TPS1, GRMZM2G049538). We then analyzed the volatile production of the maize *Zm*TPS1 loss-of-function mutant *tps1* and demonstrated the importance of this gene in production of $\beta$-myrcene and linalool. We used electroantennogram assays to determine the sensitivity of gravid *S. frugiperda* to these volatiles. We then used the *tps1* mutant to reveal differential roles of *Zm*TPS1 and the monoterpenes it produces in mediating the interactions between maize and the insect pests *S. frugiperda* and *Rhopalosiphum maidis* (corn leaf aphid) and assessed the impact of *Zm*TPS1 on the recruitment of the *S. frugidpera* parasitoid *C. marginiventris*.

## 2. Results and discussion

### 2.1. Metabolite GWAS reveals an association between ZmTPS1 and β-myrcene and linalool production

Maize has around 30 terpene synthases and blends of terpene volatiles have been observed in all maize tissues examined. However, the quantity and composition of the volatile blend depends on the maize cultivar examined (Gouinguené et al., 2001). This variability between cultivars means that the difference in production of individual volatiles in different cultivars can be used to map to the terpene synthase genes underlying the variation. In this study, we exploited this variation in a sweetcorn diversity panel to use mGWAS to map the production of the volatiles $\beta$-myrcene and linalool to a specific terpene synthase gene. To accomplish this a diversity mapping population of 347 sweetcorn varieties was infested with *S. frugiperda.* Headspace volatiles were then collected and peak areas for $\beta$-myrcene and linalool quantified by GC-MS analysis and adjusted for above ground fresh weight of the plants, to determine volatile production per gram of above ground tissue (Supplemental Table S1). This data was then analyzed using mGWAS to identify single nucleotide polymorphisms (SNPs) associated with the production of specific volatile compounds. The GWAS analysis resulted in the identification of SNPs associated with β-myrcene and/or linalool content. The genes located within 100 kb of the most significant SNPs for each trait are shown in Supplemental Table S2. A SNP in the gene *Zm*00045a007186 (*Zm*TPS1) on chromosome 2 was most strongly associated with changes in $\beta$-myrcene content (Fig. 1a). This SNP was also significantly associated with changes in linalool content (Fig. 1b). These data suggest that *Zm*TPS1 may contribute to the production of both these monoterpene volatiles as it could produce both the monoterpene $\beta$-myrcene and the acyclic monoterpenoid linalool from geranyl diphosphate (Fig. 1c).

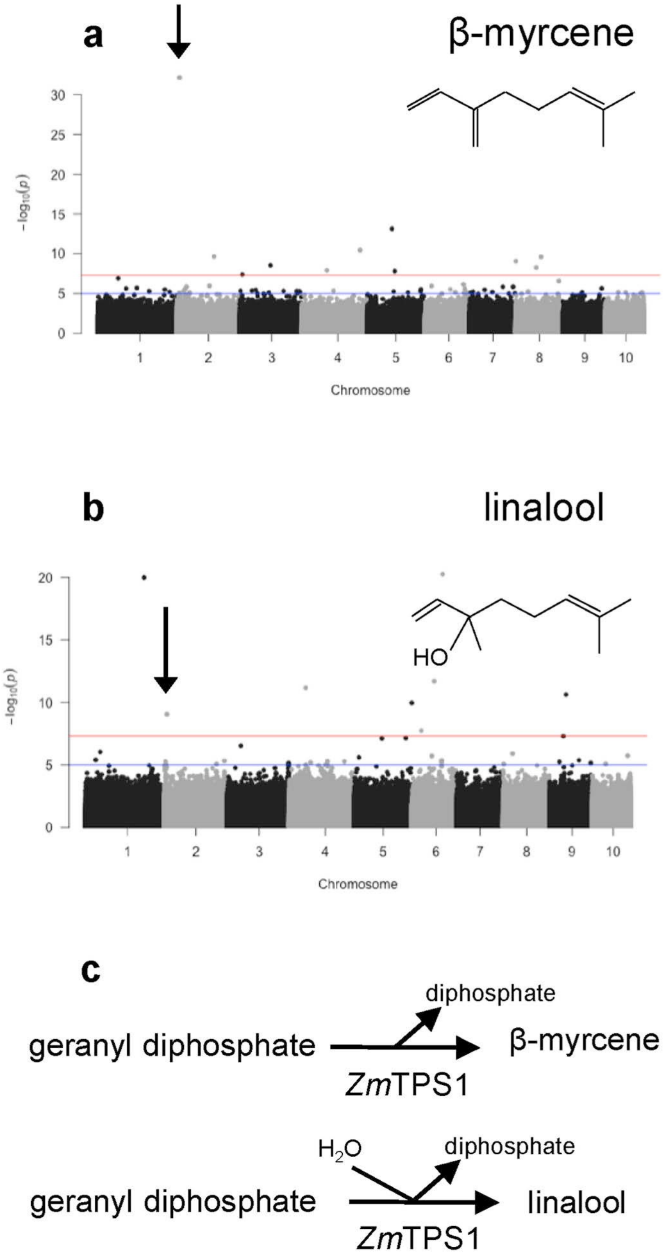

**Fig. 1. Manhattan Plots of Metabolite GWAS for β-myrcene (a) and linalool (b).** Quantification of volatile β-myrcene and linalool in a sweetcorn diversity panel infested with *S. frugiperda* (Table S1) was used for GWAS analysis. A region in chromosome 2 where a SNP is most strongly associated with changes in β-myrcene content was also significantly associated with changes in linalool content (arrows). The closest gene from the significant SNPs is *Zm*TPS1. Predicted reactions for *Zm*TPS1 mediated synthesis of β-myrcene and linalool from geranyl diphosphate (c).

The other significant SNPs for $\beta$-myrcene (Supplemental Table S2) included ones located near transcription factors on chromosomes 4 and 8, possibly linked to the regulation of $\beta$-myrcene production. As well as a SNP on chromosome 8 near *Zm*00045a028161 (*Zm*COI1b - coronatine insensitive1b), an F-Box protein involved in perception of the hormone jasmonic acid (An et al., 2019). As jasmonic acid regulates the induction of volatiles in plants, SNPs that impact COI activity could potentially alter $\beta$-myrcene production. Significant SNPs for linalool (Supplemental Table S2) included SNPs on chromosomes 5 and 6 in genomic regions containing transcription factors, suggesting that these SNPs may be linked to the regulation of linalool production. A SNP on chromosome 1 is in the vicinity of *Zm*00045a004598 that has homology to Cytochrome P450 89A2 (CYP89A2). The function of this enzyme is unknown; however, it could potentially use linalool as a substrate. Of the candidate genes identified based on the significant SNPs for and *β*-myrcene and linalool, *Zm*TPS1 has the most potential for a direct impact on the production of these volatiles and so was selected for validation.

### *2.2. The ZmTPS1 loss-of-function mutant has reduced production of linalool and β-myrcene*

To test whether the product of the *ZmTPS1* gene significantly contributes to the production of linalool and *β*-myrcene in maize, we isolated a *tps1* loss-of-function mutant from the Uniform Mu collection containing a transposon insertion in *ZmTPS1* that is known to disrupt its function (Xu et al., 2019). Headspace volatiles were collected from this *tps1* loss-of-function mutant and its corresponding wild-type (bzW22) maize plants in the presence or absence of *S. frugiperda* herbivory.

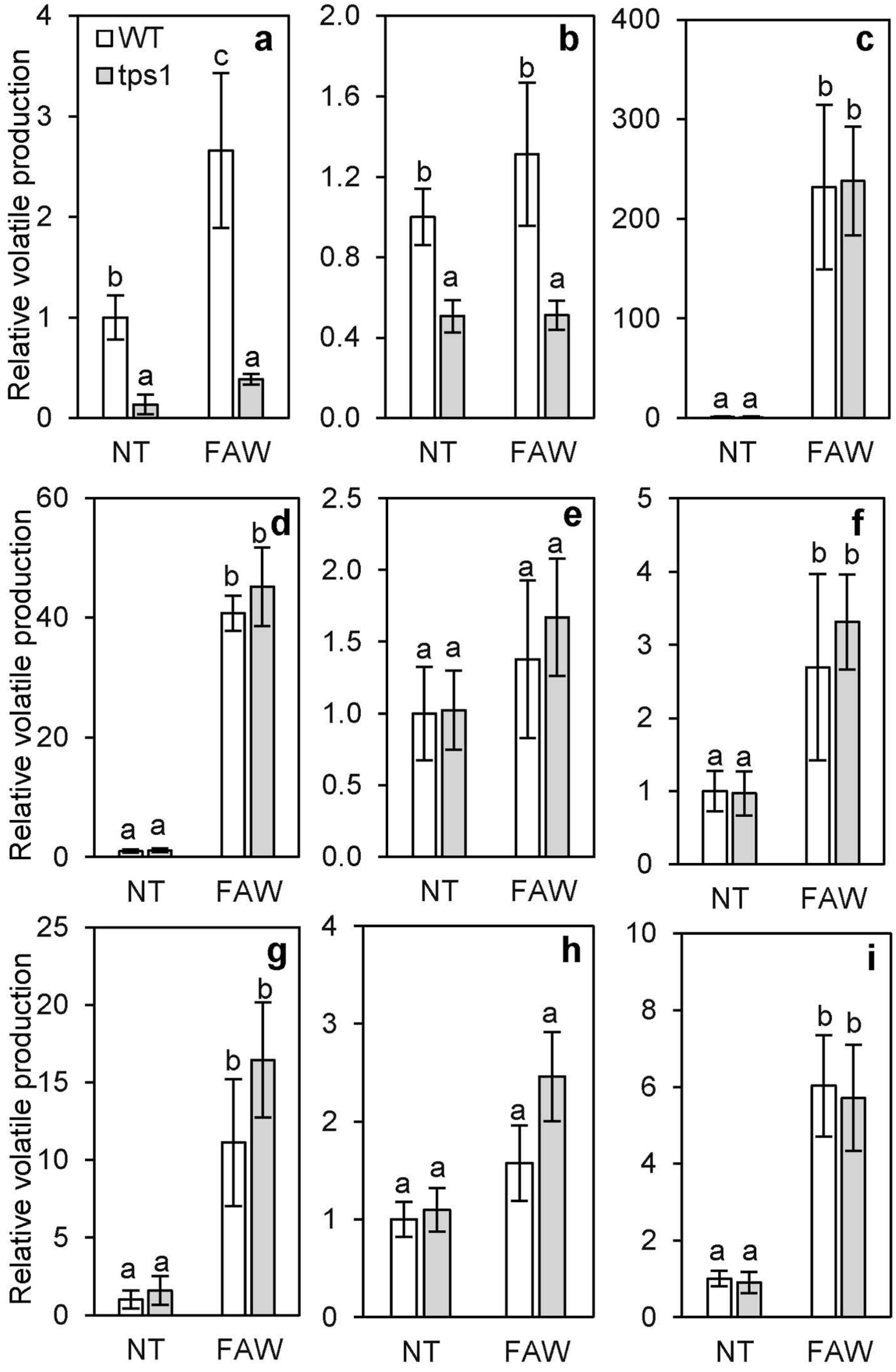

**Fig. 2. Volatile production in wild-type or *tps1* mutant plants.** Plants were non-treated (NT) or infested with *S. frugiperda* (FAW), and headspace volatiles collected for 24 h. Volatiles (a) linalool, (b) β-myrcene, (c) *(E)* β-farnesene (d) DMNT, (e) β-ocimene, (f) cyclosativene, (g) β-caryophyllene, (h) α-humulene, and (i) germacrene-D were quantified by GC-MS. Bars show mean volatile production relative to NT-WT ± SEM and different lower case letters indicate statistically significant difference ($P \leq 0.05$) by pairwise ANOVA, n = 4.

The volatile linalool was emitted from above ground tissues of non-treated wild-type maize plants at a rate of 600 ng $g^{-1}d^{-1}$, and 24 h after infestation with *S. frugiperda* neonates, the emission rate increased two and a half fold, to reach 1600 ng $g^{-1}d^{-1}$. The *tps1* mutant plants showed significantly less emission of linalool than the wild-type plants, as non-treated plants emitted 65 ng $g^{-1}d^{-1}$, and *S. frugiperda* infested plants emitted 230 ng $g^{-1}d^{-1}$ (Fig. 2a). The emission rate of *β*-myrcene in wild-type plants was not statistically inducible by herbivory than linalool, averaging 90 ng $g^{-1}d^{-1}$ in non-treated plants and 120 ng $g^{-1}d^{-1}$ in *S. frugiperda* infested plants. However, the *tps1* mutant plants had significantly lower emission rates of *β*-myrcene than wild-type, as both non-treated mutants and *S. frugiperda* infested mutants emitted around 45 ng $g^{-1}d^{-1}$ (Fig. 2b). Representative GC-MS extracted ion chromatograms showing linalool and β-myrcene for these plants and treatments are shown in supporting information (Fig. S1). These data show that *Zm*TPS1 significantly contributed to the production of both linalool and *β*-myrcene *in vivo*.

Our data show that *Zm*TPS1 is a major contributor to the production of linalool in maize inbred bzW22. Yet some linalool production is observed even in *tps1* plants indicating that *Zm*TPS1 is not the only producer of linalool in maize. *Zm*TPS2 (GRMZM2G046615), that also produces linalool (Richter et al., 2016), likely contributes to the linalool observed in the *tps1* mutant. We show that *Zm*TPS1 is responsible for half the total *β*-myrcene produced by maize inbred bzW22, suggesting that other terpene synthases play non-redundant roles in *β*-myrcene production. Based on their *in vitro* activities, candidates for these terpene synthases include *Zm*TPS6/11(GRMZM2G127087) and *Zm*TPS26 (GRMZM2G030583) (Köllner et al., 2008; Lin et al., 2008).

Interestingly, recombinant expression of *Zm*TPS1 from maize inbred B73 in *E. coli* demonstrated that *Zm*TPS1 could convert geranyl diphosphate to linalool and geraniol, and farnesyl diphosphate to (*E*)-*β*-farnesene, and (*E*)-nerolidol (Schnee et al., 2002). Yet no significant impact was observed on the emission of (*E*)-*β*-farnesene from loss of *Zm*TPS1 (Fig. 2c), and neither geraniol nor (*E*)-nerolidol are detected in the headspace of wild-type (bzW22) maize. The lack of impact of *tps1* on the emission of (*E*)-*β*-farnesene is likely due to redundancy of *Zm*TPS1 with other TPS enzymes regarding its (*E*)-*β*-farnesene production. Candidates for these enzymes include *Zm*TPS4 (GRMZM2G117319), *Zm*TPS5 (GRMZM2G074309) and *Zm*TPS10 (GRMZM2G179092) all of which can produce (*E*)-*β*-farnesene (Köllner et al., 2004; Schnee et al., 2006).

The volatile (*E*)-nerolidol was not detected in the headspace of B73 maize (Schnee et al., 2002) or in the headspace of bzW22 or *tps1* mutant maize lines. As (*E*)-nerolidol can be converted to the homoterpene (*E*)-3, 8-dimethyl-1,4,7-nonatriene (DMNT) by the maize P450 monooxygenase, CYP92C5 (Richter et al., 2016), the production of DMNT in the *tps1* mutant was also assessed. No significant difference in the production of DMNT was observed between the wild-type and *tps1* mutant lines (Fig. 2d).

Both *Zm*TPS1 and *Zm*TPS2 produce (*E*)-nerolidol *in vitro* and *Zm*TPS2 deficient maize lines produce reduced amounts of linalool, (*E*)-nerolidol, and DMNT (Schnee et al., 2002; Richter et al., 2016). In contrast, *Zm*TPS1 deficient maize produced reduced amounts of linalool and wild-type levels of DMNT, indicating that unlike *Zm*TPS2, *Zm*TPS1 does not play a significant role in the production of (*E*)-nerolidol as a precursor for DMNT in maize (Fig. 2). This lack of impact on DMNT production may be due to redundancy in (*E*)-nerolidol production with other TPSs, or due to an association between *Zm*TPS2 and CYP92C5 that leads them to have a specific and shared function in DMNT production.

To assess the impact of *Zm*TPS1 in the production of other monoterpene and sesquiterpene volatiles, the production of *β*-ocimene, cyclosativene, *β*-caryophyllene, *α*-humulene (*α*-caryophyllene), and germacrene D in the *tps1* mutant were also assessed (Fig. 2e-i). No significant difference was observed in the production of these volatiles in the *tps1* mutant compared to wild-type plants. These data provide strong evidence that *Zm*TPS1 plays a major role specifically in the production of linalool and *β*-myrcene volatiles in maize.

### 2.3. The ZmTPS1 loss-of-function mutant is less attractive to both the larval and adult stages of S. frugiperda

The larvae of *S. frugiperda* are known to be able to distinguish between damaged and undamaged maize plants using host derived volatiles and are thought to use these signals to re-locate to host plants if they are displaced (Carroll et al., 2006). Furthermore, previous analysis of linalool in Y-tube olfactometer assays demonstrated that it positively impacts the olfactory orientation responses of sixth instar *S. frugiperda* larvae, with larvae differentiating between emission rates of 2 ng $h^{-1}$ and 20 ng $h^{-1}$ linalool, suggesting that linalool can impact *S. frugiperda* host localization (Carroll et al., 2006). The non-redundant function of *Zm*TPS1 in linalool and *β*-myrcene production allowed us to utilize the *tps1* mutant to examine the impact of this gene and its products on the interactions between maize and *S. frugiperda*.

To determine if the production of linalool and *β*-myrcene by *Zm*TPS1 in maize impacts the behavior of the insect pest *S. frugiperda,* pairwise choice assays between wild-type and *tps1* mutant plants were performed using *S. frugiperda* neonates (Fig. 3a). The *S. frugiperda* neonates displayed a small but significant preference for wild-type plants over the *tps1* mutant, selecting wild-type plants 53% of the time. These data suggest that the volatiles produced by *Zm*TPS1 can be detected by the larvae and used to aid in the location of host plants.

To determine if *Zm*TPS1 derived volatiles can also impact the behavior of *S. frugiperda* adults, pairwise oviposition choice assays were performed between wild-type and *tps1* mutant plants (Fig. 3b). The *S. frugiperda* showed a significant oviposition preference for wild-type plants over the *tps1* mutant plants, with 66% oviposition on wild-type and 34% on *tps1*. These data show that *Zm*TPS1 derived products influence both larval and adult *S. frugiperda* behavior by increasing its ability to locate host plants.

### 2.4. Electrophysiological response of S. frugiperda adult females to linalool and β-myrcene

To determine the range of sensitivity of *S. frugiperda* to linalool and *β*-myrcene, dose response electroantennograms (EAGs) were performed on mated females. The antennae of virgin and gravid female *S. frugiperda* have previously been shown to be electrophysiologically responsive to linalool (Pinto-Zevallos et al., 2016). Dose-response EAGs confirmed

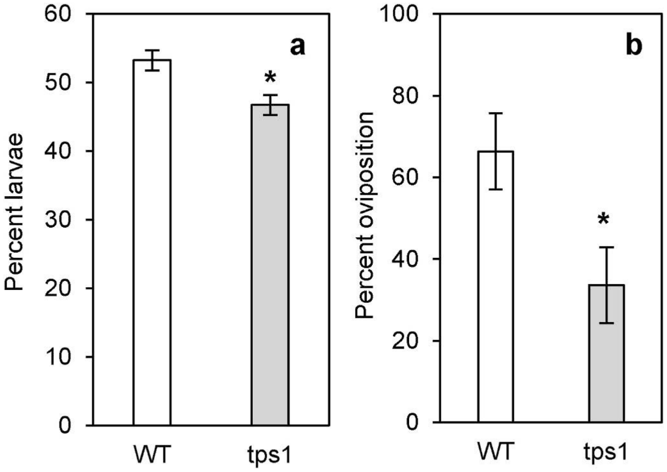

**Fig. 3. Feeding and oviposition choice of *S. frugiperda*.** Pairwise choice assays of larval feeding choice (n = 7) (a) and oviposition preference (n = 8) (b) *of S. frugiperda* on wild-type and *tps1* mutant plants. Bars show mean ± SEM and asterisks indicate statistically significant difference between genotypes $P \leq 0.05$ by paired *t*-test.

responsiveness to linalool and extended this to include a significant dose-response relationship for antennae of gravid females (K–W test, $P = 0.001$). The antennae of gravid females responding to linalool demonstrated strong responses with EAG amplitude rapidly increasing with increasing loading dose, before saturating at the highest three loading doses (50, 100, and 200 nmol) (Fig. 4a). To test if *S. frugiperda* can also perceive $\beta$-myrcene EAG analysis of gravid females with a $\beta$-myrcene standard was performed. A significant dose-response relationship was observed to $\beta$-myrcene (K–W test, $P = 0.002$) demonstrating that *S. frugiperda* can also perceive $\beta$-myrcene (Fig. 4b). The increase in EAG amplitude in response to $\beta$-myrcene was much more gradual than that observed in response to linalool, with negligible response to the three lowest loading doses (1, 10, and 20 nmol).

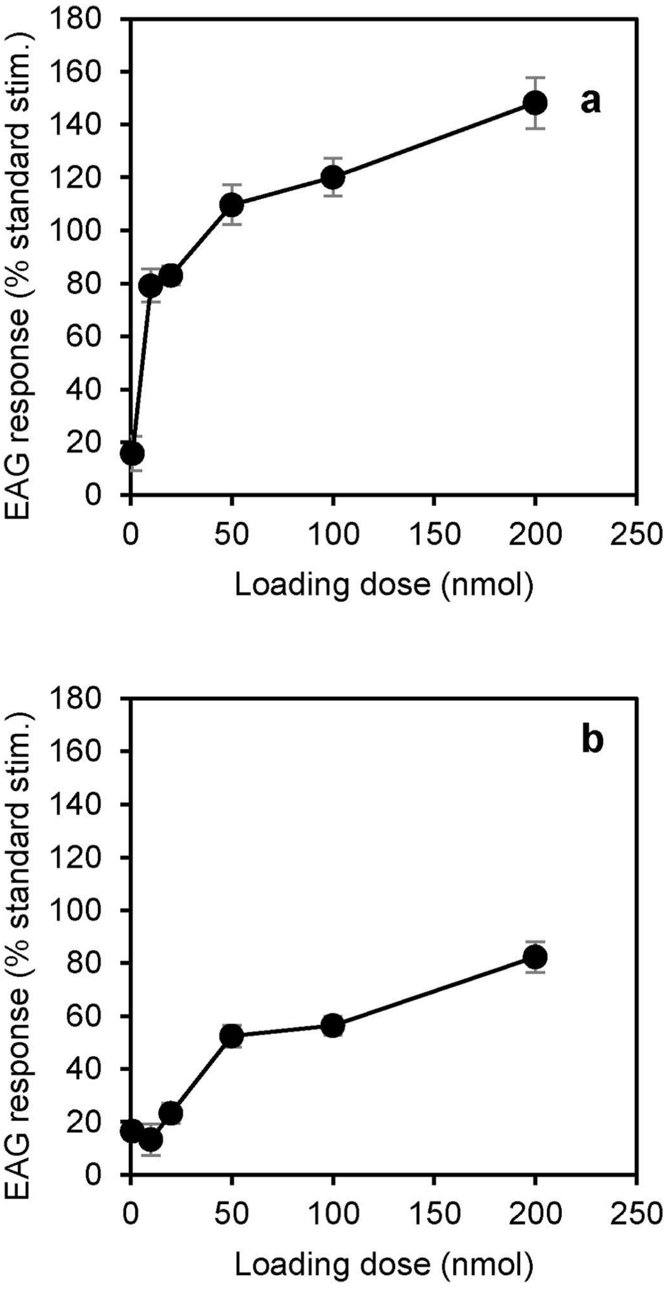

**Fig. 4. Electroantennogram (EAG) assays.** Mated female *S. frugiperda* antenna demonstrate a significant dose-response relationship for both (a) linalool ($P = 0.001$), and (b) β-myrcene ($P = 0.002$). Loading dose is defined as nmol of test compound in 20 μL of pentene applied to test filter. Graphs show mean EAG response ± S.E.M. to test compound as a percent response compared to a standard stimulus (50 nmol eugenol) set at 100%, and pentane blank set at 0%. Statistical significance was determined using the Kruskal-Wallis Rank Sum Test with an n of between 4 and 8 for each compound/concentration combination.

As the excised antenna of mated *S. frugiperda* females displayed a dose dependent response to linalool between 1 and 100 nmol, and to $\beta$-myrcene between 20 and 200 nmol the different amounts of one or both volatiles between the wild-type and *tps1* mutant are likely driving the observed differences in host choice. As wild-type maize emitted linalool at around 4 nmol $g^{-1} d^{-1}$ compared to 0.4 nmol $g^{-1}d^{-1}$ in the *tps1* mutant, and $\beta$-myrcene at 0.6 nmol $g^{-1}d^{-1}$ compared to 0.3 nmol $g^{-1}d^{-1}$ in the *tps1* mutant, it is likely that *S. frugiperda* adults can more easily distinguish the difference in linalool emission than that of $\beta$-myrcene in the plants.

As DMNT can be an oviposition deterrent for *S. frugiperda* (Yactayo-Chang et al., 2021), the loss of both linalool and DMNT in *Zm*TPS2 mutant lines could have opposing effects on *S. frugiperda* oviposition, meaning that reduced linalool via loss of *Zm*TPS1 is likely a better strategy for the development of maize that is less attractive to *S. frugiperda* than loss of *Zm*TPS2. Linalool, $\beta$-myrcene and DMNT are not the only volatiles that influence *S. frugiperda* host choice, therefore additional genetic factors will also contribute to this phenotype.

### 2.5. *ZmTPS1 gene expression is not induced in response to S. frugiperda herbivory in bzW22 maize*

*Zm*TPS1 produced linalool levels are significantly higher in *S. frugiperda* infested wild-type bzW22 plants than in non-infested plants (Fig. 2a). As transcript levels of *Zm*TPS1 can be induced in response to herbivory in some maize lines but not in others (Schnee et al., 2002), it is possible that the increase in linalool in response to herbivory in the inbred bzW22 is due to increased expression of *Zm*TPS1. To test this qRT-PCR of *Zm*TPS1 was used to examine the relative expression levels in leaves of non-treated control plants and in plants that had active *S. frugiperda* feeding (Fig. 5a). No significant impact on *Zm*TPS1 gene expression was observed in the bzW22 plants infested with *S. frugiperda* compared to non-infested control plants. These data suggest that the increased linalool production in response to herbivory is not due to increased expression of *Zm*TPS1 in this inbred. The *S. frugiperda* induced linalool may therefore be due to activity of another terpene synthase or to increased availability of the precursor geranyl diphosphate leading to increased flux through the pathway.

### 2.6. *The loss of function of tps1 has no impact on the recruitment of the parasitoid wasp Cotesia marginiventris or on S. frugiperda growth*

Our data with the *tps1* mutant indicates that maize plants that express *Zm*TPS1 are more attractive to *S. frugiperda* and therefore more at risk of infestation. The question then arises as to why maize would retain this gene. One possibility is that *Zm*TPS1 plays other functions, directly or indirectly in maize defense that offset this risk. As different volatile profiles in different maize inbred lines can impact the ability of the parasitoid *C. marginiventris* to oviposit in *S. frugiperda* (Block et al., 2018) *Zm*TPS1 may play a role in indirect defense in maize by attracting predators or parasites. To assess this we measured the impact of *tps1* loss-of-function mutant on host location by *C. marginiventris*. In pairwise choice assays between *S. frugiperda* larvae on wild-type or *tps1* mutant plants, *C. marginiventris* displayed no significant preference for parasitism, with larvae on both wild-type and *tps1* mutant plants showing 39% parasitism (Fig. 5b). *Zm*TPS1 therefore appears to play no significant role in indirect defense via the recruitment of *C. marginiventris*.

A second possibility for how *Zm*TPS1 could offset the deleterious trait of attracting *S. frugiperda* is via the production of non-volatile compounds that could function as anti-herbivore defenses. For instance, untargeted metabolomic analysis revealed that the *tps1* mutant accumulated lower amounts the linalool derivative, linalool 3-(6″-malonylglucoside), than wild type plants (Xu et al., 2019). This indicates

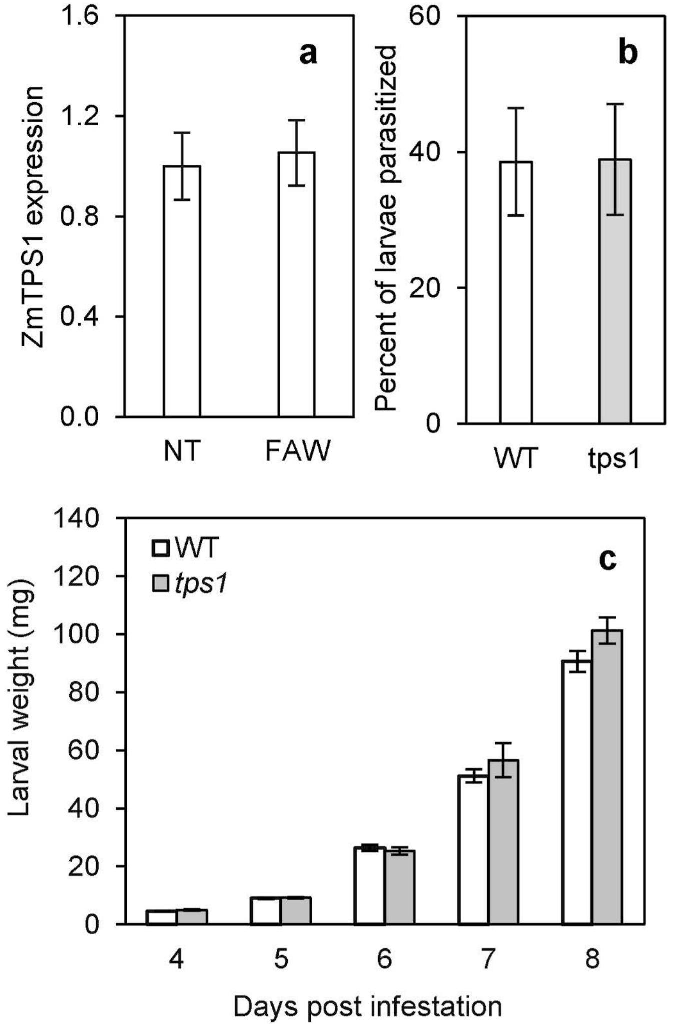

**Fig. 5. The impact of *S. frugiperda* herbivory on *ZmTPS1* expression, and assessment of *Zm*TPS1's impact on parasitoid recruitment and *S. frugiperda* growth.** Relative expression of *ZmTPS1* in *S. frugiperda* infested (FAW) and non-infested (NT) wild-type (WT) plants (n = 6), no significant difference in expression was observed with an unpaired *t*-test (a). Pairwise choice assay of *C. marginiventris* for *S. frugiperda* larvae on WT or *tps1* mutant plants (n = 9), no significant difference was observed with a paired *t*-test (b). Growth of *S. frugiperda* larvae on WT and *tps1* mutant plants (n = 60), no significant difference was observed with an unpaired *t*-test (c). Graphs show mean ± S.E.M.

that some of the linalool produced by *Zm*TPS1 may be converted into non-volatile compounds that could potentially have defensive functions. To assess if *Zm*TPS1 products or their derivatives impact *S. frugiperda* growth, larval weight of *S. frugiperda* on *tps1* mutant or wild-type plants was measured daily from 4 to 8 days post infestation (Fig. 5c). No significant difference in larval growth was observed between *S. frugiperda* on *tps1* mutant plants and those on wild-type plants. These data indicate that *Zm*TPS1 products do not have significant anti-herbivore activity against *S. frugiperda* and support the notion that *Zm*TPS1 is not detrimental for maize and its interactions with *S. frugiperda*.

### 2.7. ZmTPS1 negatively impacts R. maidis settling behavior

Linalool producing TPSs in other plant systems can play a protective role against certain pests. For instance, overexpression of *Gh*TPS12 from cotton in *Nicotiana tabacum* plants lead to increased production of linalool. In choice tests, female adults of cotton boll worm (*Helicoverpa armigera*) laid fewer eggs on these transgenic plants compared with non-transformed controls (Huang et al., 2018). In addition, the aphid *Myzus persicae* prefers feeding on wild-type leaves over leaves of linalool emitting transgenic plants expressing *Gh*TPS12 or *Fa*NES1 in *N. tabacum* or *Arabidopsis thaliana* respectively (Aharoni et al., 2003; Huang et al., 2018).

To determine whether *Zm*TPS1 can influence the behavior of piercing/sucking insects, we performed an *Rhopalosiphum maidis* (corn leaf aphid) pairwise choice assay between wild-type and *tps1* maize plants. Our results show that the number of adult aphids that settled on wild-type and *tps1* plants were comparable after 6 h, however, at 24 h after aphid release the number of adult aphids that were settled on *tps1* plants were significantly higher (62% of aphids) than those on wild-type plants (38% of aphids) (Fig. 6a). These results indicate that unlike the attractive role of *Zm*TPS1 to *S. frugiperda*, *Zm*TPS1 or *Zm*TPS1-derived volatiles may deter aphids.

Our data on the role of *Zm*TPS1 shows it has contrasting functions towards *S. frugiperda* and *R. maidis*. Since *S. frugiperda* is a chewing and *R. maidis* is a piercing/sucking insect, it is possible that this difference could occur in other insect species and raises the question as to how these opposite behaviors developed. One could suppose that *Zm*TPS1 evolved to deter insects such as *R. maidis* and that *S. frugiperda* exploited

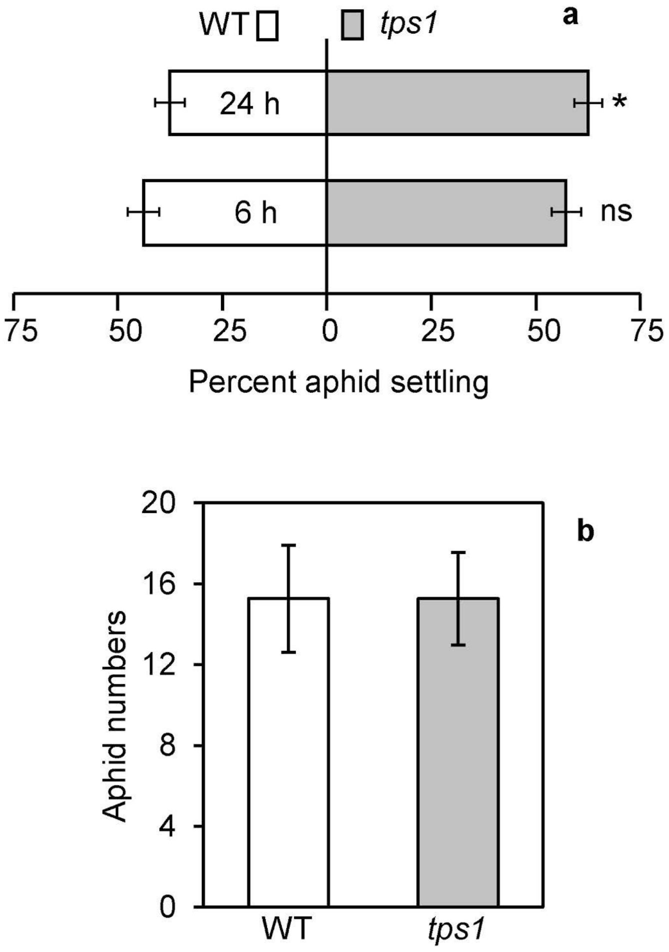

**Fig. 6. Comparison of corn leaf aphid (*R. maidis*) preference for and survival on wild-type (WT) vs *tps1* plants.** (a) Aphid pairwise choice assay between WT and *tps1* plants. Proportion of adult aphids that had settled on each plant was monitored after 6 and 24 h after aphid release (n = 15). An asterisk indicates values that are significantly different from each other ($P < 0.05$; $\chi^2$ test), while "ns" indicates no significant differences between maize lines on aphid settling**.** **(b)** Total number of *R. maidis* adults and nymphs recovered in a no-choice assay 7 days after infestation on WT and *tps1* plants (n = 11). No significant differences were observed ($P > 0.05$; Tukey's test). Graphs show mean ± S.E.M.

this trait to improve its ability to locate host plants. Future research examining the impact of *Zm*TPS1 on the behavior of other maize pests could provide information on the prevalence of these differences.

### 2.8. *ZmTPS1* does not impact *R. maidis* proliferation

Linalool is toxic to certain insects, as *in vitro* fumigation assays showed that it had contact toxicity to the rice weevil, *Sitophilus oryzae*, a stored product pest that damages the grains/seeds of several crops, including maize (Fouad et al., 2021). *Rhopalosiphum maidis* feeding on maize induces the expression of *ZmTPS2* and *ZmTPS3* in the maize inbred line B73, and the increased emission of several volatiles including linalool (Tzin et al., 2015). Interestingly, analysis of a *tps2/3*:Ds loss of function mutant showed that *Zm*TPS2/3 (a single gene in this line) is responsible for some but not all of the aphid-induced linalool emission, and that aphids reared on these mutants produce significantly less progeny than those reared on wild-type plants (Tzin et al., 2015).

To determine if *Zm*TPS1 has an impact on aphid proliferation, *R. maidis* numbers were compared on the wild-type and *tps1* plants. In this no-choice assay, aphid numbers were comparable between wild-type and *tps1* plants, indicating that *Zm*TPS1 does not adversely impact aphid proliferation (Fig. 6b). Thus, our results suggest that *Zm*TPS1 promotes aphid deterrence rather than aphid resistance in maize. These data suggest that *Zm*TPS1 and *Zm*TPS2 may function together to repel/protect against *R. maidis*, perhaps with additive effects. It would be an interesting avenue for future research to compare the impact of single and double mutants of these genes in the same genetic background.

Interestingly, *Zm*TPS1 may protect maize against certain pathogens. Linalool and myrcene have antifungal activity *in vitro* against the plant pathogens *Rhizoctonia solani, Fusarium oxysporium, Penicillium digitatum* and *Aspergillus niger*. (MareiKh et al., 2012). Furthermore, *Zm*TPS1 is one of an array of maize genes that can produce *ent*-kaurene, a precursor for the plant hormones gibberellins and for the anti-microbial phytoalexins kauralexins (Fu et al., 2016), supporting its potential involvement in anti-fungal defenses. *Zm*TPS1's multiple roles in maize defense are therefore an exciting area for future exploration.

## 3. Conclusions

The maize terpene synthase 1 gene produces volatiles linalool and *β*-myrcene. Loss of this gene leads to reduced attraction to *Spodoptera frugiperda,* but increased attraction to *Rhopalosiphum maidis*.

## 4. Experimental section

### 4.1. Headspace volatile collection and GC-MS analysis

*Zea mays* plants were grown at two per 11.4 cm pot in soil containing 45% (v/v) Canadian peat, 20% (v/v) vermiculite medium, 20% (v/v) perlite coarse, and 15% (v/v) coir from BWI Companies (Apopka, Florida, USA). Plants were grown for three weeks in a greenhouse with 12 h supplemental lighting. The temperature range in the greenhouse was 25–40 °C. One pot containing two plants was placed in each volatile collection chamber (volatile collection system model TVCSx4V; Analytical Research Systems, Gainesville, Florida, USA). Plants were infested by the addition of 10–20 *S. frugiperda* neonates in the whorl of each plant and volatiles were collected on SuperQ columns for 24 h with an incoming airflow of 1.7 L $\text{min}^{-1}$ and a vacuum from the SuperQ columns of 0.7 L $\text{min}^{-1}$. After collection the columns were removed from the chambers and an internal standard of 2 μg of nonyl acetate was added to the SuperQ columns, and volatiles were eluted from the columns with 150 μL of methylene chloride. Above ground fresh weight of the plant tissue in each chamber was then determined. Volatile compounds were analyzed and quantified on an Agilent 7890A Gas Chromatograph with an DB5-MS column (30 m × 0.250 mm x 0.25 μm) coupled to an Agilent 7000 GC Mass Spectrometer Triple Quad. Two microliters of the eluted volatiles were injected onto the GC using a splitless injection and separated with a flow of 0.94 mL $\text{min}^{-1}$ and a temperature gradient of 30 °C for 2 min followed by a temperature ramp at 10 °C $\text{min}^{-1}$ to 250 °C, and a hold for 1 min at 250 °C. Mass spectrometry was performed using an EI Source at 220 °C with an MS1 scan from m/z 45–550 with a 250 ms scan time and steps of m/z 0.1. The identities of the volatiles quantified were confirmed using authentic standards for linalool, β-myrcene, cyclosativene, farnesene, and β-caryophyllene (Sigma-Aldrich, St. Louis, Missouri, USA), humulene (Toronto Research Chemicals inc. Ontario, Canada), β-ocimene (International Flavors and Fragrances inc. New York, New York, USA), DMNT (Enamine, Monmouth Junction, New Jersey, USA), and germacrene-D (major sesquiterpene from golden rod oil). Volatile production was calculated using the total ion chromatograph peak area, normalized for recovery with nonyl acetate and adjusted for plant aboveground fresh weight.

### 4.2. Metabolomic-genome wide association study (mGWAS)

This study utilized a subset of the SweetCAP sweet corn diversity population containing 347 lines and varieties listed in (Table S1, Supporting Information). Three biological replicates of each line were infested with *S. frugiperda* neonates and used for headspace volatile collection as described above. The genotyping used in this work was presented previously. In summary, whole genome resequencing libraries for the whole SweetCAP diversity population were prepared and sequenced on an llumina NovaSeq 2 × 150 bp. Reads were aligned to the Ia453-*sh2* reference genome (Hu et al., 2021) using BWA-mem. Variants (including single-nucleotide polymorphisms and small insertion-deletion polymorphisms) were called using both GATK and Freebayes. The overlapping variants resulting from these two methods (47,160,177 variants) were selected and further filtered using GATK best practices (Van der Auwera et al., 2013) and by missingness (>30%), minor allele frequency (>1%), and LD pruning resulting in a final set of 5,080,502 variants. Genome wide association studies (GWAS) was carried out using the FarmCPU (Liu et al., 2016) implemented in the GAPIT 3 R package (Wang and Zhang, 2021). In summary, the method utilizes two models iteratively, one with fixed effects and another one with random effects. Population structure is corrected using a kinship matrix derived from the markers, but the method adjusts the kinship iteratively to include possible quantitative trait nucleotides (QTNs) to increase the statistical power (Liu et al., 2016). The kinship matrix was calculated using the default algorithm in FarmCPU. The analysis was performed using a maxLoop threshold of 10, and a QTN threshold of 10. Markers were considered significant if their p-value was smaller than the FDR-corrected 0.05 significance level. The marker effects for all the markers were made available at https://github.com/Resende-Lab/Yactayo-Chang-et-al—GWAS-Terpene-Synthase-1-

### 4.3. Isolation of *tps1* loss-of-function mutant

Seeds from the UniformMu (McCarty et al., 2005) stock UFMu-06794 in the bzW22 genetic background were provided by the Maize Genetic Stock Center (MaizeGDB.org) and screened for the presence of the mu transposon insertion (mu1004651) in the first intron of the gene *Zm*TPS1 (GRMZM2G049538, *Zm*00001d002351) using PCR with the gene specific primer P2714: 5′-AGCCATTCAGGTCAGGTACG-3′ and transposon specific primer TIR6: 5′-AGAGAAGCCAACGCCAWCGCCTCYATTTCGTC-3'. Plants containing the transposon insertion were backcrossed into bzW22 and the self-pollenated and homozygous *tps1* mutant plants were identified by the presence of the transgene and absence of a gene specific PCR product using the primer P2714 and the gene specific primer P2713: 5′-TGATGGGTCTGCATGTATGG-3'. The *tps1* loss of function nature of this transposon insertion was previously described previously (Xu et al., 2019).

### 4.4. Pairwise choice assays

The pairwise feeding choice assays for *S. frugiperda* larvae were performed with newly emerged neonates. To assess if *S. frugiperda* larvae displayed a preference between wild-type (bzW22) or *tps1* mutant plants equivalent sections of maize leaves from each plant were placed on opposite sides of a Petri dish, and approximately 30 *S. frugiperda* larvae were placed in the center of the dish. Larval choice was scored after 10 min and determined as number of larvae touching each leaf section. Each experiment was run with 24 petri dishes and the average percentage of larvae choosing each treatment was calculated. This experiment was repeated seven times. Statistical analysis of the data was performed using a paired *t*-test.

Colonies of *S. frugiperda* and *C. marginiventris* were reared and oviposition choice assays performed as described previously (Block et al., 2020). Briefly, for the *S. frugiperda* pairwise choice oviposition assay, six plants each of wild-type (bzW22) and *tps1* mutant plants (one month old) were placed in an oviposition tent using a randomized block design. The tent was 274 cm × 213 cm x 150 cm (Coleman Sundome Tent, B07ZHYS73W, Chicago, Illinois, USA) and was in a climate-controlled room at 30 °C with external air exchange. Equal numbers of mated male and female moths (6–10 each) were released into the tent at 5:00 p.m. and allowed to oviposit for 16 h in complete darkness. Oviposition preference was then assessed by determining the relative number of egg patches on wild-type plants per tent, compared to those on the *Zm*TPS1 mutant plants. The *S. frugiperda* oviposition choice assays were repeated eight independent times. Statistical analysis of the data was performed using a paired *t*-test.

For *C. marginiventris* pairwise choice assays, two wild-type and two *tps1* mutant plants (2-3-week-old) were placed into a black windowless rearing and observation cages measuring 70 cm × 70 cm × 92 cm (BioQuip Products, Rancho Dominguez, California, USA) in a greenhouse. Each plant was infested with ten first instar *S. frugiperda* larvae. The following day 20–30 mated adult *C. marginiventris* were released into the cage. Five hours later the larvae were removed from the plants and placed as one larvae per well in a 32-cell diet tray system with removable 4-cell lids (white trays, RT32W; lids, TRCV4; Frontier Agriculture Sciences, Newark, Delaware, USA) containing an *S. frugiperda*-specific diet (Southland Products, Lake Village, Arkansas, USA). The trays were marked with the identity of the plant variety the larvae were feeding upon. Once the *C. marginiventris* pupae formed, the percentage of parasitized larvae was determined for the *S. frugiperda* removed from each plant variety as the (number parasitized/[number parasitized + number not parasitized]) × 100. The *C. marginiventris* oviposition choice assays were repeated nine independent times. Statistical analysis of the data was performed using a paired *t*-test.

### 4.5. Electroantennogram assays

To examine antennal responses of mated female *S. frugiperda* to linalool and beta-myrcene, electroantennogram (EAG) assays were conducted as describe previously (Rering et al., 2018). Briefly, *S. frugiperda* pupae were sorted by sex and females were individually marked on the day of emergence before being housed with abundant males. Adult *S. frugiperda* were maintained at 27 °C and 60% RH under a 16 h light: 8 h dark photoperiod and provided a honey water solution. After 5–6 d one antenna per mated female was excised at the scape and <1 mm was trimmed from the distal end before mounting on a forked probe using electrode gel. The probe was then mounted in a constant flow of humidified air (530 mL min $^{-1}$) and allowed to stabilize before test stimuli were applied. Test odors (linalool and *β*-myrcene) were prepared in pentane as described previously (Rering et al., 2018) at each of 6 loading doses: 1, 10, 20, 50, 100, or 200 nmol of test compound in 20 μl pentane. Stimuli were delivered using a 1.0 s pulse flow, which diverted 260 mL min $^{-1}$ of the total flow through the delivery tube (total flow unchanged) and mixed the humidified air and test stimuli in a delivery tube (1.5 cm diameter) before contacting the mounted antenna. To account for variability in antennal responses between individuals and potential degradation of responses during the assay antennae were presented with a standard stimulus (50 nmol eugenol) and pentane blank at the beginning and end of each assay. Each antenna was presented with three randomly selected test odors/concentrations, resulting in n = 4–8 for each test odor/concentration combination. For analysis, pentane blanks were subtracted from each sample and antennal responses were normalized to the standard stimulus (set as 100%). Analyses were conducted using the Kruskal-Wallis Rank Sum Test in R (version 4.0.5) (R Core Team, 2021) to evaluate the effect of loading dose on antennal responses to each of the two test compounds.

### 4.6. Gene expression analysis

Three-week-old bzW22 (wild-type) maize plants were infested with approximately 20 *S. frugiperda* neonates in the central whorl for 24 h. Non-infested plants of the same age were used as no-treatment controls. Whorl tissue was harvested, and flash frozen in liquid nitrogen and total RNA was isolated using RNeasy® Plant Kit (Qiagen) according to manufacturer's instructions. Two μg of total RNA was transcribed into cDNA using Oligo(dT) primers and the RETROscript™ Reverse Transcription Kit (Ambion, Elk Grove, CA, USA). Relative expression levels were determined by quantitative real time PCR using iTaq Universal SYBR® Green Supermix (Bio-rad Laboratories, CA, USA), a CFX-96thermocycler (Bio-Rad). *tps1*-fw: 5′-GTGAAGCTGACTCCTGCCTC-3′, and *tps1*-rev: 5′-CCGATGCTTCTTGATCGTGC-3′ *Zm*TPS1 gene specific primers pairs were used to measure relative expression according to the $2^{\wedge(ddCq)}$ method with fpgs and ubcp as reference genes (fpgs-forward: 5′-ATCTCGTTGGGGATGTCTTG-3′, and fpgs-reverse: 5′-AGCACCGTTCAAATGTCTCC-3'; ubcp-forward: 5′-CAGGTGGGGTATTCTTGGTG-3′, and ubcp-reverse: 5′-ATGTTCGGGTGGAAAACCT-3' (Manoli et al., 2012).

### 4.7. Larval growth assays

For larval growth assays 12 greenhouse-grown three-week-old bzW22 (wild-type) and *tps1* mutant plants were placed in separate black windowless rearing and observation cages described above. Plants were infested by gently placing 20 to 40 newly emerged *S. frugiperda* neonates into the central whorl of each plant using a paintbrush. To obtain larval growth curves, the larvae were removed from the plants using featherweight forceps and weighed daily from 4 to 8 days post infestation. This experiment was repeated twice with similar results. Statistical analysis of the growth assay was performed using an unpaired *t*-test.

### 4.8. *Rhopalosiphum maidis* bioassays

*Rhopalosiphum maidis* colony propagation and aphid choice bioassays were conducted as described previously (Louis et al., 2015). Briefly, five adult apterous aphids were introduced on each bzW22 and *tps1* plant for the no-choice assays, and the total number of aphids (adults + nymphs) was recorded seven days after aphid release. For pairwise choice assays, 15 adult aphids were released at the center of the pot (20 cm × 20 cm) equidistant from both maize (bzW22 and *tps1*) lines. The number of adult aphids settled on each plant was counted 6 and 24 h after aphid release. Both no-choice and pairwise choice assays were repeated twice with similar results. Statistical analysis of the pairwise choice assay was performed using a $\chi^2$ test and of the no-choice assay using a Tukey's test.

## CRediT authorship contribution statement

**Jessica P. Yactayo-Chang:** Writing - original draft, Methodology, Investigation, Data curation. **Geoffrey T. Broadhead:** Writing - original draft, Methodology, Investigation, Data curation. **Robert J. Housler:** Investigation. **Marcio F.R. Resende:** Writing - original draft,

Methodology, Investigation, Funding acquisition, Formal analysis. **Kashish Verma:** Methodology, Investigation, Data curation. **Joe Louis:** Writing - original draft, Supervision, Funding acquisition, Formal analysis. **Gilles J. Basset:** Writing - review & editing, Supervision, Funding acquisition. **John J. Beck:** Writing - review & editing, Supervision, Methodology. **Anna K. Block:** Writing - original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

A link to data via github is availible in the manuscript.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2023.113957.

## References

Aharoni, A., Giri, A.P., Deuerlein, S., Griepink, F., de Kogel, W.J., Verstappen, F.W., Verhoeven, H.A., Jongsma, M.A., Schwab, W., Bouwmeester, H.J., 2003. Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. Plant Cell 15, 2866–2884.

An, L., Ahmad, R.M., Ren, H., Qin, J., Yan, Y., 2019. Jasmonate signal receptor gene family ZmCOIs restore male fertility and defense response of Arabidopsis mutant *coi1-1*. J. Plant Growth Regul. 38, 479–493. https://doi.org/10.1007/s00344-018-9863-2.

Block, A.K., Hunter, C.T., Rering, C., Christensen, S.A., Meagher, R.L., 2018. Contrasting insect attraction and herbivore-induced plant volatile production in maize. Planta 248, 105–116. https://doi.org/10.1007/s00425-018-2886-x.

Block, A.K., Vaughan, M.M., Schmelz, E.A., Christensen, S.A., 2019. Biosynthesis and function of terpenoid defense compounds in maize (*Zea mays*). Planta 249, 21–30. https://doi.org/10.1007/s00425-018-2999-2.

Block, A.K., Mendoza, J., Rowley, A., Stuhl, C., Meagher, R.L., 2020. Approaches for assessing the impact of *Zea mays* (poaceae) on the behavior of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its parasitoid *Cotesia marginiventris* (hymenoptera: braconidae). Fla. Entomol. 103, 505–513. https://doi.org/10.1653/024.103.00414.

Carroll, M.J., Schmelz, E.A., Meagher, R.L., Teal, P.E.A., 2006. Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. J. Chem. Ecol. 32, 1911–1924. https://doi.org/10.1007/s10886-006-9117-9.

Chen, F., Tholl, D., Bohlmann, J., Pichersky, E., 2011. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. Plant J. 66, 212–229. https://doi.org/10.1111/j.1365-313X.2011.04520.x.

Davis, E.M., Croteau, R., 2000. Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes, and diterpenes. Top. Curr. Chem. 209, 53–95.

Degen, T., Dillmann, C., Marion-Poll, F., Turlings, T.C.J., 2004. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. Plant Physiol 135, 1928–1938. https://doi.org/10.1104/pp.104.039891.

Ding, Y.Z., Huffaker, A., Kollner, T.G., Weckwerth, P., Robert, C.A.M., Spencer, J.L., Lipka, A.E., Schmelz, E.A., 2017. Selinene volatiles are essential precursors for maize defense promoting fungal pathogen resistance. Plant Physiol 175, 1455–1468. https://doi.org/10.1104/pp.17.00879.

Fouad, A.H., de Souza Tavares, W., Zanuncio, J.C., 2021. Toxicity and repellent activity of monoterpene enantiomers to rice weevils (*Sitophilus oryzae*). Pest Manag. Sci. 77, 3500–3507. https://doi.org/10.1002/ps.6403.

Fu, J., Ren, F., Lu, X., Mao, H., Xu, M., Degenhardt, J., Peters, R.J., Wang, Q., 2016. A tandem array of *ent*-kaurene synthases in maize with roles in gibberellin and more specialized metabolism. Plant Physiol 170, 742–751. https://doi.org/10.1104/pp.15.01727.

Gouinguené, S., Degen, T., Turlings, T., 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). Chemoecology 11, 9–16. https://doi.org/10.1007/PL00001832.

Hu, Y., Colantonio, V., Müller, B.S.F., Leach, K.A., Nanni, A., Finegan, C., Wang, B., Baseggio, M., Newton, C.J., Juhl, E.M., Hislop, L., Gonzalez, J.M., Rios, E.F., Hannah, L.C., Swarts, K., Gore, M.A., Hennen-Bierwagen, T.A., Myers, A.M., Settles, A.M., Tracy, W.F., Resende Jr., M.F.R., 2021. Genome assembly and population genomic analysis provide insights into the evolution of modern sweet corn. Nat. Commun. 12, 1227. https://doi.org/10.1038/s41467-021-21380-4.

Huang, X.Z., Xiao, Y.T., Köllner, T.G., Jing, W.X., Kou, J.F., Chen, J.Y., Liu, D.F., Gu, S. H., Wu, J.X., Zhang, Y.J., Guo, Y.Y., 2018. The terpene synthase gene family in *Gossypium hirsutum* harbors a linalool synthase *Gh*TPS12 implicated in direct defence responses against herbivores. Plant Cell Environ. 41, 261–274. https://doi.org/10.1111/pce.13088.

Köllner, T.G., Schnee, C., Gershenzon, J., Degenhardt, J., 2004. The variability of sesquiterpenes emitted from two *Zea mays* cultivars is controlled by allelic variation of two terpene synthase genes encoding stereoselective multiple product enzymes. Plant Cell 16, 1115–1131. https://doi.org/10.1105/tpc.019877.

Köllner, T.G., Schnee, C., Li, S., Svatos, A., Schneider, B., Gershenzon, J., Degenhardt, J., 2008. Protonation of a neutral (*S*)-$\beta$-bisabolene intermediate is involved in (*S*)-$\beta$-macrocarpene formation by the maize sesquiterpene synthases TPS6 and TPS11. J. Biol. Chem. 283, 20779–20788. https://doi.org/10.1074/jbc.M802682200.

Lin, C.F., Shen, B.Z., Xu, Z.N., Köllner, T.G., Degenhardt, J., Dooner, H.K., 2008. Characterization of the monoterpene synthase gene *tps*26, the ortholog of a gene induced by insect herbivory in maize. Plant Physiol 146, 940–951. https://doi.org/10.1104/pp.107.109553.

Liu, X., Huang, M., Fan, B., Buckler, E.S., Zhang, Z., 2016. Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet. 12, e1005767 https://doi.org/10.1371/journal.pgen.1005767.

Louis, J., Basu, S., Varsani, S., Castano-Duque, L., Jiang, V., Williams, W.P., Felton, G.W., Luthe, D.S., 2015. Ethylene contributes to *maize insect resistance1*-mediated maize defense against the phloem sap-sucking corn leaf aphid. Plant Physiol 169, 313–324. https://doi.org/10.1104/pp.15.00958.

Malo, E.A., Castrejón-Gómez, V.R., Cruz-López, L., Rojas, J.C., 2004. Antennal sensilla and electrophysiological response of male and female *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to conspecific sex pheromone and plant odors. Ann. Entomol. Soc. Am. 97, 1273–1284. https://doi.org/10.1603/0013-8746, 2004)097 [1273:ASAERO]2.0.CO;2.

Manoli, A., Sturaro, A., Trevisan, S., Quaggiotti, S., Nonis, A., 2012. Evaluation of candidate reference genes for qPCR in maize. J. Plant Physiol. 169, 807–815. https://doi.org/10.1016/j.jplph.2012.01.019.

Marei, G.I.Kh, Abdel Rasoul, M.A., Abdelgaleil, S.A.M., 2012. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pestic. Biochem. Physiol. 103, 56–61. https://doi.org/10.1016/j.pestbp.2012.03.004.

McCarty, D.R., Settles, A.M., Suzuki, M., Tan, B.C., Latshaw, S., Porch, T., Robin, K., Baier, J., Avigne, W., Lai, J.S., Messing, J., Koch, K.E., Hannah, L.C., 2005. Steady-state transposon mutagenesis in inbred maize. Plant J. 44, 52–61. https://doi.org/10.1111/j.1365-313X.2005.02509.x.

Nagoshi, R.N., Goergen, G., Tounou, K.A., Agboka, K., Koffi, D., Meagher, R.L., 2018. Analysis of strain distribution, migratory potential, and invasion history of fall armyworm populations in northern Sub-Saharan Africa. Sci. Rep. 8, 3710. https://doi.org/10.1038/s41598-018-21954-1.

Pinto-Zevallos, D.M., Strapasson, P., Zarbin, P.H.G., 2016. Herbivore induced volatile organic compounds emitted by maize: electrophysiological responses in *Spodoptera frugiperda* females. Phytochem. Lett. 16, 70–74. https://doi.org/10.1016/j.phytol.2016.03.005.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Rane, R., Walsh, T.K., Lenancker, P., Gock, A., Dao, T.H., Nguyen, V.L., Khin, T.N., Amalin, D., Chittarath, K., Faheem, M., Annamalai, S., Thanarajoo, S.S., Trisyono, Y. A., Khay, S., Kim, J., Kuniata, L., Powell, K., Kalyebi, A., Otim, M.H., Nam, K., d'Alençon, E., Gordon, K.H.J., Tay, W.T., 2023. Complex multiple introductions drive fall armyworm invasions into Asia and Australia. Sci. Rep. 13, 660. https://doi.org/10.1038/s41598-023-27501.

Rering, C.C., Beck, J.J., Hall, G.W., McCartney, M.M., Vannette, R.L., 2018. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. New Phytol. 220, 750–759. https://doi.org/10.1111/nph.14809.

Richter, A., Schaff, C., Zhang, Z., Lipka, A.E., Tian, F., Köllner, T.G., Schnee, C., Preiß, S., Irmisch, S., Jander, G., Boland, W., Gershenzon, J., Buckler, E.S., Degenhardt, J., 2016. Characterization of biosynthetic pathways for the production of the volatile homoterpenes DMNT and TMTT in *Zea mays*. Plant Cell 28, 2651–2665. https://doi.org/10.1105/tpc.15.00919.

Schnee, C., Köllner, T.G., Gershenzon, J., Degenhardt, J., 2002. The maize gene terpene synthase 1 encodes a sesquiterpene synthase catalyzing the formation of (*E*)-beta-farnesene, (*E*)-nerolidol, and (*E,E*)-farnesol after herbivore damage. Plant Physiol 130, 2049–2060. https://doi.org/10.1104/pp.008326.

Schnee, C., Köllner, T.G., Held, M., Turlings, T.C.J., Gershenzon, J., Degenhardt, J., 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc. Natl. Acad. Sci. U.S.A. 103, 1129–1134. https://doi.org/10.1073/pnas.0508027103.

Signoretti, A.G.C., Peñaflor, M.F.G.V., Bento, J.M.S., 2012. Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), female moths respond to herbivore-induced corn volatiles. Neotrop. Entomol. 41, 22–26. https://doi.org/10.1007/s13744-011-0003-y.

Tholl, D., 2015. Biosynthesis and biological functions of terpenoids in plants. Adv. Biochem. Eng. Biotechnol. 148, 63–106. https://doi.org/10.1007/10_2014_295.

Turlings, T.C., Tumlinson, J.H., Lewis, W.J., 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250, 1251–1253. https://doi.org/10.1126/science.250.4985.1251.

Turlings, T.C., Tumlinson, J.H., Heath, R.R., Proveaux, A.T., Doolittle, R.E., 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. J. Chem. Ecol. 17, 2235–2251. https://doi.org/10.1007/BF00988004.

Tzin, V., Fernandez-Pozo, N., Richter, A., Schmelz, E.A., Schoettner, M., Schäfer, M., Ahern, K.R., Meihls, L.N., Kaur, H., Huffaker, A., Mori, N., Degenhardt, J., Mueller, L. A., Jander, G., 2015. Dynamic maize responses to aphid feeding are revealed by a time series of transcriptomic and metabolomic assays. Plant Physiol 169, 1727–1743. https://doi.org/10.1104/pp.15.01039.

Van der Auwera, G.A., Carneiro, M.O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K.V., Altshuler, D., Gabriel, S., DePristo, M.A., 2013. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr. Protoc. Bioinformatics. 43, 11. https://doi.org/10.1002/0471250953.bi1110s43, 10.1-11.10.33.

Wang, J., Zhang, Z., 2021. GAPIT version 3: boosting power and accuracy for genomic association and prediction. Dev. Reprod. Biol. 19 (4), 629–640. https://doi.org/10.1016/j.gpb.2021.08.005.

Xu, G., Cao, J., Wang, X., Chen, Q., Jin, W., Li, Z., Tian, F., 2019. Evolutionary metabolomics identifies substantial metabolic divergence between maize and its wild ancestor, teosinte. Plant Cell 31, 1990–2009. https://doi.org/10.1105/tpc.19.00111.

Yactayo-Chang, J.P., Mendoza, J., Willms, S.D., Rering, C.C., Beck, J.J., Block, A.K., 2021. *Zea mays* volatiles that influence oviposition and feeding behaviors of *Spodoptera frugiperda*. J. Chem. Ecol. 47, 799–809. https://doi.org/10.1007/s10886-021-01302-w.