Ecological Applications, 0(0), 2021, e02289

Chemical diversity rather than cultivar diversity predicts natural enemy control of herbivore pests

KAYLEIGH C. HAURI,[1,2,6] ANDREA E. GLASSMIRE,[1,2] AND WILLIAM C. WETZEL[1,2,3,4,5]

[1]*Department of Entomology, Michigan State University, East Lansing, Michigan 48824 USA*
[2]*Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, Michigan 48824 USA*
[3]*Kellogg Biological Station, Michigan State University, Hickory Corners, Michigan 49060 USA*
[4]*Department of Integrative Biology, Michigan State University, East Lansing, Michigan 48824 USA*
[5]*AgBioResearch, Michigan State University, East Lansing, Michigan 48824 USA*

Citation: Hauri, K. C., A. E. Glassmire, and W. C. Wetzel. 2021. Chemical diversity rather than cultivar diversity predicts natural enemy control of herbivore pests. Ecological Applications 00(00):e02289. https://doi.org/10.1002/eap.2289

Abstract. Cultivar mixtures have been studied for decades as a means for pest suppression. The literature, however, shows a large variability in outcomes, suggesting that we are unable to create mixtures that consistently suppress insect pests and attract natural enemies. A key gap in our understanding of how cultivar mixtures influence pest control is that few studies have examined the plant traits or mechanisms by which cultivar diversity affects pests and their interactions with natural enemies. The diversity of plant chemistry in a cultivar mixture is one trait dimension that is likely influential for insect ecology because chemical traits alter how predators and herbivores forage and interact. To understand how plant chemical diversity influences herbivores and their interactions with predators, we fully crossed predator presence or absence with monocultures, bicultures, and tricultures of three chemotypes of tomato that differed in odor diversity (terpenes) or surface chemistry (acyl sugars) in a caged field experiment. We found that the direct effects of plant chemotype diversity on herbivore performance were strongest in bicultures and depended on herbivore sex, and these effects typically acted through growth rather than survival. The effects of chemotype diversity on top-down pest suppression by natural enemies differed between classes of chemical diversity. Odor diversity (terpenes) interfered with the ability of predators to hunt effectively, whereas diversity in surface chemistry (acyl sugars) did not. Our results suggest that phytochemical diversity can contribute to pest suppression in agroecosystems, but that implementing it will require engineering cultivar mixtures using trait-based approaches that account for the biology of the pests and natural enemies in the system.

Key words: chemical defense; cultivar mixtures; plant chemical diversity; sustainable pest management; tritrophic interactions.

INTRODUCTION

Increasing plant diversity in agroecosystems has long been promoted as a strategy for reducing pest pressure and attracting natural enemies without reliance on pesticides (Andow 1991). One way to increase plant diversity in agriculture is through the use of cultivar mixtures (Zhu et al. 2000, Raboin et al. 2012). These genetically diverse plantings have successfully reduced insect pest pressure in some cases (Tooker and Frank 2012, Grettenberger and Tooker 2015) and are logistically easier to implement than crop diversification strategies that involve multiple plant species (e.g., push-pull systems; Lin 2011). However, despite significant research on intraspecific plant diversity (Crutsinger et al. 2006, McArt and Thaler 2013, Grettenberger and Tooker 2015, 2017), cultivar mixtures have had variable success in suppressing insect pests (Reiss and Drinkwater 2018). The variable success of this strategy may exist because we have a poor understanding of the trait-based mechanisms by which cultivar diversity influences insect herbivores and alters interactions between herbivores and natural enemies (Wetzel and Thaler 2016, Wetzel and Whitehead 2020). Improving our ability to design effective cultivar mixtures necessitates information on how the effects of cultivar diversity on pests and pest–enemy interactions vary among cultivar mixtures with different types of trait diversity.

A key limitation preventing us from designing effective cultivar mixtures for pest management is that most of our knowledge about the effects of plant diversity on higher trophic levels comes from studies that manipulate the number of plant cultivars or genotypes without

---

Manuscript received 2 February 2020; revised 28 September 2020; accepted 5 October 2020. Corresponding Editor: Jason P. Kaye.

[6] E-mail: haurikay@msu.edu

examining specific traits outside general metrics of resistance or performance (Power 1991, Crutsinger et al. 2006). However, most genetically different cultivars differ in many traits simultaneously, so a fundamental gap in our understanding of the efficacy of cultivar mixtures is the lack of controlled experiments linking pest control to specific plant traits. Recent studies have adopted experimental approaches by manipulating trait diversity across plants using genetic knockouts of single alleles that increase or decrease overall defense production (Kersch-Becker and Thaler 2015, 2017, Schuman et al. 2015). These studies have shown conclusively that different defense traits cause changes in insect ecology, and a key next step will be studies that manipulate traits based on the chemical composition of plants, or their “chemotype” (Dyer et al. 2018, Kessler and Kalske 2018, Wetzel and Whitehead 2020). Plant chemicals are central to plant–pest and pest–predator interactions and can affect pests directly as they forage (Cornell and Hawkins 2003) or indirectly by altering natural enemy hunting ability (Hare 2011). If we understand how diversity in plant odors and surface chemistry affects insect populations, we could implement plant chemical diversity as a pest management strategy.

A second gap in our ability to design effective crop mixtures is that few studies have investigated how intraspecific diversity influences top-down pest suppression by predators, a key component of sustainable pest control (Moreira et al. 2016). Plant trait diversity is likely to influence natural enemies and their hunting ability directly because natural enemies use plant traits as foraging cues and interact with these traits as they move across plants (Hare 2011) and feed on herbivores (Kersch-Becker and Thaler 2015, Kersch-Becker et al. 2017). Recent studies have shown that manipulating plant diversity affects community assembly for both herbivores and predators, but few specifically manipulate predator presence. For example, chemical diversity among neighboring plants influenced insect community composition in three different lines of wild cabbage that differed in foliar glucosinolates (Bustos-Segura et al. 2017). Similarly, in wild tobacco plants that did or did not express certain chemical defense pathways, the presence of specific defense pathways improved outcomes for all plants in a plot, even those that lacked the pathway themselves (Schuman et al. 2015). These studies enhance our understanding of plant chemotype effects on community assembly but highlight the need for future work that allows us to tease apart how top-down and bottom-up effects. A study that did specifically manipulate predator presence in cultivar mixtures of soybean shows mixed effects of cultivar diversity on predators, but did not investigate chemical traits of the different cultivars (Grettenberger and Tooker 2020). Again, this leaves a gap in our understanding of how changes in the predator-prey interaction are specifically affected by chemotype diversity.

In this study, we used a field and laboratory experiment to investigate how the diversity of chemical phenotypes (chemotype diversity) within cultivar mixtures interacts with predator presence to influence pest growth and survival. We did this by tracking cabbage looper caterpillars in caged field plots of monocultures or polycultures of three tomato genotypes that differed in terpenes or acyl sugars, two important classes of chemical traits in tomato. We isolated the effects of chemotype diversity on the predator–prey interaction by including predators in half of the cages and crossing the predator treatment with the cultivar diversity treatments. We used a laboratory experiment to investigate predator preference for each chemotype in the absence of prey. Our questions were the following: (1) How does plant chemotype diversity influence herbivores, and how does this differ among chemical classes? (2) How does plant chemotype diversity influence the interactions between predator and prey, and how does this differ among chemical classes? (3) How does variation across plant chemotypes drive predator foraging behavior? Answering these questions will enhance our ability to breed and design sets of cultivars that make efficient mixtures.

METHODS

Plants and insects

We investigated the effects of intraspecific chemotype diversity by establishing plots with different combinations of tomato chemotypes. We used three chemotypes from a set of introgression lines created by crossing cultivated “commercial” tomato (*Solanum lycopersicum* cv. M82, Solanaceae) with a wild tomato (*Solanum pennellii*). Each introgression line is genetically identical to the commercial genotype except in specific, known segments of a chromosome, which are replaced with the corresponding section of the *S. pennellii* genome. Many traits in each of these lines, including the chemical defense traits terpenes and acyl sugars, have been described and quantified (Schilmiller et al. 2010). This allowed us to curate cultivar mixtures that varied in zero, one, or two classes of plant defense (monocultures, bicultures, and polycultures, respectively).

Our three chemotypes included the commercial line, a line that differs from the commercial line in acyl sugars (IL 1-3); and a line that differs from the commercial line in terpenes (IL 10-3; Table 1). Both introgression lines were similar morphologically to the commercial line and indistinguishable by eye. Acyl sugars are sticky compounds exuded by glandular trichomes, located on the stem and leaf surfaces. They are direct defenses that deter herbivores via chemical and mechanical processes (Goffreda et al. 1989). The line we used with altered acyl sugars differed in the type of acyl sugar it has, meaning the acyl sugar lacked an acetyl group in the chain and had a sucrose rather than a glucose core (Table 1). Terpenes are volatile compounds released by plants and often act as both direct and indirect defenses that repel herbivores and attract predators (Gershenzon and

Table 1. Tomato genotypes, chemotypes, and total replicates for each plant treatment used in the field experiment.

Diversity level	Chemotype	Genotype	Chemical experience in comparison to commercial	Total control replicates	Total predator replicates
Monoculture	commercial	M82	NA	18	17
Monoculture	terpene	10-3	A 75–80% reduction in sesquiterpenes compared to commercial genotype.†	16	15
Monoculture	acyl sugar	1-3	Acyl sugars have chain lengths of 3, lacking an acetyl group. Chain is connected to a sucrose base. Has an additional monoterpene.†	15	13
Biculture	commercial + terpene	two M82, one 10-3	Insects experience either a change in terpenes (moving from commercial to terpene or terpene to commercial) or no change (commercial to commercial).	18	19
Biculture	commercial + acyl sugar	two M82, one 1-3	Insects experience either a change in acyl sugars (moving from commercial to acyl sugar or acyl sugar to commercial) or no change (commercial to commercial).	17	17
Polyculture	commercial, acyl sugar, terpene	M82, 10-3, 1-3	Insects experience either a change in terpenes or acyl sugars when moving from plant to plant.	16	14

†Schilmiller et al. (2010).

Dudareva 2007). The tomato line with altered terpenes used in this experiment had 75–80% reduced sesquiterpene abundance compared to the commercial line (Schilmiller et al. 2010, Table 1).

Our herbivore was *Trichoplusia ni* (Lepidoptera: Noctuidae), the cabbage looper, a generalist pest on tomatoes and many other crop species (Shorey et al. 1962). We obtained *T. ni* from a commercial insectary (Benzon Research, Carlisle, Pennsylvania, USA) and maintained them on general noctuid diet (Benzon Research) until we used them in experiments. Our predator was *Podisus maculiventris* (Hemiptera: Pentatomidae), the spined soldier bug, a generalist biocontrol predator that is native to much of North America, including our field site. Our colony was initiated with wild-caught individuals, supplemented with nymphs from Rincon-Vitova Insectaries (Ventura, California), and reared in the greenhouse on mealworms (*Tenebrio molitor*) and a tomato line not used in the experiment.

Field experiment

We tested the effects of different levels and types of plant chemotype diversity on the interaction between *T. ni* and *P. maculiventris* by fully crossing predator presence with chemotype diversity treatments in a field experiment at Kellogg Biological Station (Hickory Corners, Michigan, USA). Our chemotype diversity treatments included three monocultures, two bicultures, and one polyculture. The monocultures represented each of our three tomato lines (commercial, altered terpenes, or altered acyl sugars). Monocultures present insects with no genetically based chemotype diversity among plants, thus, allowing us to isolate the effects of each chemotype individually on *T. ni* and its interactions with *P. maculiventris*. We had two biculture treatments with two chemotypes each: commercial and altered terpenes or commercial and altered acyl sugars. Last, the polyculture treatment contained one plant of each of the three chemotypes and therefore presented insects with chemical diversity across two compound classes (terpenes and acyl sugars). For each of these plant treatments, sets of three 5-week-old greenhouse-grown tomatoes were transplanted into the field in a triangle shape with 20-cm spacing centered in 1-m^2 plots in black plastic mulch with dripline irrigation, with each plot covered by a 1-m mesh cage (Lumite).

One week after planting and caging, we measured plant height and number of leaves (Appendix S1) and released 15 pre-weighed second-instar *T. ni* into the center of each plot. Approximately 30 minutes after release, *T. ni* reached the plants and began feeding, and we released one third-instar, one fourth-instar, and one adult *P. maculiventris* into cages in the predator-present treatment. We exhaustively censused each cage for caterpillar and predator survival and location once per week for three weeks. We replaced any dead *P. maculiventris* found in cages. On week 3 when *T. ni* were pupating, we harvested all remaining insects. Pupae were collected, sexed, and individually massed. We also collected plant herbivory data (Appendix S1). Because *T. ni* are multivoltine in many areas where they are crop pests (Ehler 1977, Chamberlain and Kok 1986), we performed two temporal rounds of the experiment, one in July and one in August. In total we had between 13 and 19 replicates for all plant treatments (Table 1).

We examined the effects of our treatments on *T. ni* survival and pupal mass in the presence and absence of predators using mixed models in the lme4 package (Bates et al. 2015, R Core Team 2017). We analyzed pupal mass using linear mixed models and survival using binomial generalized linear mixed models. *T. ni* males and females differ in pupal mass (Shorey et al. 1962), thus we analyzed them separately. We accounted for correlations between caterpillars in the same cage and round using plot and round as random effects. We tested hypotheses about the effects of each treatment and their interaction by comparing models with treatment effects to models without treatment effects using likelihood ratio tests (Bolker et al. 2009). We used the lsmeans function in the package emmeans for pairwise comparisons between individual treatments.

We addressed our first question about the direct effects of chemotype diversity on pest performance by analyzing data only from plots without predators. First, we examined the effects of each chemotype individually on herbivore growth and survival by comparing performance across our three monocultures in the absence of predators. Second, we examined the effects of overall amount of chemotype diversity, ignoring chemotype identities, by using amount of chemotype diversity (monoculture, biculture, or polyculture) as the only fixed-effect predictor. Third, we compared the effects of diversity across our two chemical classes by including a predictor for all six non-predator treatments (all monocultures, altered terpene biculture, altered acyl sugar biculture, and full polyculture). Fourth, we looked for effects of variation in diversity within a specific chemical class by comparing performance within two separate group of plant treatments: commercial monoculture, altered acyl sugar monoculture, and altered acyl sugar biculture (for acyl sugar diversity); or commercial monoculture, altered terpenes monoculture, and altered terpenes biculture (for terpene diversity). Finally, we looked at the effects of adding a second type of chemical diversity to a plot with only one type of chemical diversity by comparing performance between each biculture and the full polyculture.

We addressed our second question about the effects of chemotype diversity on the herbivore–predator interaction by analyzing our full data set including plots with and without predators. First, we examined the effects of each chemotype individually on herbivore growth and survival by comparing performance across our three monocultures using both chemotype and predator presence as fixed-effect predictors. Second, we tested for interactions between predators and diversity level. Third, we tested interactions between predators and chemotype identity. Fourth, we compared the effects of diversity across our two chemical classes by including a predictor for all six chemotype identity treatments (all monocultures, altered terpene biculture, altered acyl sugar biculture, and full polyculture) as well as predator presence or absence. Fifth, we looked for interactions between predator presence and effects of variation in diversity within a specific chemical class by comparing performance within two separate group of plant treatments: commercial monoculture, altered acyl sugar monoculture, and altered acyl sugar biculture (for acyl sugar diversity); or commercial monoculture, altered terpenes monoculture, and altered terpenes biculture (for terpene diversity). Finally, we looked at predator interactions with the effects of adding a second type of chemical diversity to a plot with only one type of chemical diversity by comparing performance between each biculture and the full polyculture.

Laboratory choice experiment

We examined the extent to which results from the field experiment could have been driven by the predator foraging preferentially on specific plant chemotypes in bicultures and polycultures using a choice experiment. We measured *P. maculiventris* preferences by placing them individually into the center of mesh cages (30.5 × 30.5 × 61 cm) with two 7-week-old tomato plants. Each cage had either a commercial chemotype and an altered acyl sugar chemotype, a commercial chemotype and an altered terpene chemotype, or an altered acyl sugar chemotype and an altered terpene chemotype. After a 20-minute acclimation period, we recorded insect location and behavior six or more times per day for 72 h. Before starting the experiment, we damaged each plant by removing every fifth leaf and running a serrated tracing wheel 10 times across the three youngest leaflets. Mechanical damage causes tomato to release volatile cues that are used by predators in host location (Korpita et al. 2014). We excluded 15 cages in which insects were never observed on plants, leaving us with 10 commercial and altered acyl sugar, 10 commercial and altered terpene, and 7 altered acyl sugar and altered terpene combinations. We estimated *P. maculiventris* preference probabilities using binomial generalized linear mixed models in the lme4 package (Bates et al. 2015, R core team 2017) with cage as a random effect.

RESULTS

Field experiment

How does chemotype diversity influence herbivores?.—We investigated the bottom-up effects of each chemotype, levels of chemotype diversity, and chemical class of chemotype diversity on herbivores by analyzing plots without predators. Plants were an approximately equal growth stages for both rounds of the experiment, with similar heights (Appendix S1: Fig. S1) and about two more compound leaves on average per plant in round 1 compared to round 2 (Appendix S1: Fig. S2) before caterpillars were added. When we examined the effects of each chemotype on herbivore growth and survival in monoculture, we found that herbivore survival was similar across the three chemotypes in monoculture (χ^2 = 1.10, df = 2, P = 0.58; Fig. 1A). The plant chemotype that was present in monoculture plots affected male *T. ni* mass (χ^2 = 7.26, df = 2, P = 0.03) but not females (χ^2 = 3.76, df = 2, P = 0.15; Fig. 1). Males were largest in the terpene chemotype monocultures (Fig. 1); there

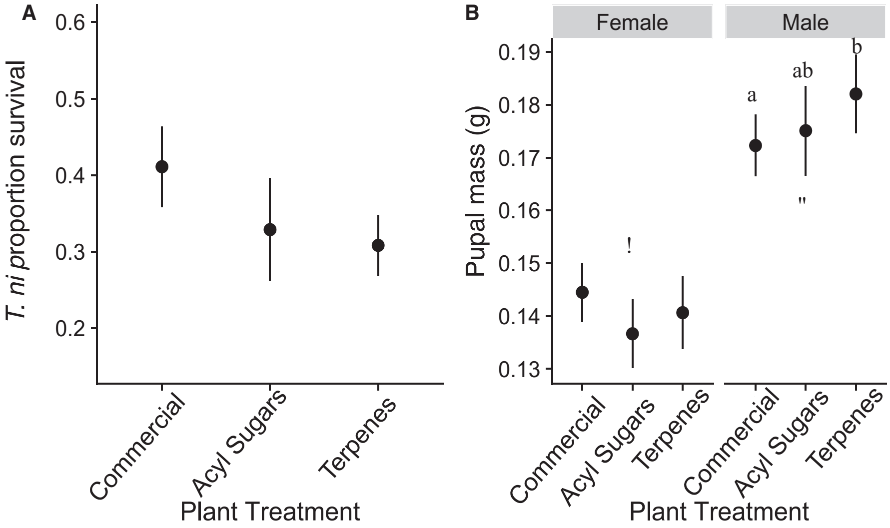

Fig. 1. (A) Survival and (B) pupal mass in control monocultures. Plot shows mean ± SE. Survival was not significantly different between plant treatments, although survival in the commercial chemotype was slightly higher than either of the introgression lines, which varied in acyl sugars or terpenes. Overall survival in control monocultures was 33.45%. Pupal mass was significantly different between males and females, which is typical for *Trichoplusia ni* (Shorey et al. 1962). Due to this difference, we analyzed male and female pupal mass results separately. Plant treatment did not influence female pupal mass in control monocultures, but did influence males. Uppercase letters represent significant ($P \leq 0.05$) differences between sexes. Lowercase letters represent significant differences ($P \leq 0.05$) within sexes.

was an 11.0% difference in pupal mass between the largest and smallest monocultures for males.

Next, we examined the effect of chemotype diversity by analyzing the three cultivar diversity levels (monoculture, biculture, and polyculture) in the absence of predators, isolating bottom-up effects on herbivores. There was no effect of overall level of chemotype diversity on *T. ni* survival in control cages ($\chi^2 = 1.16$, df = 2, $P = 0.56$). We also found that level of chemotype diversity did not influence proportion of damaged leaves (Appendix S1: Fig. S3). Cultivar diversity in control cages had a stronger effect on male pupal mass than females, and male *T. ni* pupal mass showed a nonlinear pattern with increasing diversity ($\chi^2 = 6.93$, df = 2, $P = 0.03$). Pupal mass was lowest in biculture and highest in polyculture (Fig. 2). Individuals in the biculture were 14.1% smaller than those in the polyculture and males in monocultures were of intermediate size. Individual plant treatment also affected male pupal mass ($\chi^2 = 12.23$, df = 5, $P = 0.03$; Fig. 3). Females, in contrast, were less sensitive to diversity level than males and diversity level did not significantly explain pupal mass ($\chi^2 = 1.73$, df = 2, $P = 0.42$), nor did individual plant treatment ($\chi^2 = 4.63$, df = 5, $P = 0.46$).

How does chemotype diversity alter the effect of predators on herbivores?—We examined how chemotype diversity influenced top-down pest control by comparing *T. ni* survival and pupal mass between control and predator plots with the same level of overall cultivar diversity (monocultures, bicultures, and polycultures). First, we found predators strongly reduced survival ($\chi^2 = 33.29$, df = 1, $P < 0.05$; Fig. 4) and proportion of damaged leaves on plants (Appendix S1: Fig. S4), and that this effect did not vary significantly with the number of chemotypes in a plot.

In contrast, the specific class and level of chemotype diversity altered the effects of predators on male pupal mass, with certain classes and levels of chemotype diversity reducing the effects of predators and others increasing the effects of predators (Fig. 5). Overall, the effect of the predator × diversity level and predator × plant chemotype identity interaction for male pupal mass were nonsignificant ($\chi^2 = 2.78$, df = 2, $P = 0.25$ and $\chi^2 = 9.52$, df = 5, $P = 0.09$; Fig. 5). Plant treatments within monocultures did not show an interactive effect with predators ($\chi^2 = 1.05$, df = 2, $P = 0.59$). When we analyzed the effects of bicultures and polycultures, predator presence increased male pupal mass in the terpene diversity biculture by 15.5%, whereas predators reduced male pupal mass in the full polyculture by 8.0% (predator × diversity interaction for bicultures and polycultures: $\chi^2 = 7.68$, df = 2, $P = 0.02$). This result suggests that predators may have been attacking larger larvae more often in the full polyculture compared to the terpene biculture. However, predator presence decreased male pupal mass fairly consistently in both the acyl sugar biculture and the polyculture, a reduction of 6.8% compared to 8.0%. This indicates that predators were attacking larvae of roughly the same size in both polyculture and biculture with acyl sugar diversity. This interaction did not hold when looking only at one

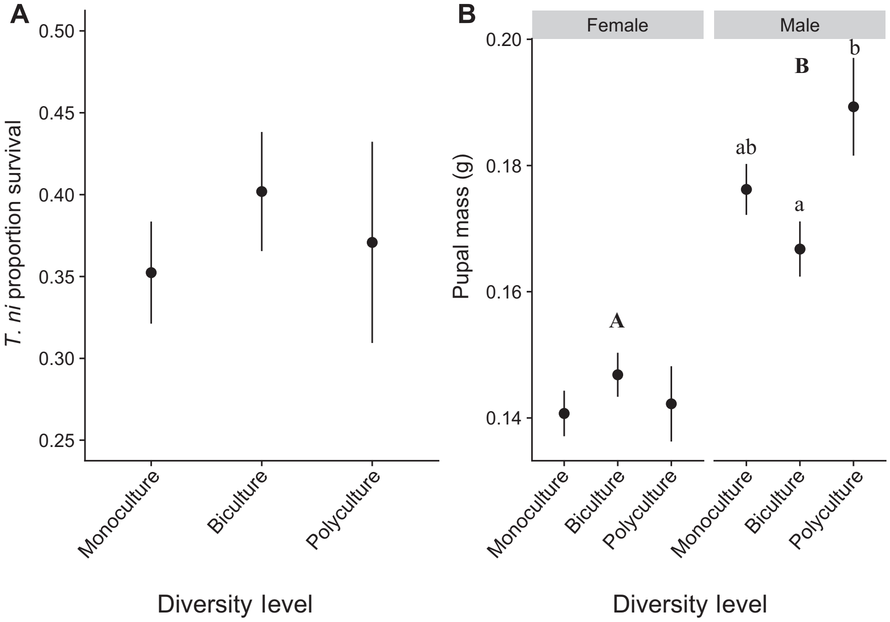

FIG. 2. *T. ni* (A) survival and (B) pupal mass by overall diversity level. Plot shows mean ± SE. Level of diversity (monoculture, biculture, or polyculture) did not have an effect on survival in control cages. Level of overall diversity did not have a significant effect on female pupal mass, but did influence males. Uppercase letters represent significant ($P \leq 0.05$) differences between sexes. Lowercase letters represent significant ($P \leq 0.05$) differences within sexes.

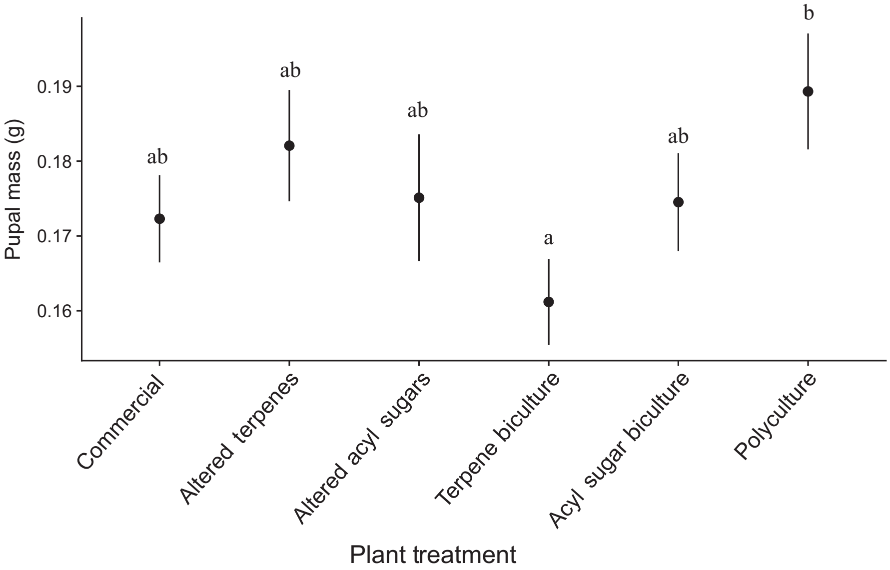

FIG. 3. Male *T. ni* pupal mass in control cages by individual plant treatment. Plot shows mean ± SE. Plant treatment had a significant effect on male pupal mass. Groups with different lowercase letters have significantly different ($P \leq 0.05$) average pupal mass.

biculture and its constituent monocultures ($\chi^2 = 0.21$, df = 2, $P = 0.89$ for the commercial chemotype, the acyl sugar chemotype, and the commercial chemotype + the acyl sugar chemotype; $\chi^2 = 2.14$, df = 1, $P = 0.14$ for the commercial chemotype, the terpene chemotype, and the commercial chemotype + the terpene chemotype).

Females, on the other hand, consistently had higher pupal mass in predator treatments (Fig. 5; $\chi^2 = 8.36$, df = 1, $P < 0.05$), but there was no effect on female pupal mass of the interaction between of diversity level or plant treatment with predator presence ($\chi^2 = 0.95$, df = 2, $P = 0.62$ and $\chi^2 = 1.02$, df = 5, $P = 0.96$,

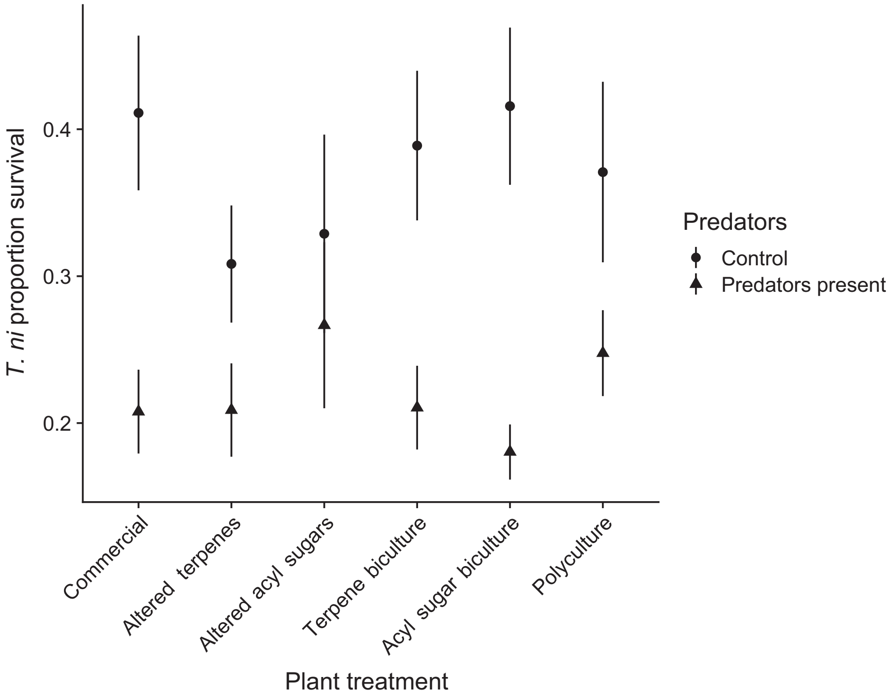

FIG. 4. Proportion *T. ni* survival in control and predator cages by plant treatment. Plot shows mean ± SE. Predators had a significant effect on survival.

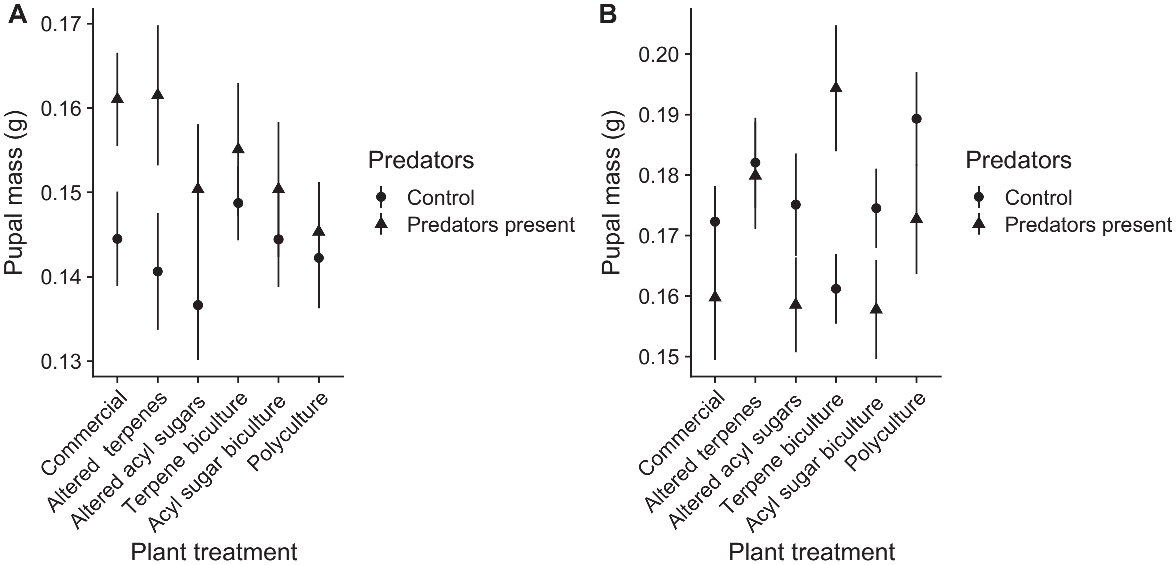

FIG. 5. *T. ni* pupal mass by plant treatment with and without predators for (A) females and (B) males. Plot shows mean ± SE. Female *T. ni* consistently had higher pupal mass in predator treatments. Although predators had an overall effect on female pupal mass, there was no interactive effect between pupal mass and level of diversity or pupal mass and plant treatment. This was true for monocultures only and bicultures + polycultures. For male *T.ni*, predators and plant treatment had a marginally significant interactive effect on pupal mass. There is no significant interaction between plant treatments and predators when looking at monocultures only, but a strong interaction between plant treatments and predators when looking at bicultures and polycultures only.

respectively). Because females are smaller than males and *P. maculiventris* prefers larger prey, they likely faced lower competition in predator treatments and may have been located or sought out less frequently by predators.

Lab choice experiment

Do predators show a preference for plant chemotype?.— Our laboratory choice experiment indicated that

P. maculiventris had weak preferences for the commercial chemotype and weak avoidance of the altered terpene chemotype. In the commercial + terpene biculture, we observed *P. maculiventris* resting on the commercial chemotype 84.5% of the time (CI, 49.8–99.8%). In the commercial + acyl sugar biculture, they spent a similar amount of time on each chemotype (66.3% on commercial; CI, 10.0–97.2%). In the acyl sugar + terpene biculture, predators were observed 89.0% of the time on the acyl sugar chemotype, but again, the confidence interval was large (CI, 25.1–100.0%).

Discussion

We examined the effects of two types and three levels of plant chemotype diversity on an herbivore and its interaction with a predator. We did this by presenting herbivores and predators with monocultures, bicultures, or polycultures of three tomato chemotypes that differed primarily in terpene odor composition or acyl sugar composition. Our results indicated that the direct effects of cultivar diversity were nonlinear, depended on herbivore sex, acted through growth rather than survival, and that plant herbivory damage closely mirrored survival. We found that the effects of predators on herbivore performance depended on the specific chemotypic mixture of plants, rather than on the overall amount of genetic diversity. Finally, these diversity effects occurred even though herbivore performance was similar among monocultures of the three chemotypes, indicating that there are emergent effects of plant chemotype diversity on the predator–prey interaction.

Our finding that there was a greater effect of plant chemotype diversity on males than females suggests that physiological differences between sexes can alter diversity effects within a species. In the presence of active hunters such as *P. maculiventris*, herbivores often reduce movement to avoid predation (Hermann and Thaler 2014). As a result, the predators may have forced *T. ni*, especially males, which are larger and consume more plant tissue during development, to consume more chemically suboptimal regions of a leaf (Shelton 2005) than they would in the absence of predators, when they are freer to move about the plant. When predators are not present, males may benefit more from moving and feeding on multiple chemotypes, potentially allowing them to dilute their intake of toxins from a single chemotype (Bernays et al. 1994). Females, in contrast, may be less affected by chemotypic diversity because they consume less plant tissue and may switch plants less frequently. Cultivar diversity that affects one sex disproportionately could alter population dynamics by reducing mating success: previous studies of lepidopteran species have shown that early emergence (Saastamoinen et al. 2013) and larger size (Wiklund and Kaitala 1995, Makee and Saour 2001) are good predictors of male mating success. Many lepidopteran crop pests (including *T. ni*) are multivoltine (Ehler 1977, Chamberlain and Kok 1986), which could cause the effects of plant diversity to compound over a growing season.

We found that male pupal mass exhibited a nonlinear response to chemotype diversity, with the lowest pupal mass in biculture and highest pupal mass in polyculture. One explanation for this nonlinearity is that cultivar diversity has both costs and benefits to an herbivore: chemotype diversity allows for toxin dilution, but it also makes the environment more complex. Feeding on multiple toxins could affect caterpillar foraging and subsequently growth by reducing aversion to a plant with a specific toxin, not allowing any specific secondary metabolite to get to a noxious level inside the caterpillar, or by consuming secondary metabolites that have different modes of action and therefore a non-additive effect (Bernays et al. 1994). This is evident in a recent study in the same system, where *T. ni* caterpillars performed best in plant neighborhoods that were chemically different (Glassmire et al. 2020). Increasing complexity may make it more difficult to locate and consume high quality plant tissue. Together, these factors could explain the nonlinear relationship between amount of chemotype diversity and male pupal mass; adding a single plant with different odors to a patch (bicultures) may confuse caterpillars and make it more difficult for them to find high quality tissue without providing enough cultivar diversity to allow toxin dilution to happen. However, adding a third chemotype that differs in surface chemistry could then facilitate toxin dilution (Bernays et al. 1994) in polyculture.

Our result that growth was more sensitive than survival to cultivar diversity has at least two explanations. First, because we were unable to sex caterpillars that died, it is possible that male and female survival were affected in contrasting ways that were masked in our data set. Second, it may be a true biological result that diversity affects growth more than survival. For larger males, which consume more plant tissue during development, either having clear chemical signals to locate the best feeding areas (monoculture) or a high diversity of compounds leading to toxin dilution (polyculture) may lead to better outcomes than a mix of the two (biculture), whereas females who feed less and therefore need to switch less from plant to plant do not see the same costs and benefits to diversity. Herbivory damage mirrored survival rather than growth. This is most likely because our insect densities were optimized for quantifying effects of diversity on insect ecology and too low to reveal nuanced differences in plant damage; even plants with the highest levels of damage had just over 25% of leaves with feeding damage. We did see that monocultures on average had higher damage than bicultures and polycultures, and the biculture with reduced terpenes had lower feeding damage than the biculture without, although variability was high in both cases. Predator presence also reduced herbivory damage. A management implication is that the most appropriate cultivar mixture may change depending on whether the key concern is managing pest population dynamics over multiple generations or growing seasons, in which case it may be useful to select cultivars that reduce pest survival and growth, or reducing immediate defoliation, in which case it may be best to pick a mixture that promotes predator presence, since predator presence predicted herbivory levels.

We also found that the effects of cultivar diversity on predator-prey interactions depended on the chemical traits involved. Males in the terpene diversity biculture were larger in the presence of predators than without predators, while males in the acyl sugar diversity biculture and the polyculture were smaller than males in control treatments. Previous cultivar diversity studies have found that crop genotypic diversity can affect predator–prey and tritrophic interactions (Wetzel et al. 2018) and that the effects of plant genotypic diversity can depend on the identity of genotypes present (Grettenberger and Tooker 2017), but these studies did not look at specific traits. This study extends that work and shows that outcomes vary among plant chemotypes and that those chemotypes can have direct effects on predators and their hunting success.

One hypothesis for why the effect of predators on male *T. ni* pupal mass differed between the altered terpenes biculture, altered acyl sugars biculture, and polyculture is that predators hunted less efficiently in treatments with terpene diversity compared to treatments with acyl sugar diversity. *P. maculiventris* preferentially feeds on larger prey (DeClercq and Degheele 1994), so lower average pupal masses may indicate contexts in which predators were foraging more successfully. The altered terpene chemotype releases a lower abundance of sesquiterpenes than the commercial, and terpenes are often used by natural enemies to locate prey (Vuorinen et al. 2004, Schnee et al. 2006, Vieira et al. 2019). Locating prey might be more difficult in an odor environment where different plants are signaling different information about herbivore presence. If true, having less information about prey location and amount of feeding may have reduced predators' ability to locate the large males and instead cause predators to consume whichever caterpillar they encountered first; the altered terpene plants offered caterpillars a refuge from predation by *P. maculiventris*. This is supported by our laboratory experiment with bicultures and mechanically damaged plants. *P. maculiventris* spent more time on the commercial chemotype compared to the altered terpene chemotype, possibly because they received fewer chemical cues from those plants. It is also possible that the altered acyl sugars, a physical defense, were easier for *P. maculiventris* to traverse than the acyl sugars of the commercial line, so predators foraged more effectively on plants of the altered acyl sugar chemotype.

Overall, our results suggest that increasing intraspecific plant diversity can be a viable strategy for ecological pest management, but that it is not simply a matter of maximizing diversity. Instead, the successful use of intraspecific diversity likely depends on finding the right types and amounts of diversity for a given set of pests and natural enemies. Researchers have explored the use of plant diversity for pest management for decades (Altieri et al. 1984, Andow 1991, Reiss and Drinkwater 2018), but few studies have examined the traits by which cultivar mixtures affect insect ecology (Tooker and Frank 2012). Most studies have focused on the effects of cultivar richness, and those that have examined traits have focused on general metrics of resistance or performance rather than specific plant traits (Underwood 2009, Grettenberger and Tooker 2017, 2020). While these studies have enhanced our understanding of the effects of plant genetic diversity on insect communities, our work suggests that selecting cultivars using a trait-based approach that harnesses the power of chemical ecology could yield more effective mixtures. The approach we suggest will require a focus on at least two areas: (1) an understanding of the key traits that differ among cultivars, (2) how those traits are expressed in monoculture vs. polyculture, and (3) an understanding of how those traits interact with the biology of a system's pests and natural enemies.

Understanding key traits that differ among cultivars is critical because our work suggests that it is not the level of plant diversity but instead the specific plant traits that determine the effects of cultivar diversity on insects. For example, in our biculture with terpene diversity, but not our biculture with diversity in acyl sugars, male *T. ni* had lower pupal mass compared to polycultures, which led to the highest male pupal mass. This finding is supported by a recent review that concludes that number of plant genotypes can be insufficient to produce diversity effects in agricultural mixtures (Grettenberger and Tooker 2015). Specific plant traits have been taken into account when designing interspecific push-pull systems (Khan et al. 2000, 2010) with success, and studies have demonstrated the importance of specific floral traits, rather than floral availability, for pollinators and parasitoids (Wäckers 2004, Campbell et al. 2012). A major implication of our study is that we may be able to use intraspecific plant diversity more effectively for pest management if we apply the trait-based, mechanistic approach that has made some push-pull systems so successful.

Importantly, plant traits can also be expressed differently in genetic monoculture vs. polyculture. In a study of wheat and bird cherry-oat aphid, aphid performance was lower in polyculture than monoculture overall, but certain varieties responded differently in a diverse neighborhood than they did in monoculture (Grettenberger and Tooker 2016). Furthermore, an abiotic stressor (in this case, drought) reduced the effect of plant diversity on aphid performance. Therefore, crafting effective mixtures will require testing how cultivars perform with different potential neighbors as well as how specific traits affect insect pests and natural enemies independently, and how much those effects depend on the environmental context. While these factors lengthen the list of contexts and traits that should be assessed before choosing a specific mixture for agricultural use, it is encouraging that we saw the strongest diversity effects with a mixture of just two cultivars; while the challenge of assessing mixtures in a variety of contexts is daunting, it is not insurmountable.

In addition to how plants express target traits, cultivar mixtures will also need to be tailored to the biology of target insects, including their physiology, foraging behavior, and movement ability, as well as how they respond to plant traits. In particular, the amount of plant tissue insects consume and how far they move during feeding will affect how they experience and interact with trait diversity within a field and across a landscape (Pearse et al. 2018, Haan et al. 2020). Indeed, the differences we found in how cultivar diversity affects male and female *T. ni* could have been driven by the male's larger size, need to consume more, and greater movement. If size differences within an insect species could lead to differential responses to intraspecific plant diversity, then size differences among insect species could be a key factor to consider when applying intraspecific plant diversity for ecological management. It will also be critical to consider the foraging biology of natural enemies. In our study, at least part of how cultivar diversity altered top-down control by predators was likely related to the predator's avoidance of one specific chemotype and preference for the other cultivars, which may have shaped how successful they were as they moved and hunted among plants. That cultivar may have offered a refuge to herbivores in the presence of predators even as it physiologically challenged herbivores in the absence of predators. A key implication of these results is that ecological pest management with intraspecific plant diversity may be less effective in systems with strong pressure from many different species of herbivore pests because foraging ecology can vary so drastically between species, potentially making it difficult to devise cultivar mixtures that work against each pest without disrupting natural enemy foraging.

However, as always, context may be key. Our experimental system, reflective of many agricultural systems, had a small number of pest species and natural enemies. This low diversity likely limited the number of ways plant diversity could scale up to influence arthropod communities and plant damage. This may explain why our results differed in some ways from classic studies of plant genotypic diversity with open plots and high arthropod richness. These studies have generally found that increasing plant genotypic diversity on average increases natural enemy diversity and reduces plant damage (Crutsinger et al. 2006, Johnson et al. 2006). This comparison suggests that the importance of plant traits and trait-based diversity strategies for pest management may depend on arthropod species richness. In systems with high arthropod richness in the regional species pool, those classic studies indicate that increasing genotypic plant diversity, irrespective of traits and chemical ecology, may reliably enhance natural enemy diversity and reduce herbivore damage. In systems with low arthropod richness, where plant diversity effects act through arthropod density not arthropod diversity, however, our findings indicate that genotypic diversity may be a less important ecological predictor than trait diversity. In these low diversity systems, our work suggests that using chemical ecology to develop a trait-based, mechanistic understanding of species interactions has potential to allow intraspecific plant diversity to be a powerful tool for the ecological management of pest species.

ACKNOWLEDGMENTS

We thank W. Jawad, L. Zehr, H. Dole, J. Lillard, M. Frick, A. Levardsen, E. Mall, and A. Benson for help in the field and lab. We thank S. Whitehead, D. Landis, M. Weber, Z. Szendrei, and two anonymous reviewers for valuable comments and feedback. N. Afflito, J. Thaler, B. Jarrett, and M. Szucs kindly provided *P. maculiventris.* This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2018-67013-28065 from the USDA National Institute of Food and Agriculture, the W. K. Kellogg Biological Station, and MSU AgBioResearch. The Kellogg Biological Station contribution number for this document is 2169. K. Hauri, A. Glassmire, and W. Wetzel designed the experiments. K. Hauri conducted the experiments and wrote the first draft. K. Hauri and W. Wetzel conducted the analyses. All authors edited the manuscript.

LITERATURE CITED

Altieri, M. A., D. K. Letourneau, and S. J. Risch. 1984. Vegetation diversity and insect pest outbreaks. Critical Reviews in Plant Sciences 2:131–169.

Andow, D. A. 1991. Vegetational diversity and arthropod population response. Annual Review of Entomology 36:561–586.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.

Bernays, E. A., K. L. Bright, N. Gonzalez, and J. Angel. 1994. Dietary mixing in a generalist herbivore: tests of two hypotheses. Ecology 75:1997–2006.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S.-S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–135.

Bustos-Segura, C., E. H. Poelman, M. Reichelt, J. Gershenzon, and R. Gols. 2017. Intraspecific chemical diversity among neighbouring plants correlates positively with plant size and herbivore load but negatively with herbivore damage. Ecology Letters 20:87–97.

Campbell, A. J., J. C. Biesmeijer, V. Varma, and F. L. Wäckers. 2012. Realising multiple ecosystem services based on the response of three beneficial insect groups to floral traits and trait diversity. Basic and Applied Ecology 13:363–370.

Chamberlain, J. R., and L. T. Kok. 1986. Cabbage lepidopterous pests and their parasites in Southwestern Virginia. Journal of Economic Entomology 79:629–632.

Cornell, H. V., and B. A. Hawkins. 2003. Herbivore responses to plant secondary compounds: A test of phytochemical coevolution theory. American Naturalist 161:507–522.

Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313:966–968.

DeClercq, P., and D. Degheele. 1994. Laboratory measurement of predation by *Podisus maculiventris* and *P. sagitta* (Hemiptera: Pentatomidae) on beet armyworm (Lepidoptera: Noctuidae). Journal of Economic Entomology 87:76–83.

Dyer, L. A., et al. 2018. Modern approaches to study plant–insect interactions in chemical ecology. Nature Reviews Chemistry 2:50–64.

Ehler, L. E. 1977. Natural enemies of cabbage looper on cotton in the San Joaquin Valley. Hilgardia 45:73–106.

Gershenzon, J., and N. Dudareva. 2007. The function of terpene natural products in the natural world. Nature Chemical Biology 3:408–414.

Glassmire, A. E., L. N. Zehr, and W. C. Wetzel. 2020. Disentangling dimensions of phytochemical diversity: alpha and beta have contrasting effects on an insect herbivore. Ecology 101: e03158.

Goffreda, J. C., M. A. Mutschler, D. A. Ave, W. M. Tingey, and J. C. Steffens. 1989. Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennellii*. Journal of Chemical Ecology 15:2137–2147.

Grettenberger, I. M., and J. F. Tooker. 2015. Moving beyond resistance management toward an expanded role for seed mixtures in agriculture. Agriculture, Ecosystems & Environment 208:29–36.

Grettenberger, I. M., and J. F. Tooker. 2016. Inter-varietal interactions among plants in genotypically diverse mixtures tend to decrease herbivore performance. Oecologia 182:189–202.

Grettenberger, I. M., and J. F. Tooker. 2017. Variety mixtures of wheat influence aphid populations and attract an aphid predator. Arthropod-Plant Interactions 11:133–146.

Grettenberger, I. M., and J. F. Tooker. 2020. Cultivar mixtures of soybeans have inconsistent effects on herbivore and natural-enemy populations. Agriculture, Ecosystems and Environment 292:106835.

Haan, N. L., Y. Zhang, and D. A. Landis. 2020. Predicting landscape configuration effects on agricultural pest suppression. Trends in Ecology and Evolution 35:175–186.

Hare, J. D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annual Review of Entomology 56:161–180.

Hauri, K. 2020. Chemical diversity rather than cultivar diversity predicts natural enemy control of herbivore pests. Figshare, data set. https://doi.org/10.6084/m9.figshare.13110821.v1

Hermann, S. L., and J. S. Thaler. 2014. Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. Oecologia 176:669–676.

Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. Ecology Letters 9:24–34.

Kersch-Becker, M. F., A. Kessler, and J. S. Thaler. 2017. Plant defences limit herbivore population growth by changing predator–prey interactions. Proceedings of the Royal Society B 284:20171120

Kersch-Becker, M. F., and J. S. Thaler. 2015. Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids. Journal of Animal Ecology 84:1222–1232.

Kessler, A., and A. Kalske. 2018. Plant secondary metabolite diversity and species interactions. Annual Review of Ecology, Evolution, and Systematics 49:115–138.

Khan, Z. R., C. A. O. Midega, T. J. A. Bruce, A. M. Hooper, and J. A. Pickett. 2010. Exploiting phytochemicals for developing a 'push-pull' crop protection strategy for cereal farmers in Africa. Journal of Experimental Botany 61:4185–4196.

Khan, Z. R., J. A. Pickett, J. van den Berg, L. J. Wadhams, and C. M. Woodcock. 2000. Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. Pest Management Science 56:957–962.

Korpita, T., S. Gómez, and C. M. Orians. 2014. Cues from a specialist herbivore increase tolerance to defoliation in tomato. Functional Ecology 28:395–401.

Lin, B. B. 2011. Resilience in agriculture through crop diversification: Adaptive management for environmental change. BioScience 61:183–193.

Makee, H., and G. Saour. 2001. Factors influencing mating success, mating frequency, and fecundity in *Phthorimaea operculella* (Lepidoptera: Gelechiidae). Environmental Entomology 30:31–36.

McArt, S. H., and J. S. Thaler. 2013. Plant genotypic diversity reduces the rate of consumer resource utilization. Proceedings of the Royal Society B 280(1762):20130639.

Moreira, X., L. Abdala-Roberts, S. Rasmann, B. Castagneyrol, and K. A. Mooney. 2016. Plant diversity effects on insect herbivores and their natural enemies: current thinking, recent findings, and future directions. Current Opinion in Insect Science 14:1–7.

Pearse, I. S., R. Paul, and P. J. Ode. 2018. Variation in plant defense suppresses herbivore performance. Current Biology 28:1981–1986.

Power, A. G. 1991. Virus spread and vector dynamics in genetically diverse plant populations. Ecology 72:232–241.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www. R-project.org

Raboin, L. M., A. Ramanantsoanirina, J. Dusserre, F. Razasolofonanahary, D. Tharreau, C. Lannou, and M. Sester. 2012. Two-component cultivar mixtures reduce rice blast epidemics in an upland agrosystem: Cultivar mixtures and blast in upland rice. Plant Pathology 61:1103–1111.

Reiss, E. R., and L. E. Drinkwater. 2018. Cultivar mixtures: a meta-analysis of the effect of intraspecific diversity on crop yield. Ecological Applications 28:62–77.

Saastamoinen, M., S. Ikonen, S. C. Wong, R. Lehtonen, and I. Hanski. 2013. Plastic larval development in a butterfly has complex environmental and genetic causes and consequences for population dynamics. Journal of Animal Ecology 82:529–539.

Schilmiller, A., F. Shi, J. Kim, A. L. Charbonneau, D. Holmes, A. Daniel Jones, and R. L. Last. 2010. Mass spectrometry screening reveals widespread diversity in trichome specialized metabolites of tomato chromosomal substitution lines: *Solanum trichome* chemistry. Plant Journal 62:391–403.

Schnee, C., T. G. Köllner, M. Held, T. C. J. Turlings, J. Gershenzon, and J. Degenhardt. 2006. The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proceedings of the National Academy of Sciences USA 103:1129–1134.

Schuman, M. C., S. Allmann, and I. T. Baldwin. 2015. Plant defense phenotypes determine the consequences of volatile emission for individuals and neighbors. eLife 4:e04490.

Shelton, A. L. 2005. Within-plant variation in glucosinolate concentrations of *Raphanus sativus* across multiple scales. Journal of Chemical Ecology 31:1711–1732.

Shorey, H. H., L. A. Andres, and R. L. Hale. 1962. The Biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). I. Life History and Behavior. Annals of the Entomological Society of America 55:591–597.

Tooker, J. F., and S. D. Frank. 2012. Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. Journal of Applied Ecology 49:974–985.

Underwood, N. 2009. Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. Journal of Animal Ecology 78:839–847.

Vieira, E. A., R. Arruda, K. F. Massuda, P. Cardoso-Gustavson, E. F. Guimarães, and J. R. Trigo. 2019. Volatiles released by damaged leaves of *Piper mollicomum* (Piperaceae) act as cues for predaceous wasps: evidence using plasticine dummies as herbivore model. Arthropod-Plant Interactions 13:593–601.

Vuorinen, T., A. Nerg, M. A. Ibrahim, G. V. P. Reddy, and J. K. Holopainen. 2004. Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO_2 and orientation behavior of the natural enemies. Plant Physiology 135:1984–1992.

Wäckers, F. L. 2004. Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. Biological Control 29:307–314.

Wetzel, W. C., N. C. Afflito, and J. S. Thaler. 2018. Plant genotypic diversity interacts with predation risk to influence an insect herbivore across its ontogeny. Ecology 99:2338–2347.

Wetzel, W. C., and J. S. Thaler. 2016. Does plant trait diversity reduce the ability of herbivores to defend against predators? The plant variability-gut acclimation hypothesis. Current Opinion in Insect Science 14:25–31.

Wetzel, W. C., and S. R. Whitehead. 2020. The many dimensions of phytochemical diversity: linking theory to practice. Ecology Letters 23:16–32.

Wiklund, C., and A. Kaitala. 1995. Sexual selection for large male size in a polyandrous butterfly: the effect of body size on male versus female reproductive success in *Pieris napi*. Behavioral Ecology 6:6–13.

Zhu, Y., et al. 2000. Genetic diversity and disease control in rice. Nature 406:718–722.

SUPPORTING INFORMATION

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2289/full

DATA AVAILABILITY STATEMENT

Data available on Figshare (Hauri 2020): https://doi.org/10.6084/m9.figshare.13110821.v1