EFFECTS OF PLANT CHEMICAL DIVERSITY ON PREDATOR-PREY INTERACTIONS IN AN AGROECOSYSTEM

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ABSTRACT

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A central question in agroecology is how to manage herbivore pests without relying on expensive and environmentally harmful pesticides. One potential method that has shown promise is to use plant cultivars with different chemical profiles, which may be more difficult for herbivore pests to deal with physiologically or make it more difficult to locate the best host plants. However, few studies have focused on the influence of specific traits and how they influence the predator-prey interaction. We performed a series of field and laboratory experiments to determine the effect of different amounts and types of plant chemical diversity on a predator-prey interaction, and how the spatial arrangement of plant chemical diversity affects insect foraging. In the first study, we found that the type of chemical diversity was more important than the overall amount of diversity for a system of *Trichoplusia ni* (Lepidoptera: Noctuidae), a generalist herbivore, and *Podisus maculiventris* (Hemiptera: Pentatomidae), a biocontrol predator. Diversity effects tended to act more strongly through growth rather than survival, and males were more strongly affected than females. In the second study we found that chemical diversity reduced the spread of herbivory within a plot, but that the arrangement of that plant diversity independent of amount reduced total feeding and was correlated with higher predator presence. Overall, successfully using plant chemical diversity to reduce pest pressure will require using the right types and amounts of diversity in arrangements that are biolocially relevant for target herbivores and predators.

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CHAPTER 1:

Introduction

A major challenge in agroecology is managing insect pests without relying solely on expensive and environmentally harmful pesticides. This problem has been exacerbated as farms in the United States shift towards larger-scale production and become more specialized (O'Donoghue 2011) leaving them susceptible to pest outbreaks, which are common in large-scale monocultures (Altieri et al. 1984, Andow 1991). Reducing genetic monocultures through the use of intraspecific cultivar mixtures is one way to increase diversity in cropping systems, potentially reducing pest outbreaks. Cultivar mixtures have been successfully used to manage disease spread in a variety of crops (Zhu et al. 2000, Mundt 2002, Newton and Guy 2011), and while using mixtures to manage insect pests has also gained traction in recent years, consistent reduction of pest pressure has not been achieved (Grettenberger and Tooker 2015).

The variance of pest suppression success with cultivar mixtures shows that we are still not completely effective at designing cultivar mixtures to reduce pest pressure; the question remains why some mixtures are so successful while others fall short. One potential avenue to improve pest suppression outcomes is to focus on the way that both herbivorous and predatory insects are experiencing the mixtures and design them for maximum impact to target pests. For example, when insects feed on different genetic lines of plants, they are not experiencing the overall genetic diversity, but rather diversity in traits that those gene variants control. One set of plant traits that we know decrease loss to insect herbivory are chemical defense traits. Chemical defenses are an especially promising target for further investigation because not only do they affect herbivores directly feeding on the plant (Ali and Agrawal 2012, Dyer et al. 2018), but

chemical defense also impacts predators and parasitoids through indirect defense such as releasing volatile cues (Gershenzon and Dudareva 2007, Hare 2011, Kersch-Becker and Thaler 2015), and these natural enemies are responsible in large part for reducing insect pest populations in the field.

It will be important to investigate which specific chemical traits reduce pest pressure as well as how those traits are being experienced by insects. For example, there are still conflicting results regarding the benefit of diet diversity to insects, which includes plant chemistry. It is possible that more diet diversity is worse from an insect's perspective, because it is more difficult for herbivores to deal with physiologically (Wetzel et al. 2016, Pearse et al. 2018); alternatively, a noisier chemical environment could allow herbivores to dilute the toxins they do consume (Bernays et al. 1994) and make it more difficult for natural enemies to forage (Schröder and Hilker 2008). In fact, a variable diet could even lead to more loss of herbivory through compensatory feeding when insects are on a low-quality plant (Lee et al. 2004, Berner et al. 2005). Additionally, the way that diversity is experienced by insects might also matter: it seems clear that a field with one cultivar on one half and a second cultivar on the other half will be experienced differently by insects than one where the two are interspersed, and indeed models support that arrangement in addition to amount of diversity has an effect on insect populations (Riolo et al. 2015). This is especially true for less mobile insects, including economically important larval pests, but at what patch size is chemical diversity most effective at reducing pest pressure and promoting natural enemies? A wealth of literature has established the importance of spatial arrangement at the landscape scale (Martin et al. 2016, Haan et al. 2020), but does arrangement still matter at the plot scale?

We performed field and laboratory experiments to address two main questions: 1) How do differing amounts of phytochemical diversity influence a generalist herbivore and its interactions with a biocontrol predator? 2) How does the presence and spatial arrangement of phytochemical diversity affect insect herbivores and the ambient predator community? Our results will inform the design of more effective cultivar mixtures and enhance our ability to manage insect pests in agroecosystems.

CHAPTER 2:

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

ABSTRACT

Cultivar mixtures have been studied for decades as a means for pest suppression. However, the literature 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 interaction 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, dicultures, 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 dicultures 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 the right type and level of diversity will be required to promote predator hunting success and reduce herbivore populations.

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 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 examining traits (Power 1991, Crutsinger 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 (Schuman et al. 2015, Kersch-Becker and Thaler 2015, Kersch-Becker et al. 2017). 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 influences 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 how plant chemotypes affect 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 chemotype diversity—diversity in the secondary metabolites and chemical composition of plants—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: 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, dicultures, 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 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) and maintained them on general noctuid diet (Benzon Research, Carlisle Pennsylvania) 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, MI). Our chemotype diversity treatments included three monocultures, two dicultures, 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 diculture treatments with two chemotypes each: commercial and altered terpenes or commercial and altered acyl sugars. Lastly, 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, Inc.).

One week after planting and caging, we released 15 pre-weighed 2nd instar *T. ni* into the center of each plot. Approximately 30 min after release, *T. ni* reached the plants and began feeding, and we released one 3rd instar, one 4th 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 two 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. Because *T. ni* are multivoltine in many areas where they are crop pests (Ehler 1977, Chamberlin 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, diculture, 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 diculture, altered acyl sugar diculture, 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 diculture (for acyl sugar diversity); or commercial monoculture, altered terpenes monoculture, and altered terpenes diculture (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 diculture and the full polyculture.

We addressed our second question about the effects of chemotype diversity on the herbivore–predator interaction by analyzing our full dataset 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 diculture, altered acyl sugar diculture, 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 diculture (for acyl sugar diversity); or commercial monoculture, altered terpenes monoculture, and altered terpenes diculture (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 diculture 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 dicultures and polycultures using a choice experiment. We measured *P. maculiventris* preferences by placing them individually into the center of mesh cages (30.5 x 30.5 x 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-min 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 5th leaf and running a serrated tracing wheel ten 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. 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 ($\chi^2 = 1.10$, df = 2, p = 0.58; Figure 1a). Which plant chemotype was present in monoculture plots affected male *T. ni* mass ($\chi^2 = 7.26$, df = 2, p = 0.03) but not females ($\chi^2 =$

3.76, df = 2, p = 0.15) (Figure 1). Males were largest in the terpene chemotype monocultures (Figure 1); there 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, diculture, 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). 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 diculture and highest in polyculture (Figure 2). Individuals in the diculture 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) (Figure 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, dicultures, and polycultures). We found that predators strongly reduced survival ($\chi^2 = 33.29$, df = 1, $p < 0.05$; Figure 4) and that this effect did not vary significantly with amount of chemotype diversity.

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 (Figure 5). Overall, the effect of the predator x diversity level and predator x 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, $p = 0.09$, Figure 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 dicultures and polycultures, predator presence increased male pupal mass in the terpene diversity diculture by 15.5%, whereas predators reduced male pupal mass in the full polyculture by 8.0% (predator x diversity interaction for dicultures 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 diculture. However, predator presence decreased male pupal mass fairly consistently in both the acyl sugar diculture 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 diculture with acyl sugar diversity. This interaction did not hold when looking only at one diculture 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 (Figure 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, $p = 0.96$, 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 diculture, we observed *P. maculiventris* resting on the commercial chemotype 84.5% of the time (CI: 49.8%-99.8%). In the commercial + acyl sugar diculture, they spent a similar amount of time on each chemotype (66.3% on commercial, CI: 10.0%-97.2%). In the acyl sugar + terpene diculture, 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, dicultures, 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, and acted through growth rather than 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 emerging early (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, Chamberlin 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 diculture 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). 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 (dicultures) 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 dataset. 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 (diculture), 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.

We also found that the effects of cultivar diversity on predator-prey interactions depended on the chemical traits involved. Males in the terpene diversity monoculture were larger

in the presence of predators than without predators, while males in the acyl sugar diversity diculture and the polyculture were smaller than males in control treatments, and this pattern was even more exaggerated in the monocultures with predators present. 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 diculture, altered acyl sugars diculture, 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 (Declerq 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 their ability to locate the large males and instead consume whichever caterpillar they encountered first. This is supported by our laboratory experiment with dicultures 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.

Our results suggest that it is possible to manage insect pests using plant chemotype diversity, but that chemotype diversity is a tool more like a scalpel than a mallet: to be effective, the biology of key players must be the central driver. In hindsight, this should not surprise us—different types of chemical defenses have different modes of action and do not all affect herbivores and predators the same way. Broadly, we can sharpen our metaphorical scalpel by understanding how the foraging behavior of both predators and herbivores shapes the type of chemotype diversity that will be most effective in promoting or suppressing their presence and foraging success.

A major component in how much diversity will be experienced by an insect over its lifetime is dispersal distance. Previous studies have shown that plant diversity has the strongest influence on herbivore responses when it is closely matched to the insect's dispersal distance (Pearse et al. 2018, Haan et al. 2020). Our results emphasize how even minor differences in foraging and size can lead to different affects on herbivore responses: within our species, larger males were affected more strongly than smaller females. This pattern in itself will likely be widespread, since only 5% of insect species monomorphic (Stillwell et al. 2010). However, even more exaggerated size and dispersal distances exist between species. Therefore, utilizing plant chemotype diversity to manage herbivores must involve closely matching the plant diversity level with the dispersal distance of the target herbivore. For a very mobile herbivore, perhaps larger patches of diversity will have the strongest effects on herbivore performance; for a less mobile herbivore, within-plant diversity may be more effective for reducing herbivore growth.

Additionally, hunting strategy will influence how effective chemotype diversity can be, especially for promoting natural enemies. For example, we saw a strong avoidance by predators of our chemotype with reduced terpenes. Terpenes are a major component of many plant volatiles, including crop plants; in fact, terpenes are the largest group of secondary metabolites (Gershenzon and Dudareva 2007). Additionally, as a herbivore-induced plant volatile, we expect that their presence would not only affect predators that actively search plants such as *P. maculiventris* but specialist predators such as parasitoids that are often attracted to terpene volatiles (Gershenzon and Dudareva 2007, Vogler et al. 2009). Acyl sugars, on the other hand, did not seem to hinder the foraging of *P. maculiventris*, and so offer a promising option for herbivore suppression without adversely affecting biocontrol; but if the main agent of biocontrol in a system is a parasitoid, altering acyl sugars may offer no benefit to biological control. The more knowledge we have about insect responses to plant chemotypes, the more specifically we can craft an effective mixture. Like nearly all ecology, effective cultivar mixtures will not be one size fits all.

CHAPTER 3:

Plant chemical diversity and its spatial arrangement have distinct but complementary effects on insect foraging

ABSTRACT

The importance of landscape spatial arrangement on insect community structure has been well established, but fewer studies have investigated the effects of diversity on the plot scale. This is particularly important because studies of disease have shown that altering spatial arrangement of plant cultivars can be a highly effective management tool for reducing disease spread, but the effect on insect pests is largely unknown; therefore, we may be neglecting an effective, pesticide-free pest management option. Theoretical work has shown that spatially varying plant quality should lead to a higher ratio of predators to herbivores over the long term. One major component of plant quality that is a strong candidate for manipulation is chemical defense. In order to determine the effects of the spatial arrangement of chemical diversity on insect pests and the ambient predator community, we released a generalist herbivore into monoculture plots of one of two chemically distinct tomato varieties or a diculture of the two varieties with varying patch size. We then monitored herbivore movement and feeding from second instar to pupation. We found that chemical diversity in a plot reduced spread of feeding, but only the diculture with larger patch size had reduced total herbivory. Herbivore survival in the diculture with larger patch size was nearly half of survival in the diculture with small patch size or monocultures, and overall herbivore feeding was reduced by approximately 25%. This pattern was mirrored by predator presence, which was highest in dicultures with larger patch

size. Our results indicate that spatial arrangement, not just chemical diversity, matters for insect pests and predators, even at the scale of a single plot.

INTRODUCTION

A surge of recent work has indicated that chemical diversity within a plant population influences insects (e.g., Moreira et al. 2016, Bustos-Segura et al. 2017, Massad et al. 2017, Pearse et al. 2018). Both herbivores and predators directly interact with plant chemical defense while foraging (Cornell and Hawkins 2003, Hare 2011, Dyer et al. 2018), and the presence of chemical diversity can lead to changes in foraging patterns (Power 1991) and predator-prey interactions (Wetzel et al. 2018, Wetzel and Whitehead 2020). Experiencing plant chemical diversity may have physiological effects on insects, either positive (Unsicker et al. 2008) or negative (Pearse et al. 2018), and these physiological effects are a potential mechanism reducing insect herbivore performance in genetically diverse plant communities (Crutsinger 2006).

Additionally, some studies have suggested that not only the amount of chemical diversity, but also the way that it is experienced may present a challenge for insects. Theoretical work has shown that interspersing high and low quality plants in a field should lead to higher parasitoid:prey ratios over the long term compared to simply dividing a field in half, even if the overall amount of chemical diversity is the same (Riolo et al. 2015). Furthermore, models and experimental work with daphnia have shown that the temporal structure of diet resource fluctuations can affect population outcomes (Koussoroplis et al. 2019). Similar results have been found by a recent laboratory diet study which shows that experiencing high levels of a toxin in

diet early rather than later led to slower development times for caterpillars, even though the total amount and variability of toxin was the same overall (Pearse et al. 2018). This study suggests…

This concept is supported by studies at the landscape scale which have suggested that not only does the presence of diversity matter for insect communities, but the spatial arrangement of that diversity matters as well (Martin et al. 2016, Haan et al. 2020). Landscape arrangement is expected to be most important when the scale of insect dispersal is closely matched to the scale of patch size (Haan et al. 2020), suggesting that spatial arrangement on the plot level may require relatively larger patch sizes to be effective as well. Due to the limited mobility of many insects, especially as larvae, altering the spatial arrangement of plants influences the temporal sequence by which herbivores experience diversity. Therefore, plot-level spatial arrangements of plant diversity will likely have a large effect on insect population outcomes, and is a key gap in our understanding.

Additionally, few studies at the plot scale have investigated how insect behavior contributes to performance outcomes in the face of different spatial arrangements of chemical diversity. Chemical cues are known to influence insect behavior (Hare 2011, Kersch-Becker and Thaler 2015, Kersch-Becker et al. 2017), and changing the amount and arrangement of chemical diversity in a plot could lead to increased or decreased movement of insects, changing their foraging patterns and time spent feeding. Furthermore, natural enemies are influenced by chemical cues and changes in their density and foraging success could explain herbivore population outcomes. Predators may experience volatiles in a more chemically complex environment as either masking or enhancing target odors (Schröder and Hilker 2008). Additionally, changing herbivore movement behavior, which has been reported in systems with increased plant diversity (Power 1991), might make herbivores more vulnerable to predators.

We integrated field and laboratory experiments to determine the effects of the presence and arrangement of chemical diversity on a generalist herbivore and the predator community. We did this by placing 30 *Trichoplusia ni*, the cabbage looper, in plots of one or two tomato varieties planted in rows and monitoring their movement and feeding, as well as predator presence, over the course of a week—approximately the length of time from second instar to pupation. We performed a laboratory experiment to determine the physiological effects of diet switching at varying intervals and to tease apart top-down and bottom-up effects of xxx on herbivores. Our specific questions were: 1) How does chemical diversity and its spatial arrangement influence the foraging of herbivores? 2) How do chemical diversity and spatial arrangement affect herbivore movement and survival? 3) How do predators interact with different levels and arrangements of diversity? 4) How does switching between plant chemotypes at different rates influence herbivores physiologically? Addressing these questions will help us understand how spatial arrangement of plant diversity influences insects at the scale that is most relevant for many species.

METHODS

Plants and insects

To investigate the effects of plant chemical diversity and its arrangement on a generalist herbivore, we used two tomato (Solanaceae) genotypes: a commercially produced genotype (*Solanum lycopersicum* cv. M82) and a genotype similar to the commercial one except that is has an altered terpene profile resulting from a substitution from *S. pennellii* on one chromosome. The

altered terpene genotype has a 75-80% reduction in sesquiterpenes compared to the commercial genotype (Schilmiller et al. 2010). Terpenes are primarily experienced by insects as volatile cues and influence both herbivore and predator feeding behavior (Gershenzon and Dudareva 2007). We grew plants in a greenhouse for 5 weeks before transplanting them into the field.

Our focal herbivore was a generalist pest, *Trichoplusia ni* (Lepidoptera: Noctuidae; Shorey et al. 1962). Eggs were purchased from an insectary (Benzon Research, Carlisle, Pennsylvania) and reared in the laboratory until the second instar, at which point they were transferred to plant tissue. For the field experiment, we reared larvae in a growth chamber at 28°C and a 16:8 L:D photoperiod on general noctuid diet (Benzon Research) until deployment in the field. For the laboratory experiment, we reared *T.ni* at room temperature and on general noctuid diet (Benzon Research) until transfer to plant tissue.

Field experiment

We conducted a field experiment at Michigan State University's Kellogg Biological Station, Hickory Corners, MI, to compare the two plant genotypes and to investigate their different spatial configurations on herbivore feeding and movement. We had three treatments all consisting of four plants in a row: monocultures of one genotype, dicultures with alternating plant genotypes within a row (single), and dicultures with alternating plant genotypes after every other plant so that two of the same genotype are placed next to each other within a row (double). This meant that all four dicultures contained the same amount of plant chemical diversity, but in different spatial arrangements, allowing us to independently measure the effects of chemotypic

diversity from its spatial arrangement. Half of the plots within each diculture treatment had the commercial genotype in the starting position, while the other half had the altered terpenes genotype in the starting position. We performed two rounds of the field experiment, one planted and harvested in July and one planted and harvested in August of 2019. Within each round, the planting of plots was separated into three temporal blocks, so that each block was planted and monitored 1-2 days apart. Full replicate information can be found in Table 2.

In each plot, 5-week-old tomatoes were planted into 1 m long plots covered with black plastic and with dripline irrigation. Plants within plots were spaced 20 cm apart and plots were separated by a 1 m black plastic section in a field 18m x 125 m; plots were lined up in five long rows. One week after transplanting plants into the field, 30 second instar *T.ni* were placed onto the first plant using a paintbrush. After placement, caterpillars were free to move among plants in the plot. All plots were uncaged and exposed to the ambient insect community.

We visually searched the entire foliage of all plants and recorded the number and locations of *T. ni,* as well as all other arthropods within each plot 1 week after caterpillar release. For the second (August 2019) round of the experiment, we added two additional searches of plants at 24h and 48h after caterpillar release. We also performed one night sampling for each block by visually censusing between 21:00h and 24:00h and recording the identity, number and location of all arthropods in a subset of plots. While we performed three censuses during the first round, censuses were not performed on a strict 24h, 48h, and one week schedule for each block as they were in the second round; therefore, we used predator data from those censuses but not caterpillar location data, since we were interested in total number of predator visits to plots but caterpillar movement at specific timepoints to understand how they moved through a plot. One week after release, some caterpillars had begun to pupate, therefore we destructively sampled

plants and visually estimated leaf area removed by herbivory one leaf at a time using a 0.5 cm^2 grid. Total number of recaptured caterpillars was recorded for each plot, as well as which plant caterpillars were found on.

Laboratory experiment

We conducted a no-choice laboratory experiment to investigate the effects of plant chemical diversity variation on caterpillar performance. As in the field experiment, these treatments were designed to test effects of chemical variation on herbivores, removing caterpillar choice and predation effects. Caterpillars were fed tomato leaves in one of three arrangements: 1) monoculture, where they were fed on either the commercial or altered terpenes genotype for 8 days; 2) 'slow' diculture (hereafter, "Slow"), where they were fed on one genotype for 4 days and then the second genotype for 4 days; or 'fast' diculture, where they switched genotypes every 2 days for a total of 4 days per genotype (hereafter, "Fast") (Figure 6). As with the field experiment, within dicultures half of the replicates started on the commercial genotype and half started on the altered terpenes genotype. Tissue was harvested from tomato plants grown and maintained in the greenhouse. Plants were approximately eight weeks old at the time of the experiment, and leaflets were only collected from a plant on a single day to minimize induction of plant defenses. Full replicate numbers can be found in Table 3.

We placed individual second instar *T. ni* in 10 cm petri dishes kept in the lab with single tomato leaflets and allowed them to feed *ad libitum* after measuring their initial mass. Leaflets were kept fresh by placing them in Eppendorf tubes filled with an 0.4% agarose:water solution

(UltraPure Agarose, Invitrogen; and BD Bacto™Agar, Fisher Scientific). Every two days, tomato leaflets were removed and replaced with fresh plant tissue. We selected leaflets >7 cm in length that were not part of the lowest or highest two leaves of the plant in order to standardize plant tissue age. Additionally, we used only healthy leaflets (avoided yellowing, thrips damage, etc). We applied predatory nematodes in the soil (NemAttack™, Arbico Organics; and Thripex, Koppert Biological Systems) to prevent thrips and parasitoid wasps (Enermix, Koppert Biological Systems) to control whiteflies. We did not observe thrips on any plants, but did experience whiteflies on the tomatoes in round 2; we achieved good control of the whiteflies with the parasitoid wasps.

We quantified leaf area removed by herbivory using the image analysis software LeafByte (Getman-Pickering et al. 2020) each time caterpillars were moved to a new leaflet: days 2, 4, 6, and 8. To quantify caterpillar performance, we took two approaches. First, we massed each caterpillar's frass (wet weight) on days 6 and 8. We used this to calculate the efficiency of conversion of ingested food (ECI), which measures how much of the food a caterpillar consumes is converted into increased mass (Waldbauer 1968). We did not weigh frass on days 2 and 4 because its mass was too small to be registered by our scale with accuracy. Second, after 8 days, caterpillar final mass was recorded and insects were frozen. We then calculated change in mass for each caterpillar. We performed two rounds of the experiment, one in January and one in February of 2020, each with 10 replicates per treatment for a total of 20 replicates per treatment.

Statistical analyses

Field experiment

All statistical analyses were conducted in R (Bates et al. 2015, R Core Team 2017). We used Generalized Linear Mixed Models (GLMMs) to assess relationships between treatments and plant damage, herbivore movement, and herbivore performance. Models were implemented using the package *lme4* (Bates et al. 2015) or *glmmTMB* (Brooks et al. 2017). We used likelihood ratio tests to compare models and test hypotheses (Bolker et al. 2009).

To explore how our treatments affected damage to plants, we asked how leaf area removed by herbivory varied by plant position (from the initial plant) and whether this relationship differed among chemical diversity treatments (monoculture vs. diculture; ignoring spatial arrangement/grain size) or spatial arrangement of chemical diversity (small-grain or large-grain diculture). We tested for an effect of the presence of diversity on herbivore feeding by comparing models with and without a factor representing monoculture or diculture, with an interaction between that factor and plant position. This allowed us to see if presence of diversity changed the relationship between herbivory and distance from initial plant—the spatial spread of herbivory across the plot. We tested for an effect of the spatial arrangement of diversity by comparing similar models with and without a factor representing treatment (small grain or large grain diculture) on a dataset including diculture plots only. This allowed us to separate out the effects of spatial arrangement independent of chemical diversity by only comparing plots with the same level of chemical diversity.

We used total leaf area removed by herbivory to determine the effects of chemical diversity on total plant damage per plot. We compared models with the same factors as in spread

of herbivory, but used total damage in each plot rather than damage per plant as our response variable. We compared null models to models including either a monoculture and diculture factor or a set of diculture-only plots, again allowing us to compare the effects of chemical diversity and spatial arrangement independent of each other.

To explore how these treatments affected larval survival, we determined whether the number of caterpillars recaptured during harvest differed by plant treatment (monoculture, small-grain diculture, or large-grain diculture). We used models with the number of *T.ni* found vs. the number of *T.ni* not recovered and plant number as interactive fixed effects and compared those models to similar models that were additive rather than interactive.

We asked how many caterpillars either remained on a plant (the number located on that plant) or departed from that plant (the sum of caterpillars on subsequent plants) to understand effects of chemical diversity on caterpillar movement. We compared models with and without a factor for plant genotype (either genotype of the focal plant or the subsequent plant: commercial or altered terpenes) to see whether genotype drove departure rates for caterpillars early in the experiment.

We addressed our final question about how chemical diversity and its arrangement influenced the predator community by asking how predator abundance at the whole-plot level differed by time of day (day or night) or plant treatment (monoculture, large-grain diculture, or small-grain diculture). We compared models with and without arrangement type as a factor determining predator abundance. We modeled the relationship between daytime and nighttime predator counts separately due to the different level of census data collection. We also asked whether time of day influenced predator abundance by comparing models with and without time of day as a fixed effect.

For models investigating spread of herbivory, we accounted for non-independence of caterpillars in the same plot and round by using these factors as nested random effects, since temperature (round) and feeding of other caterpillars (plot) might affect feeding levels of individual caterpillars. For survival models and models of caterpillar movement, we used plot as a random effect, since we used only data from our second round. For models of predator presence, we used round as a random effect. For all models predicting herbivore damage, we log-transformed total leaf area removed by herbivory at the per-plot scale because by plot there were no zero values, and square-root transformed herbivory at the per-plant scale because there were some plants without any damage (zeroes present). We performed these transformations to achieve normally-distributed residuals.

Laboratory experiment

To address our final question about effects of chemical diversity on caterpillar performance, we used linear mixed models in the *lme4* package (Bates et al. 2015, R Core Team 2017). We analyzed survival using a binomial generalized linear mixed model comparing the number of caterpillars that survived with the number of caterpillars that died with diet switching type (monoculture, fast, slow) as a fixed effect and round as a random effect. Mass change and leaf area removed were analyzed similarly, but with a linear mixed model rather than a generalized linear mixed model. Lastly, efficiency of conversion of ingested food (ECI) was square root transformed to achieve normality of residuals and then analyzed with a linear mixed model with diet switching type as a fixed effect and round as a random effect.

RESULTS

Field experiment

How does chemical diversity and its arrangement influence the foraging of herbivores?

In order to determine the effect of chemical diversity on leaf area removed by caterpillars, we analyzed monoculture or diculture plots separately—ignoring spatial arrangement or starting plant. The presence of chemical diversity resulted in a lower spread of herbivory from the plant where caterpillars were initially deployed (Fig 2; $\chi^2 = 8.72$, df = 1, $p < 0.05$). Slopes in plots with chemical diversity were 66% more negative than slopes in monoculture plots (CI: 22% to 109%, Figure 7A). Chemical diversity also led to decreased herbivory: decultures had a 30% reduction in total area removed by herbivory per plot (Fig 2B; $\chi^2 = 3.71$, df = 1, $p = 0.054$). This effect was largely driven by the large grain diculture (Fig 2B).

To determine how spatial arrangement of chemical diversity influenced herbivores, we compared spread of area removed by herbivory and total area removed by herbivory in small and large grain diculture plots. Spatial arrangement independent of chemical diversity did not significantly influence the spread of herbivory (Fig 2; $\chi^2 = 0.01$, df = 1, $p = 0.92$) but there was a 26% reduction in total area removed by herbivory per plot in large grain dicultures compared to small grain dicultures (Fig 2; $\chi^2 = 4.94$, df = 1, $p = 0.03$).

How do chemical diversity and spatial arrangement affect herbivore movement and survival?

We determined the impact of plant chemical diversity and spatial arrangement on caterpillar survival 1 week post release (harvest) in round 2, where we had the most complete census data. Caterpillar survival at harvest closely mirrored feeding patterns (Figure 8; $\chi^2 = 5.81$, df = 2, p = 0.054). Survival at one week was fairly similar between monocultures (19.8%) and small grain dicultures (23.1%), while large grain dicultures—which also had lowest total feeding—had the lowest survival (12.7%, a reduction of 45.1% compared to the small grain diculture). After one week post release (harvest), caterpillars were found in different parts of each plot based on plant treatment (Figure 8; $\chi^2 = 5.76$, df = 2, p = 0.056). In monocultures, location was equally distributed among plants, whereas caterpillars in dicultures were located more frequently on the middle two compared to the outer two plants.

During the first 24 hours of our experiment, the genotype of plant 1—the caterpillar's starting plant—did influence departure (Figure 9; $\chi^2 = 4.13$, df = 1, p = 0.04), but the genotype of plant 2 (the next plant in the row) did not (Figure 9; $\chi^2 = 2.75$, df = 1, p = 0.10). However, by 48 hours, the caterpillars' choice to depart from plant 1 was not determined by the genotype of plant 1 (Figure 9; $\chi^2 = 0.022$, df = 1, p = 0.88), but was determined by the genotype of plant 2 (Figure 9; $\chi^2 = 5.42$, df = 1, p = 0.02).

How do predators interact with different levels and arrangements of diversity?

During the daytime, total predators observed in plots differed by plant arrangement type (Figure 10; $\chi^2 = 5.73$, df = 2, $p = 0.057$). Predator presence was lowest in monocultures during the day (0.41 cumulative predators observed per plot, CI: 0.24-0.69), with intermediate levels in small grain dicultures (0.52 cumulative predators observed per plot, CI: 0.32-0.86), and highest levels in large grain dicultures (0.88 cumulative predators observed per plot, CI: 0.59-1.32). This represents a 69% increase in predator abundance in large grain diculture plots compared to small grain diculture plots, even though they have identical levels of chemical diversity, and a 116% increase over monocultures. We observed 2.67 times as many predators at night compared to daytime censuses ($\chi^2 = 17.66$, df = 1, $p < 0.05$; Table 2), but there was no difference in predator presence by plant arrangement type at night ($\chi^2 = 1.78$, df = 2, $p = 0.41$).

Laboratory experiment

How does switching between plant chemotypes at different rates influence herbivores physiologically?

Diet switching treatment did not affect survival ($\chi^2 = 0$, df = 2, $p = 1$) or caterpillar mass change ($\chi^2 = 0.78$, df = 2, $p = 0.68$). There were no statistical differences in the cumulative leaf area removed by herbivory ($\chi^2 = 2.95$, df = 2, $p = 0.23$), or efficiency of conversion of ingested food (ECI; $\chi^2 = 1.35$, df = 2, $p = 0.51$).

DISCUSSION

We examined the effects of plant chemical diversity and its arrangement in space and time on the performance and behavior of a generalist herbivore and the predator community. We did this by performing a field experiment where we placed caterpillars in rows of four tomato plants of a single genotype or of two genotypes of varying patch size and tracked their movement and feeding until pupation, as well as the distribution and abundance of predators. We followed up with a laboratory experiment that investigated physiological effects of diet switching at different rates on individual caterpillars. We found that the presence of chemical diversity in a plot reduced spread of feeding, but spatial arrangement affected total amount of feeding, indicating that spatial arrangement matters not only at a landscape scale, but at a plot scale. Physiologically, we did not find any effects of switching diet between the two tomato genotypes on *T. ni* caterpillars. However, we found that predators reached higher densities in plots where chemical diversity was present in larger patch sizes, suggesting that natural enemies – rather than physiological effects of diet switching - are a likely mechanism for lower rates of survival and feeding in those plots.

There are at least three potential mechanisms for the effects of spatial arrangement of chemical diversity on insects: physiological effects of diet switching, behavioral changes of herbivores, or altered movement behavior of natural enemies in plots of different spatial arrangements. Although several studies have shown that experiencing diet variability can decrease herbivore performance (Riolo et al. 2015, Wetzel et al. 2016, Pearse et al. 2018), we did not find any physiological effects on caterpillars in lab trails manipulating the temporal sequence of feeding on two tomato genotypes with different chemistries. Survival in our study was identical between treatment groupings (monoculture, fast diculture, and slow diculture), and we

also found no effects of diet treatment on change in pupal mass or area removed by herbivory. Therefore, it seems unlikely that direct physiological effects can explain our results.

However, the role of behavior is less clear. In our laboratory experiment, caterpillars were only provided a single leaf at a time. In the field, some element of behavior was clearly at play: not only did chemical diversity slow the spread of herbivory across the plot, it also resulted in caterpillars being less likely to leave plants of the commercial genotype compared to the altered terpenes genotype at 24 hours. At 48 hours, caterpillars were more likely to have left the first plant if the second plant was the altered terpenes genotype. *T. ni* caterpillars are highly mobile; in our experiment, approximately 1% of experimental caterpillars had reached the final plant in our plot after 24 hours, so caterpillars experienced multiple plants that they could choose from over the course of the experiment. Experiencing chemical diversity early could discourage caterpillars from continuing to move, since they are not guaranteed to find an equally optimal plant, which could explain the decreased spread of feeding in dicultures compared to monocultures. However, because there were no physiological differences between the two genotypes, behavior alone does not seem to explain differences in survival and feeding.

Over the course of our experiment, natural enemies were present at higher densities in large grain dicultures compared to the monocultures or small grain dicultures. More predators were present during night sampling than the day sampling, but no differences emerged between arrangement types and predator presence at night. It is possible that day active predators had a larger effect because *T.ni* are much more active during daylight hours (Johnson et al. 1987), making them more vulnerable to predation. Because our design did not include any predator exclusion treatments, it is impossible to know whether predators were more drawn to large grain dicultures due to their chemical signals or a higher rate of herbivore success. In some cases, areas

with more natural enemies also have more herbivores, so enemies may be seeking out prey but not reducing overall herbivory (Martin et al. 2016). In our study this did not seem to be the case. Not only did large grain diculture plots have lower *T. ni* survival rates, but their herbivory rates were low as well. A high rate of herbivory but low survival could indicate that caterpillars were thriving in large grain plots and higher levels of feeding attracted predators, but this was not the case in our plots. It seems most likely that natural enemies reduced caterpillar survival early, that their increased presence reduced herbivore foraging behavior (Hermann and Thaler 2014), or both.

If natural enemies are largely responsible for the reduction in feeding and survival in the large grain diculture, it is not immediately apparent why they would prefer the large grain diculture over the small grain diculture, since both treatments contained the same amount of chemical diversity overall. The main difference between the two tomato genotypes is their production of terpenes: the altered terpenes genotype has a 75-80% reduction in sesquiterpenes compared to the commercial genotype (Schilmiller et al. 2010). Terpene volatiles are utilized by predators and parasitoids in prey location (Vuorinen et al. 2004, Schnee et al. 2006, Vieira et al. 2019). In the small grain diculture, higher-emitting commercial plants are separated by lower-emitting altered terpene plants, whereas in the large-grain diculture, higher-emitting plants are more clumped. It is possible that this is perceived by natural enemies as a stronger signal of herbivory. However, this does not explain why monocultures had the lowest predator presence. Perhaps because of the more even spread of herbivory in monocultures, plants did not reach a threshold level of herbivory as early as in dicultures, where spread of herbivory was lower. Once predators were in plots, they may remain and forage there, since the prey density was quite

high—leaving predators less likely to move to monoculture plots once damage levels and herbivore-induced plant volatile production increased.

Our study shows that the impacts of chemical diversity are dependent on the spatial arrangement of genotypes driveing insect populations on the plot scale, and that these outcomes may be largely due to attraction of and control by natural enemies. These results help explain some mechanisms by which plant diversity may be influencing insect populations and communities in both natural and agricultural systems.

CHAPTER 4:

Conclusions and future directions

We examined the effects of different types, levels, and arrangements of chemical defense diversity on a generalist herbivore and its interactions with predators. We did this through field experiments, where we placed *T. ni* in caged plant contexts of monocultures, dicultures, or polycultures of three tomato chemotypes, or open plots of two tomato chemotypes planted in monocultures or dicultures of varying patch size. We tracked *T. ni* movement, herbivory, survival, and pupal mass, as well as the presence of the predator community during the duration of our experiments. We also investigated specific mechanisms through laboratory experiments, to determine predator preference for specific tomato genotypes as well as physiological effects of diet switching on herbivores. Our results indicated that dicultures had the strongest effect on *T. ni*, caterpillar and predator outcomes were dependent on specific plant traits and arrangements, and that outcomes for plants and herbivores may be driven by natural enemies.

One clear result from our experiments was the effectiveness of dicultures on *T. ni* feeding and survival. Male *T. ni* had lower pupal mass in dicultures compared to monocultures or polycultures, and caterpillars overall had lower survival and feeding in our open chemical diversity plots—again, dicultures—compared to open monocultures. In this case, more diversity was not better from a pest management perspective. However, relying on dicultures alone for pest control might be impractical, at least in the short term. The direct plant effects that we did see in *T. ni* were typically growth related and affected males more than females. Lower male pupal mass does have the potential for long-term population effects: males that emerge early (Saastamoinen et al. 2013) and are larger (Wiklund and Kaitala 1995, Makee and Saour 2001) tend to have more mating success in many Lepidopteran species, so smaller males (in this case,

emerging from dicultures) could contribute less genetic material to the next generation. However, lower male pupal mass would not likely reduce pest populations in the short term. In our laboratory experiment, for example, we found that survival was identical over a period of eight days for *T. ni* that fed on two chemically distinct tomato lines or a single line. We also found no differences in mass gain. Therefore, direct plant effects may not be effective alone from a pest management perspective. It is worth noting that outcomes may have been different if we used plants with higher production of chemical defense than normal: previous work with tomatoes, aphids, and lady beetles has shown that predator effects are more powerful in plants with lower levels of jasmonic acid production, whereas plants with high levels of JA production directly limit herbivore population growth but have lower levels of predation (Kersch-Becker and Thaler 2015, Kersch-Becker et al. 2017). Our three lines had different types of plant defense compared to the commercial (acyl sugar bases) or reduced defenses (reduced sesquiterpenes), so may have relied more heavily on natural enemies to suppress insect pests than a high resistance line.

Natural enemies, on the other hand, showed a clear reduction of pest populations in both field experiments. When they were introduced to cages with herbivores, they reduced *T. ni* populations strongly regardless of plant context. The ambient predator community reduced *T. ni* populations by about 25% in the diculture with larger patches of chemical diversity compared to the diculture with smaller patches or the monoculture. These effects, which translated to lower feeding damage and survival, could have a much more timely impact on pest populations. Predators preferred certain tomato lines and avoided others in our system, a result which is in line with previous studies: we know that predators are attracted to some volatile blends more than others (Ninkovic et al. 2011, Grettenberger and Tooker 2017), and forage more effectively

on lines that lack certain chemical defenses such as waxy blooms (Eigenbrode 2004). Therefore, it does seem clear that certain plant contexts promote predator presence and effective foraging. Perhaps in the future this knowledge could be used to construct more chemotypically complex agroecosystems, with certain genotypes preferred by predators as spatial corridors to promote their movement into the field, where insect pests otherwise escape predation.

Our results suggest that cultivar mixtures, especially dicultures, have the potential to be effective management tools for reducing insect pest pressure if they are used in the right context. Mixtures will be most effective if they are chosen for specific traits that are expected to reduce herbivore performance or promote natural enemy presence. The grain size of patch arrangement should be relevant to the life history of the insects expected to be most important in the system. Lastly, mixtures will be most effective in contexts with standing natural enemy populations, since the strongest immediate effects on pest reduction come from natural enemies. Encouragingly, planting these mixes—even in particular spatial arrangements—is feasible for many crops. For example, commercially produced transplanters allow for seedlings to be manually fed into the machine in whatever order the planter desires, and are suitable for a variety of crops including tomato, tobacco, peppers, cabbage, cauliflower, aubergines, and many more (Z. Szendrei, personal communication; and Fedele Agricultural Machinery Manufacturer, Italy). Additionally, these results shed light on plant-insect interactions in general. Although large-scale trends and landscape arrangements matter for insects, the environmental conditions they experience especially at low mobility stages can significantly shape insect trajectories, survival, and performance.

APPENDIX

Tables and Figures

Table 1. Plant treatments. 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	75-80% reduction in sesquiterpenes compared to commercial genotype[1]	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[1]	15	13
Diculture	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
Diculture	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

[1]Schilmiller et al. 2010

Table 2. Plant treatments and replicates: field experiment. Plants were arranged in plots of 4 tomato plants in a single row. 30 *T.ni* caterpillars were released on plant 1 and allowed to move freely for a period of one week.

Diversity level and grain size	**Plant treatment**	**Plant 1 (+30 *T.ni*)**	**Plant 2**	**Plant 3**	**Plant 4**	**Round 1 Replicates**	**Round 2 Replicates**	**Total Replicates**
Monoculture	Commercial	Comm.	Comm.	Comm.	Comm.	12	6	18
Monoculture	Altered terpenes	Alt. terpenes	Alt. terpenes	Alt. terpenes	Alt. terpenes	14	6	20
Small grain diculture	Commercial Start	Comm.	Alt. terpenes	Comm.	Alt. terpenes	13	8	21
Small grain diculture	Altered terpenes start	Alt. terpenes	Comm.	Alt. terpenes	Comm.	14	7	21
Large grain diculture	Commercial start	Comm.	Comm.	Alt. terpenes	Alt. terpenes	13	10	23
Large grain diculture	Altered terpenes start	Alt. terpenes	Alt. terpenes	Comm.	Comm.	15	7	22

Table 3. Plant treatments and replicates: laboratory experiment. Treatments are identical to the field experiment, but each plant has been replaced with a 2-day increment of exposure to a specific chemotype. Each replicate represents an individual *T.ni* in a petri dish with a leaflet of the listed chemotype.

Diversity level and grain size	Plant treatment	Day 1-2	Day 3-4	Day 5-6	Day 7-8	Round 1 Replicates	Round 2 Replicates	Total Replicates
Monoculture	Commercial	Comm.	Comm.	Comm.	Comm.	10	10	20
Monoculture	Altered terpenes	Alt. terpenes	Alt. terpenes	Alt. terpenes	Alt. terpenes	10	10	20
Small grain diculture	Commercial Start	Comm.	Alt. terpenes	Comm.	Alt. terpenes	10	10	20
Small grain diculture	Altered terpenes start	Alt. terpenes	Comm.	Alt. terpenes	Comm.	10	10	20
Large grain diculture	Commercial start	Comm.	Comm.	Alt. terpenes	Alt. terpenes	10	10	20
Large grain diculture	Altered terpenes start	Alt. terpenes	Alt. terpenes	Comm.	Comm.	10	10	20

Table 4. Predators observed. Predators were observed in the field at six sampling dates for the day samples (three in round one, three in round two) and two sampling dates for the night samples (1 in round one, one in round two).

Census time	Order or subphylum	Family	Number observed
Day	Araneae	Unknown	46
	Coleoptera	Coccinellidae	9
	Coleoptera	Lampyridae	4
	Coleoptera	Unknown	3
	Hemiptera	Nabidae	3
	Homoptera	Unknown	1
	Hymenoptera	Formicidae	12
	Hymenoptera	Unknown	3
	Myriapoda	Chilopoda	1
	Opiliones	Unknown	3
Night	Araneae	Unknown	32
	Coleoptera	Unknown	11
	Hemiptera	Unknown	1
	Hymenoptera	Formicidae	12
	Hymenoptera	Unknown	2
	Opiliones	Unknown	2
Total	Araneae	Unknown	78
	Coleoptera	Coccinellidae	9
	Coleoptera	Lampyridae	4
	Coleoptera	Unknown	14
	Hemiptera	Nabidae	3
	Hemiptera	Unknown	1
	Homoptera	Unknown	1
	Hymenoptera	Formicidae	24
	Hymenoptera	Unknown	5
	Myriapoda	Chilopoda	1
	Opiliones	Unknown	5

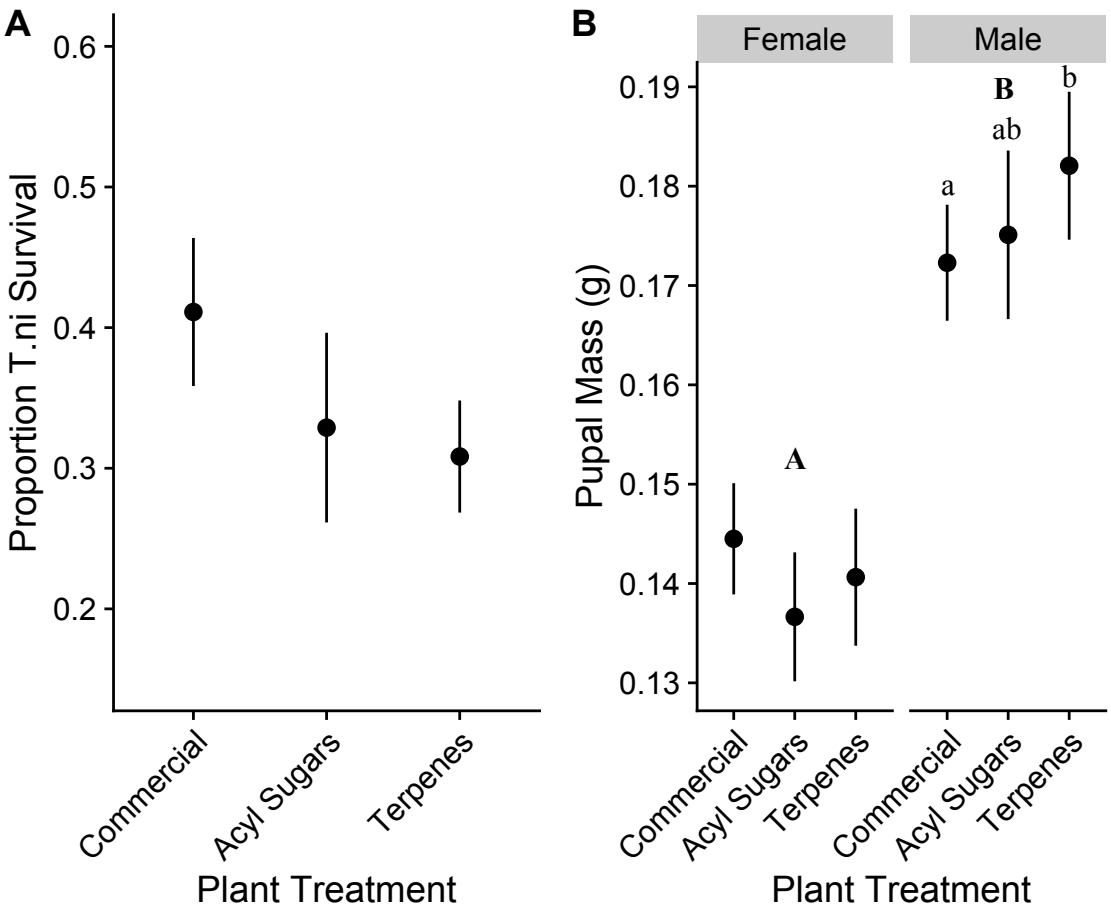

Figure 1. Survival proportion and pupal mass in control monocultures. Plot shows mean +/- SE. Survival (panel A) was not significantly different between plant treatments, although survival in the commercial chemotype was slightly higher than either of the introgression lines, which varied in acyl sugars or terpenes. Overall survival in control monocultures was 33.45%. Pupal mass (panel B) was significantly different between males and females, which is typical for *T. 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.

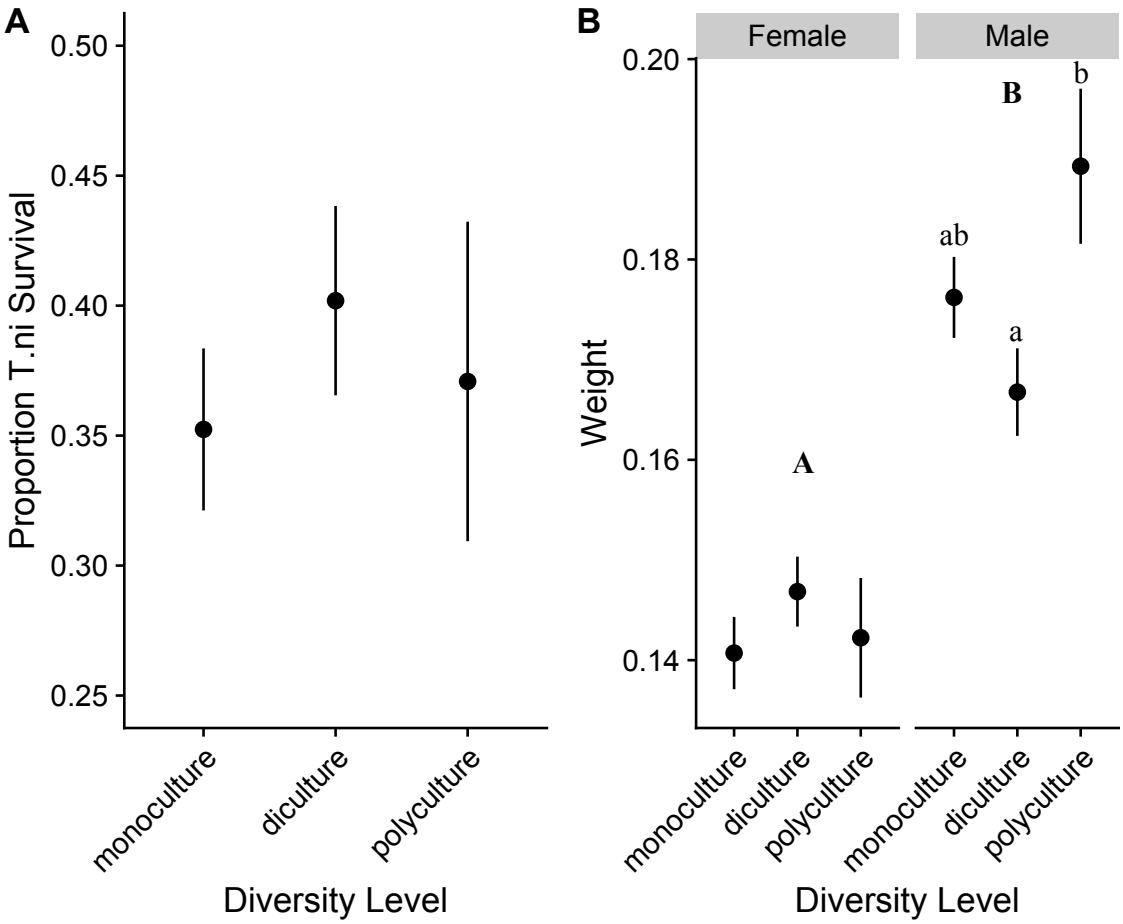

Figure 2. *T. ni* survival and pupal mass by overall diversity level. Plot shows mean +/- SE. Level of diversity (monoculture, diculture, or polyculture, panel A) did not have an effect on survival in control cages. Level of overall diversity did not have a significant effect on female pupal mass, but did influence males.

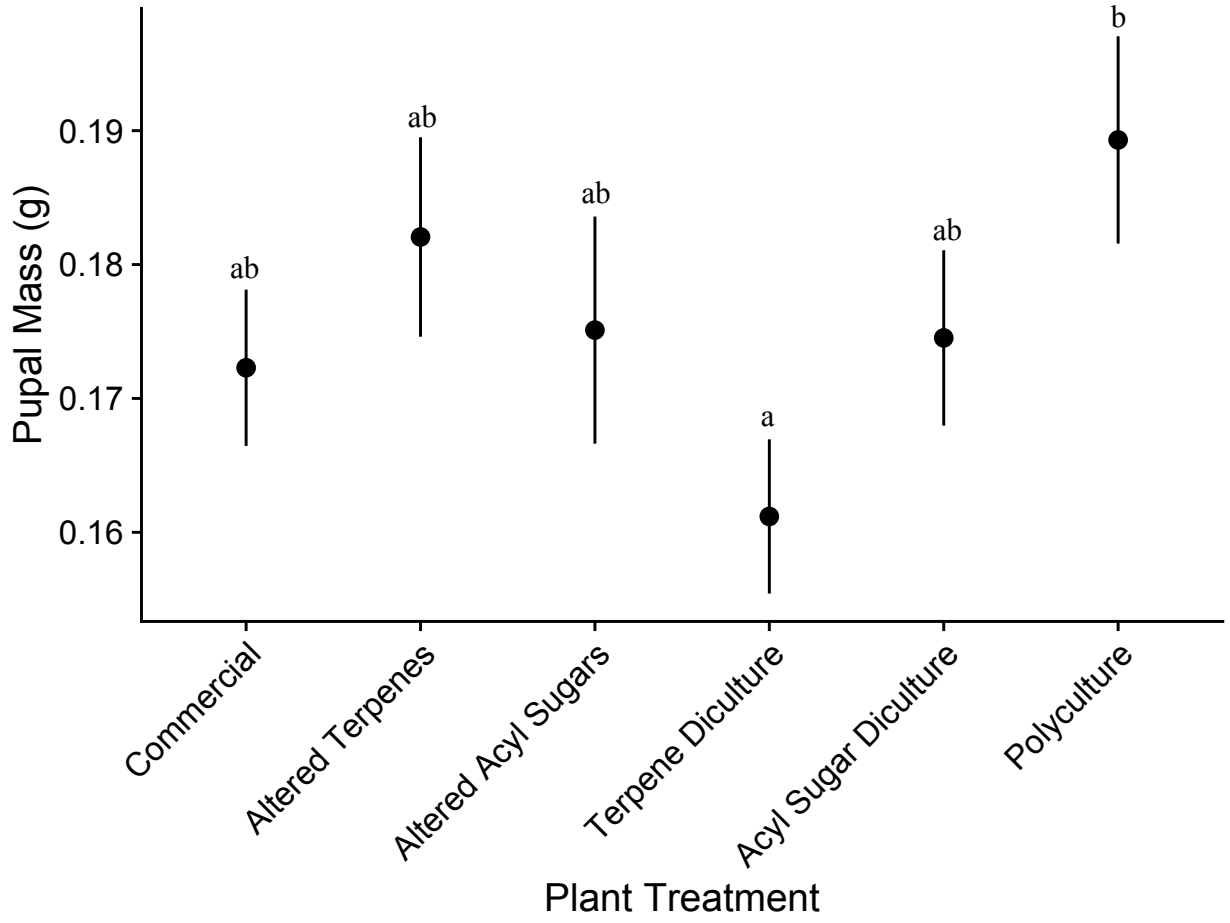

Figure 3. Male *T. ni* pupal weight in control cages by individual plant treatment. Plot shows mean +/- SE. Plant treatment had a significant effect on male pupal mass.

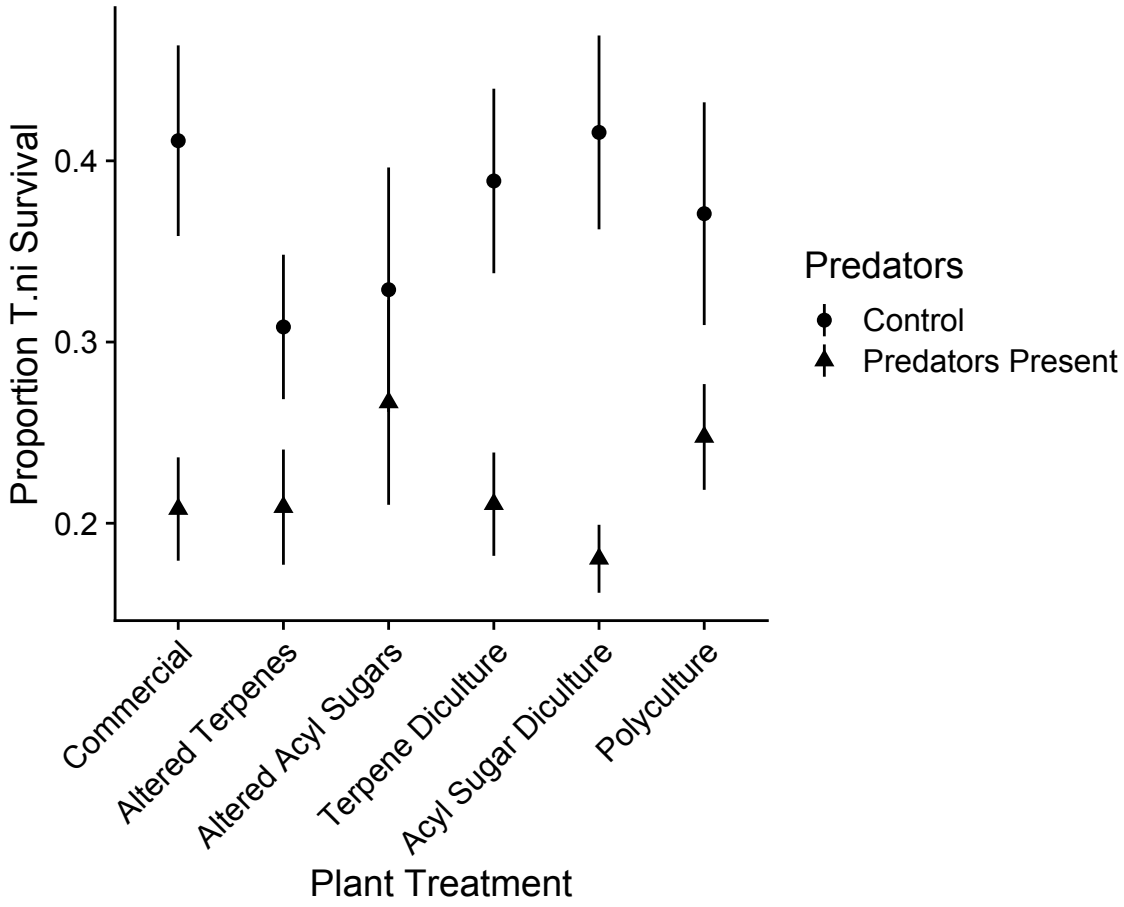

Figure 4. Proportion *T. ni* survival in control and predator cages by plant treatment. Plot shows mean +/- SE. Predators had a significant effect on survival.

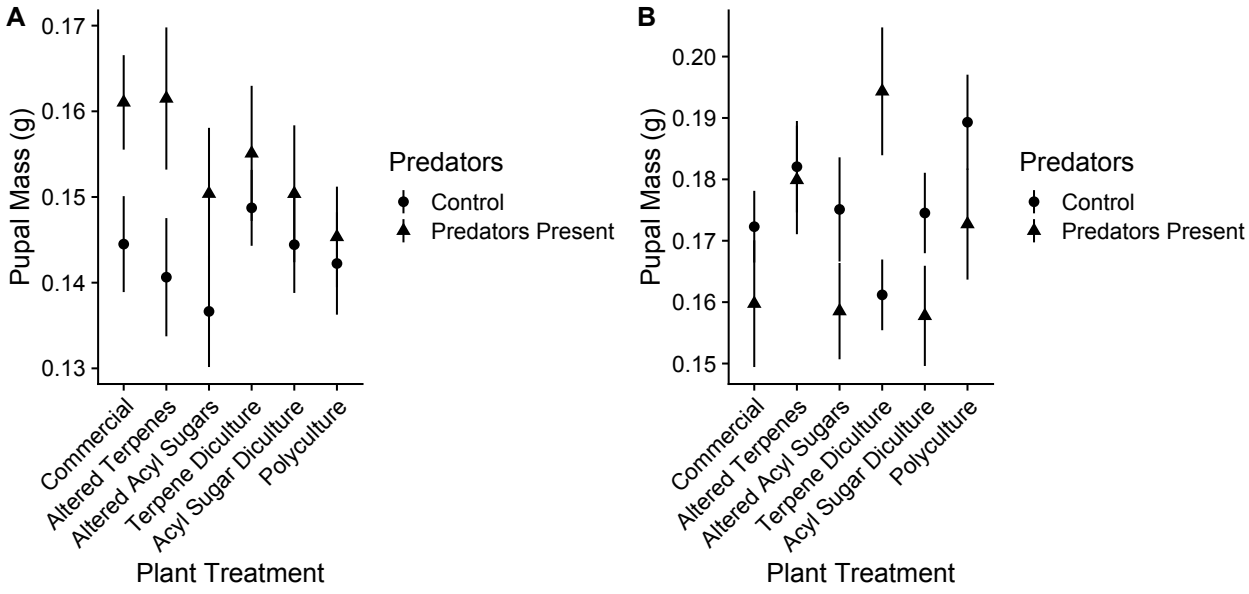

Figure 5. *T. ni* pupal mass by plant treatment with and without predators. Plot shows mean +/- SE. Female *T.ni* (panel A) consistently had higher pupal mass in predator treatments. Although predators had an overall effect on female pupal mass, there was no interactive effect between pupal mass and level of diversity or pupal mass and plant treatment. This was true for monocultures only and dicultures + polycultures. For male *T.ni* (panel B), 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 dicultures and polycultures only.

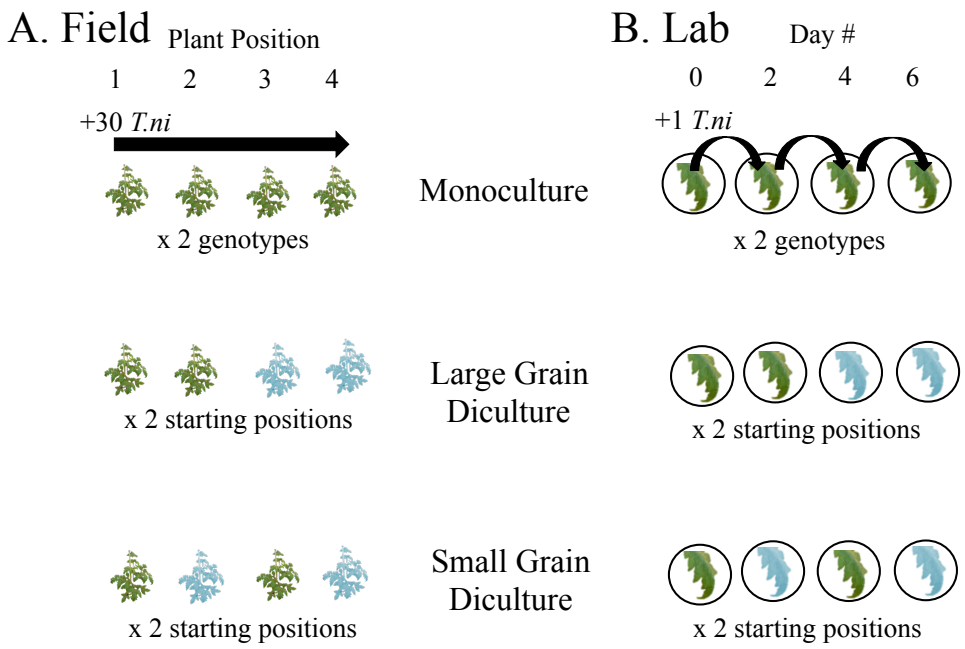

Figure 6. Experimental setup. In field setup (panel A), 30 caterpillars were deposited in plots of 4 tomatoes planted in a single row and censused three times over the course of one week. Approximately half of the monoculture treatments (38 in field experiment) consisted of the commercial genotype, and the other half consisted of the altered terpenes genotype. For dicultures, approximately half of each treatment (45 large grain, 42 small grain in field) had the commercial genotype in the starting position followed by altered terpenes, and half had the opposite arrangement. In laboratory setup (panel B, 40 replicates for each treatment), individual caterpillars were placed in petri dishes and moved to a new food source every two days for eight days.

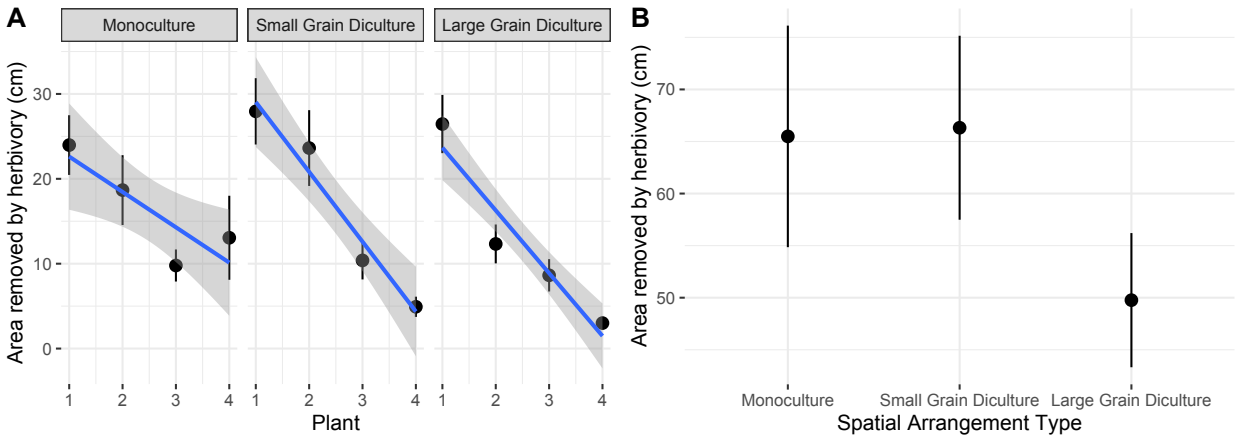

Figure 7. Effects of chemical diversity on herbivore foraging in a field experiment. Mean ± SEM tomato leaf area (cm^2) removed by caterpillars that dispersed from the release plant (1) to subsequent plants (2-4) in the plot after 7 days (A). Grey shaded area indicates standard error of the mean from a generalized mixed model (A). Mean ± SEM of tomato leaf area (cm^2) removed by caterpillars from plants arranged in plots that differed in spatial arrangement and level of chemical diversity (B).

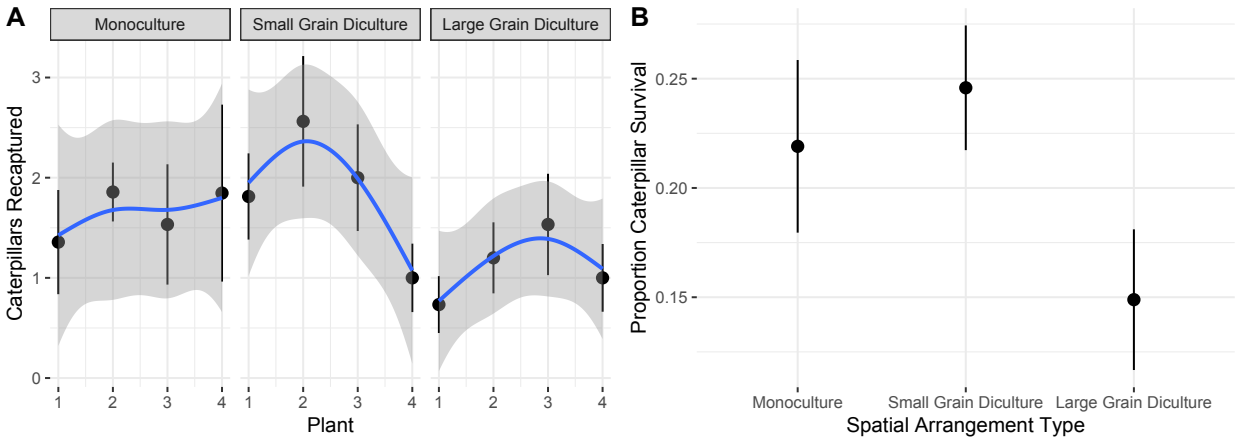

Figure 8. Caterpillar survival and location after one week. Mean ± SEM caterpillars recaptured by plant 1 week post release in tomato plots that differed in chemical diversity in a field experiment (A). Caterpillars were released on plant 1 and allowed to disperse to subsequent plants. Plants were destructively sampled and recaptured live caterpillars were counted. Missing caterpillars out of the initial 30 released were assumed dead. Mean ± SEM caterpillar survival by plot (B).

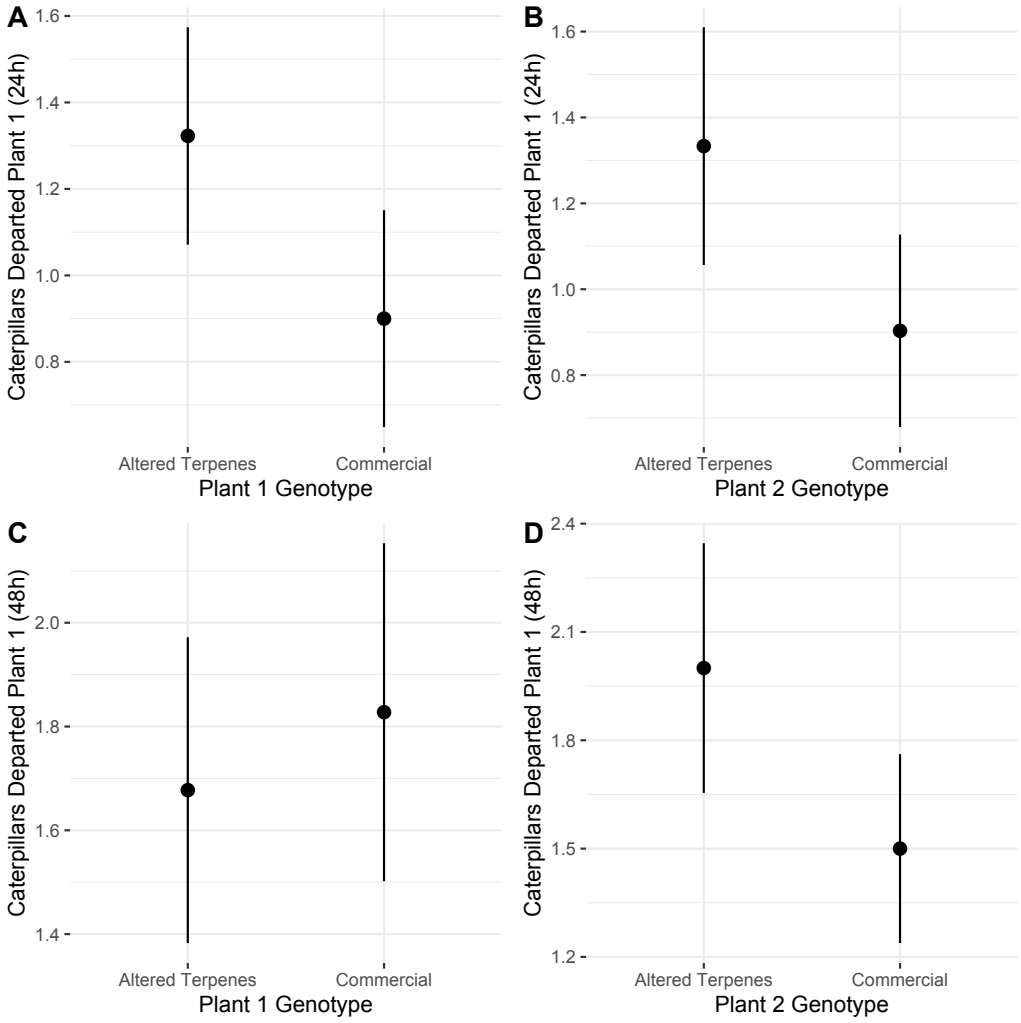

Figure 9. Caterpillar departure rates by plant genotype. Mean ± SEM caterpillars departing plant 1 (starting plant) at 24 and 48 hours depending on whether starting plant or subsequent plant was commercial or altered terpene genotype.

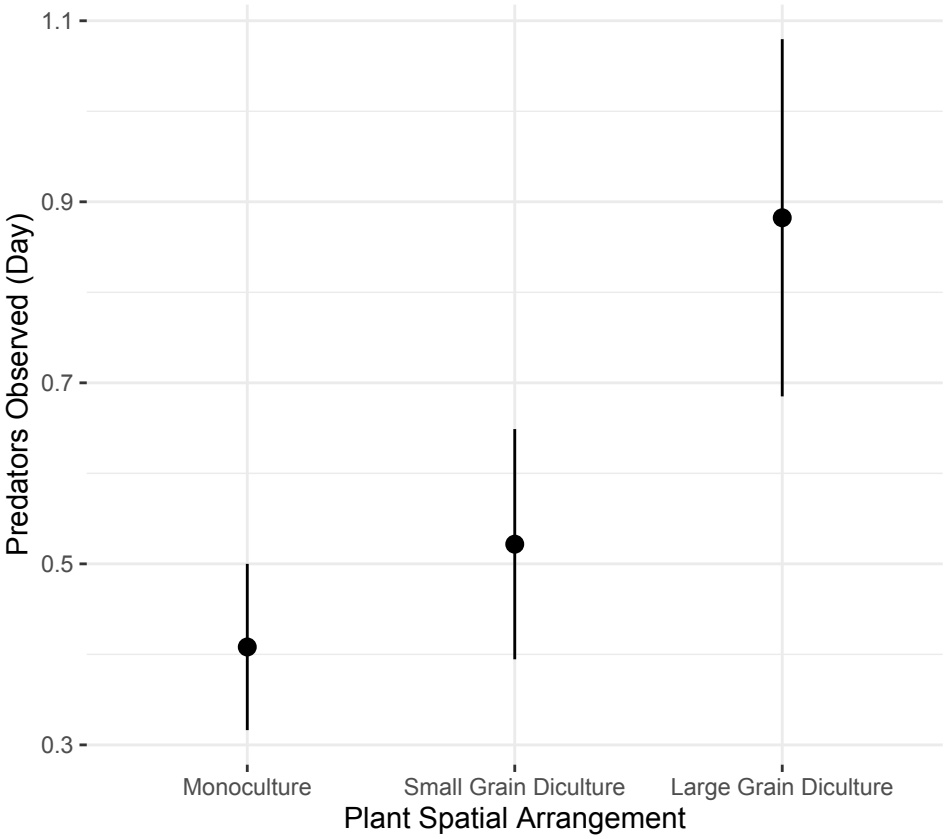

Figure 10. **Predators observed by treatment.** Cumulative mean ± SEM number of predators observed in tomato plots with different levels of chemical diversity and spatial arrangement, across three daytime censuses per round.

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