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RESEARCH ARTICLE

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

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Abstract

1. Variability in plant traits such as nutrients and defences can challenge insect herbivores searching for a host plant. Cultivar mixtures are designed to harness this variability to reduce herbivore damage in agroecosystems but have had mixed success.
2. We examine how the spatial frequency of plant trait variability—a fundamental but rarely examined feature of variability—influences insect foraging and survival. We released a generalist herbivore into monocultures of two chemically distinct tomato varieties or dicultures of the two varieties with two spatial frequencies of chemical diversity and tracked herbivore movement and feeding damage.
3. We found the pattern of herbivore feeding damage was more spatially concentrated in both diculture treatments than in either monoculture, indicating that the presence of chemical diversity, regardless of its spatial frequency, influences herbivore foraging.
4. In contrast, total amount of feeding damage was reduced by 25% in dicultures where genotypes were grouped compared to monocultures or dicultures with alternating genotypes, which had similar levels of damage.
5. Similarly, herbivore survival in the low-frequency diculture was nearly half the survival in the high-frequency diculture or monocultures.
6. *Synthesis and applications*. Whereas previous work investigates how the amount of chemical diversity in a plant population influences insect ecology, our results indicate that local spatial distribution of diversity is equally important. A key implication of this work is the potential to design cultivar mixtures spatially, at the movement scale of target organisms, to create more effective pest management landscapes and promote sustainable agriculture.

KEYWORDS
chemical defence, cultivar mixtures, insect movement, integrated pest management, plant chemical diversity, spatial arrangement

1 | INTRODUCTION

Intraspecific trait variability accounts for approximately 25% of variation within plant communities (Siefert et al., 2015). Despite its ubiquity, plant trait variability has typically been seen as a challenge for herbivorous insects looking for a single host plant (Denno & McClure, 1983; Herrera, 2009). This perspective has motivated the development and use of cultivar mixtures that aim to suppress insect pests (Perrin & Phillips, 1978; Tooker & Frank, 2012). Even though subsequent research has grown, our understanding of the difficulty that plant variability can pose for insect herbivores (Hauri et al., 2021; Pearse et al., 2018; Wetzel et al., 2016; Wetzel & Thaler, 2016), and has demonstrated that variability can scale up to influence populations and communities (Bustos-Segura et al., 2017; Crutsinger, 2006; Glassmire et al., 2016), cultivar mixtures have had mixed success suppressing pests and enhancing yield in field settings (Reiss & Drinkwater, 2018). One limitation is that studies have focused on the amount of variability but overlooked its spatial pattern. Because many herbivorous insects have limited mobility, especially as larvae, how they experience plant trait diversity depends fundamentally on its pattern of occurrence (Wetzel & Whitehead, 2020). Although the importance of spatial configuration for insect ecology and management has been well established in landscape ecology (Haan et al., 2020; Jonsson et al., 2010; Tscharntke et al., 2012), to our knowledge studies have not examined how the spatial configuration of crop cultivars within a field influences insect herbivores and their feeding.

Although we have a poor understanding of the mechanisms by which cultivar mixtures influence insects in the field, research on temporal resource variability suggests that frequency may play a major role. Recent theory and empirical work with the model organism *Daphnia* indicates that the effects of temporal resource variability depend highly on the frequency of the variability relative to the timing of organismal processes (Koussoroplis et al., 2019). This suggests that the effects of spatial variability on a consumer cannot be understood without considering how the spatial frequency, or the patch or grain size, of the variability relates to the movement patterns of the consumer (Pearse et al., 2018). Studies of forced insect diet switching through time show that variability in plant nutrients (Stockhoff, 1993) and defences (Pearse et al., 2018) have major effects on insect performance and can cascade up to the third trophic level (Paul et al., 2021). Forced diet switching laboratory studies are a powerful way to isolate the physiological effects of variability, but they prevent insects from responding behaviourally. Because behaviour and movement are key ways that plant variability influences insect herbivores (Schultz, 1983) and their interactions with predators (Wetzel & Thaler, 2016), improving our understanding of how cultivar mixtures influence insect ecology and our ability to use cultivar mixtures for pest management necessitates studies that examine plant variability and its frequency in field settings where movement and behaviour are possible.

Whether or not spatial frequency alters the effects of chemical variability on insects would have very different consequences for our understanding of chemical ecology and how we could use cultivar mixtures for sustainable pest management. If frequency does not influence the effects of plant variability on insects, it could imply that insects quickly adjust to new host plants, or that herbivores integrate over the variability between plant hosts. In that case, herbivores would essentially experience the average plant phenotype across each neighbourhood, and performance differences would depend on whether the average phenotype in a monoculture or mixed plot is more or less toxic to an herbivore. If, in contrast, the spatial frequency of diversity does alter its effects on insects, such that performance varies between plots with identical levels but different spatial arrangements of plant variability, the implications would depend on if higher frequency were beneficial or detrimental to insects. If insects have higher performance when the spatial frequency of plant variability is high, it could imply that the insects use host plant diversity to balance their toxin intake (Glassmire et al., 2020). In contrast, if higher spatial frequency exacerbates the negative effects of plant variability on insect herbivores, it could suggest that insect digestive physiology is challenged by frequent diet changes due to a cost of acclimation (Pearse et al., 2018; Wetzel & Thaler, 2016) or that fine-scale plant variability challenges foraging behaviour (Bernays, 2001).

We determined the effects of the presence and frequency of plant chemical diversity on a generalist herbivore and the predator community using field and laboratory experiments that manipulated the presence and frequency of chemical variability. We did this by placing 30 cabbage looper caterpillars *Trichoplusia ni* in plots of one or two genetic lines of tomato and monitoring their movement and feeding, as well as predator presence, until pupation. One of the tomato lines was a commercial variety, while the other was a backcrossed introgression, identical except for a single chromosomal introgression from wild relative *Solanum pennellii* (Eshed & Zamir, 1995), which causes a 75%–90% reduction in sesquiterpenes (Schilmiller et al., 2010). We isolated the effects of chemical frequency on physiological from behavioural effects using a no-choice diet switching lab experiment. Our specific questions were as follows: (a) How does chemical diversity and its spatial arrangement influence the foraging, movement and survival of herbivores? (b) How do predators interact with different levels and arrangements of diversity? (c) How does switching between plant chemotypes at different rates influence herbivores physiologically? Addressing these questions will help us understand how plant chemical diversity and its frequency may be shaping insect performance and interactions with other species at the local scale.

2 | MATERIALS AND METHODS

2.1 | 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 isogenic to the commercial genotype except that it has an altered terpene profile resulting from a substitution from *S. pennellii* on one chromosome (PIL 10-3, Eshed & Zamir, 1995). The altered terpene genotype has a 75%–90% reduction of sesquiterpenes only, specifically germacrene D, α-humulene and caryophyllene (Schilmiller et al., 2010). Sesquiterpenes are important volatile cues for insects and influence both herbivore and predator feeding behaviour. There are not significant differences in acyl sugar or monoterpene levels or types (Schilmiller et al., 2010). M82 and PIL 10-3 have similar glandular trichomes, likely related to their similar acyl sugar profiles (Schilmiller et al., 2010). Based on these known chemotypes, we assume that sesquiterpenes are the main trait showing inter-cultivar variation, though it is possible there are undiscovered differences. We grew plants in a greenhouse for 5 weeks before transplanting them into the field. No permits were required for the fieldwork we performed.

Our focal herbivore was a generalist pest, *Trichoplusia ni* (Lepidoptera: Noctuidae; Shorey et al., 1962). No ethical approval was required to work with *T. ni*. Eggs were purchased from an insectary (Benzon Research) 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 (Southland Products Inc.) until deployment in the field. For the laboratory experiment, we reared *T. ni* at room temperature and on general noctuid diet (Southland Products Inc.) until transfer to plant tissue.

2.2 | 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 used an orthogonal design with frequency nested within diversity to allow us to separate the effects of diversity and frequency. There were three treatments all consisting of four plants in a row: monocultures of one genotype (commercial genotype: 18 replicates; altered terpenes genotype: 20 replicates; total monoculture replicates: 38, Figure 1), dicultures with alternating plant genotypes within a row, with approximately half the replicates starting on each genotype ('ABAB' pattern; commercial start, 21 replicates; altered terpenes start, 21 replicates; total: 42 replicates, Figure 1) and dicultures with alternating plant genotypes in sets of two, again with half starting on each genotype ('AABB' pattern; commercial start, 23 replicates; altered terpenes start: 22 replicates; total: 45 replicates, Figure 1). 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. 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 1–2 days apart and then monitored at the same length of time after its planting date.

In each plot, 5-week-old tomatoes were planted into 1 m long uncaged 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 18 m × 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. We alternated direction of the plot (East–West or West–East) every plot. After placement, caterpillars were free to move among plants.

We visually searched the entire foliage of all plants and recorded the number and locations of *T. ni*, as well as all other

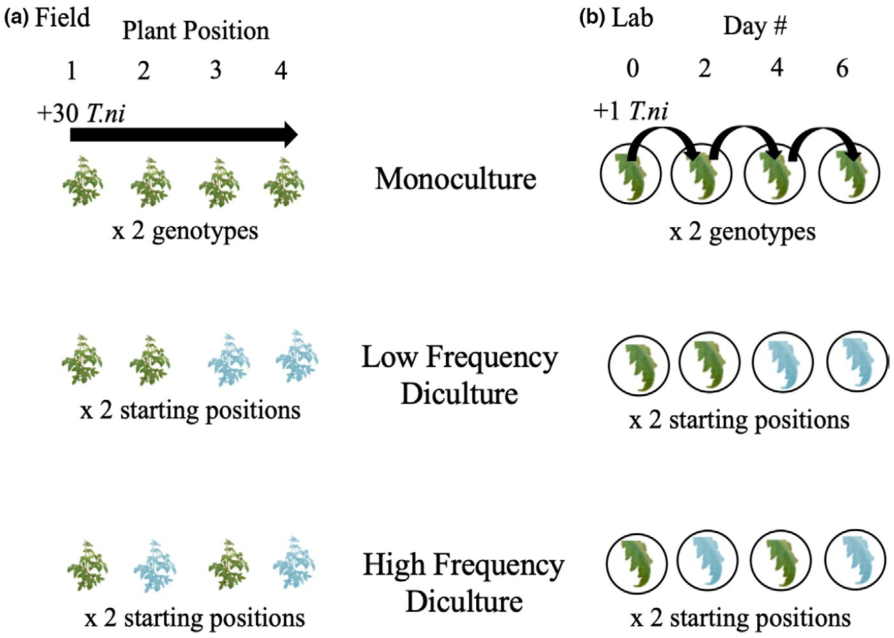

FIGURE 1 Experimental setup. In the field (a), 30 caterpillars were placed in a row of four tomatoes and censused three times over the course of 1 week. For dicultures, approximately half of each treatment began with the commercial genotype followed by altered terpenes, and half had the opposite arrangement. In the laboratory experiment (b), individual caterpillars were placed in petri dishes and moved to a new food source every 2 days for 8 days

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 24 and 48 h after caterpillar release. While we performed three censuses during the first round, censuses were not performed on a strict 24, 48 h, and 1-week schedule 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. Caterpillar departure behaviour for these timepoints is found in Appendix S1. We also performed one night sampling for each block by visually censusing between 21:00 h and 24:00 h and recording the identity at least to order, number and location of all arthropods in a subset of plots. One week after release, some caterpillars had begun to pupate; therefore, we destructively sampled plants and visually estimated leaf area removed by herbivory using a 0.5 cm^2 grid. Total number and plant location of recaptured caterpillars was recorded for each plot.

2.3 | 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: (a) monoculture, where they were fed on either the commercial or altered terpenes genotype for 8 days; (b) ‘slow’ diculture 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 (Figure 1). As with the field experiment, within dicultures half of the replicates started on the commercial genotype and half started on the altered terpenes genotype (20 replicates of each starting position, for a total of 40 monoculture replicates, 40 ‘slow’ replicates, and 40 ‘fast’ replicates). Tissue was harvested from tomato plants grown and maintained in the greenhouse. Plants were approximately 8 weeks old at the time of the experiment, and leaflets were collected from a plant on a single day to minimize induction of plant defences.

We placed individual second instar *T. ni* in 10 cm petri dishes 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 2 days, tomato leaflets were removed and replaced with fresh plant tissue. We selected leaflets >7 cm in length that were not on the lowest or highest two leaves of the plant to standardize plant tissue age. Additionally, we used only healthy leaflets (avoided yellowing, thrips damage, etc). We applied predatory mites 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. 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.

2.4 | Statistical analyses

2.4.1 | 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). We discuss *p* values $0.1 > p > 0.05$ as marginally significant and $p < 0.05$ as statistically significant.

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) or spatial arrangement of chemical diversity (high-frequency or low-frequency 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 whether presence of diversity changed the spatial distribution of within-plot 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 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 within-plot distribution 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. Similar models for both within-plot distribution and amount of herbivory were performed using exact chemical diversity experience rather than arrangement type as a factor and can be found in the supplement. More analyses of how plant chemistry influenced herbivore departure behaviour are found in Appendix S1.

To explore how these treatments affected larval survival, we determined whether the number of caterpillars recaptured during harvest differed by plant treatment (monoculture, high-frequency diculture or low-frequency diculture). We used models with the number of *T. ni* found versus 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 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, low-frequency diculture or high-frequency diculture). We compared models with and without arrangement type as a factor determining predator abundance. We modelled 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. In all models, we used round as a random effect. We also performed a quantile regression using tau values of 0.2. 0.5, and 0.8 to ask how total *T. ni* recaptured at harvest predicted cumulative predator presence (see Appendix S1).

For models investigating within-plot distribution 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.

2.4.2 | 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 analysed 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 analysed 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 analysed with a linear mixed model with diet switching type as a fixed effect and round as a random effect.

3 | RESULTS

3.1 | Field experiment

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

To determine the effect of chemical diversity alone on leaf area removed by caterpillars, we first grouped plots by ‘monoculture’ or ‘diculture’, ignoring the frequency of chemical diversity (Figure 1). The presence of chemical diversity (‘diculture’ plots) resulted in a clumped within-plot distribution of herbivory from the plant where caterpillars were initially released, with a significant plant location × chemical diversity interaction ($\chi^2 = 8.72$, $df = 1$, $p < 0.05$). Herbivory was concentrated on early plants in chemical diversity plots compared to monocultures, indicated by 66% more negative model slopes of decreasing herbivory from starting plant to final plant within plots with chemical diversity compared to slopes in monoculture plots (95% CI: 22%–109%). Consistent with past work showing a slight beneficial effect of cultivar mixtures, we found marginally significant decreased herbivory in chemically diverse plots: dicultures had a 30% reduction in total area removed by herbivory per plot ($\chi^2 = 3.71$, $df = 1$, $p = 0.054$). To determine how the spatial arrangement of chemical diversity influenced herbivory, we compared within-plot distribution of herbivory and total area removed by herbivory in high- and low-frequency diculture plots. We found a significant 26% reduction in total area removed by herbivory per plot in low-frequency dicultures compared to high-frequency dicultures (Figure 2b; $\chi^2 = 4.94$, $df = 1$, $p = 0.03$), indicating that the variability present in the cultivar mixture had a greater suppressive effect on herbivore feeding when it was present at low frequency. The spatial arrangement of chemical diversity, however, did not significantly influence the within-plot distribution of herbivory (Figure 2a; $\chi^2 = 0.01$, $df = 1$, $p = 0.92$), suggesting that the presence of chemical diversity influences how much herbivores move while feeding, whereas the arrangement of diversity influences the total amount of feeding.

3.1.2 | 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 after release in

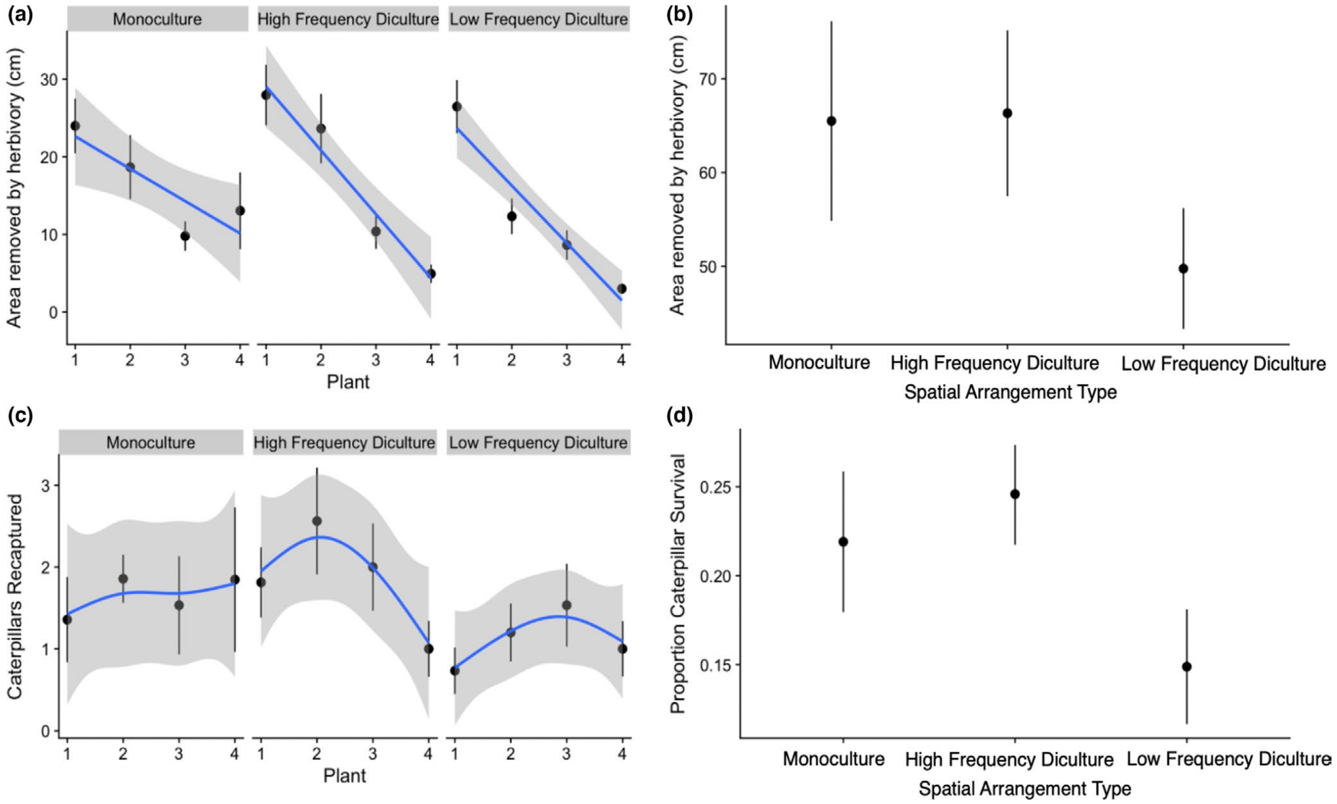

FIGURE 2 Effects of chemical diversity on herbivore foraging and survival in the field experiment. (a) Mean ± *SEM* tomato leaf area (cm^2) removed by caterpillars after 7 days by monoculture and diculture frequency treatment. Lines and shaded areas are predictions added using geom_smooth (method = lm). (b) 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. (c) Mean ± *SEM* caterpillars on each plant 1 week post-release by monoculture and diculture frequency treatment. Caterpillars were released on plant 1 and allowed to disperse to subsequent plants. Lines and shaded areas are predictions added using geom_smooth (method = gam). (d) Mean ± *SEM* caterpillar survival by monoculture and diculture frequency treatment

the second round of the field experiment, when we took the most complete census data. While treatment was a marginally significant predictor of survival (Figure 2d; $\chi^2 = 5.81$, $df = 2$, $p = 0.054$), with similar survival in monocultures (19.8%) and high-frequency dicultures (23.1%), low-frequency dicultures had 45.1% lower survival (12.7%) than the high-frequency dicultures (lsmeans, *z*-ratio = 2.39, $p = 0.05$). Surviving caterpillars in monocultures were equally distributed among plants, whereas in dicultures there was a marginally significant arrangement × plant number interaction (Figure 2c; $\chi^2 = 5.76$, $df = 2$, $p = 0.056$).

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

Daytime predator presence was lowest in monocultures (0.41 cumulative predators observed per plot, 95% CI: 0.24–0.69), with intermediate levels in high-frequency dicultures (0.52 cumulative predators observed per plot, CI: 0.32–0.86), and highest levels in low-frequency dicultures (0.88 cumulative predators observed per plot, CI: 0.59–1.32). Although these differences were marginally significant (Figure 3; $\chi^2 = 5.73$, $df = 2$, $p = 0.057$), they represent a 69% increase in predator abundance in low-frequency diculture plots compared to high-frequency 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 ($\chi^2 = 17.66$, $df = 1$, $p < 0.05$; Appendix S1, Table S1), and there was no difference in predator presence by plant arrangement type at night ($\chi^2 = 1.78$, $df = 2$, $p = 0.41$). Additional analyses in Appendix S1 suggest that predators may be more attracted to low-frequency dicultures at high levels of herbivory.

3.2 | Laboratory experiment

3.2.1 | How does chemical diversity and its frequency influence herbivores physiologically?

Diet switching frequency did not affect caterpillar survival ($\chi^2 = 0$, $df = 2$, $p = 1$) or growth ($\chi^2 = 0.78$, $df = 2$, $p = 0.68$). Cumulative leaf area removed by herbivory ($\chi^2 = 2.95$, $df = 2$, $p = 0.23$) and efficiency of conversion of ingested food (ECI; $\chi^2 = 1.35$, $df = 2$, $p = 0.51$) also did not differ by frequency treatment. This suggests that field results

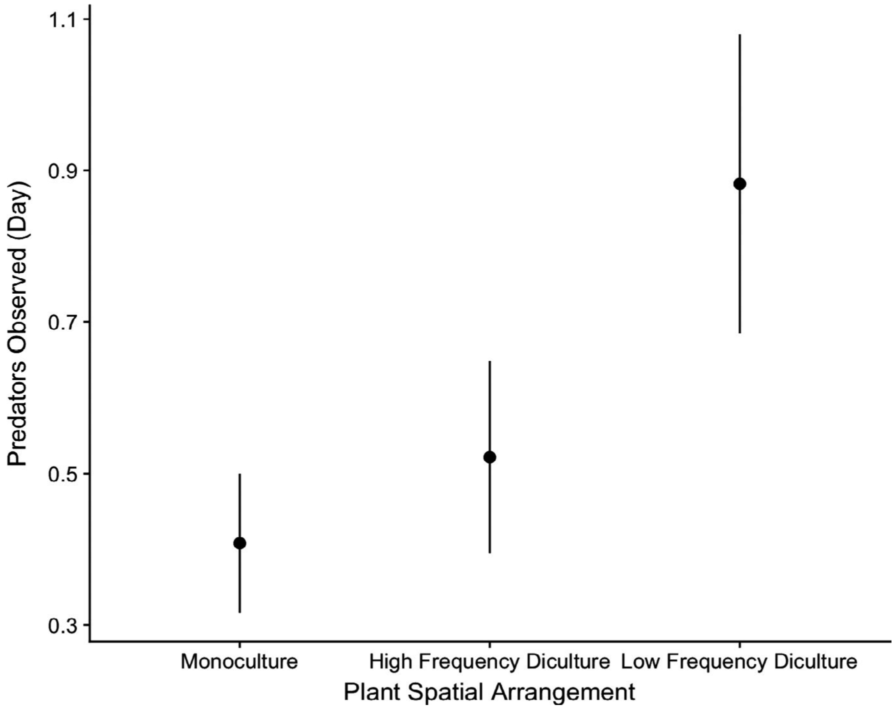

FIGURE 3 Cumulative mean ± *SEM* number of predators observed in plots with different levels of chemical diversity and spatial arrangement across three daytime censuses per round

may have been mediated through caterpillar behaviour, predation or both, rather than a physiological effect. Additional analyses of caterpillar performance and herbivory by monoculture and starting plant are found in Appendix S1.

4 | DISCUSSION

We found that the presence of plant chemical variability in a plot resulted in an aggregated distribution of herbivore damage, and that a lower spatial frequency of that variability led to reduced herbivore feeding and increased mortality. Our results suggest that the frequency of plant diversity—independent of the amount of diversity—matters to insects at the field scale. In our no-choice assay we did not find direct, physiological effects of diet switching at low versus high frequency. Additionally, we did not find differences in predator presence between plot types, although there was a trend towards higher predator density in plots where chemical diversity was present in larger patch sizes. These results suggest that the effects of the spatial frequency of plant variability on the distribution and abundance of herbivory and herbivores were mediated through changes in insect behaviour, for herbivores, predators or both. A key implication of this work, which we discuss below, is that the effectiveness of cultivar mixtures may be largely dependent on how effectively an arrangement is designed to maximize the effects of plant chemical signals.

The reduced movement we observed in plots with chemical diversity could be driven by caterpillar's attempts to manage their consumption of toxins. In the field, we saw clear evidence that chemical diversity caused a more clumped distribution of feeding damage. Additionally, at 48 hours, caterpillars were more likely to have left the first plant if the second plant was the reduced terpenes genotype. In some contexts, *T. ni* may benefit from a high turnover of chemical diversity between plants, potentially because it allows them to balance their chemical intake (Glassmire et al., 2020). Perhaps the caterpillars chose to stay in an area where they had guaranteed chemical diversity that they could use to balance their diet, whereas in monocultures they continued to move to search out this diversity.

The lack of physiological differences between caterpillars feeding on the two diet switching frequency treatments in our laboratory study suggests that differences in herbivore damage and density in the field were a result of behavioural responses to our plant variability treatments, likely driven by the major difference in sesquiterpene production between these tomato lines. Lepidopteran larvae use olfactory cues during foraging (Huang et al., 1990; Roessingh et al., 2007) and tomato plants release additional β-caryophyllene after just hours of *T. ni* feeding (Miresmailli et al., 2010). In our laboratory assay, however, chemical cues may have been unimportant because caterpillars could not choose, natural enemies were not present, and leaves were excised from undamaged plants. In the field, feeding on plants with reduced β-caryophyllene could have allowed *T. ni* to act as stealthy feeders. On the other hand, before feeding, they may perceive an altered terpenes plant as being less damaged than it actually is. Because plant chemical defence is complex, reduced herbivory cues such as β-caryophyllene does not necessarily mean that the overall plant quality was higher. Plots with more frequent variability would allow *T. ni* to quickly move between 'stealthy' plants and 'accurate' plants. The relative benefit of these factors may change over time: studies with *Nicotiana attenuata* and *Manduca sexta* have established that plants gain an advantage from inducing defences at the stage where larvae begin to feed significantly, around the third instar, causing larvae to move to a neighbouring plant (Backmann et al., 2019; Van Dam et al., 2000; Van Dam et al., 2001). The larger the patch without volatile signals, the farther the caterpillars would have to travel to sense accurate damage information, forcing caterpillars to spend resources on movement and exposing them to predation.

Terpene volatiles are also utilized by predators and parasitoids in prey location (Schnee et al., 2006; Vieira et al., 2019; Vuorinen et al., 2004) and may explain the predator abundance trends in our treatments. In the high-frequency diculture, higher-emitting commercial plants are separated by lower-emitting reduced terpene plants, whereas in the low-frequency diculture, higher-emitting plants are more clumped. Predators may be locating prey more efficiently when direct plant neighbours have reduced odours; β-caryophyllene specifically is known to be an attractant to some natural enemies (Flint et al., 1979). Quantile regression supports this hypothesis: for plots with near average numbers of predators, predator presence does increase with caterpillar presence (Figure S2). This makes sense if predators are responding to volatile signals from the plants induced by herbivore feeding, or if they are actively searching for plots with a larger density of prey. However, these results do not explain why monocultures had the lowest predator presence. Perhaps plants did not reach a threshold level of herbivory as early as in dicultures, where distribution of herbivory was more concentrated. Once predators were in plots, they may remain and forage there, since the prey density was high—leaving predators less likely to move to monoculture plots once damage levels and herbivore-induced plant volatile production increased. Statistical models have shown that in a fine-grain landscape parasitoids can supplement their populations in low-quality patches with high-quality patches, providing better biocontrol (Riolo et al., 2015); perhaps a similar process was occurring in chemically diverse plots. Cultivar mixtures could potentially be designed to concentrate herbivory, thus attracting natural enemies at a lower overall level of pest pressure.

Our findings suggest that one reason cultivar mixtures have yielded mixed results (Reiss & Drinkwater, 2018) is because the arrangement of diversity itself is a key factor that has been overlooked. We propose a new generation of research on cultivar mixtures that seeks to engineer specific spatial arrangements of crop trait diversity that target insect pests, rather than focusing on overall genetic diversity. As technological advances make planting strategies that manipulate frequency of plant diversity more and more feasible, the arrangement of cultivar mixtures could be adjusted to the injury threshold for a particular crop, creating a powerful and flexible tool for pest management in sustainable agriculture. Though they will need to be tailored to specific crops and planting contexts—for example, deciding whether concentrating or distributing damage is more beneficial—these results offer us a new way to manipulate plant diversity to influence insect pests and natural enemies.

When herbivores and predators cannot accurately perceive the quality of a plant based on external signals such as volatiles, they may make inefficient behavioural choices. These inaccurate host signals are part of the reason that commercial lures have had mixed success in the field (Kaplan, 2012). To use local-scale ecology for pest management—for example, directing predators into a field interior to promote biocontrol, or designing trap plant systems to attract pests away from crops—future work on cultivar mixture development should use spatial patterns that interact with the movement scale of target organisms. Additional benefits of altering spatial frequency, such as promoting and maintaining plant diversity, have been established in non-agricultural contexts (Porensky et al., 2012). Our results suggest the same approach could also lead to greater ecosystem services, or yield gains separate from herbivory reduction, in agroecosystems. There is a well-established body of work at the landscape level showing how configuration of landscape types in addition to composition can have significant effects on pest and predator outcomes (Haan et al., 2020; Jonsson et al., 2010; Tscharntke et al., 2012). Our results indicate that analogous approaches should be implemented at the within-field level.

AUTHORS' CONTRIBUTIONS

K.C.H. and W.C.W. designed the field experiment, and K.C.H., W.C.W. and B.R. designed the laboratory experiment; K.C.H. and B.R. conducted the experiments; K.C.H. wrote the first draft. K.C.H. and W.C.W. conducted the analyses. All authors edited the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available via Figshare https://doi.org/10.6084/m9.figshare.c.5562450.v1 (Hauri et al., 2022).

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