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RESEARCH ARTICLE

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Intraspecific correlations between growth and defence vary with resource availability and differ within and among populations

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Abstract

1. A paradigm in the plant defence literature is that defending against herbivores comes at a cost to growth, resulting in a growth–defence trade-off. However, while there is strong evidence for growth–defence trade-offs across species, evidence is mixed within species.
2. Several mechanisms can account for this equivocal support within species, but teasing them apart requires examining growth–defence relationships both within and among populations, an approach seldom employed.
3. We examined correlations between plant biomass (growth) and terpene production (defence) within and among populations of *Monarda fistulosa*, a perennial herb. We sampled populations from Montana and Wisconsin, regions that differ in resource availability characterized by different summer precipitation and associated abiotic conditions that influence plant productivity.
4. We found negative, neutral and positive growth–defence correlations, depending on the scale examined. Negative correlations occurred across populations originating from divergent regions, positive correlations occurred across populations originating from within the high-resource region and neutral correlations were found within single populations.
5. Collectively, these results challenge the general expectation of ubiquitous trade-offs and support emerging views that resource availability (as it affects productivity) shapes the evolution of defence at different scales.

KEYWORDS

herbivory, local adaptation, resistance, resource availability hypothesis, trade-off, trait correlations

1 | INTRODUCTION

Life-history trade-offs form an important foundation for understanding trait evolution, constraints, and how organisms adapt to and interact with their abiotic and biotic environments. Within species, plant defence traits are particularly variable both within and across populations but predicting intraspecific variation in plant defence remains challenging (Agrawal, 2020). Theoretical and empirical studies have typically focused on variation either within or across populations, but not both. For example, theory predicts that within populations growth and defence should trade-off because of physiological limits or prioritization of either growth or defence that optimizes fitness (Herms & Mattson, 1992; Züst & Agrawal, 2017). There is some support for growth–defence trade-offs within populations, suggesting allocation costs of producing defences (Koricheva, 2002; Massad et al., 2011). While these costs are far from universal, they are most commonly detected in individuals growing under resource-limited conditions (Cipollini et al., 2014; Koricheva, 2002; Sampedro et al., 2011). Across populations, most studies focus on how variation in herbivore pressure across large spatial gradients shapes the evolution of plant defence (Moreira et al., 2018; Pennings et al., 2009; Woods et al., 2012). Generally, these studies have not considered how growth rate might constrain defence. For the few studies that have examined growth–defence correlations across populations, less than 20% of studies found evi- dence for trade-offs (Hahn & Maron, 2016) and several studies have found positive correlations between growth and defence (Hahn et al., 2019; Lehndal & Ågren, 2015; Méndez-Espinoza et al., 2018). Understanding why there are such mixed patterns at different lev- els of biological organization has emerged as a critical challenge to ecologists and evolutionary biologists (Agrawal, 2020; Moreira et al., 2018; van Noordwijk & de Jong, 1986).

Recently, Peiman and Robinson (2017) outlined a framework that can lead to a deeper understanding of the mechanisms that influence evolutionary patterns of trait covariation within species. Critically, this framework entails a detailed analysis of patterns of functional trait variation both within populations and among pop- ulations that occur across divergent selective environments (i.e. resource availability or predator pressure). For example, if two traits are strongly negatively correlated among genotypes within a population, regardless of the environmental context from which they originate, this would suggest strong genetic constraints on trait evolution. However, if among-population patterns differ from within-population patterns or populations from divergent environ- mental contexts differ in their patterns of trait correlations, envi- ronmental factors may shape patterns of trait expression more so than genetic constraints (Armbruster & Schwaegerle, 1996; Peiman & Robinson, 2017). Although this approach holds great potential for increasing insight into how resource availability influences trait evo- lution across levels of ecological organization; to our knowledge, this general framework has not yet been employed to evaluate growth– defence correlations across scales.

The resource environment could potentially shape growth and defence correlations (either positively or negatively) within versus among populations through several mechanisms (Figure 1). For example, Figure 1a depicts a scenario where growth and defence traits are negatively correlated, both within and among populations. This pattern is generally expected to evolve because growth and defence are both energetically costly and allocating resources to one process necessarily diverts resources away from other processes (Herms & Mattson, 1992). Similarly, a negative correlation could be found across populations from divergent resource environments, resulting in strong divergence of traits between the populations (Figure 1b). This scenario may represent local adaptation to environmental con- ditions across populations driven by costs of producing defences (Agrawal, 2020; Coley et al., 1985). However, correlations may be negative or positive within any given population (i.e. one population exhibits a positive correlation among individuals), which would sug- gest that genetic constraints are not strong, at least given sufficient genetic variation within populations (within-population variation is assumed to be smaller than among-population variation in these scenarios). In contrast to scenarios that exhibit growth–defence trade-offs across populations (Figure 1a,b), populations with more access to resources may evolve both greater growth *and* defence (Figure 1c). For example, if populations are distributed across a re- source or herbivory gradient (or if genetic drift produces population- level differentiation), growth and defence may be positively (or not) correlated across populations (Hahn & Maron, 2016), despite under- lying negative correlations within populations (Figure 1c). In this in- stance, differences in resource acquisition among populations could mask underlying trade-offs within populations (van Noordwijk & de Jong, 1986). Clearly more scenarios are possible and we direct read- ers to recent reviews discussing how trade-offs might vary across scales for further reading (Agrawal, 2020; Hahn & Maron, 2016; Lopez-Goldar & Agrawal, 2021; Peiman & Robinson, 2017).

Here we explicitly use Peiman and Robinson's (2017) framework to address an important goal of plant defence theory, which is to reconcile growth and herbivore defence trait relationships within versus among populations that occur in two different resource environments (Agrawal, 2020). We ask how growth and defence trait correlations compare across three distinct levels of organization: (a) across populations from two divergent resource environments, (b) across populations occurring within similar environments and (c) among half-siblings within two populations that occur at opposite ends of a resource availability gradient. We address these questions using a common garden experiment with source material from 12

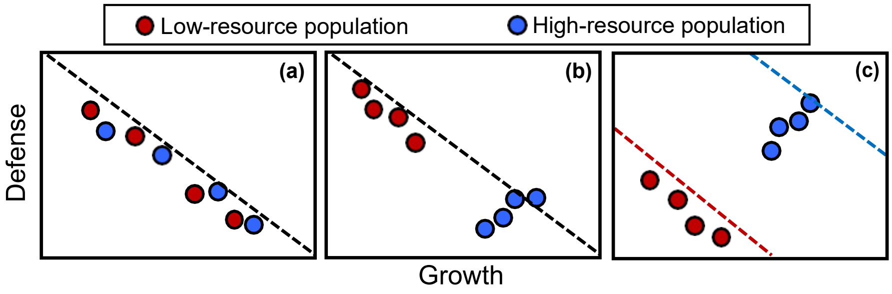
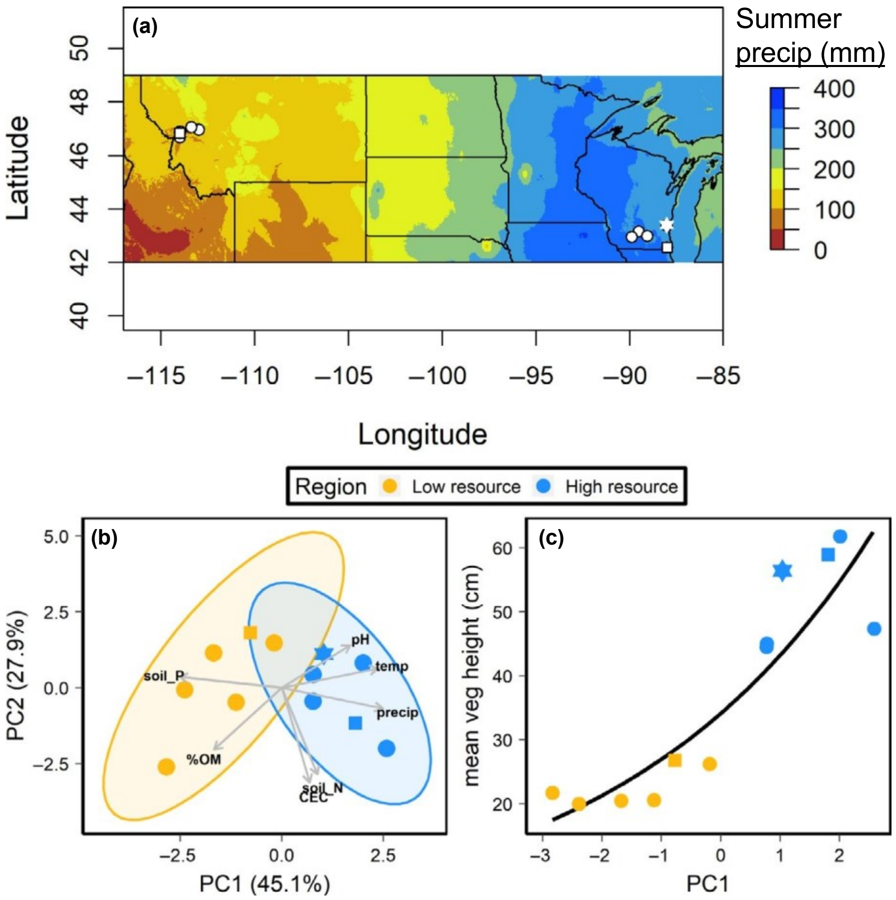


FIGURE 1 Hypothetical patterns of correlations for growth and defence traits within and among populations. Data are assumed to come from a common garden experiment. Different colours represent two populations within a single species and dots indicate individual genotypes within those populations. See main text for a description of the three scenarios

FI G U R E 2 (a) Map of the 12 study sites. Note that some points partially overlap. (b) Principal component analysis of environmental variables. Shaded polygons show 95% confidence ellipses for each region. (c) Mean vegetation height (cm) at each site plotted against the PC1 score. Square symbols indicate the two populations that had additional replicates. Common garden location, also a collection site, is indicated by a star in all panels



populations of the perennial herb *Monarda fistulosa* (Lamiaceae), six originating from high-resource environments and six from low-resource environments (Figure 2a). Finally, we measured growth and herbivory patterns in the field to help inform our inferences from the common garden study.

2 | MATERIALS AND METHODS

2.1 | Study system

Monarda fistulosa is a perennial herb widely distributed throughout grasslands in much of North America. Most pollination occurs through outcrossing, as self-fertilization is highly unlikely (Cruden et al., 1984). Thus, most seeds within an inflorescence are likely full- or half-siblings. *Monarda fistulosa* produces terpenoid compounds in peltate glandular trichomes on the surface of leaves and floral structures, predominately in two chemotypes that produce mostly thymol or carvacrol (Keefover-Ring, 2015). Both of these monoterpenes have been shown to effectively deter a range of pathogens and herbivores in other labiate species (Linhart & Thompson, 1999) and have differential effects on the survival and performance of a coleopteran herbivore that is a specialist on *M. fistulosa* (Keefover-Ring, 2015).

We collected seed of *M. fistulosa* from 12 populations located in two regions with distinct environmental conditions (Figure 2a). Six populations were located in intermountain grasslands in western Montana, characterized by low productivity and low summer precipitation (hereafter, 'low-resource region'). Populations were separated by a minimum 3.25 km and a maximum of 84 km (mean = 42.1 km). The two closest sites were on different mountain sides separated by a residential valley. Sites in western Montana were never cultivated but were likely grazed over the last century. The plant communities were dominated by native bunchgrasses, primarily *Festuca campestris* and *Pseudoroegneria spicata*, and native perennial forbs (e.g. *Achillea millefolium*, *Balsamorrhiza saggitorium*, *Geum triflorum*, *Erigeron pumillus*, *Lupinus serritorium*, etc.). The other six populations were located in tallgrass prairie remnants and old field habitats in southern Wisconsin, characterized by higher productivity and summer precipitation (hereafter 'high-resource region'). We selected remnant tallgrass sites or abandoned pasture in Wisconsin with no history of cultivation, although some sites have been historically grazed. Populations were separated by a minimum of 310 m and a maximum of 160 km (mean = 95.9 km). The two closest populations were in small, disjunct remnant prairie patches on different facing slopes of different hillsides historically separated by agricultural lands. The plant communities are dominated by native grasses (e.g. *Andropogon gerardii*) and native perennial forbs (e.g. *Achillea millefolium*, *Solidago* spp., *Silphium* spp., *Asclepias* spp., etc.). Within each population, we collected seed heads from up to 16 maternal plants (range: 8–16 maternal plants from each population, mean = 13 maternal plants per population). Only one seed head (i.e. inflorescence) was collected per maternal plant. Plants were separated by at least 2 m and we avoided sampling multiple clonal ramets that appeared to be part of the same individual.

2.2 | Quantifying site characteristics

Our goal was to contrast growth–defence relationships among populations from two distinct regions with different resource environments. Populations from Montana were on the low-resource end, as this region experiences relatively low summer rainfall, a short growing season and lower plant production. In contrast, Wisconsin populations are from a higher resource region, which is characterized by relatively high summer rainfall, a longer growing season and overall higher plant production. To quantify these differences in abiotic conditions across the regions, we collected several environmental variables that characterize important axes of the resource environment and therefore typically influence plant growth and overall productivity (Field et al., 2015). At each site, we collected 10 soil samples (at a depth of 0–15 cm), pooling samples within sites. We then had these samples analysed for physical and chemical properties (Ward Laboratory Inc.). For soil variables, we focused on pH, percent organic matter, cation exchange capacity, soil nitrate- nitrogen and Bray soil phosphorus to quantify potential differences in soil nutrient resources varied across regions. We also extracted mean annual temperature and summer precipitation data for each site from the WorldClim-Bioclim database (Hijmans et al., 2005) since both precipitation and temperature are resources that strongly influence overall plant productivty (O'Donell & Ignizio, 2012). Additionally, we estimated productivity at each site by establishing 20 1 m^2 quadrats at haphazardly selected locations along 50m transects and measured vegetation height in the four corners of the quadrat. Vegetation height was strongly correlated with the amount of photosynthetically active radiation (PAR) reduced by the vegetation (PAR above – PAR below the canopy) measured using a LiCor quantum sensor at one site in each region ($r = 0.78$, $t = 6.6$, $df = 27$, $p < 0.0001$), suggesting that plant height is a reasonable surrogate for above-ground productivity.

2.3 | Common garden experiment

During spring 2019, we grew *M. fistulosa* from collected seed in a greenhouse. Seeds were sown in a 1:1:1 mixture of field-collected soil, sand and Turface within 400 ml pots. We grew two replicate individuals (i.e. siblings) from each maternal plant collected from the 12 populations. For two populations, one representative population from each region, we grew five siblings from each maternal plant. Five siblings allow for a more robust estimate of genetically based trait values for each maternal plant by allowing to average out random environmental variation across the replicate siblings (Rausher, 1992). Some seeds did not germinate, so for the 10 populations with lower sibling replication we ended up with approximately 1.9 siblings per maternal plant. For the two populations with additional sibling replication, we averaged 4 plants from 15 maternal plants. We grew all plants in the greenhouse for approximately 2.5 months and then in May 2019 we transplanted them into a common garden located within the high-resource region (Figure 2a). The common garden site was an old field located approximately 250 m from a seed collection site and was dominated by pasture grasses (e.g. *Festuca arundinacea*) with many species of native forbs (e.g. *Asclepias verticillata*, *Erigeron strigosus*, *Solidago altissima*). *Monarda fistulosa* was growing nearby, but not within the garden plot. The vegetation was mowed prior to planting. Plants received supplemental water initially after planting but were not watered subsequently. In total, the common garden contained 360 plants collected from 157 maternal individuals from 12 populations (see Table S1).

2.4 | Trait measurements

We used total dry biomass at the end of the experiment as a metric of allocation to growth. All above-ground biomass was clipped at the soil surface on 15 August 2019, oven-dried at 50°C for 48 hr and weighed to the nearest 0.1 mg. We opted for this destructive metric because growth rate, as measured by non-destructive size measurements, was not strongly asymptotic and was correlated with final dried biomass (Appendix S1). The plants received only minor amounts of herbivory in the garden and so we did not record leaf herbivory on these plants. Additionally, *M. fistulosa* is a long-lived perennial plant and no plants produced flowers this first year.

We collected leaf samples from 324 plants in the common garden on 10 July 2019 for chemical analysis. From each plant, we collected the highest fully expanded leaf. Due to variation in leaf size, the samples were submerged into 0.5, 0.75, 1.0 or 1.5 ml of an internal standard solution (0.2 μl/ml of *m*-xylene in *n*-hexane). The samples were brought back to the laboratory and sonicated in an ice bath for 15 min, allowed to extract for 1 week at ambient temperature, and then the leaves removed and dried to a constant weight. We stored the extracts at −20°C until chemical analysis. The terpene composition was analysed using a Thermo Scientific Trace 1310 gas chromatography coupled with a Thermo ISQ LT single quadrupole mass spectrometer (GC-MS) using a DB-5 capillary column (30 m × 0.25 mm and 0.25 μm film thickness). We used helium as the carrier gas at 1 ml/min with the injector temperature set at 250°C and oven conditions of an initial temperature of 40°C for 5 min, followed by a ramp to 200°C at 3°C/min. Compounds in the chromatograms were identified with retention time, mass spectra of pure standards when available and linear retention indices of an *n*-alkane series (Adams, 2007). We used standard curves of pure compounds where available to quantify compound concentrations in the extracts and sample dry weight (DW) to calculate final compound levels (mg compound g^{-1} DW). We identified and measured the amounts of 25 monoterpenes, two sesquiterpenes, an alkenyl alcohol (1-octen-3-ol) and an allylbenzene (eugenol) from the essential oil of the foliage. We summed these compounds to calculate total terpene concentration (mg/g) per sample. Concentrations of the two dominant terpenes, thymol and carvacrol (note that a plant usually produces mostly thymol or carvacrol and only small amounts of the other), comprised on average 55% and 60%, respectively, of the total concentrations and were highly correlated with total amounts (r^2 = 0.92).

2.5 | Field surveys

During our visits to the field sites in July and August of 2018 to collect seed for the common garden experiment, we also measured plants in situ to quantify differences in plant height and herbivore damage between the regions. At each site, 10–16 haphazardly chosen individual plants were surveyed (mean = 13.2 plants per site). On each plant, stem height was measured from the soil surface to the top of the plant (highest piece of foliage or reproductive structure) to the nearest cm. We estimated leaf chewing damage visually on five haphazardly selected leaves per plant. The five leaves were averaged to get a mean proportion of leaf chewing damage per plant. Although we do not know the source of the chewing damage, it was most likely caused by generalist herbivores such as grasshoppers or specialized lepidopterans such as *Pyraustra signatalis* or *Coleophora monardella*. We also examined 1–3 seed heads per plant to assess damage to the seed heads (although note that only one head was collected from each plant for the common garden experiment). Two larval insect species were commonly found causing damage to developing seeds, *Pyrausta signatalis* (Family Crambidae) and an unidentified weevil (Family Curculionidae). Other sources of herbivory were uncommon, although we occasionally noticed aphids, spittlebugs and stippling on the leaves on <3% of the plants surveyed.

2.6 | Statistical analysis

2.6.1 | Differences in site characteristics between regions

To analyse the differences in soil properties and climatic conditions across the regions, we first conducted a principal component analysis on the soil and climatic variables. For soil variables, we included pH, percent organic matter, cation exchange capacity, soil nitrate-nitrogen and Bray soil phosphorus. For climate variables, we used mean annual temperature and summer precipitation. We also examined how these environmental variables, represented by the first axis of the PCA, correlated with vegetation height, a metric of productivity, at the 12 study sites using linear regression.

2.6.2 | Addressing primary research questions

We used standardized major axis regressions (SMAR) to address our primary research questions. SMAR is conceptually similar to ordinary least squares regression and analysis of covariance in that they estimate how intercepts (*a*) and slopes (*b*) between two variables might differ between groups. However, SMAR are particularly well suited for correlations between traits because the coefficients account for measurement uncertainty in both the *x* and *y* variables. The parameter estimation is mathematically more similar to multivariate approaches like principal component analysis (Warton et al., 2012). To address our first question examining trade-offs across populations from two divergent resource environments, we calculated population means for biomass and total terpene concentration for the 12 populations. We then constructed SMAR models to evaluate growth and defence correlations across all 12 populations to test whether there was a significant relationship between the two variables by examining the estimated slope parameter (*b*). To address our second question examining trade-offs across populations occurring within similar regions, we compared the intercept (*a*) and slope (*b*) parameters between the two regions. To address our third question examining trade-offs among siblings within populations from different resource environments, these same analyses were conducted using the two populations for which we had additional replication within maternal plants. Traits of siblings (*n* = 3–5 per maternal plant) were averaged and we used maternal plant means for this analysis. SMAR models were implemented in the smatr::sma() function in R (Warton et al., 2012). Exploratory analyses revealed that spatial autocorrelation was low among populations within both regions. Including a spatial covariance matrix in a similar modelling framework did not substantially change the results and did not affect the interpretation. Therefore, we did not correct for potential spatial autocorrelation among populations within each region.

2.6.3 | Field surveys of plant size and herbivory

We used linear mixed models to compare stem height of naturally occurring plants between regions. Site was included as a random effect in the model to account for multiple plants being measured per site. We used a linear mixed model to compare the proportion leaf damage per naturally occurring plant between regions. The response variable, mean proportional leaf damage per plant, was logit transformed prior to analysis and site was included as a random effect. The logit transformation is suitable for proportional data (Warton & Hui, 2011) and provided a better fit to the data than a generalized model with a beta distribution (results not shown). We used a generalized linear mixed model to compare the proportion of seed heads with damage between the regions. The response variable was the number of seed heads with versus without insect damage, modelled with a binomial error distribution and a logit link function. Site was included as a random effect. Linear mixed models were implemented using the lme4::lmer() function and the generalized linear mixed model was implemented using the lme4::glmer() function (Bates et al., 2015). Residual plots were visually assessed to ensure reasonable fits. The lmerTest::ANOVA() function (Kuznetsova et al., 2016) was used to obtain *F*- and *p*-values from linear models, using the Kenward-Roger method to estimate denominator degrees of freedom. The car::ANOVA() function (Fox & Weisberg, 2019) was used to obtain Wald χ^2 and *p*-values for the generalized model. Estimated marginal means were obtained from each model using the emmeans::emmeans() function (Lenth, 2020). Transformed response variables, either manually or via the link function, were back-transformed to the original scale prior to interpretation and graphing.

3 | RESULTS

3.1 | Site characteristics

The first axis of the PCA conducted on the soil and climatic variables explained 45.1% of the total variation in the abiotic environment between low- and high-resource sites. PC1 mainly summarized climatic variables (mean annual temperature and summer precipitation, which loaded strongly positively) and soil phosphorus (which loaded strongly negatively; Figure 2a) and differed strongly between regions (linear model: $F_{1,10}$ = 34.5, *p* = 0.0002). Higher resource Wisconsin sites were characterized by warmer temperatures and greater precipitation, whereas the low-resource Montana sites had lower precipitation but higher soil phosphorous (Figure 2b). PC1 strongly correlated with log-transformed vegetation height across both regions ($F_{1,10}$ = 53.4, *p* < 0.0001, R^2 = 0.84, Figure 2c). Greater productivity is likely driven by greater total amount of precipitation as well as the tim- ing of precipitation. In Montana, the warmest summer months (July and August) are very dry, truncating the growing season (Figure S1). In Wisconsin, the warmest months are also months that receive the greatest precipitation (Figure S1). The second axis (PC2) mainly summarized cation exchange capacity, soil nitrogen and percent organic matter, which all loaded strongly negatively (Figure 2b) and were not related to regional differences in veg- etation height ($F_{1,10}$ = 0.01, *p* = 0.93, R^2 = 0.0) and did not differ between regions ($F_{1,10}$ = 0.3, *p* = 0.62).

3.2 | Are growth and defence correlated across populations Q1) from divergent resource environments and Q2) within similar resource environments?

Population means for biomass and total terpene concentration were negatively correlated when pooled across both regions (SMA regression: *b* = −1.05 [95% CI: −1.59, −0.69], *p* = 0.001, R^2 = 0.64). Populations originating from the low-resource region (MT) had lower biomass and greater total terpene concentrations compared to populations from the high-resource region (WI; Figure 3a). When examined by region, the relationship between biomass and terpene concentrations differed in slopes (*b*) between regions al- though not significantly (likelihood ratio statistic = 2.45, *p* = 0.118).

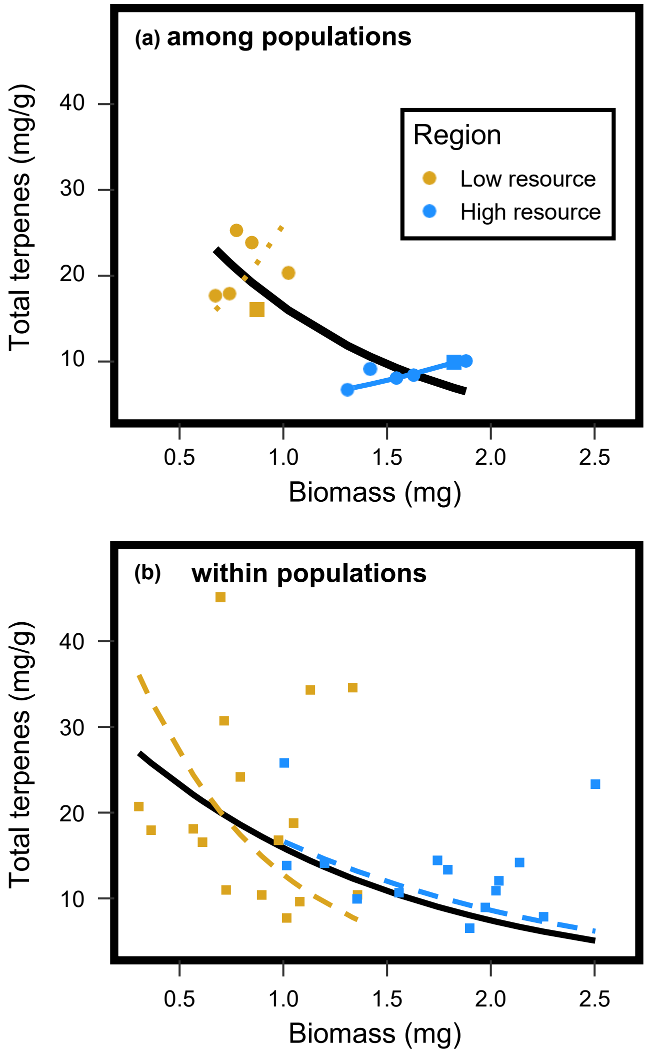


FIGURE 3 Correlations between growth (above-ground dry mass biomass) and defence (total terpene concentration) of *Monarda fistulosa* plants grown in a common garden in southeastern Wisconsin, USA. (a) Correlations for populations means. Square symbols indicate the two focal populations that had additional individuals per maternal plant represented in the common garden. (b) Correlations for maternal plant means for two populations. Plants originated from either the low-resource region (MT) or from the high-resource region (WI). Lines indicate the best-fit regression from standardized major axis regression models (SMAR). Black lines are SMAR best-fit regressions pooled across both regions, coloured lines are fit for relationships within a region. Solid lines indicate a significant relationship ($p \le 0.05$); dashed lines indicate non-significant relationship ($p > 0.05$). Note that total terpene concentrations were log-transformed for analysis but plotted here on the original scale

The correlation between biomass and total terpene concentration among the populations originating from the low-resource region was not significant (MT: *b* = 1.47 [95% CI: 0.59, 3.67], R^2 = 0.01, *p* = 0.83). However, among the populations from the high-resource region (WI), there was a positive correlation between biomass and total terpene concentration (*b* = 0.72 [95% CI: 0.58, 0.90], R^2 = 0.69, *p* = 0.041, Figure 3a).

3.3 | Q3: Are growth and defence correlated among half-siblings within two populations that occur at opposite ends of a resource gradient?

Maternal plant means for biomass and total terpene concentration were negatively correlated when data from the two focal populations (one from each region) were pooled (*b* = −0.76 [95% CI: −1.05, −0.55], *p* = 0.003, R^2 = 0.27). The population originating from the low-resource region had lower biomass and greater terpene concentrations compared to the high-resource population (Figure 3b). When testing for differences between the two regions, the slopes (*b*) for the populations from each region differed (likelihood ratio statistic = 4.3, *p* = 0.038). While neither population had a significant correlation between growth and defence, populations from the low-resource region had a more negative slope (*b* = −1.49 [95% CI: −2.54, −0.87], *p* = 0.46, R^2 = 0.04) compared to the high-resource region (*b* = −0.66 [95% CI: −1.16, −0.37], *p* = 0.25, R^2 = 0.11, Figure 3b).

3.4 | Field surveys of plant height and herbivore damage

Plants were nearly twice as tall at field sites in the high-resource region (emmean = 72.3 cm ±4.16 *SE*) than plants in the low-resource field sites (41.6 cm ± 4.17; $F_{1,9.99}$ = 27.2, *p* = 0.0004, R^2_m = 0.53, R^2_c = 0.74, Figure 4a). Proportion leaf damage was similar between regions ($F_{1,9.99}$ = 1.3, *p* = 0.27, R^2_m = 0.04, R^2_c = 0.37), with both regions having approximately 3.3% (±0.5%) leaf damage (Figure 4b). Proportion of seed heads damaged was nearly twice as high in the high-resource region (59.6% ± 5.0%) compared to the low-resource region (27.1% ± 4.6%; χ^2 = 19.0, *p* < 0.0001, R^2_m = 0.24, R^2_c = 0.29, Figure 4c).

4 | DISCUSSION

Trade-offs can strongly constrain trait evolution (Agrawal, 2020; Stearns, 1989). For plants, it is commonly assumed that producing defences against herbivores is costly and trade-offs with growth. While there is substantial evidence that growth–defence trade-offs occur among species (Defossez et al., 2018; Endara & Coley, 2011), evidence supporting trade-offs within species is mixed (Cipollini et al., 2014; Hahn & Maron, 2016; Koricheva, 2002; Massad et al., 2011). We applied a recently proposed comparative framework involving measuring traits of many individuals from divergent populations to shed light on the level of biological organization(s) where growth and defence traits evolve independently or are constrained by each other (Peiman & Robinson, 2017). Our specific goals were to examine growth–defence correlations: (a) across populations from divergent environments, (b) across populations within similar environments and (c) among half-siblings within two populations. In our common garden experiment, we documented different patterns of growth–defence correlations at all three levels of biological organization. Our results most closely match Scenario B (Figure 1b). Below, we discuss the implications for understanding the factors that drive the evolution of plant defence at different scales.

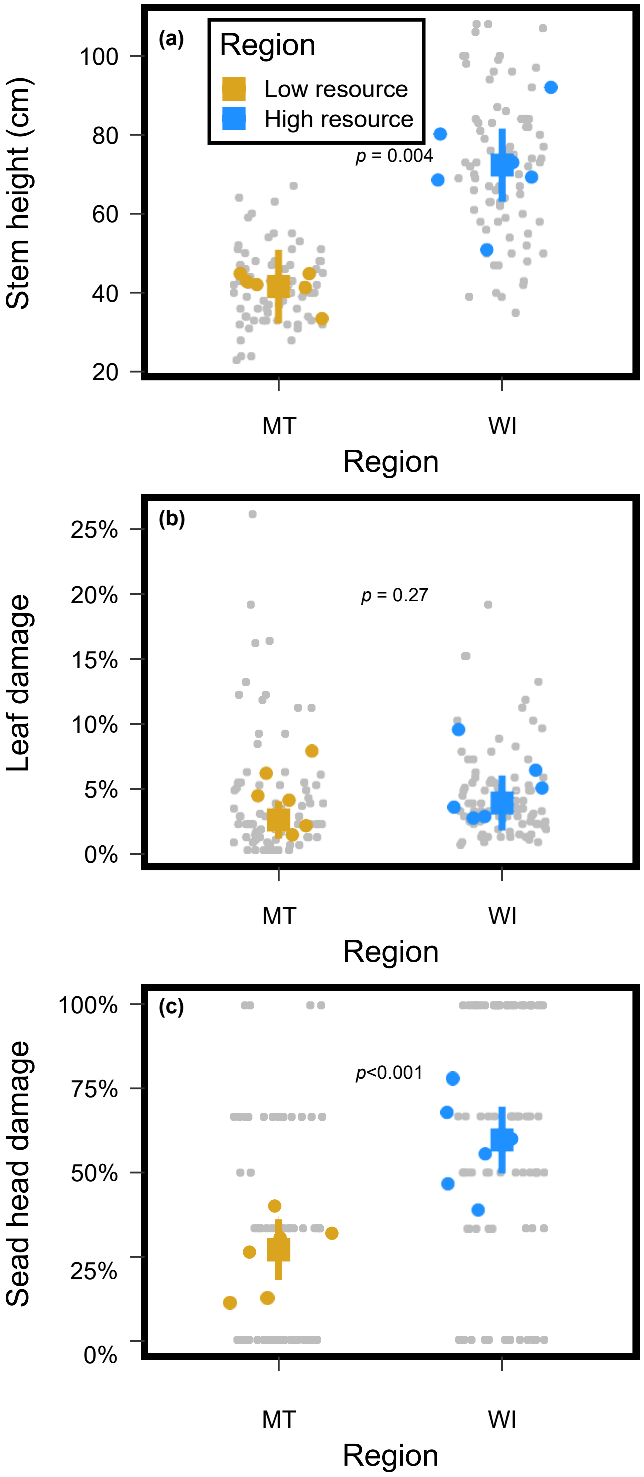


FIGURE 4 Surveys of (a) stem height, (b) leaf damage and (c) seed head damage measured on *Monarda fistulosa* plants growing naturally in low- (MT) and high-resource (WI) regions. Large squares are estimated marginal means, bars are 95% confidence intervals and small grey dots show individual data points. Note that data for (b) and (c) were transformed for analysis, but back-transformed to the original scale for plotting

Among populations, we found that differences in resource availability between the two regions likely dictated the nature of the growth–defence trade-off. *Monarda fistulosa* populations from the low-resource region had slower growth (as measured by end of season above-ground biomass) and higher defence relative to populations from the high-resource region that exhibited faster growth but lower defence (Figure 3). Low- and high-resource populations were separated by over 2,000 km, and thus gene flow between these populations was likely negligible. Additionally, the climatic conditions experienced by these populations differed substantially, as do other abiotic and biotic variables. However, the most likely driver of resource availability and productivity between the regions is summer precipitation (Figure 2b). Stem height measured on naturally occurring plants at field sites followed the same pattern as biomass measured in the garden, suggesting strong genetic variation in growth related to resource availability. Limited gene flow coupled with strongly contrasting environmental conditions has been often shown to produce local adaptation and population differentiation (Blanquart et al., 2013; Clausen et al., 1941). Interestingly, the slow growth, high defence of *M. fistulosa* in low-resource environments and vice versa is similar to cases where this trade-off is most apparent, which is from comparisons among species (Coley et al., 1985; Endara & Coley, 2011). We also found that leaf damage was low (3%– 4%) but similar between the regions, suggesting that herbivore pressure (at least leaf chewers) does not strongly vary between regions, which is also an assumption of Coley et al.'s (1985) resource availability hypothesis. In contrast, seed head damage was much greater in the high-resource region, which could have a strong and direct influence on fitness as well as evolution of defences. While leaves and flowers of *M. fistulosa* produce similar terpene compounds (Keefover-Ring, 2013), our common garden plants did not flower during this study and so we were not able to assess differences in flower chemistry. Although Coley et al.'s (1985) influential hypothesis was originally intended to describe among species patterns, our data suggest that if populations occur across substantially divergent productivity or resource environments, similar patterns can be detected within a species.

When considering populations distributed across a continuous gradient within a region, growth–defence trade-offs are often expected to be relaxed (Hahn & Maron, 2016; Koffel et al., 2018). When we examined growth and defence correlations across populations within each of the two resource regions, there was no growth–defence trade-off in the low-resource region and a *positive* growth–defence correlation among the high-resource populations (Figure 2a). Both patterns have frequently been observed among populations of single species (Hahn & Maron, 2016). We hypothesize that the lack of growth–defence trade-offs or even positive growth–defence relationships within species could arise when sampled populations occur in highly productive, resource-rich habitats, where herbivory is often more intense (Hahn et al., 2019; Hahn & Maron, 2016; Koffel et al., 2018). In our system, there was a reasonable amount of variation in leaf damage rates among sites within each resource region (Figure 4b). For example, the 'region' fixed effect explained only about 4% of the variation in leaf damage, whereas the random effect of site explained an additional 33% of the variation ($R^2_m = 0.04$, $R^2_c = 0.37$). Thus, the positive correlation between growth and defence could be due to a lack of resource constraint on the evolution of defence coupled with greater selection imposed by herbivores on defensive traits among populations within a region. By examining correlations both within and among populations from divergent resource regions, our results are a step towards resolving the conundrum of why patterns of growth and defence are so highly mixed in the literature.

Within populations, growth–defence trade-offs are also expected among individuals within populations, although evidence for this is mixed (Cipollini et al., 2014; Massad et al., 2011; Stamp, 2003). In our study, we did not find evidence for negative correlations among maternal plants from either the low- or high-resource population, despite considerable variation in both growth and defence traits within populations (Figure 3b). This finding suggests that growth and defence production are not strongly genetically constrained in this species. Often, costs are only thought to be detectable under low-resource conditions (Koricheva, 2002; Kruger et al., 2020; Sampedro et al., 2011). We conducted our common garden experiment in the higher resource region, which could have potentially masked trade-offs within populations if resources alleviate the costs of producing defences. Additionally, we were not truly able to replicate the resource region, in that we only had one low- and one high-resource region, even though we had replicate populations representing each region. One way to generalize how resource availability affects phenotypic traits would be by examining low- and high-resource populations from multiple species, where each species would represent an independent adaptation to a particular resource region. Therefore, future reciprocal transplant experiments capable of testing the G × E interaction for multiple species would provide a robust test of how plasticity might influence growth–defence correlations among the differentiated populations.

By comparing trait correlations both within and among populations, we were able to provide a more robust description of what may be driving underlying patterns of trait covariance than would have been possible by examining variation at only one scale. The overarching message of our study is that negative growth–defence trade-offs were only found under certain circumstances and universal trade-offs should not be expected. In fact, we argue that they should only be expected under certain, fairly limited conditions as there are several explanations for why trade-offs may not exist. For example, the costs of producing some chemical defences are not high (Koricheva et al., 2004; Neilson et al., 2013), and classes of chemicals with certain biosynthetic pathways may be more costly than others (Koricheva et al., 2004; Züst & Agrawal, 2017). The production costs of terpenoids and associated glandular trichomes are thought to be high (Gershenzon, 1994; Koricheva et al., 2004), yet we only found negative correlations at the highest level of biological organization (i.e. between regions). In our samples, total terpene concentration was only 0.6%–3.5% of dry tissue mass (Figure 3), which may not strongly draw resources from other physiological processes. Costs of defence may more commonly manifest in ways other than reductions in growth, such as ecological costs (Cipollini et al., 2014; Strauss et al., 2002). Additionally, because most chemicals are regulated by many loci of small to moderate effect (Holeski et al., 2014; Lowry et al., 2019), the genetic architecture may be unlikely to impose strong growth–defence trade-offs (Houle, 1991). Collectively, our results challenge the general expectation of ubiquitous trade-offs but more broadly support the view that resource availability shapes the evolution of defence at different scales.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

P.G.H. and J.L.M. conceived the study; P.G.H., K.K.-R., L.M.N.N. and J.L.M. collected the data; K.K.-R. and L.M.N.N. contributed the reagents and analytical methods; P.G.H. analysed the data; P.G.H. wrote the paper with input from J.L.M.; K.K.-R. and L.M.N.N. contributed critically to editing the manuscript.

DATA AVAILABILITY STATEMENT

Data and code are available in Dryad Digital Repository https://doi.org/10.5061/dryad.x0k6djhjq (Hahn et al., 2021).

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