Title: Context dependence shapes the evolution of glyphosate and herbivory resistance in *Ipomoea purpurea*

Authors: Grace M. Zhang[1] and Regina S. Baucom[1]
[1]Ecology and Evolutionary Biology Department, University of Michigan

Corresponding author: Grace M. Zhang (gmzhang@umich.edu), Ecology and Evolutionary Biology Department, 4034 Biological Sciences Building, University of Michigan, Ann Arbor, MI 48109

Data availability: Data will be made publicly available upon manuscript acceptance.

Author contributions: GMZ and RSB designed the field experiment. GMZ performed the experiment and analyzed the data. GMZ and RSB wrote the manuscript.

Funding: This work is supported by the AFRI Predoctoral Fellowships Program, project award no. 2024-67011-42912, from the U.S. Department of Agriculture's National Institute of Food and Agriculture. This work was also supported by funding from the University of Michigan.

Conflicts of interest: None declared.

Acknowledgements: We thank N. Hammond, G. Lu, and M. Vaughan-Coston for assisting with the field experiment and data processing, M. Palmer and the Matthaei Botanical Gardens for field support, and V. Iriart for providing feedback for the manuscript.

ABSTRACT

Plant defense evolution has often been examined under single-stressor scenarios, overlooking the complex, interacting stressors of natural environments. Recent research incorporating multiple stressors has largely emphasized physiological and molecular responses rather than evolutionary dynamics, leaving such dynamics less explored. To address this gap, we examined the potential for fitness costs and benefits to the herbicide glyphosate and insect herbivory in *Ipomoea purpurea* (common morning glory). Through a factorial field experiment, we manipulated glyphosate and the insecticide spinosad to estimate selection on glyphosate and herbivory resistance. We found that glyphosate resistance was under positive selection with no detectable fitness costs, whereas herbivory resistance showed neither costs nor benefits. Notably, positive correlational selection favored higher glyphosate and herbivory resistance in the combined-stressor environment, suggesting these traits may be genetically linked. Further, the strength of positive selection on glyphosate resistance depended on the environmental context of herbivory. Together, these results indicate that selection for glyphosate resistance predominantly drives evolution in this system and may promote increased herbivory resistance. By applying a fitness cost-benefit framework to multiple interacting stressors, our study underscores the context-dependent nature of selection on plant defense traits and highlights how combined stressors can shape their evolutionary trajectories.

KEYWORDS: glyphosate resistance, herbivory resistance, *Ipomoea purpurea*, costs, benefits

INTRODUCTION

A central question in evolutionary biology is how organisms adapt to complex, stressful environments. The evolution of resistance to damage—whether from natural enemies, abiotic stressors, or anthropogenic agents—provides some of the clearest demonstrations of adaptation in action. Classic studies of resistance have yielded insights into the genetic basis of adaptation, the costs of defense, and the trade-offs that constrain evolutionary trajectories (Bergelson & Purrington, 1996; Strauss et al., 1999; Warwick, 1991). In plants, for example, the framework of assessing fitness costs and benefits emerged from literature regarding plant defense against herbivory, namely whether such costs and benefits existed for herbivory resistance in *Ipomoea purpurea* (common morning glory) (Simms & Rausher, 1987, 1989). Subsequent studies extended to other plant systems like *Arabidopsis thaliana* (mouse-ear cress) (Mauricio, 1998), as well as to other natural biotic stressors such as fungal pathogens (Simms & Rausher, 1993; Tian et al., 2003) and competition (Chaney & Baucom, 2014), and climatic stressors like frost (Agrawal et al., 2004). This framework has also been applied to abiotic stressors such as the herbicide glyphosate (Baucom & Mauricio, 2008; Debban et al., 2015).

Our understanding of the evolutionary trajectory of defense based on potential costs and benefits remains largely grounded in a single-stressor framework. For example, the majority of empirical work quantifies the costs and benefits of resistance in the presence or absence of one stressor at a time, or at best, sequentially. This approach overlooks the ecological reality that organisms do not experience stressors in isolation. Instead, they inhabit environments characterized by overlapping biotic and abiotic pressures that interact—in additive or non-additive ways—to shape both the expression and fitness consequences of resistance (Côté et al., 2016; Zandalinas & Mittler, 2022). Further, when multiple stressors are considered, it is generally in the context of how one stressor influences the effects of another. For example, studies have documented how abiotic stressors such as herbicide exposure can alter biotic stressors like insect herbivory in various plant species (Dewar et al., 2000; Johnson & Baucom, 2022; Lipok, 2009). Other studies consider how multiple stressors may alter plant physiological and molecular responses (Desaint et al., 2021; Zandalinas et al., 2018). Such studies rarely consider the evolutionary responses of plants to overlapping stressors. Thus, we lack a clear understanding of how resistance to one stressor affects performance under additional challenges, and whether the associated costs and benefits are additive, synergistic, or mitigated in combined environments.

A notable exception comes from Gassmann and Futuyma, who examined how insect herbivory modified the costs of resistance to the herbicide triazine in *Amaranthus hybridus* (smooth pigweed) (Gassmann & Futuyma, 2005). Strikingly, they found that the fitness cost of triazine resistance increased by 360% when plants were exposed to the herbivore *Disonycha glabrata* (pigweed flower beetle). This heightened cost arose from a phenotypic trade-off: triazine-resistant plants were more vulnerable to herbivore damage and less capable of recovering from

it. In this system, the cost of triazine resistance was not fixed, but contingent on the biotic context. Comparable context-dependence of defense has been observed in other systems as well. *Arabis perennans* (perennial rockcress) demonstrated a fitness cost associated with resistance to the herbivore *Plutella sylostella* (diamondback moth) that only manifested among populations of *A. perennans* that were poor competitors in the presence of *Bouteloua gracilis* (blue grama) (Siemens et al., 2003). Similarly, *I. purpurea* plants subjected to drought conditions produced fewer fruits in response to simulated folivory in comparison to plants that received ample water (Atala & Gianoli, 2009). These examples underscore that the costs and benefits of resistance are environmentally contingent and can shift across ecological gradients. Yet such factorial tests remain rare, and we still lack a general framework predicting when resistance to multiple stressors in different environmental contexts will impose additive, synergistic, or mitigating effects on fitness. Specifically, it remains unclear whether resistance to one stressor constrains or facilitates responses to another, and how such interactions shape the overall costs and benefits of adaptation. Understanding these interactions is essential for predicting how resistance evolves and persists in heterogeneous, multi-stressor environments.

We address this gap by examining how plants evolve under dual selective pressures mediated by anthropogenic chemicals: the herbicide glyphosate, which exerts strong selective pressure, and the insecticide spinosad, which decreases the presence—and therefore the potential selective pressure—of insect herbivores. Previously, we showed that glyphosate application can alter plant-herbivore interactions in *I. purpurea*, leading to higher levels of herbivory and shifts in feeding patterns (Zhang & Baucom, 2024), and that glyphosate resistance is positively correlated with herbivory resistance. Building on these findings, we conducted a factorial common garden experiment to explore the evolution of resistance to glyphosate and herbivory, examining selection on each trait and how patterns of selection differ across environmental contexts (Figure 1). Specifically, we ask: (1) Are there fitness costs and benefits associated with glyphosate and herbivory resistance? (2) Does correlational selection act on resistance to both stressors, suggesting shared genetic mechanisms? And (3) what does structural equation modelling reveal about how glyphosate and spinosad may directly and indirectly affect resistance and fitness? By embedding a multi-stressor perspective within the cost-benefit framework, our study advances understanding of the ecological and evolutionary dynamics of adaptation under realistic, environmentally complex conditions.

MATERIALS AND METHODS

Study system

Ipomoea purpurea (L.) Roth (Convolvulaceae), the common morning glory, is an annual weed frequently found in agricultural fields and disturbed soil beds in the United States. The species begins germination in the early spring and through the growing season. It grows rapidly, capable

of producing over 8,000 seeds in a season before being killed by the first winter frost (Chaney & Baucom, 2014). In Michigan, *I. purpurea* is fed upon by generalist coleopteran and lepidopteran herbivores such as *Popillia japonica* (Japanese beetle) and *Trichoplusia ni* (cabbage looper) (Zhang & Baucom, 2024). *I. purpurea* has previously been shown to have natural resistance to insect herbivory that is under positive selection (Rausher & Simms, 1989). Populations of the species have also naturally evolved resistance to the herbicide glyphosate, likely through detoxification and cell transport mechanisms (Baucom & Mauricio, 2004; Gupta et al., 2023; Kuester et al., 2015).

Field experiment

On June 9, 2022, we planted replicate *I. purpurea* seeds from 45 family lines in a common garden field plot at the Matthaei Botanical Gardens in Ann Arbor, Michigan. These lines were generated from random crosses between individuals (*i.e.*, maternal lines) sampled from naturally glyphosate-resistant and -susceptible populations in the US Southeast (Kuester et al., 2015). The randomly-selected 24 individuals were sampled as seed from six populations and selfed once before being crossed. All family lines generated from the crosses were also selfed once before being put into the field in order to generate sufficient sample sizes. Four replicate seeds from each family were randomly planted in each of four treatment environments—1) glyphosate-present, spinosad-absent, 2) glyphosate-absent, spinosad-absent, 3) glyphosate-present, spinosad-present, and 4) glyphosate-absent, spinosad-present—that were repeated in two blocks, for a total of 1440 individual seeds. We watered daily and used a combination of weed fabric and hand-weeding to control weeds. Germination started a few days after planting and was complete within ten days.

To suppress insect herbivory in the spinosad-present plots, we applied a field dose of 0.30 kg ai/L of the insecticide spinosad (Garden Insect Spray, Monterey Lawn and Garden Products, Inc., Fresno, CA, USA) to the plants. Spinosad is derived from a naturally-occurring soil bacterium *Saccharopolyspora spinosa* (Bacteria: Actinobacteridae) (Mertz & Yao, 1990). Introduced in 1997, spinosad is an organic insecticide that targets the nicotinic acetylcholine and gamma amino butyric acid (GABA) receptor sites of insect nervous systems, causing them to twitch to death (Zhang et al., 2021). We conducted a preliminary growth room experiment comparing the effects of the insecticides Bt (*Bacillus thuringiensis* subsp. *kurstaki*, Monterey Lawn and Garden Products, Inc., Fresno, CA, USA), spinosad, and esfenvalerate (Bug Buster II, Monterey Lawn and Garden Products, Inc., Fresno, CA, USA) to assess which would be the most appropriate insecticide for this study (see Figure S1 for details). We found that while both Bt and spinosad application did not affect plant biomass in the preliminary experiment, Bt interacted synergistically with glyphosate to increase biomass. We thus decided to proceed with spinosad—its broad-spectrum insecticidal effects was also more appropriate for our field site

than Bt, which is highly specific to lepidopterans (Hertlein et al., 2011; Lambert & Peferoen, 1992; Mayes et al., 2003).

We applied spinosad every other week for a total of five applications, starting two weeks after the seeds were planted. We counted the number of leaves on all plants five weeks after planting in order to estimate plant size (Debban et al., 2015). Six weeks after planting, we applied the herbicide glyphosate (Roundup WeatherMax, Bayer Corp., Whippany, NJ, USA) at the sublethal dose of 1.0 kg ai/ha to the glyphosate-present treatment plots using a handheld CO_2-pressurized sprayer (Spraying Systems Co., Wheaton, IL, USA) at a rate of 187 L/ha at 30 psi with a stride pace of 90 paces/min (Kuester et al., 2015). Because it was necessary to measure individual-level fitness later in the experiment, we chose a dose lower than the recommended field dose for *Ipomoea spp.* of 1.54 kg ai/ha (Monsanto, 2017). Our chosen dose of 1.0 kg ai/ha was in line with past studies that aimed to merely stress the plants rather than eradicate them and is well within the range of recommended field doses of glyphosate for weed control (Duke et al., 2012; Vázquez et al., 2021). We scored damage two weeks after glyphosate application by dividing the number of damaged (*i.e.*, yellowed, browned, and/or shriveled) leaves on each plant by the total number of leaves in order to calculate the proportion of damaged leaves (per Baucom & Mauricio, 2008). We also took note of which plants had died due to glyphosate application.

Five weeks after the glyphosate application, we collected four leaves from each plant for herbivory damage estimates. Leaves were sandwiched between paper towels and kept cool on ice to be brought back to the lab, where they were scanned with a Canon CanoScan LIDE 400 flatbed scanner (Canon USA, Melville, NY, USA) to generate high-resolution images at 600 dpi. Herbivory damage was estimated as the percentage of tissue missing per leaf using the iOS app LeafByte (Getman-Pickering et al., 2020), then averaged across the four leaves for each plant. In line with our previous work, we elected to be conservative with our herbivory damage estimates and excluded ambiguous damage that could be attributed to glyphosate from our estimates (Zhang & Baucom, 2024). Additionally, we noticed that many plants had leaves exhibiting patches of rust fungus; thus we scored the presence/absence of the fungus on each leaf as a binomial (1 - present, 0 - absent) and averaged those values across the four leaves for each plant. Finally, we collected seeds from all plants in two complete rounds in order to estimate fitness.

Germination assay of field-collected seeds

We conducted a germination assay using the field-collected seeds to determine if fitness costs and benefits of resistance may have manifested in terms of germination rate, in line with previous work that showed a fitness cost associated with seed germination in this species (Van Etten et al., 2016). Five seeds per plant were randomly selected and placed in a 60mm $\times$ 15 mm petri dish, with one dish per plant. The seeds were submerged in filtered water, and petri dishes were placed on lab benches to allow seeds to germinate in ambient lab lighting and temperature

(around 21°C). We scored germination on Days 3 and 7. If water did not penetrate the seed coat by Day 7, we scarified the seeds to encourage germination, then continued to score germination on Days 10 and 14. The germination assays were ended for all seeds on Day 14. The total number of seeds that germinated was used as the fitness estimate.

Statistical analyses

We operationally defined resistance as 1-*p*, where *p* is the amount of damage experienced by an individual plant. Thus, when calculating glyphosate resistance, *p* refers to the proportion of leaves damaged by glyphosate per plant (Baucom & Mauricio, 2008). Likewise, when calculating herbivory resistance, *p* is the average percentage of leaf tissue missing due to herbivory for each plant (Rausher & Simms, 1989).

All analyses were conducted using R 4.3.2 (R Core Team, 2023). For the field experiment, we excluded any individual plants that did not germinate (N = 229), as well as plants in the glyphosate-absent treatments that were inadvertently damaged by glyphosate (N = 43). We also removed any plants that died before herbivory data and/or seed number could be recorded. Ultimately, we had 258 plants in the glyphosate-present, spinosad-absent treatment, 233 plants in the glyphosate-absent, spinosad-absent treatment, 262 plants in the glyphosate-present, spinosad-present treatment, and 255 plants in the glyphosate-absent, spinosad-present treatment, for a total of 1008 plants. We used the package *bestNormalize* (Peterson, 2021) to identify the appropriate transformation for each response variable.

To ensure that spinosad had the desired treatment effect of reducing herbivory damage, we used the package *lme4* (Bates et al., 2015) to build linear mixed models, with spinosad and glyphosate included as fixed effects. Leaf count (as a proxy for plant size) and the proportion of collected leaves showing rust fungus were included as covariates in all models, and the Satterthwaite approximation was used to determine the degrees of freedom of the fixed-effect variables. Family line and block were included as random effects unless stated otherwise. To test the significance of random effects, we removed each one and used the χ^2 test of difference (df = 1) to compare the simplified model to the full model. We constructed the following model using all experimental plants:

herbivory damage ~ spinosad + glyphosate + leaf count + rust + 1|family + 1|block

Preliminary analyses did not show any evidence of interactions between family line and either of the two treatments, nor was there any evidence of an interaction between the two treatments, so we did not include those terms in the full model.

Our preliminary analyses further indicated that spinosad application may have had an effect on glyphosate resistance. We thus built the following linear mixed model using just the plants treated with glyphosate to further explore the relationship:

glyphosate resistance ~ spinosad + leaf count + rust + (1 + spinosad|family) + 1|block

We specifically tested for an interaction between spinosad and family line while letting the slopes and intercepts vary because we were interested in whether the effect of spinosad application on glyphosate resistance showed genetic variation.

We also assessed if there was evidence for genetic variation for glyphosate and herbivory resistance by including family line as a fixed effect in the following linear mixed model:

resistance ~ family + leaf count + rust + 1|block,

where the resistance was either glyphosate or herbivory resistance. For either resistance, we tested for genetic variation first among all plants that had been exposed to that stressor to determine overall variation in the trait, then in both the presence and the absence of the other stressor to account for any treatment effects of the second stressor.

Genotypic resistance selection analyses

To determine if there were fitness costs and benefits associated with glyphosate and herbivory resistance, we conducted selection analyses on the two forms of resistance in each of the four treatment environments. If there was positive selection for a form of resistance in the presence of the corresponding stressor, then we would conclude there was a fitness benefit associated with that form of resistance. The converse was also examined—negative selection for a form of resistance in the absence of the stressor would suggest there was a fitness cost associated with resistance to that stressor. We examined fitness costs and benefits of resistance traits in terms of both seed count and germination.

In accordance with past studies, we used a modified form of Lande and Arnold's method of detecting selection using partial regression coefficients (Lande & Arnold, 1983; Mauricio & Mojonnier, 1997; Rausher, 1992). Instead of performing selection analyses on phenotypic trait values, we used genotypic means of trait values in order to eliminate environmental biases (Rausher, 1992; Stinchcombe et al., 2002). Additionally, we used the residuals of untransformed values of relative fitness and resistance after removing the effects of block to further remove environmental effects from the analyses. Relative fitness was calculated by dividing the fitness of each plant by the average fitness of all plants in the experiment, in terms of both seed count and germination. After calculating genotypic means of relative fitness and resistance trait values,

we standardized the resistance traits such that they had an average of zero and a standard deviation of one. Relative fitness was then regressed on each form of resistance to determine the pattern of selection for that particular trait. Directional selection was estimated using only linear terms, while nonlinear (*i.e.*, stabilizing/disruptive) selection was estimated using models containing both linear and quadratic terms. All selection gradients for nonlinear selection were reported as twice the quadratic regression coefficient.

To detect correlational selection between the two forms of resistance in each environment, we regressed relative fitness on the interaction term between the resistance traits, as well as all linear and nonlinear terms. We then used the *fields* package (Nychka et al., 2021) to generate a fitness landscape fitted with thin-plate splines. We further tested for genetic correlations between the two forms of resistance in each environment by calculating Pearson's correlation coefficient.

Direct and indirect effects on fitness

We assessed the direct and indirect effects of glyphosate and spinosad treatments on glyphosate resistance, herbivory resistance, and fitness by constructing a structural equation model (SEM) using the package *piecewiseSEM* (Lefcheck, 2016). Using data from plants across all treatments, we included glyphosate treatment, spinosad treatment, glyphosate resistance, herbivory resistance, and fitness in terms of seed count as variables, with family line and block included as random effects. We elected to omit germination as a fitness measure in this model because we did not detect any relationship between the resistance traits and germination in the selection analyses. The following relationships were modeled and the Fisher's C statistic was used to determine model fit:

Glyphosate resistance ~ glyphosate + spinosad + 1|family + 1|block

Herbivory resistance ~ glyphosate + spinosad + 1|family + 1|block

Seed count ~ glyphosate + spinosad + glyphosate resistance + herbivory resistance + 1|family + 1|block

We did not include a relationship between glyphosate resistance and herbivory resistance in the final model. Doing so would have resulted in a saturated model, which would not have allowed us to assess model fit. Additionally, the saturated model reported that the relationship between the two resistances was not significant, and including the relationship resulted in a model with a higher AIC score (saturated: 6408.696 vs. current model: 6402.301), which suggests a worse fit. Thus, we proceeded with the SEM that omitted this relationship.

RESULTS

Treatments affect both herbivory and leaf damage

We wanted to ensure that glyphosate and spinosad application were effective, such that plants were damaged but not killed in the presence of glyphosate and herbivory was reduced in the presence of spinosad respectively. To this end, we examined the effects of spinosad on herbivory damage and glyphosate on plant death and leaf damage. We found that spinosad application significantly reduced herbivory (untreated: 0.53%, treated: 0.15%; $F_1 = 174.06$, $p < 0.001$; Figure 2a, Table S1). Additionally, although this difference was not statistically significant, glyphosate application increased herbivory (untreated: 0.30%, treated: 0.37%; $F_1 = 2.14$, $p = 0.144$; Figure 2b, Table S1), which is in line with our previous work (Zhang & Baucom, 2024). We found that glyphosate application killed only ten plants, but caused leaf damage across plants (on average, 37.7% of leaves per plant were damaged) and reduced total seed production (mean seed number in glyphosate-treated plants: 290 vs. untreated plants: 1723).

Interestingly, spinosad treatment affected glyphosate resistance. Plants that were exposed to both glyphosate and the spinosad showed higher levels of resistance to glyphosate than plants that were just exposed to glyphosate (spinosad: 69.43% leaves showed no glyphosate damage, no spinosad: 55.14% leaves showed no glyphosate damage; $F_1 = 71.83$, $p < 0.001$; Figure 2c, Table S2). There was no interaction between spinosad treatment and family line, however, indicating that families generally experienced similar levels of increased glyphosate resistance in the presence of the spinosad ($\chi^2_1 = 0.81$, $p = 0.666$; Table S2). In line with the results of the preliminary insecticide experiment in the growth room, we found that spinosad application in the field did not affect mean plant size in terms of leaf number six weeks after the seeds were planted (untreated: 26.52 leaves; treated: 26.47 leaves).

Genetic variation for resistance to glyphosate and herbivory

We investigated whether there was genetic variation for the two forms of resistance. We found evidence of genetic variation for glyphosate resistance across all plants in glyphosate-present environments regardless of spinosad treatment ($F_{44} = 1.46$, $p = 0.033$; Table S3), as well as glyphosate-treated plants that were not treated with spinosad ($F_{44} = 1.67$, $p = 0.009$; Table S3). There was no genetic variation for glyphosate resistance detected among plants that were treated with both glyphosate and spinosad ($F_{44} = 0.99$, $p = 0.492$; Table S3).

We did not find evidence of genetic variation underlying herbivory resistance among plants that were not treated with spinosad ($F_{44} = 0.61$, $p = 0.977$; Table S4). Nor did we find evidence of genetic variation for herbivory resistance in either the glyphosate-present or glyphosate-absent environments (glyphosate-present: $F_{44} = 0.70$, $p = 0.924$; glyphosate-absent: $F_{44} = 0.89$, $p = 0.664$; Table S4).

Genotypic selection for glyphosate and herbivory resistance by environment

We tested for genotypic selection acting upon glyphosate and herbivory resistance in each of the four treatment environments in order to assess fitness costs and benefits of each form of resistance depending on the treatment environment (Figure 1). We did not detect any form of selection, linear or nonlinear, acting on either glyphosate or herbivory resistance when germination was used as the fitness measurement (Figure S2), so here we only report the results of selection analyses using seed count as fitness estimate.

In the glyphosate-present, spinosad-absent environment (*i.e.*, herbivores were present), we detected positive selection for glyphosate resistance, meaning that there is a fitness benefit associated with that trait ($\beta = 0.11$, $F_1 = 18.69$, $p < 0.001$; Figure 3a; Table S5). However, we found no such fitness benefit for herbivory resistance ($\beta = 0.03$, $F_1 = 1.12$, $p = 0.295$; Figure 3a; Table S5). We also identified a fitness peak for high levels of both glyphosate and herbivory resistance (*i.e.*, correlational selection: $\gamma = 0.06$, $F_1 = 5.10$, $p = 0.030$; Figure 4; Table S6), although the two traits are not genetically correlated ($r^2 = 0.13$, $p = 0.383$). We did not find any other form of nonlinear selection in this environment for either glyphosate resistance ($\gamma = 0.02$, $F_1 = 0.47$, $p = 0.495$; Table S6) or herbivory resistance ($\gamma = 0.02$, $F_1 = 0.20$, $p = 0.655$; Table S6).

In the glyphosate-absent, spinosad-absent environment, we expected to find a fitness cost associated with glyphosate resistance and a fitness benefit associated with herbivory resistance (Figure 1). Contrary to our expectations, we did not find a cost associated with glyphosate resistance ($\beta = 0.09$, $F_1 = 2.01$, $p = 0.163$) or a benefit of herbivory resistance ($\beta = -0.08$, $F_1 = 1.72$, $p = 0.197$; Figure 3b; Table S5). There was also no evidence of correlational selection for the two resistance traits ($\gamma = 0.02$, $F_1 = 0.09$, $p = 0.765$; Table S6) or genetic correlation between them ($r^2 = 0.09$, $p = 0.547$), nor was there nonlinear selection for either glyphosate resistance ($\gamma = 0.06$, $F_1 = 0.38$, $p = 0.543$; Table S6) or herbivory resistance ($\gamma < 0.001$, $F_1 < 0.001$, $p = 0.992$; Table S6).

In the glyphosate-present, spinosad-present environment (*i.e.*, herbivory was reduced), we expected to find a fitness benefit of glyphosate resistance and a fitness cost of herbivory resistance (Figure 1). There was evidence for potential positive selection for glyphosate resistance ($\beta = 0.06$, $F_1 = 3.42$, $p = 0.071$), but no evidence for selection acting on herbivory resistance ($\beta = 0.01$, $F_1 = 0.11$, $p = 0.739$; Figure 3c; Table S5). We did not find any evidence of correlational selection acting on the two forms of resistance ($\gamma = -0.06$, $F_1 = 2.83$, $p = 0.101$; Table S6) or any genetic correlation between the traits ($r^2 = 0.11$, $p = 0.479$) in this environment. There was also no evidence of nonlinear selection for either glyphosate resistance ($\gamma = -0.006$, $F_1 = 0.01$, $p = 0.921$; Table S6) or herbivory resistance ($\gamma = 0.06$, $F_1 = 1.45$, $p = 0.235$; Table S6).

In the glyphosate-absent, spinosad-present environment, we expected to find fitness costs associated with both glyphosate and herbivory resistance (Figure 1). Contrary to our expectations, we did not find evidence for a cost to glyphosate resistance ($\beta = 0.08$, $F_1 = 3.07$, $p = 0.087$) or herbivory resistance ($\beta = 0.03$, $F_1 = 0.35$, $p = 0.558$; Figure 3d; Table S5). There was no evidence for correlational selection for glyphosate and herbivory resistance ($\gamma = 0.08$, $F_1 = 3.00$, $p = 0.091$; Table S6), nor was there a genetic correlation ($r^2 = 0.18$, $p = 0.246$). We also did not find evidence of nonlinear selection acting on either glyphosate resistance ($\gamma = -0.02$, $F_1 = 0.14$, $p = 0.712$; Table S6) or herbivory resistance ($\gamma = -0.02$, $F_1 = 0.14$, $p = 0.705$; Table S6).

Direct and indirect effects of treatments on resistance and seed production

We next wanted to determine the direct and indirect effects of the two forms of resistance on fitness—how do the dual treatments of glyphosate and spinosad affect resistance to glyphosate and herbivory, and how does that impact fitness? To answer these questions, we constructed an SEM using all experimental plants, specifically testing the effects that glyphosate and spinosad have on both glyphosate resistance and herbivory resistance, as well as seed number. We also tested for relationships between the resistances and fitness. The constructed SEM had a Fisher's C statistic of 0.007 ($p = 0.996$; Figure 5).

The SEM showed that as expected, glyphosate treatment negatively impacted glyphosate resistance (standardized $\beta = -0.745$, $df = 984$, $p < 0.001$). This is necessarily the case because unexposed plants would not have experienced glyphosate damage and therefore would have perfect resistance. Additionally, glyphosate treatment negatively impacted seed production (standardized $\beta = -0.550$, $df = 923$, $p < 0.001$), but had no effect on herbivory resistance. Predictably, spinosad treatment led to decreased herbivory (*i.e.*, increased herbivory resistance; standardized $\beta = 0.380$, $df = 978$, $p < 0.001$) as well as increased glyphosate resistance (standardized $\beta = 0.155$, $df = 981$, $p < 0.001$), mirroring the results of the treatment effects above. Plants exposed to spinosad are by necessity more resistant to herbivory because they experience less herbivory damage. There was no relationship between spinosad treatment and seed production.

By constructing an SEM, we were able to assess the overall impact that glyphosate and spinosad treatment had on seed production by breaking down the relationships into direct, indirect, and total effects. Glyphosate's impact on seed production was primarily driven by its direct effect (total effect (-0.761) = direct effect (-0.550) + indirect effect through glyphosate resistance (-0.745 * 0.283 = -0.211)). On the other hand, spinosad did not have any direct effects on seed production, but weakly but positively impacted fitness instead through glyphosate resistance (total effect (0.044) = indirect effect through glyphosate resistance (0.155 * 0.283 = 0.044)). Thus, the impacts that glyphosate and spinosad have on fitness are either directly imparted by glyphosate treatment, or mediated through resistance to glyphosate.

DISCUSSION

We conducted a factorial field experiment to examine how concurrent exposure to a herbicide (glyphosate) and an insecticide-mediated herbivory environment (*via* spinosad) shapes the evolution of resistance traits in *Ipomoea purpurea*. Three main patterns emerge. First, glyphosate is the dominant selective agent, consistently reducing fitness and favoring higher resistance in glyphosate-present environments. Second, herbivory resistance confers neither detectable fitness costs nor benefits, and lacks genetic variation under the conditions of this study. Third, spinosad unexpectedly alters glyphosate resistance and its evolutionary dynamics, increasing resistance and modifying the strength of selection. Together, these results demonstrate that resistance evolution is strongly context-dependent and that additional stressors can reshape both the expression of resistance traits and the selection acting upon them.

Glyphosate as the primary driver of resistance evolution

Glyphosate application, even at a sublethal dose, imposed strong fitness consequences, delaying development and substantially reducing seed production. Consistent with previous work in this system (Baucom & Mauricio, 2008), glyphosate resistance experienced positive selection in glyphosate-present environments, indicating a clear fitness benefit of increased resistance. Importantly, we detected genetic variation for glyphosate resistance across plants exposed to glyphosate, suggesting continued evolutionary potential (Zhang & Baucom, 2024).

Despite strong selection, we detected no fitness costs of glyphosate resistance measured as seed production or germination. This absence of detectable costs contrasts with prior studies that identified costs in seed viability, germination, or plant size (Debban et al., 2015; Van Etten et al., 2016). Differences in experimental design likely explain this discrepancy. For example, in our prior work we used both field-collected seeds and selfed seeds to examine the potential for fitness costs (Van Etten et al., 2016). We additionally used a higher dose of herbicide to identify the most resistant populations (*e.g.*, 1.7 kg ai/ha; Kuester et al., 2015). Here, in contrast, we generated experimental individuals from crosses among randomly-sampled maternal lines, and we assessed resistance at a lower glyphosate dose. We have previously hypothesized that apparent fitness costs in this system are due to physical linkage between resistance alleles and cost alleles, such that recombination could have uncoupled resistance from deleterious seed traits (Gupta et al., 2023; Van Etten et al., 2020). Further, the lower dose of glyphosate may have been insufficient to reveal subtle fitness penalties. Thus, although glyphosate resistance clearly enhances fitness in the presence of herbicide, its associated costs appear highly conditional on dose, genetic background, and experimental environment (Neve et al., 2014).

The structural equation model (SEM) reinforces this conclusion—glyphosate reduced fitness

primarily through a strong direct effect, with an additional indirect effect mediated by glyphosate resistance. These results highlight glyphosate as the principal driver of evolutionary change in this system.

Herbivory resistance shows no evolutionary response under current conditions

In contrast to glyphosate resistance, herbivory resistance to foliar damage accrues neither fitness costs nor benefits in *I. purpurea*. Moreover, herbivory did not directly affect fitness in the SEM. The lack of selection on herbivory resistance is in line with previous work in this species (Simms & Rausher, 1987, 1989), but the lack of genetic variation for the trait is not (Rausher & Simms, 1989).

Several factors could explain these results. Overall herbivory levels were low (<1% foliar damage on average), potentially limiting the strength of selection. In comparison, in a similar study, Tiffin and Rausher measured a mean foliar herbivory level of 3.4% across *I. purpurea* plants in the herbivory-present treatment (Tiffin & Rausher, 1999), suggesting that the intensity of herbivory in our study may have been insufficient for fitness costs or benefits of resistance to manifest. Moreover, because complete exclusion of insect herbivores is rarely achievable in field experiments, we employed spinosad as a pragmatic and agriculturally relevant approach to reducing herbivory. Given that spinosad application did not affect plant size or seed production, it is unlikely that reduced herbivory meaningfully altered fitness or masked costs associated with herbivory resistance. Finally, while prior work took place in North Carolina, our study took place in Michigan, a latitudinally-different site with different herbivores—for example, in a previous study we identified a majority of generalist herbivores such as *Popillia japonica* (Japanese beetle) (Zhang & Baucom, 2024), while prior work reported herbivory from specialist herbivores like *Chaetocnema confinis* (sweet potato flea beetle) and *Deloyala guttata* (tortoise beetle) (Simms & Rausher, 1987, 1989). Although our location lies outside the geographic focus of much previous work, wild *I. purpurea* populations are present in southeastern Michigan and northern Ohio, indicating that the patterns observed here reflect biologically realistic interactions within part of the contemporary range of the species.

Spinosad alters glyphosate resistance and selection

One of the most unexpected findings was that spinosad application increased glyphosate resistance and indirectly increased fitness through this effect. Plants exposed to spinosad exhibited higher glyphosate resistance, as well as reduced genetic variation and weaker evidence of positive selection for that resistance. These patterns suggest that spinosad modifies the expression and evolutionary dynamics of glyphosate resistance.

A plausible explanation is physiological priming via shared detoxification pathways. Resistance

to spinosad in insects can be conferred *via* metabolic detoxification, particularly by upregulating enzymes such as monooxygenases, cytochrome P450s, and uridine diphosphate glucosyltransferases (UGTs) (Bastarache et al., 2023; Sparks et al., 2012; Wang et al., 2024). Notably, plants have metabolic detoxification processes that trigger the same classes of enzymes (Edwards et al., 2011; Gharabli et al., 2023), and importantly, the same processes likely confer resistance to glyphosate in *I. purpurea* (Gupta et al., 2023). Thus, it is possible that the application of spinosad may prime plants to become more resistant to glyphosate by activating the same detoxification pathways. Moreover, spinosad has been detected in leaf tissue of both *Solanum lycopersicum* (tomato) and *Allium cepa* (onion) (Moretti et al., 2021; Van Leeuwen et al., 2005). If *I. purpurea* can similarly take up spinosad, the insecticide may activate detoxification pathways, priming the plants to respond quickly to the herbicide and better protect from damage.

Our design does not allow us to fully disentangle the effects of spinosad from reduced herbivory, but the SEM suggests that spinosad acts directly on glyphosate resistance rather than indirectly through herbivory. This result illustrates how additional stressors can alter not only trait expression but also the availability of genetic variation and the strength of selection.

Correlational selection links glyphosate and herbivory resistance

Although we detected no univariate selection on herbivory resistance, we found correlational selection favoring jointly higher levels of glyphosate and herbivory resistance in the presence of both stressors (*i.e.*, spinosad was absent). This pattern suggests that as glyphosate resistance evolves, herbivory resistance may also increase—even in the absence of detectable genetic variation for herbivory resistance itself. This interpretation aligns with our previous SEM-based work, which inferred a causal relationship between glyphosate and herbivory resistance (Zhang & Baucom, 2024). One explanation is that the two resistance traits share underlying genetic mechanisms.

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme upstream of metabolic pathways that synthesize aromatic amino acids—the precursors of many secondary metabolites involved in chemical defense against herbivores (Amrhein et al., 1980; Fuchs et al., 2021; Mithöfer & Boland, 2012). The likely mechanism of glyphosate resistance in *I. purpurea*—metabolic detoxification coupled with enhanced transport (Gupta et al., 2023)—could affect herbivory resistance in two ways. First, detoxification may reduce the number of glyphosate molecules that reach and inhibit EPSPS (Baek et al., 2021), thereby mitigating glyphosate's indirect suppression of anti-herbivore defense compound biosynthesis. Second, loci underlying detoxification pathways (*e.g.*, cytochrome P450s, glycosyltransferases, and ABC and phosphate transporters) may have pleiotropic effects on herbivory resistance. For example, UGTs regulate the glycosylation of phytohormones central to herbivory defense—such as

abscisic acid, jasmonates, and salicylic acid (Dimunová et al., 2022; Gharabli et al., 2023). If glyphosate and herbivory resistance are linked due to pleiotropy, the relationship between the traits will persist, and correlational selection will likely shape the evolution of those traits concurrently over time (Svensson et al., 2021).

In our previous work, we found a causal relationship between glyphosate and herbivory resistance, such that higher levels of glyphosate resistance should lead to higher levels of herbivory resistance (Zhang & Baucom, 2024). In this work, however, we did not detect a relationship between the two traits in an SEM. Nonetheless, positive correlational selection in the glyphosate-present, spinosad-absent environment suggests that the two traits are linked. This discrepancy between the two experiments may be due to differences between field conditions. For example, the rate of glyphosate that was applied in Zhang and Baucom was the field dose of 1.5 kg ai/ha for *I. purpurea*, which caused considerable death amongst the glyphosate-treated plants (44.95% plants died). In contrast, for this study we applied a lower rate of glyphosate at 1.0 kg ai/ha, which killed only ten plants. The dose of glyphosate in this current experiment may be too low for the relationship between glyphosate resistance and herbivory resistance to manifest in the SEM, which summarized relationships across all treatment environments in the experiment. Additionally, the previous study considered only glyphosate-present and -absent environments, with herbivory present regardless of glyphosate treatment. Thus, herbivory as mediated by insecticide application was not included as a treatment, which changes the structure of the SEM.

In contrast, this current study considered four distinct environments (Figure 1), and the SEM was constructed to holistically summarize the impacts that both glyphosate and spinosad had on fitness in all environments, which included plants from an environment where neither stressor was present. Given that correlational selection between glyphosate and herbivory resistance was detected in just the environment where both stressors are present, it is possible that data from the other environments masked the relationship between the two forms of resistance in the SEM from the current study.

Leveraging multi-stressor systems to parse plant evolutionary trajectories

Historically, plant evolutionary ecology has evaluated resistance traits one stressor at a time, following the framework established for foliar herbivory resistance (Simms & Rausher, 1987) and later extended to pathogens, competitors, frost, and herbicides (Agrawal et al., 2004; Baucom & Mauricio, 2004; Chaney & Baucom, 2014; Simms & Triplett, 1994). Yet plants rarely face stressors in isolation; they encounter multiple threats simultaneously. Recent work has therefore begun to examine multi-stressor environments, particularly in the context of pollution. For example, additive effects of several abiotic stressors severely reduced *Arabidopsis thaliana* growth, whereas single stressors had minimal impact (Zandalinas et al., 2021), and

reviews highlight the complex signaling crosstalk underlying plant responses to combined biotic and abiotic stress (Nawaz et al., 2023). However, most studies focus on physiological and molecular responses rather than evolutionary outcomes (Gupta et al., 2016; Pandey et al., 2015; Prasch & Sonnewald, 2013), and few address how multiple stressors influence evolutionary trajectories (Gassmann & Futuyma, 2005).

Measuring genetic variation for resistance traits and applying selection analyses in multi-stressor, multi-environment contexts, as in our study, allows us to predict how plants may evolve in response to interacting stressors—patterns that are highly species- and stressor-specific (Strauss et al., 2002). Our results show that the weedy *I. purpurea* can evolve increased resistance to both glyphosate and herbivory when both stressors are present, with glyphosate resistance acting as the primary driver. However, the presence of spinosad can generally increase glyphosate resistance and alter how selection acts on the trait. Beyond revealing the environmental dependence of glyphosate's fitness benefits, our findings may inform management strategies for this agricultural weed.

In conclusion, our study examined how plants evolve under multiple stressors—specifically glyphosate and insect herbivory as mediated by spinosad—and identified glyphosate, not herbivory, as the primary driver of context-dependent evolutionary change in this system. Although we focused on two resistance traits, many other plant traits may also respond to these stressors or influence the evolution of resistance and fitness. Likewise, we did not assess every stressor present in natural environments or all possible interactions among them. The unexpected influence of spinosad on glyphosate resistance illustrates how additional, unmeasured factors can shape evolutionary trajectories. While it is impossible to capture every interaction in complex field environments, evaluating fitness effects across multiple stressors and environmental contexts offers a powerful way to understand how plants adapt to real-world conditions. Extending the classic fitness cost-benefit framework to multi-stressor factorial designs can therefore improve our ability to predict how shifting, multifaceted environments guide the evolution of plant defenses.

LITERATURE CITED

Agrawal, A. A., Conner, J. K., & Stinchcombe, J. R. (2004). Evolution of plant resistance and tolerance to frost damage. *Ecology Letters*, *7*(12), 1199–1208.

Amrhein, N., Brigitte Deus, Peter Gehrke, & Hans Christian Steinrücken. (1980). The site of the inhibition of the shikimate pathway by glyphosate: II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro*. *Plant Physiology*, *66*(5), 830–834.

Atala, C., & Gianoli, E. (2009). Effect of water availability on tolerance of leaf damage in tall morning glory, *Ipomoea purpurea*. *Acta Oecologica*, *35*(2), 236–242.

Baek, Y., Bobadilla, L. K., Giacomini, D. A., Montgomery, J. S., Murphy, B. P., & Tranel, P. J. (2021). Evolution of glyphosate-resistant weeds. *Reviews of Environmental Contamination and Toxicology*, *255*, 93–128.

Bastarache, P., Bouafoura, R., Omakele, E., Moffat, C. E., Vickruck, J. L., & Morin, P., Jr. (2023). Spinosad-associated modulation of select cytochrome P450s and glutathione S-transferases in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Archives of Insect Biochemistry and Physiology*, *112*(3), e21993.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Baucom, R. S., & Mauricio, R. (2004). Fitness costs and benefits of novel herbicide tolerance in a noxious weed. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(36), 13386–13390.

Baucom, R. S., & Mauricio, R. (2008). Constraints on the evolution of tolerance to herbicide in the common morning glory: resistance and tolerance are mutually exclusive. *Evolution; International Journal of Organic Evolution*, *62*(11), 2842–2854.

Bergelson, J., & Purrington, C. B. (1996). Surveying patterns in the cost of resistance in plants. *The American Naturalist*, *148*(3), 536–558.

Chaney, L., & Baucom, R. S. (2014). The costs and benefits of tolerance to competition in *Ipomoea purpurea*, the common morning glory. *Evolution; International Journal of Organic Evolution*, *68*(6), 1698–1709.

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings. Biological Sciences*, *283*(1824), 20152592.

Debban, C. L., Okum, S., Pieper, K. E., Wilson, A., & Baucom, R. S. (2015). An examination of fitness costs of glyphosate resistance in the common morning glory, *Ipomoea purpurea*. *Ecology and Evolution*, *5*(22), 5284–5294.

Desaint, H., Aoun, N., Deslandes, L., Vailleau, F., Roux, F., & Berthomé, R. (2021). Fight hard or die trying: when plants face pathogens under heat stress. *The New Phytologist*, *229*(2), 712–734.

Dewar, A. M., Haylock, L. A., Bean, K. M., & May, M. J. (2000). Delayed control of weeds in glyphosate□tolerant sugar beet and the consequences on aphid infestation and yield. *Pest Management Science*, *56*(4), 345–350.

Dimunová, D., Matoušková, P., Podlipná, R., Boušová, I., & Skálová, L. (2022). The role of UDP-glycosyltransferases in xenobiotic resistance. *Drug Metabolism Reviews*, *54*(3), 282–298.

Duke, S. O., Lydon, J., Koskinen, W. C., Moorman, T. B., Chaney, R. L., & Hammerschmidt, R. (2012). Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *Journal of Agricultural and Food Chemistry*, *60*(42), 10375–10397.

Edwards, R., Dixon, D. P., Cummins, I., Brazier-Hicks, M., & Skipsey, M. (2011). New perspectives on the metabolism and detoxification of synthetic compounds in plants. In P. Schröder & C. D. Collins (Eds.), *Organic Xenobiotics and Plants: From Mode of Action to Ecophysiology* (pp. 125–148). Springer Netherlands.

Fuchs, B., Saikkonen, K., & Helander, M. (2021). Glyphosate-modulated biosynthesis driving plant defense and species interactions. *Trends in Plant Science*, *26*(4), 312–323.

Gassmann, A. J., & Futuyma, D. J. (2005). Consequence of herbivory for the fitness cost of herbicide resistance: photosynthetic variation in the context of plant-herbivore interactions. *Journal of Evolutionary Biology*, *18*(2), 447–454.

Getman-Pickering, Z. L., Campbell, A., Aflitto, N., Grele, A., Davis, J. K., & Ugine, T. A. (2020). LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. *Methods in Ecology and Evolution / British Ecological Society*, *11*(2), 215–221.

Gharabli, H., Della Gala, V., & Welner, D. H. (2023). The function of UDP-glycosyltransferases in plants and their possible use in crop protection. *Biotechnology Advances*, *67*, 108182.

Gupta, A., Dixit, S. K., & Senthil-Kumar, M. (2016). Drought stress predominantly endures *Arabidopsis thaliana* to *Pseudomonas syringae* infection. *Frontiers in Plant Science*, *7*, 808.

Gupta, S., Harkess, A., Soble, A., Van Etten, M., Leebens-Mack, J., & Baucom, R. S. (2023). Interchromosomal linkage disequilibrium and linked fitness cost loci associated with selection for herbicide resistance. *The New Phytologist*, *238*(3), 1263–1277.

Hertlein, M. B., Thompson, G. D., Subramanyam, B., & Athanassiou, C. G. (2011). Spinosad: A new natural product for stored grain protection. *Journal of Stored Products Research*, *47*(3), 131–146.

Johnson, N. M., & Baucom, R. S. (2022). Dicamba drift alters plant–herbivore interactions at the agro□ecological interface. *Ecosphere*, *13*(11), e4274.

Kuester, A., Chang, S.-M., & Baucom, R. S. (2015). The geographic mosaic of herbicide resistance evolution in the common morning glory, *Ipomoea purpurea*: Evidence for resistance hotspots and low genetic differentiation across the landscape. *Evolutionary Applications*, *8*(8), 821–833.

Lambert, B., & Peferoen, M. (1992). Insecticidal promise of *Bacillus thuringiensis*. *Bioscience*, *42*(2), 112–122.

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution; International Journal of Organic Evolution*, *37*(6), 1210–1226.

Lefcheck, J. S. (2016). piecewiseSEM : Piecewise structural equation modelling in r for ecology,

evolution, and systematics. *Methods in Ecology and Evolution / British Ecological Society*, *7*(5), 573–579.

Lipok, J. (2009). Dual action of phosphonate herbicides in plants affected by herbivore--model study on black bean aphid *Aphis fabae* rearing on broad bean *Vicia faba* plants. *Ecotoxicology and Environmental Safety*, *72*(6), 1701–1706.

Mauricio, R. (1998). Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *The American Naturalist*, *151*(1), 20–28.

Mauricio, R., & Mojonnier, L. E. (1997). Reducing bias in the measurement of selection. *Trends in Ecology & Evolution*, *12*(11), 433–436.

Mayes, M. A., Thompson, G. D., Husband, B., & Miles, M. M. (2003). Spinosad toxicity to pollinators and associated risk. *Reviews of Environmental Contamination and Toxicology*, *179*, 37–71.

Mertz, F. P., & Yao, R. C. (1990). *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar mill rum still. *International Journal of Systematic Bacteriology*, *40*(1), 34–39.

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology*, *63*, 431–450.

Monsanto. (2017). *Roundup Weathermax Complete Directions for Use*. https://assets.greenbook.net/16-46-57-15-03-2018-63003R9-40_RuWMAX_524-537_SpecimenLabel_2017.pdf

Moretti, E. A., Taylor, A. G., Wickings, K., & Nault, B. A. (2021). Insights into how spinosad seed treatment protects onion from onion maggot (Diptera: Anthomyiidae). *Journal of Economic Entomology*, *114*(2), 694–701.

Nawaz, M., Sun, J., Shabbir, S., Khattak, W. A., Ren, G., Nie, X., Bo, Y., Javed, Q., Du, D., & Sonne, C. (2023). A review of plants strategies to resist biotic and abiotic environmental stressors. *The Science of the Total Environment*, *900*(165832), 165832.

Neve, P., Busi, R., Renton, M., & Vila-Aiub, M. M. (2014). Expanding the eco-evolutionary context of herbicide resistance research. *Pest Management Science*, *70*(9), 1385–1393.

Nychka, D., Furrer, R., Paige, J., & Sain, S. (2021). *fields: Tools for spatial data*. University Corporation for Atmospheric Research. https://github.com/dnychka/fieldsRPackage

Pandey, P., Ramegowda, V., & Senthil-Kumar, M. (2015). Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. *Frontiers in Plant Science*, *6*, 723.

Peterson, R. (2021). Finding optimal normalizing transformations via bestNormalize. *The R Journal*, *13*(1), 310.

Prasch, C. M., & Sonnewald, U. (2013). Simultaneous application of heat, drought, and virus to *Arabidopsis* plants reveals significant shifts in signaling networks. *Plant Physiology*, *162*(4), 1849–1866.

Rausher, M. D. (1992). The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution; International Journal of*

Organic Evolution, *46*(3), 616–626.

Rausher, M. D., & Simms, E. L. (1989). The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. *Evolution; International Journal of Organic Evolution*, *43*(3), 563–572.

R Core Team. (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Siemens, D. H., Lischke, H., Maggiulli, N., Schürch, S., & Roy, B. A. (2003). Cost of resistance and tolerance under competition: the defense-stress benefit hypothesis. *Evolutionary Ecology*, *17*(3), 247–263.

Simms, E. L., & Rausher, M. D. (1987). Costs and benefits of plant resistance to herbivory. *The American Naturalist*, *130*(4), 570–581.

Simms, E. L., & Rausher, M. D. (1989). The evolution of resistance to herbivory in *Ipomoea purpurea*. II. Natural selection by insects and costs of resistance. *Evolution; International Journal of Organic Evolution*, *43*(3), 573–585.

Simms, E. L., & Rausher, M. D. (1993). Patterns of selection on phytophage resistance in *Ipomoea purpurea*. *Evolution; International Journal of Organic Evolution*, *47*(3), 970–976.

Simms, E. L., & Triplett, J. (1994). Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution; International Journal of Organic Evolution*, *48*(6), 1973–1985.

Sparks, T. C., Dripps, J. E., Watson, G. B., & Paroonagian, D. (2012). Resistance and cross-resistance to the spinosyns – A review and analysis. *Pesticide Biochemistry and Physiology*, *102*(1), 1–10.

Stinchcombe, J. R., Rutter, M. T., Burdick, D. S., Tiffin, P., Rausher, M. D., & Mauricio, R. (2002). Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *The American Naturalist*, *160*(4), 511–523.

Strauss, S. Y., Rudgers, J. A., Lau, J. A., & Irwin, R. E. (2002). Direct and ecological costs of resistance to herbivory. *Trends in Ecology & Evolution*, *17*(6), 278–285.

Strauss, S. Y., Siemens, D. H., Decher, M. B., & Mitchell-Olds, T. (1999). Ecological costs of plant resistance to herbivores in the currency of pollination. *Evolution; International Journal of Organic Evolution*, *53*(4), 1105–1113.

Svensson, E. I., Arnold, S. J., Bürger, R., Csilléry, K., Draghi, J., Henshaw, J. M., Jones, A. G., De Lisle, S., Marques, D. A., McGuigan, K., Simon, M. N., & Runemark, A. (2021). Correlational selection in the age of genomics. *Nature Ecology & Evolution*, *5*(5), 562–573.

Tian, D., Traw, M. B., Chen, J. Q., Kreitman, M., & Bergelson, J. (2003). Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. *Nature*, *423*(6935), 74–77.

Tiffin, P., & Rausher, M. D. (1999). Genetic constraints and selection acting on tolerance to herbivory in the common morning glory. *Am. Nat.*, *154*, 700–716.

Van Etten, M., Lee, K. M., Chang, S.-M., & Baucom, R. S. (2020). Parallel and nonparallel genomic responses contribute to herbicide resistance in *Ipomoea purpurea*, a common agricultural weed. *PLoS Genetics*, *16*(2), e1008593.

Van Etten, M. L., Kuester, A., Chang, S.-M., & Baucom, R. S. (2016). Fitness costs of herbicide

resistance across natural populations of the common morning glory, I*pomoea purpurea*. *Evolution; International Journal of Organic Evolution*, *70*(10), 2199–2210.

Van Leeuwen, T., Dermauw, W., van de Veire, M., & Tirry, L. (2005). Systemic use of spinosad to control the two-spotted spider mite (Acari: Tetranychidae) on tomatoes grown in rockwool. *Experimental & Applied Acarology*, *37*(1-2), 93–105.

Vázquez, M. B., Moreno, M. V., Amodeo, M. R., & Bianchinotti, M. V. (2021). Effects of glyphosate on soil fungal communities: A field study. *Revista Argentina de Microbiologia*, *53*(4), 349–358.

Wang, J., Wan, Y., Zhang, Y., Yuan, J., Zheng, X., Cao, H., Qian, K., Feng, J., Tang, Y., Chen, S., Zhang, Y., Zhou, X., Liang, P., & Wu, Q. (2024). Uridine diphosphate glucosyltransferases are involved in spinosad resistance in western flower thrips *Frankliniella occidentalis* (Pergande). *Journal of Hazardous Materials*, *466*(133575), 133575.

Warwick, S. (1991). Herbicide resistance in weedy plants: Physiology and population biology. *Annual Review of Ecology, Evolution, and Systematics*, *22*, 95–114.

Zandalinas, S. I., & Mittler, R. (2022). Plant responses to multifactorial stress combination. *The New Phytologist*, *234*(4), 1161–1167.

Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., & Gómez-Cadenas, A. (2018). Plant adaptations to the combination of drought and high temperatures. *Physiologia Plantarum*, *162*(1), 2–12.

Zandalinas, S. I., Sengupta, S., Fritschi, F. B., Azad, R. K., Nechushtai, R., & Mittler, R. (2021). The impact of multifactorial stress combination on plant growth and survival. *The New Phytologist*, *230*(3), 1034–1048.

Zhang, G. M., & Baucom, R. S. (2024). Herbicidal interference: glyphosate drives both the ecology and evolution of plant-herbivore interactions. *The New Phytologist*, *245*(2), 807–817.

Zhang, Y., Liu, X., Yin, T., Li, Q., Zou, Q., Huang, K., Guo, D., & Zhang, X. (2021). Comparative transcriptomic analysis of two *Saccharopolyspora spinosa* strains reveals the relationships between primary metabolism and spinosad production. *Scientific Reports*, *11*(1), 14779.

FIGURES

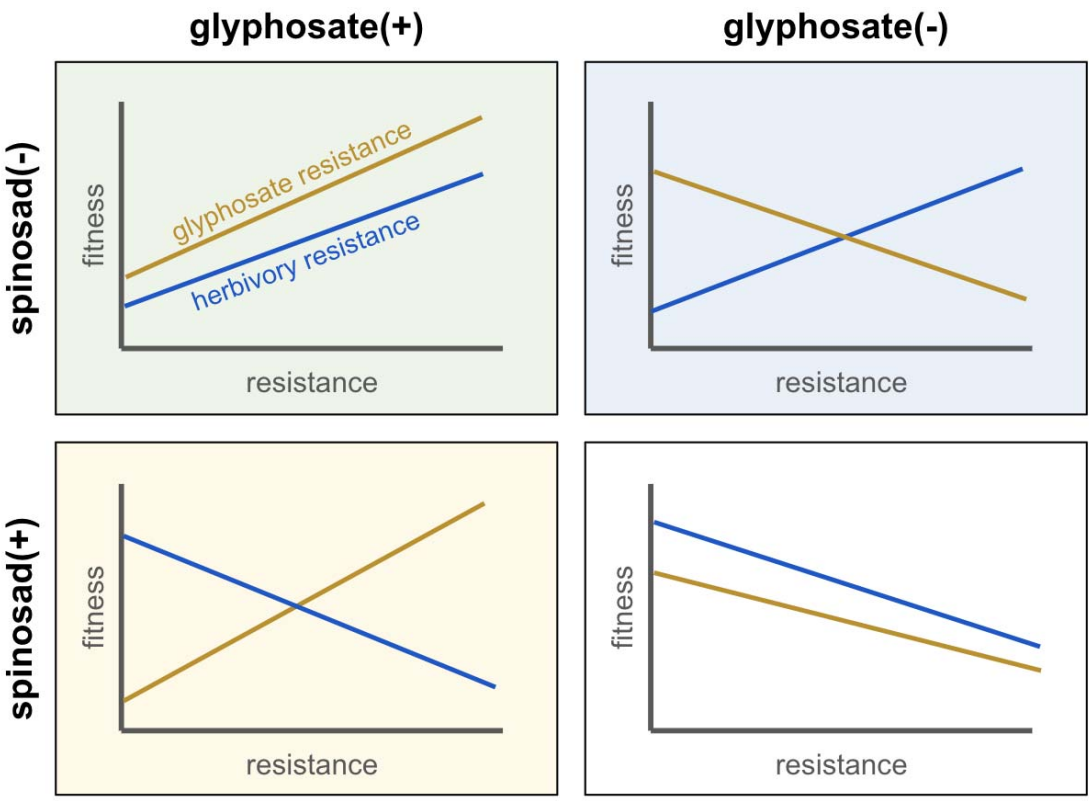

Figure 1. Factorial design of the common garden study, showing predictions of the fitness costs and benefits of glyphosate and herbivory resistance (gold and dark blue lines respectively) in the form of selection analyses in the four treatment environments: glyphosate-present, spinosad-absent (green), glyphosate-absent, spinosad-absent (blue), glyphosate-present, spinosad-present (yellow), and glyphosate-absent, spinosad-present (white).

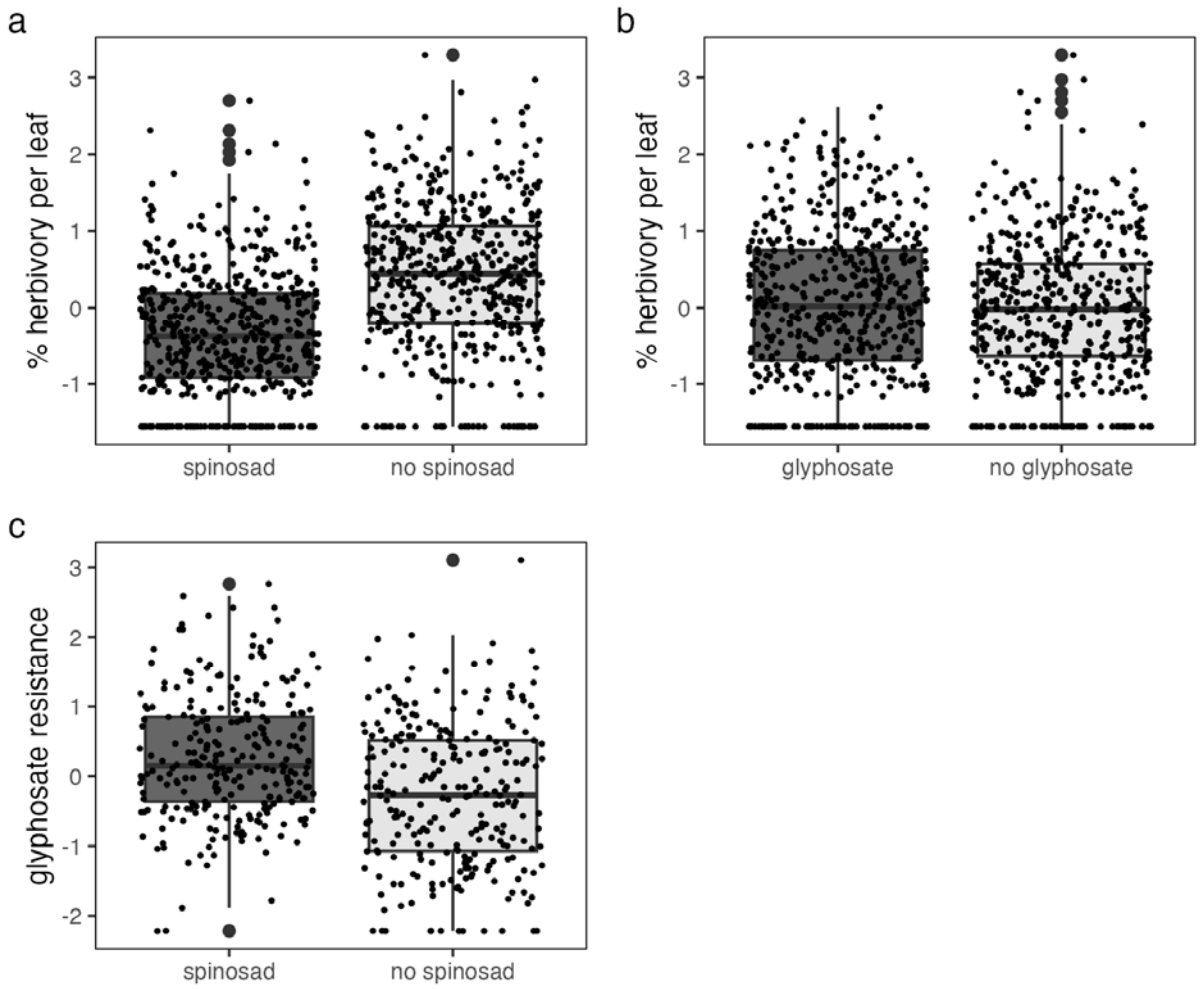

Figure 2. Treatment effects of glyphosate and spinosad application. Each point is an individual plant. (a) Spinosad-treated plants experienced less herbivory damage (treated plants: 0.15%, untreated plants: 0.53%; $F_1 = 174.06$, $p < 0.001$). (b) Glyphosate treatment did not have a significant effect on the amount of herbivory damage (treated plants: 0.37%, untreated plants: 0.30%; $F_1 = 2.14$, $p = 0.144$). (c) Spinosad-treated plants had higher levels of glyphosate resistance (treated plants: 69.43%, untreated plants: 55.14%; $F_1 = 71.83$, $p < 0.001$).

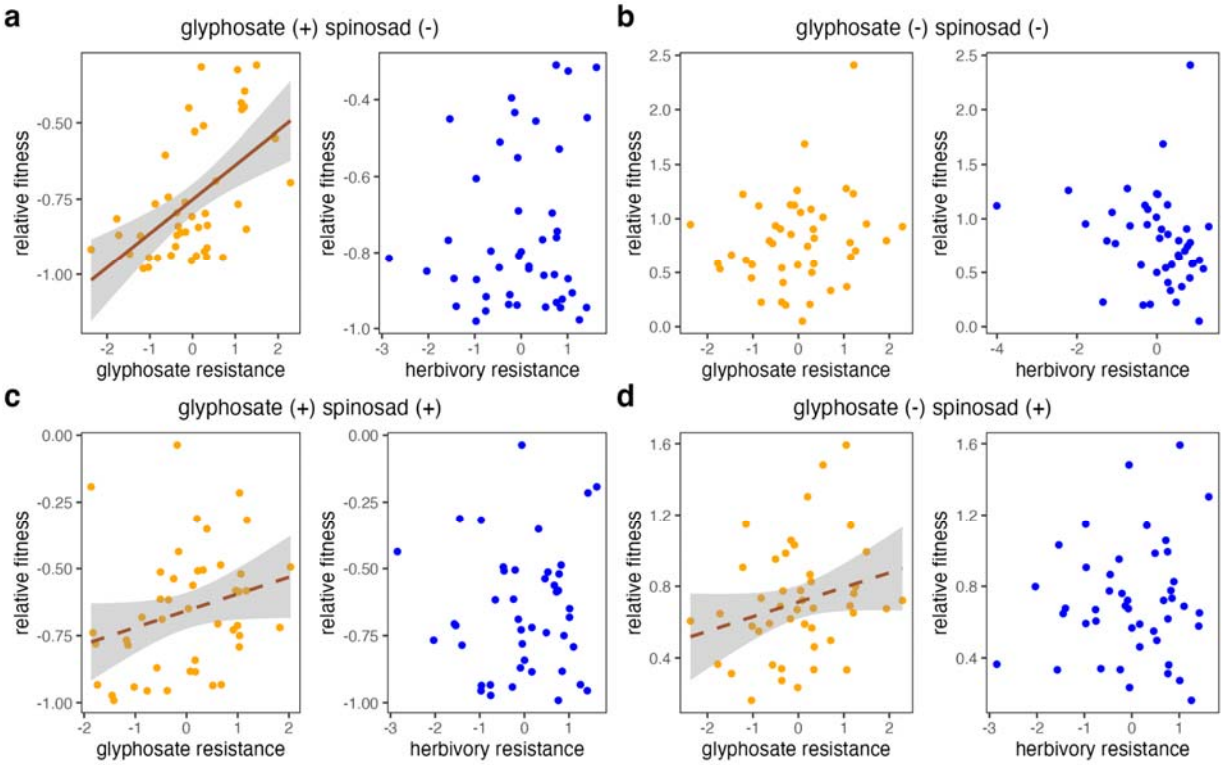

Figure 3. Selection analyses of resistance to glyphosate and herbivory in the four treatment environments, using seed count as the fitness estimate. (a) In the glyphosate-present, spinosad-absent environment, there was positive selection for glyphosate resistance ($\beta = 0.11$, $F_1 = 18.69$, $p < 0.001$). (b) In the glyphosate-absent, spinosad-absent environment, there was no selection acting on either glyphosate or herbivory resistance. (c) In the glyphosate-present, spinosad-present environment, there was potentially positive selection for glyphosate resistance ($\beta = 0.06$, $F_1 = 3.42$, $p = 0.071$). (d) In the glyphosate-absent, spinosad-present environment, there was no cost of glyphosate resistance, but rather potential positive selection ($\beta = 0.08$, $F_1 = 3.07$, $p = 0.087$).

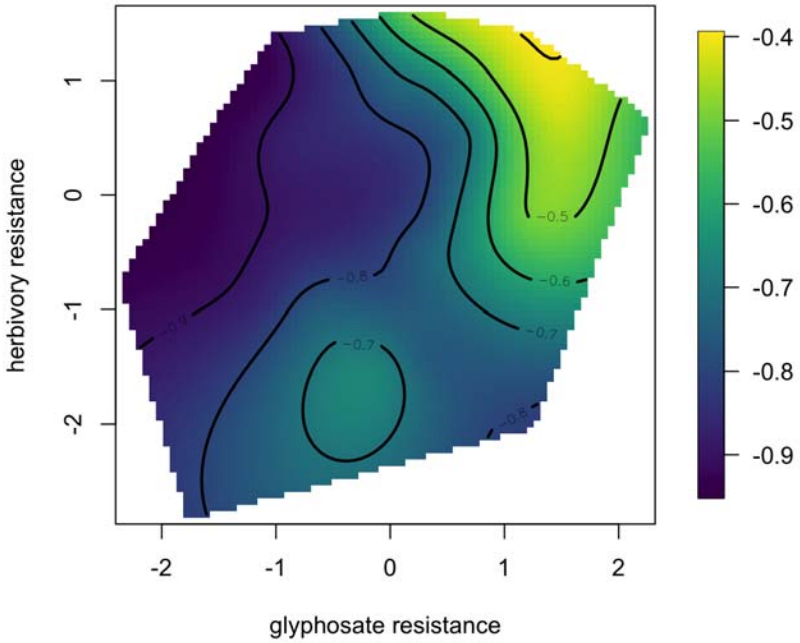

Figure 4. In the glyphosate-present, spinosad-absent environment (*i.e.*, herbivores are present), there is correlational selection for high levels of both glyphosate and herbivory resistance (γ = 0.06, F_1 = 5.10, p = 0.030).

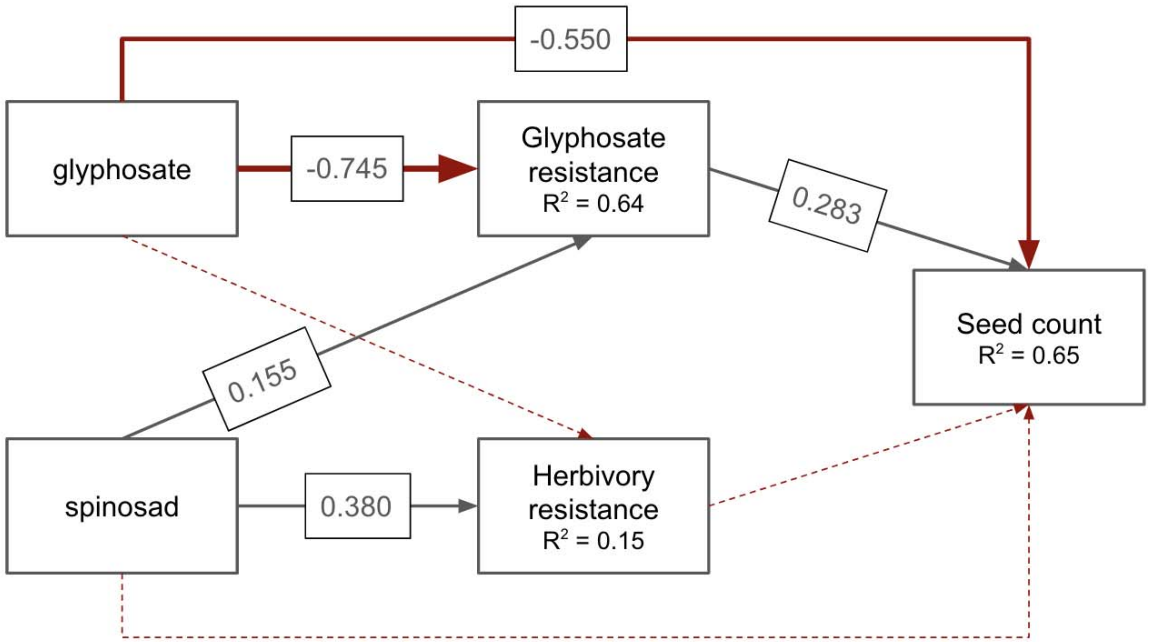

Figure 5. Structural equation model (SEM) of the relationships between glyphosate treatment, spinosad treatment, glyphosate resistance, herbivory resistance, and seed number. All models in the SEM are linear mixed models, with family line and block included as random effects. Boxes represent the measured variables and arrows show the unidirectional relationships between variables. Gray arrows indicate positive relationships and red arrows represent negative relationships. The thickness of the arrows reflect the magnitude of the standardized β coefficients, which are shown along the arrows. All solid arrows show statistically significant paths ($p < 0.001$), while dotted arrows show nonsignificant paths. Conditional r^2 values are included within the box of each response variable. This model fit well, with a Fisher's C score of 0.007 ($p = 0.996$).