Interspecific variation in resistance and tolerance to herbicide drift reveals potential consequences for plant community coflowering interactions and structure at the agro-eco interface

Veronica Iriart[1*], Regina S. Baucom[2], and Tia-Lynn Ashman[1]

[1] Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA;

[2] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA

[*] For correspondence. Email vei2@pitt.edu

- **Background and Aims** When plant communities are exposed to herbicide 'drift', wherein particles containing the active ingredient travel off-target, interspecific variation in resistance or tolerance may scale up to affect community dynamics. In turn, these alterations could threaten the diversity and stability of agro-ecosystems. We investigated the effects of herbicide drift on the growth and reproduction of 25 wild plant species to make predictions about the consequences of drift exposure on plant-plant interactions and the broader ecological community.
- **Methods** We exposed potted plants from species that commonly occur in agricultural areas to a drift-level dose of the widely used herbicide dicamba or a control solution in the glasshouse. We evaluated species-level variation in resistance and tolerance for vegetative and floral traits. We assessed community-level impacts of drift by comparing species evenness and flowering networks of glasshouse synthetic communities comprised of drift-exposed and control plants.
- **Key Results** Species varied significantly in resistance and tolerance to dicamba drift: some were negatively impacted while others showed overcompensatory responses. Species also differed in the way they deployed flowers over time following drift exposure. While drift had negligeable effects on community evenness based on vegetative biomass, it caused salient differences in the structure of coflowering networks within communities. Drift reduced the degree and intensity of flowering overlap among species, altered the composition of groups of species that were more likely to coflower with each other than with others, and shifted species roles (e.g., from dominant to inferior floral producers and vice versa).

- **Conclusions** These results demonstrate that even low levels of herbicide exposure can significantly alter plant growth and reproduction, particularly flowering phenology. If field-grown plants respond similarly, then these changes would likely impact plant-plant competitive dynamics and potentially plant-pollinator interactions occurring within plant communities at the agro-ecological interface.

Key words: coflowering, flowering time, herbicide, dicamba, network, phenology, pesticide, community, resistance, tolerance, weeds, wildflowers, agro-eco interface, anthropogenic stress, interspecific variation, drift, herbicide drift, dicamba drift.

INTRODUCTION

Wild plant communities at the agro-ecological interface (Bernardo *et al.*, 2018) are important reservoirs of plant diversity and support the maintenance of agro-ecosystems (Requier *et al.*, 2015; Ouvrard *et al.*, 2018). These assemblages of native and introduced species (Burdon and Thrall, 2007) contribute to a range of ecosystem services, including nutrient cycling (Altieri *et al.*, 1999), habitat structuring (Brooker, 2006), and food production (Marshall *et al.*, 2003; Beismeijer *et al.*, 2006). In particular, these communities produce critical floral resources that sustain pollinators, especially while crops are not in bloom (Holzaschuh *et al.*, 2008; Karamaouna *et al.*, 2019; Kati *et al.*, 2021).

Anthropogenic stressors associated with farming such as agrochemical pollution, however, have led to a 50% decrease in wild plant diversity over the past 70 years (Bretagnolle and Gaba, 2015). This loss combined with concurrent widespread declines in pollinator richness and abundance (Kluser and Peduzzi, 2007; Potts *et al.*, 2010) threaten the sustainability of agro-ecosystems and thereby the services they provide for human health, including the pollination of up to 75% of the world's leading food crops (Klein *et al.*, 2007). Thus, understanding the mechanisms by which agrochemical usage impacts wild plant communities is a key concern.

Non-target exposure to herbicides via drift pollution is a leading form of anthropogenic stress at the agro-ecological interface (Marshall *et al.*, 2003; Boutin *et al.*, 2014; Schütte *et al.*, 2017). Despite drift-levels being typically very low (~0.5-10% of the field application rate [Grover *et al.*, 1972; Egan *et al.*, 2014; Olszyk *et al.,* 2017]), they can significantly affect the growth and flowering of various plants. Depending on the herbicide, drift to susceptible plants can reduce vegetative size and growth as well as decrease or delay flower production (reviewed in Iriart *et al.*, 2020; later Ramos *et al.*, 2021 and Strandberg *et al.*, 2021).

However, some plants may be resistant to herbicide drift, i.e. able to prevent or limit damage incurred; while others may be tolerant, i.e. unable to prevent damage but able to buffer negative effects on fitness. Although herbicide resistance and tolerance can be defined in other ways as well (e.g., Neve and Powles, 2005; Devine 2005; Vieira *et al.* 2020), we follow the approach by Baucom and Mauricio (2004; 2008) and Baucom (2019) in this paper to define them within an evolutionary ecology framework (Table 1). In addition, at low concentrations, herbicides that mimic plant growth hormones such as auxin (e.g., 2,4-D, dicamba) may stimulate growth (Allender *et al.*, 1997; Guardiola and García-Luis, 2000; Belz and Duke, 2014), leading to drift effects more akin to overcompensation than herbicide damage (Table 1, Garcia and Eubanks, 2019). Plant-defense theory posits that because resources are finite, there will be a trade-off between resistance and tolerance (Fineblum and Rausher, 1995; Debban *et al.*, 2015; Mikaberidze and McDonald, 2020). Thus, within a community, there could be considerable interspecific variation in resistance or tolerance to herbicide drift, leading to community-level changes that affect higher-level processes.

These community-level repercussions could occur via changes in vegetative or flowering dynamics (reviewed in Iriart *et al.*, 2020). Sensitive species may not survive whereas resistant or tolerant ones may achieve greater vegetative biomass – outcomes that can affect community structure, i.e., species evenness (Hald, 1999; Mayerová *et al.*, 2018). For example, Egan *et al.* (2014) found that applications of ~1% of the field rate of the herbicide dicamba led to declines in forbs but left grasses unaltered, thereby changing the evenness of field plant communities. But this metric alone may not reflect interspecific variation in the timing of, and investment in, flowering which could in-turn change floral community structure.

Previous studies have shown the utility of network analyses to assess the impact of anthropogenic stressors on multi-species interactions (e.g., Hoffmiester *et al.*, 2015; Filipe-Lucia, 2020); therefore, we propose that a network approach can be used to inform on how herbicide drift affects floral community structure by documenting changes in patterns of coflowering (Arceo-Gomez *et al.*, 2018). For instance, if multiple plant species in a community are sensitive to drift and respond to exposure by shifting flowering phenology, then these changes could lead to less frequent and/or weaker coflowering interactions, indicating a reduction in the average diversity and abundance of floral resources available at a given time. In turn, this outcome may lead to decreased reproductive success among species due to reduced facilitation for pollinator visitation, especially when plant species share pollinators (Ghazoul, 2006). Moreover, variation in flowering response to herbicide drift could alter the composition of coflowering modules, i.e. groups of species that are more likely to overlap in flowering (Olesen *et al.*, 2007), or cause shifts in floral community dominance. Tolerant or overcompensated species may become novel network hubs that, by producing flowers consistently and coflowering with many species, are important for community stability (Bader *et al.*, 2007).

Altogether, these species-level impacts could scale up to affect community-level properties, especially connectance (the total number of links [coflowering interactions] relative to the total possible; Dunne *et al.*, 2002) and modularity (the difference in the fraction of links occurring within groups of species and the expected amount if links were distributed randomly; Olesen *et al.*, 2007; Brandes *et al.*, 2008). On a larger scale, these fluctuations could disrupt patterns of plant-plant competition or facilitation for pollinators that are mediated through trends in coflowering (e.g., Waser, 1979). Consequently, we propose a network perspective can provide a richer evaluation of the impacts of herbicide drift than separately examining components of coflowering, such as the timing, duration, or date of peak flowering (e.g., Poole and Rathcke 1979; Parra-Tabla and Vargas 2004; Forrest *et al.*, 2010).

While previous work reviewed studies of herbicide exposure on plants and called for more holistic approaches (Iriart *et al.*, 2020), it also emphasized the lack of a comprehensive understanding of how diverse plant species respond to sublethal herbicide levels and how these responses could influence community structure. To fill this gap in knowledge, in this study, we grew plants from 25 species collected from the agro-eco interface in a glasshouse environment and exposed half to a drift-level rate of an herbicide. We evaluated interspecific variation in herbicide drift tolerance and resistance and determined their impact on community metrics, such as species evenness or coflowering structure. We chose dicamba as our focal herbicide, a synthetic auxin, whose use to control eudicot plants has surged in the United States (Knezevic *et al.*, 2018; USGS, 2021a) and has been linked to unprecedented numbers of off-target exposures (US EPA, 2021).

Specifically, we ask five questions: 1) Do species vary in resistance or tolerance to dicamba drift? 2) Is there a trade-off between resistance and tolerance across species? 3) Does dicamba drift alter the probability of flowering, day of first flower, duration of flowering, and/or flower size, and if so, do these flowering responses vary among species? 4) Does dicamba exposure lead to community-level changes in species evenness or in metrics of coflowering interaction for a glasshouse synthetic community? And finally, 5) can changes in community-level interactions due to dicamba drift be explained by resistance or tolerance among species?

MATERIALS AND METHODS

Study species

We collected seeds from 25 agro-eco species (described in Table 2) in 2018 from 1-3 populations growing near soybean or fallow fields in southwest Kentucky and northeast Tennessee, USA (Supplementary data Table S1). Species occurred at varying frequencies across all surveyed sites, with the rarest species (*G. canadense*, *S. canadensis*, and *A. theophrasti*) observed about 4% of the time and the most common (*S. spinosa*, *I. lacunosa*, and *E. serotinum*) about 55% of the time (Iriart, Baucom, and Ashman, unpublished data). Species were mainly insect-pollinated (Table 2). Although three species were primarily wind-pollinated, insects can visit them when pollen sources are limited (Saunders, 2018; Ashman pers. obs.). Dicamba use at the time of seed collection was estimated to be high (> 1.1 L km^{-1}) according to the United States Geological Survey (USGS, 2021b) and conversations with local farmers (Ashman and Baucom, pers. obs.). Although we did not acquire detailed information about the history of dicamba in the area, our framework for defining resistance and tolerance sought to characterize standing variation for resistance and tolerance, rather than previous evolutionary histories (Table 1).

Experiment Set-Up

For each species, we planted 3-10 seeds in each of 22 pots (11.4 cm × 11.4 cm × 10.2 cm) filled with a 3:1 mixture of unfertilized Old castle C/B soil (45% Canadian Sphagnum Peat Moss, 35% Aged Pine Bark, 15% Perlite, 5% Vermiculite; BFG Supply Co., Burton, OH) and Germination Mix (65% Canadian Sphagnum Peat Moss, 25% Perlite, 10% Vermiculite; BFG Supply Co., Burton, OH) in the University of Pittsburgh glasshouse. We transplanted some seedlings (66 out 479) to new pots to make up for those with zero germination and thinned pots with multiple seedlings to one seedling per pot. Final sample sizes were 22 plants per species except nine species which had 6-21 plants. The average daily temperature was 25.6°C ± 1.6 and daylength ranged from 12-16 hours throughout the experiment (20 May – 8 Nov. 2019). We supplied water as needed and fertilized plants once with 0.2 g of Osmocote 14 N -14 P-14 K (ICL Specialty Fertilizers, Ltd., Dublin OH).

Herbicide Treatments

We divided 16-day old plants into two groups with 3-10 plants per species, then treated them with one of two levels of dicamba (3,6-dichloro-o-anisic acid, Albaugh, LLC, Ankeny, IA): 0% ('control') or 1% ('drift') of the field application rate of 561 g of active ingredient per hectare (Albaugh, 2018). The drift treatment represented a particle drift rate, i.e., when herbicidal particles travel away from application sites by wind (Felsot *et al.*, 2011). While the control and drift treatments related directly to our research questions, we also treated a third group with 2 plants per species with 100% of the field application rate of dicamba to confirm the effectiveness of our dicamba stock and to identify any unaffected species (i.e., alive at 145 days post-treatment of this high dosage). All treatments included 'Preference' surfactant (non-ionic surfactant blend, WinField Solutions, St. Paul, MN) at 0.1% v/v and were applied with a handheld multi-purpose sprayer with an adjustable nozzle (Chapin International Inc., Batavia, NY, USA; Model #1002; operating pressure = 40-60 PSI; flow rate = 1.5-2.3 L minute^{-1}) set to a medium-fine mist. Plants were sprayed until they were just wet. We randomized plants by treatment and species across each bench in the glasshouse.

Data Collection

Twenty-four hours prior to applying herbicide treatments, we counted the total number of leaves and measured the longest leaf with a digital caliper to the nearest 0.1 mm and used the product of these to estimate 'pre-treatment plant size.' We used this metric to estimate plant size because it could easily be standardized across our species set which included plants of various heights and life forms (Table 2). Forty-eight hours after treatment, we assessed damage by enumerating the number of leaves showing typical symptoms of dicamba injury, i.e., leaf cupping or twisting (Foster and Griffin, 2018; Griffen *et al.*, 2013). Given our general definition of resistance (Table 1), an instantaneous measure of damage ('proportion of undamaged leaves' = 1 — # damaged leaves 48 hours after treatment/total # leaves pre-treatment) is appropriate. This measure also accounts for the fact that synthetic auxins may positively or negatively affect growth at low concentrations (Gianfagna, 1995; Kelley and Riechers, 2007; Grossman, 2010) and initial leaf damage could impact downstream flower production (Mothershead and Marquis, 2000; Jacobsen and Raguso, 2018).

Since we could not measure reproductive success directly across all species given that the majority required insect pollination (Table 2), we measured two standard fitness proxies to assess tolerance (Table 1): 1) 'short-term growth' estimated from plant size at 21 days post-treatment and 2) 'final biomass' of shoots harvested at 145 days post-treatment, dried at 70°C for at least 48 hours, and weighed to the nearest 0.01 g (Mettler AE200 Analytical Balance, Mettler-Toledo International Inc., Columbus, OH). Plant size and biomass are known to positively correlate with fecundity, especially for plants of the same age as those in our study (reviewed in Younginger *et al.,* 2017).

We recorded the 'day of first flower' and counted the total number of open flowers (i.e., 'floral display') per plant 2-3 times per week from 15 July to 8 Nov. Thus, 'flowering duration' reflected the count in days from the first day flowers were present to the last day flowers were present or at the end of experiment (for four species; see Supplementary data Table S2 for details). On each flowering plant, we collected 2-5 of the first 10 open flowers, dried them on silica gel and weighed each to the nearest 0.1 mg (Mettler AE200) to obtain dry 'biomass per flower.' For *E. annuus*, *P. lanceolata*, *P. virginica*, and *T. officionale*, which had extremely small (~3-25 mm long) flowers clustered into heads or spikes, we counted and sampled biomass at the flowerhead or spike-level.

Statistical Analyses

We performed all statistical analyses, unless otherwise specified, in R version 3.6.1 (R Core Team 2019), using linear, mixed-effects linear, and generalized linear models via the *lm*, *lmer,* and *glm* functions, respectively, from the *lme4* package (Bates *et al.*, 2015; see Supplementary data Table S3 for a full list and description of all linear models). All models included data from the control and drift treatments only. We graphically inspected residuals of all response variables for normality using the *ggqqplot* function (*ggpubr* package; Kassambara, 2019) and performed square-root or log-transformation as needed to meet model assumptions. If graphical assessments and kolmogorov-smirnov tests (*ks.test* function; R stats Library) confirmed non-normality of both transformed and original scales of measurement, then we performed the nonparametric Wilcoxon Mann-Whitney Test (*wilcox.test* function; R stats library). We tested significance of fixed effects with type III sums of squares using the *Anova* function (*car* package; Fox and Weisberg, 2019) and of correlations using Pearson's product moment correlation coefficients (Sharma, 2005) using the *cor* function from the R stats library. All figures, unless otherwise specified, were created using the *ggplot2* package (Wickham, 2016).

Species-level analyses

To analyze the response variable 'proportion of undamaged leaves', we performed a linear model where only plants in the drift treatment were analyzed and species was the sole explanatory variable, because control-treated plants showed no evidence of leaf damage. For all other dependent variables, including short-term growth, final biomass, day of first flower, flowering duration, floral display, and biomass per flower, we ran linear models which contained the explanatory variables: species, treatment, and the species × treatment interaction. Models assessing effects on short-term growth and final biomass included 'pre-treatment plant size' as a covariate. The short-term growth model additionally included the random effect 'transplanted' (a binary variable) to account for any effects of transplantation (see Methods).

We used a generalized linear model with the Poisson distribution (Katti and Rao 1968) to analyze herbicide effects on flowering duration. We also ran two models, with and without short-term growth as a covariate, for flowering duration as well as floral display, to determine the extent to which drift effects on flowering production and duration were dependent on growth effects. Some species were removed from some models due to leaf drop (short-term growth: *D. illinoensis*), or nonflowering/inadequate replication (see Table 2 for species that were removed for day of first flower, flowering duration, floral display, and biomass per flower analyses).

To characterize species-specific resistance, given species was a significant predictor of 'proportion undamaged leaves', we ran independent sample *t*-tests to determine whether species' estimated marginal means (Searle *et al.*, 1980) significantly differed from one (Table 1). We used the *emmeans* (*emmeans* package; Lenth, 2020) and *test* functions to calculate estimated marginal means and perform *t*-tests, respectively.

To characterize species-specific tolerances, given a significant species × treatment effect for tolerance variables (Table 1), we calculated contrast estimates for each species using the *contrast* function (Abdi and Williams, 2010; *emmeans* package). Contrast estimates reflected the difference in the estimated marginal means for tolerance between treatments (e.g., growth in drift subtracted by that in control). Thus, we used the degree to which species estimates differed from zero to describe species' tolerances to drift (Table 1). We also used contrast estimates to detail species' responses to drift via flowering time, duration, floral display and biomass per flower.

To explore whether there was a tradeoff between resistance and tolerance on either time scale across species, we estimated correlations between standardized estimated marginal means for resistance and contrast estimates for tolerance variables using z-scores. To elucidate whether long-term responses to dicamba drift could be predicted from short-term responses, we regressed z-scores of short-term tolerance on long-term tolerance.

To test whether dicamba drift affected the probability of flowering and whether it varied by species, we performed a chi-squared test of independence and a chi-squared test of homogeneity (Stuart, 1955), respectively. Some species were excluded from this analysis due to low replication (see Table 2 for details).

To account for shared evolutionary histories, we created a phylogenetic tree (Supplementary data Methods S1, Fig. S1) and used it to conduct associated phylogenetic models for all response variables. However, phylogenetically-controlled models performed worse than models which did not account for phylogeny based on Akaike Information Criterion (AIC) model selection (AIC values were two or more units higher; Supplementary data Table S4; Akaike, 1973); thus, we present results from the latter models only.

Community-level analyses

To assess the effects of dicamba drift at the community scale, we considered data from all plants in the drift treatment as one synthetic community and all control plants as another.

To compare the control community against the drift for species evenness, we used species mean final biomass data to estimate each community's Shannon's Equitability Index (E_H; Kent, 1992):

$$E_{\mathrm{H}} = [\ \textstyle\sum_{i=1}^{S} p_i \ln(p_i)\ ]\ /\ \ln(S)\ .$$

Here, S is the number of species in the community and p_i is the relative proportion of species i.

To evaluate dicamba drift effects on community-wide patterns of coflowering, we estimated a coflowering index for every pair of plant species within each community using the daily number of open flowers. This index was adapted from Schoener's index (*SI*) of niche overlap (Schoener, 1970) as applied to flowering (following Arceo-Gomez *et al.*, 2018):

$$SI = 1 - \tfrac{1}{2}\textstyle\sum_k \left| p_{ik-}\ p_{jk} \right|$$

where p_{ik} and p_{jk} are the proportion of open flowers by species i and j, respectively, occurring on day k. *SI* ranges from 0 (no flowering overlap, i.e. the absence of potential interaction) to 1 (complete flowering overlap, i.e. maximum potential interaction). By inputting *SI* values into the program Gephi, version 9.2 (Bastian *et al.*, 2009), we constructed weighted, unipartite networks for both communities (see Fig. 1 for a schematic that contrasts two hypothetical coflowering networks).

We characterized several species-level network properties, including degree (the average number of times that plant species interact with each other by coflowering; Fig. 1), strength (average *SI* value for coflowering, i.e. the intensity and duration of flowering overlap; Fig. 1), weighted degree (degree weighed by strength; i.e., the mean sum of interaction strengths across species), and betweenness centrality (the relative importance of species to network stability as measured by the average percentage of shortest paths in the coflowering network that must go through a species; Fig. 1) and assessed how these were impacted by community type (control and dicamba drift) using Wilcoxon Mann-Whitney tests. We conducted each of these analyses twice, once where each community included the 'full' set of 22 species that flowered in at least one treatment and again where each network included only the 'subset' 19 species that had at least one flowering plant in both treatments (see Table 2 for species that were removed from full and subset network analyses). In this way, we gauged whether network differences were due to species-level differences in flowering propensity in full networks or due to changes in flowering pattern alone in subset networks.

Further, we identified community-level flowering properties by estimating network connectance and modularity using Gephi (the Blondel *et al.* [2008] optimization algorithm at a 1.0 resolution estimated modularity).

To test whether observed differences in species evenness, connectance, and modularity between communities were significant, we simulated two random communities of 25 species by taking random samples of datapoints without replacement from our mean final biomass data or *SI* data for flowering. We then counted the number of times out of at least 100 iterations that the difference in E_H, connectance, or modularity between two random communities was greater than or equal to the actual difference between the control and drift community.

To address whether changes in critical species-level coflowering metrics (Fig. 1) that occurred between the drift and control communities are related to resistance or tolerance to dicamba drift, we calculated the change in these metrics between the two full networks (i.e., metric value in drift network subtracted by that in control) for each species. We then estimated correlations between these changes and resistance, short-term tolerance, and long-term tolerance (Table 1).

RESULTS

Do species vary in resistance or tolerance to dicamba drift?

We found that species was a significant predictor of resistance to dicamba drift ($F_{24,188}$, $P < 0.001$; Supplementary data Table S3). Most species (21 out of 25) showed significant signs of dicamba-related injury as the proportion of undamaged leaves 48 hours after treatment ranged from 0.85 to 0.26 (Fig. 2A; Supplementary data Fig. S2A, Table S5). Remarkably, four species showed no signs of damage: *S. spinosa, C. virginica, A. theophrasti,* and *I. lacunosa* (Fig. 2A).

Dicamba drift did not have a uniform effect on growth or biomass across species ($P > 0.7$ for both; Supplementary data Fig. S3). Rather, its effect on both measures was highly influenced by species (treatment × species interaction: all $P < 0.001$; Supplementary data Table S3). Species-level variation in tolerance occurred at both time scales. One quarter of the species were intolerant at 21 days post-treatment (Fig. 2B; Supplementary data Table S6). On the longer timeframe, however, most species showed tolerance; but several still showed significant reductions in biomass (by 13-39%; Fig. 2C; Supplementary data Table S7). Interestingly, two species overcompensated in response to drift exposure — drift-treated *P. philadelphica* plants grew significantly larger (by 50%) in the short term than controls and *P. virginica* (by 25%) in the long term (Fig. 2B-C; Supplementary data Fig. S2B-C, Fig. S4).

All species except four were killed by 100% of the field application rate of the herbicide dicamba (three eudiocots: *O. stricta*, *P. lanceolata*, and *P. virginica*; and one monocot: *C. virginica*).

Is there a trade-off between resistance and tolerance?

Resistance and long-term tolerance were not significantly correlated across species ($r = 0.26$, d.f. = 23, $P = 0.22$; Supplementary data Fig. S5A). However, short-term tolerance did predict long-term tolerance ($r^2 = 0.25$, d.f. = 22, $P = 0.01$; Supplementary data Fig. S5B).

Does dicamba drift affect flowering?

Flowering time was marginally significantly delayed due to drift (by 8 days, $F_{1,224} = 3.31$, $P = 0.07$, Supplementary data Fig. S6A). It was also affected by species ($F_{16,224} = 26.70$, $P < 0.001$) and its interaction with treatment ($F_{16,224} = 7.38$, $P < 0.001$; Supplementary data Table S3). Two out of the 17 species were significantly delayed in producing their first flower relative to controls (Fig. 2D; Supplementary data Table S8), *T. officinale* and *T. pratense* (41 and 47 days later, Fig. 2D); while one, *I. lacunosa*, was significantly accelerated in flowering (11 days earlier, Fig. 2D; Supplementary data Fig. S7A). All others showed only modest or no effects. Beyond flowering initiation, species ($X^2 = 2152.34$, d.f. = 16, $P < 0.001$) and treatment ($X^2 = 6.38$, d.f. = 1, $P = 0.012$) were significant predictors of flowering duration. On average, drift shortened flowering duration by six days, but a significant treatment × species interaction ($X^2 = 335.72$, d.f. = 16, $P < 0.001$) also suggested that this result varied significantly in intensity and direction depending on species identity (Supplementary data Fig. S6B, Fig. S7B, Table S3). Contrast analyses revealed that about 50% of species flowered for a shorter period of time (by 4 – 41 days) in the drift treatment relative to the control, while a small portion flowered for longer (by 8 –12 days), and the remainder were unchanged (Fig. 2E; Supplementary data Table S2). These results were mostly unaffected when short-term growth was included as a covariate in the analysis (Supplementary data Fig. S8). In this case, short-term growth was likewise a significant predictor of flowering duration ($X^2 = 38.52$, d.f. = 1, $P < 0.001$) along with treatment ($X^2 = 6.33$, d.f. = 1, $P = 0.012$), species ($X^2 = 2189.94$, d.f. = 16, $P < 0.001$) and their interaction ($X^2 = 361.95$, d.f. = 16, $P < 0.001$). For two species *(I. lacunose and E. serotinum),* differences between treatments became significant after accounting for short-term growth. The fact that significant changes in flowering duration for species were maintained in this way suggests that the drift effect went beyond what was mediated by plant size.

Neither drift, nor its interaction with species, significantly affected the probability of flowering (treatment effect: $X^2 = 1.03$, d.f. = 1, $P = 0.31$; species × treatment effect: $X^2 = 0.65$, d.f. =

13, P = 1.0) or size of floral display (treatment effect: $F_{1,203}$ = 0.341, P = 0.56; species × treatment effect: $F_{16, 203}$ = 0.734, P = 0.76). Floral display, however, did vary among species ($F_{16,203}$ = 76.02, P < 0.001; Supplementary data Table S3). These results remained consistent when short-term growth was a covariate in the model, as it was not a strong predictor of floral display ($F_{1,202}$ = 1.38, P = 0.24).

Species ($F_{16,197}$ = 204.62, P < 0.001) and its interaction with treatment ($F_{16,197}$ = 1.82, P < 0.05), but not treatment alone ($F_{1,197}$ = 2.79, P = 0.10; Supplementary data Fig. S6C) affected biomass per flower (Supplementary data Table S3). One species (*A. palmeri*) responded to drift by producing 50% smaller flowers (P < 0.01), but all other species were not significantly affected (Fig. 2F; Supplementary data Table S9, Fig. S9).

Does dicamba exposure lead to community-level changes in species evenness or in metrics of coflowering interaction for a glasshouse synthetic community?

Potential community-level effects of drift exposure were assessed by assembling control and dicamba drift-treated plants into two separate 'synthetic' communities. Evenness based on biomass of control and dicamba drift-treated synthetic communities was not affected by dicamba drift (P = 0.98; Supplementary data Fig. S10).

In contrast, dicamba drift significantly decreased average degree (by 23%), strength (by 32%), and weighted degree (by 30%) of coflowering community networks (Table 3; Fig. 3A-B). These shifts resulted in a reduction in overall connectance (23% less) and increase in modularity (49% more) of the drift-exposed flowering community (Table 3). These changes were larger than expected by chance alone (P < 0.01). Analyses constrained to have at least one flowering plant per species in *both* treatments (i.e., subset networks with n =19 species) showed similar results although slightly fewer statistically significant differences (Supplementary data Fig. S11A-B; Table S10). Not only was connectivity reduced by dicamba drift, but the identity of the most important species in the community shifted (Fig. 3C-D)—while the control network contained numerous species with the highest betweenness centrality value of 3 (*T. pratense, C. halicacabum, D. carota,* and *P. pennsylvanica*), the drift network only contained two species with high betweenness centrality values of 16 (*C. halicacabum*) and 11 (*A. theophrasti*), and all other species values were at least 66% lower than that (Fig. 3C-D). Although less extreme, the subset network showed similar trends (Supplementary data Fig. S11C-D).

Are drift-induced changes in coflowering interactions or roles within the community correlated with resistance or tolerance across species?

The change in some species-level network parameters were correlated with long-term tolerance or resistance (tolerance-weighted degree: r = 0.46, d.f. = 20, P = 0.033; Fig. 4A; tolerance-betweenness centrality: r = 0.41, d.f. = 20, P = 0.061; Fig. 4B; resistance-betweenness centrality: r = 0.419, d.f. = 20, P = 0.052) but not others (resistance-weighted degree: r = -0.018, d.f. = 20, P = 0.93). We also tested for significant correlations between short-term tolerance and changes in network parameters and found no significant relationships (P > 0.6). Thus, drift effects on vegetative growth in the long term are more indicative of downstream effects on species' coflowering dynamics within their plant community than those on the short term.

DISCUSSION

This study demonstrated considerable variation in resistance and tolerance to dicamba drift among species common to the agro-ecological interface. Few of the species even showed overcompensation in response to this herbicide. Drift effects extended to flowering traits by impacting day of first flower, flowering duration, and flower size for some species but not others. These variable species-level effects transcended to community-level impacts, especially for coflowering structure in our glasshouse communities. Specifically, dicamba drift significantly decreased and weakened coflowering interactions and the direction of change in species roles within the community could be predicted from species' degree of tolerance.

Among-species variation in resistance and tolerance to dicamba at a very low, drift-level rate is consistent with previous findings of interspecific variation in LD_{50} for dicamba (Boutin *et al.*, 2014; Olszyk *et al.*, 2015). However, we identified new species with potential resistance to dicamba drift. For example, while we expected *C. virginica* to show resistance (Fig. 2A), because it is a monocot and dicamba is designed to target eudicot species, we did not anticipate finding resistance for four additional species (*S. spinosa*, *A. theophrasti*, *I. lacunosa*, and *P. lanceolata*; Fig. 2A). In addition, while the majority of species showed significant signs of dicamba damage at 48 hours post-treatment, only one-quarter of species demonstrated significant fitness losses in the short or long term, i.e. decreases in size at 21 days post-herbicide treatment and final biomass (Fig. 2B-C). This result suggests that some species may be capable of recovering from initial damage due to dicamba drift exposure over time. Such an outcome has been documented before with sub-lethal levels of herbicides, including dicamba (Carpenter and Boutin, 2010; Ramos *et al.*, 2021).

To our knowledge, our study is also the first to demonstrate that dicamba drift can have significant positive effects on growth for some species (Fig. 2B-C). This response has previously been observed with low-dose applications of other synthetic auxins on crops to stimulate growth (Agustí *et al.*, 2002; Gianfagna, 1995). We suspect these species may have overcompensated in response to the moderate stress induced by drift exposure, similar to what can be caused by other herbicides or herbivory (Agrawal, 2000; Belz and Duke, 2014; Vieira *et al.*, 2020). Alternatively, since auxins are known to stimulate cell elongation in shoots and initiate the formation of new leaves, these species might use low doses of dicamba to increase growth (Liscum *et al.,* 2014; Xiong and Jiao 2019). Thus, while sublethal herbicide stress is typically expected to affect plants in a neutral or negative manner (reviewed in Iriart *et al.*, 2020), this finding suggests that exposure to dicamba drift might enhance fitness for at least a handful of species, potentially influencing competitive dynamics in agro-ecosystems.

Despite the noted interspecific variation in resistance and tolerance to dicamba drift and past research showing that shifts in evenness from sensitive species to tolerant ones can occur in herbicide-exposed communities (reviewed in Iriart *et al.*, 2020; later Qi *et al.*, 2020), we did not detect such a change in our synthetic communities (Supplementary data Fig. S10). Instead, we found that the drift treatment, while strong enough to significantly affect some species' growth, was not potent enough to affect species evenness based on biomass in the glasshouse environment where plants were not competing for resources.

Additionally, while previous work showed trade-offs between resistance and tolerance to stressors such as herbicides (Baucom and Mauricio, 2008), we did not find this negative correlation in response to dicamba drift across species (Supplementary data Fig. S5A). The lack of a trade-off could potentially be explained by variation in mechanisms of resistance. While resistance evolution to herbicides like glyphosate are commonly attributed to mutations in herbicide-targeted biosynthetic pathways, synthetic auxins do not target a specific pathway. Consequently, resistance evolution to auxinic herbicides is more complex and multiple resistance mechanisms have been found (Mithila *et al.*, 2011; Goggin *et al.*, 2016; Goggin *et al.,* 2018). Thus, if experimental plants varied in resistance mechanisms, this variation may have traded off with other life-history traits beyond tolerance (e.g. reduced seed set, mutualistic interactions, or increased disease susceptibility; Vila-Aiub *et al.*, 2009 and Baucom, 2019; Cousens and Fournier-Level, 2018). The lack of a relationship between resistance and tolerance may also suggest that instantaneous measures of damage do not reflect impacts on plant fitness, although tolerance reflected in short-term growth is a good proxy for long-term tolerance, i.e. final biomass (Supplementary data Fig. S5B).

Our results fill the gap in knowledge of the effects of sublethal herbicide exposure on floral traits and reveal striking effects of interspecific variation on these outcomes. While Bohnenblust *et* *al.* (2016) found that dicamba drift decreased flower production and delayed flowering in two agro-eco species (*Medicago sativa* and *Eupatorium perfoliatum* L.), we did not detect an overall trend in flower production. Our results do, however, corroborate Bohnenblust et al. (2016) in terms of drift delaying flowering for some species.

The most striking result was the wide range of flowering phenological responses to dicamba drift, including flowering initiation and duration. As expected, the species that experienced the largest decrease in flowering duration under drift conditions were also those that were the most delayed in day of first flower and vice versa (Fig. 2D-E). The four-month 'season' in our glasshouse community is analogous to what these species experience in nature. Therefore, these detected shifts in flowering phenology are likely to have important ecological implications. For instance, extreme delays in flowering onset may lead to reduced pollination or insufficient time to accumulate resources and maximize investment in seed production following pollination. Meanwhile, accelerations in flowering can cause phenological mismatch between flowering period and pollinator emergence (e.g., Kudo and Ida, 2013). Further, while an increase in flowering duration could benefit plants by increasing the potential for reproduction if pollinators are present (Barber *et al.*, 2015), a decrease in flowering duration could have the opposite effect, leading to a decrease in reproductive output (Jin *et al.*, 2015).

In our synthetic communities, we uncovered that interspecific variation in the deployment of flowers following herbicide exposure can lead to profound changes in coflowering network properties. In particular, simulations showed that the dicamba drift community was significantly less connected but more modular than the control community, meaning drift exposure resulted in less flowering overlap and more exaggerated differences in flowering time among species. Moreover, drift reduced the frequency of flowering time overlaps and the quantity of open flowers overlapping (network degree and strength; Fig. 3; Table 3). Most important perhaps is that the identities of species within modules was changed. For example, species whose flowering durations were significantly lengthened or shortened due to drift (e.g., *A. palmeri* and *C. virginica*'s; Fig. 2E) experienced a drastic change in the composition of their interacting module partners between networks (Fig. 3). If these patterns hold under field conditions, then they could impact heterospecific pollen transfer, pollen limitation, and/or resources for pollinators (Ashman *et al.*, 2004, Ashman and Arceo-Gómez, 2013; Fang and Huang 2013; Vitt *et al.*, 2020; Arceo-Gómez, 2021).

Species roles within the two communities also changed and these were correlated with their tolerance to dicamba drift (Fig. 4). Specifically, the most tolerant species either increased or maintained their ability to provide strong and plentiful connections (i.e., high weighted degree values) under dicamba drift, whereas the least tolerant species incurred the greatest devaluation in coflowering interactions between communities (Fig. 4A). By the same token, the species that experienced the largest increase in their role as network hubs (i.e., greatest change in betweenness centrality due to drift), were all tolerant whereas the least tolerant species decreased considerably in importance from the control to the drift community (Fig. 4B). Thus, it is possible that wild coflowering networks affected by dicamba drift may likewise experience shifts in flowering dominance in favor of more drift-tolerant species.

These results add significantly to the growing body of novel work employing network analysis to characterize complex ecological interactions and monitor anthropogenic impacts on natural communities (Gray *et al.*, 2014; Watts *et al.*, 2016; Leite *et al.*, 2018). Specifically, our findings support previous work describing human-mediated impacts on connectance (e.g., Doré *et al.*, 2020), modularity (e.g. Larson *et al.*, 2016), or the identities of dominant species (e.g., O'Gorman *et al.*, 2012). Thus, we argue that by providing a rich characterization and evaluation of an herbicide-stressed plant community, network analysis allowed us to make refined predictions about the consequences of herbicide drift for pollinator-mediated plant-plant interactions.

One potential outcome of plant communities becoming more modular and less connected due to herbicide drift may be decreased facilitation, especially if plant species jointly attract shared pollinators (Moeller, 2004; Ghazoul, 2006; Mitchel *et al.,* 2009). On the contrary, if pollinators are limited, then plant species in less connected, more modular communities may experience less competition for pollinators, since they are able to occupy different flowering niches (i.e., modules) and therefore more evenly engage with pollinators (Waser, 1978; Rathcke, 1988; Liao *et al.*, 2011; Albor *et al.*, 2019). The network perspective also allows for identification of species key to community structure and stability under herbicide-stressed and control communities (Fig. 3). Specifically, our results suggest herbicide-stressed communities are more vulnerable to breakdown, since species that can produce flowers consistently throughout the growing season and thereby serve as pollination bridges while other species are not flowering (Arceo-Gomez *et al.*, 2018) would be scarce relative to unstressed communities. Further, the extinction of these species could be detrimental (Bascompte and Jordano, 2007; Martín Gonzàlez *et al.*, 2010).

Beyond pollinator-mediated plant-plant interactions, the consequences of herbicide drift on plant communities have important implications for pollinators as well, because patterns of coflowering reflect nectar and pollen resource availability. Thus, if herbicide drift results in less connected plant communities with limited key flowering species, then pollinators will have less abundant and diverse floral resources available to them on average as well as less plant species to utilize as resource bridges during significant resource gaps (Timberlake and Memmot, 2019).

In conclusion, our study provides strong evidence that herbicide pollution, even at extremely low drift concentrations, can have significant consequences for agro-eco plant species, the coflowering interactions between them, and potentially the pollinators that would visit them. However, it is important to note that plant species may respond differently to herbicide exposure depending on the context: for example, *A. theophrasti* showed higher sensitivity to dicamba drift in a recent field study than what we report here, and these results also varied by year (Johnson and Baucom, *In press*). Moreover, while we gained insight into how interspecific variation in response to dicamba drift could affect communities using 'synthetic' communities, these differed from real plant communities in important ways that could affect outcomes. Unlike natural communities, our plants were approximately the same age, grown in pots, and randomly distributed throughout a constant glasshouse environment. Our controlled design, however, enabled us to isolate and characterize the effects of dicamba drift on broad ecological phenomena, particularly biomass accumulation and coflowering interactions, thereby allowing us to make predictions that now can be tested in natural plant communities.

In particular, our work highlights both unanswered questions and prompts new ones concerning drift in the wild, such as: does variation in resistance or tolerance lead to persistent shifts in plant community composition over time (e.g. Baucom, 2009)? And do shifts in coflowering interactions caused by herbicide drift significantly affect pollinator visitation patterns? The adoption of our multi-species ($\geq$ 25 species) community model into long-term field experiments with opportunities for direct plant-plant and plant-pollinator interactions would provide these key insights.

ACKNOWLEDGEMENTS

We thank former University of Pittsburgh undergraduate students G. Blanco, and S. O'Neill for their help in data collection, L. Follweiler for administering herbicide treatments, and anonymous reviewers and the editor for comments that improved the paper. VI, RSB, and TLA conceived the study, and VI and TLA designed it. VI performed the study under supervision by TLA. VI analyzed the data, and wrote the first draft of the manuscript, wherein RSB and TLA contributed revisions.

FUNDING

This work was supported by NSF DEB-1834496 and USDA 2017-09529/1016564 to TLA and RSB. VI was additionally supported by the University of Pittsburgh Dietrich School of Arts and Sciences First-Year and K. Leroy Irvis Fellowships.

LITERATURE CITED

Abdi H, Williams J. 2010. Contrast analysis. In: Salkind N, ed. *Encyclopedia of Research Design*. Thousand Oaks, CA, USA: Sage, 244-251.

Agrawal AA. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends in Plant Science* 5: 309–313.

Agustí M, Zaragoza S, Iglesias DJ, Almela V, Primo-Millo E, Talón M. 2002. The synthetic auxin 3,5,6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. *Plant Growth Regulation* 36: 141–147.

Akaike H. 1973. Maximum likelihood identification of Gaussian autoregressive moving average models. *Biometrika* 60: 255–265.

Albor C, García-Franco JG, Parra-Tabla V, Díaz-Castelazo C, Arceo-Gómez G. 2019. Taxonomic and functional diversity of the co-flowering community differentially affect *Cakile edentula* pollination at different spatial scales. *Journal of Ecology* 107: 2167–2181.

Albaugh. 2018. *Specimen Label: Dicamba DMA Salt*. Ankeny, IA, USA.

Allender WJ, Cresswell GC, Kaldor J, Kennedy IR. 1997. Effect of lithium and lanthanum on herbicide induced hormesis in hydroponically-grown cotton and corn. *Journal of Plant Nutrition* 20: 81–95.

Altieri MA. 1999. The ecological role of biodiversity in agroecosystems. *Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes* 74: 19–31.

Arceo-Gómez G. 2021. Spatial variation in the intensity of interactions via heterospecific pollen transfer may contribute to local and global patterns of plant diversity. *Annals of Botany* 128: 383–394.

Arceo-Gómez G, Ashman TL. 2011. Heterospecific pollen deposition: Does diversity alter the consequences? *New Phytologist* 192: 738–746. doi: 10.1111/j.1469-8137.2011.03831.x

Arceo-Gómez G, Kaczorowski RL, Ashman TL. 2018. A network approach to understanding patterns of coflowering in diverse communities. *International Journal of Plant Sciences.* 179: 569–582.

Ashman TL, Knight TM, Steets JA, *et al.* 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.

Ashman TL, Arceo-Gómez G. 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany* 100: 1061–1070.

Bader DA, Kintali S, Madduri K, Mihail M. 2007. Approximating betweenness centrality. In: Bonato A, Chung FRK, eds. *Algorithms and Models for the Web-Graph*, Vol 4863. Heidelberg: Springer, 124–137.

Barber NA, Gorden NLS. 2015. How do belowground organisms influence plant–pollinator interactions? *Journal of Plant Ecology* 8: 1–11.

Bascompte J, Jordano P. 2007. Plant-animal mutualistic networks: The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 38: 567–593.

Bastian M, Heymann S, Jacomy M. 2009. *Gephi: an open source software for exploring and manipulating networks.* https://gephi.org/publications/gephi-bastian-feb09.pdf.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1-48.

Baucom RS. 2019. Evolutionary and ecological insights from herbicide-resistant weeds: What have we learned about plant adaptation, and what is left to uncover? *New Phytologist* 223: 68–82.

Baucom RS, Busi R. 2019. Evolutionary epidemiology in the field: A proactive approach for identifying herbicide resistance in problematic crop weeds. *New Phytologist* 223: 1056-1058.

Baucom RS, Mauricio R. 2004. Fitness costs and benefits of novel herbicide tolerance in a noxious weed. *Proceedings of the National Academy of Sciences* 101: 13386–13390.

Baucom RS, Mauricio R. 2008. Constraints on the evolution of tolerance to herbicide in the common morning glory: Resistance and tolerance are mutually exclusive. *Evolution* 62: 2842–2854.

Belz RG, Duke SO. 2014. Herbicides and plant hormesis. *Pest Management Science* 70: 698–707.

Bernardo P, Charles-Dominique T, Barakat M, *et al.* 2018. Geometagenomics illuminates the impact of agriculture on the distribution and prevalence of plant viruses at the ecosystem scale. *ISME Journal* 12: 173–184.

Biesmeijer JC, Roberts SPM, Reemer M, *et al.* 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351–354.

Blondel VD, Guillaume JL, Lambiotte R, Lefebvre E. 2008. Fast unfolding of communities in large networks. *Journal of Statistical Mechanics: Theory and Experiment*: P10008.

Bohnenblust EW, Vaudo AD, Egan JF, Mortensen DA, Tooker JF. 2016. Effects of the herbicide dicamba on nontarget plants and pollinator visitation. *Environmental Toxicology and Chemistry* 35: 144–151.

Boutin C, Aya KL, Carpenter D, Thomas PJ, Rowland O. 2012. Phytotoxicity testing for herbicide regulation: Shortcomings in relation to biodiversity and ecosystem services in agrarian systems. *Science of The Total Environment* 415: 79–92.

Boutin C, Strandberg B, Carpenter D, Mathiassen SK, Thomas PJ. 2014. Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? *Environmental Pollution* 185: 295–306.

Brandes U, Delling D, Gaertler M, *et al.* 2008. On modularity clustering. *IEEE Transactions on Knowledge and Data Engineering* 20: 172–188.

Bretagnolle V, Gaba S. 2015. Weeds for bees? A review. *Agronomy for Sustainable Development* 35: 891–909.

Brooker RW. 2006. Plant–plant interactions and environmental change. *New Phytologist* 171: 271–284.

Burdon JJ, Thrall PH. 2008. Pathogen evolution across the agro-ecological interface: Implications for disease management. *Evolutionary Applications* 1: 57–65.

Carpenter D, Boutin C. 2010. Sublethal effects of the herbicide glufosinate ammonium on crops and wild plants: Short-term effects compared to vegetative recovery and plant reproduction. *Ecotoxicology* 19: 1322–1336.

Cousens RD, Fournier-Level A. 2018. Herbicide resistance costs: What are we actually measuring and why? *Pest Management Science* 74: 1539–1546.

Debban CL, Okum S, Pieper KE, Wilson A, Baucom RS. 2015. An examination of fitness costs of glyphosate resistance in the common morning glory, *Ipomoea purpurea*. *Ecology and Evolution* 5: 5284-5294.

Devine MD. 2005, Why are there not more herbicide-tolerant crops? *Pesticide Management Science*, 61: 312-317.

Doré M, Fontaine C, Thébault E. 2021. Relative effects of anthropogenic pressures, climate, and sampling design on the structure of pollination networks at the global scale. *Global Change Biology* 27: 1266–1280.

Dunne JA, Williams RJ, Martinez ND. 2002. Food-web structure and network theory: the role of connectance and size. *Proceedings of the National Academy of Sciences* 99: 12917–12922.

Egan JF, Mortensen DA. 2012. Quantifying vapor drift of dicamba herbicides applied to soybean. *Environmental Toxicology and Chemistry* 31: 1023–1031.

Egan JF, Bohnenblust E, Goslee S, Mortensen D, Tooker J. 2014. Herbicide drift can affect plant and arthropod communities. *Agriculture, Ecosystems and Environment* 185: 77–87.

Fang Q, Huang SQ. 2013. A directed network analysis of heterospecific pollen transfer in a biodiverse community. *Ecology* 94: 1176–1185.

Felipe-Lucia MR, Soliveres S, Penone C, *et al.* 2020. Land-use intensity alters networks between biodiversity, ecosystem functions, and services. *Proceedings of the National Academy of Sciences of the United States of America* 117: 28140–28149.

Felsot AS, Unsworth JB, Linders JBHJ, *et al.* 2010. Agrochemical spray drift; assessment and mitigation—a review. *Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants, and Agricultural Wastes* 46: 1–23.

Fineblum WL, Rausher MD. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. *Nature* 377: 517–520.

Forrest J, Inouye DW, Thomson JD. 2010. Flowering phenology in subalpine meadows: Does climate variation influence community co-flowering patterns? *Ecology* 91: 431-440.

Foster MR, Griffin JL. 2018. Injury criteria associated with soybean exposure to Dicamba. *Weed Technology* 32: 608–617.

Fox J, Weisberg S. 2019. *An R companion to applied regression, third edition*. Thousand Oaks, CA, USA: Sage.

Garcia LC, Eubanks MD. 2019. Overcompensation for insect herbivory: A review and meta-analysis of the evidence. *Ecology* 100: e02585.

Ghazoul J. 2006. Floral diversity and the facilitation of pollination. *Journal of Ecology* 94: 295–304.

Gianfagna T. 1995. Natural and synthetic growth regulators and their use in horticultural and agronomic crops. *Plant Hormones*: 751–773.

Goggin DE, Cawthray GR, Powles SB. 2016. 2,4-D resistance in wild radish: Reduced herbicide translocation via inhibition of cellular transport. *Journal of Experimental Botany* 67: 3223–3235.

Goggin DE, Kaur P, Owen MJ, Powles SB. 2018. 2,4-D and dicamba resistance mechanisms in wild radish: subtle, complex and population specific?, *Annals of Botany* 122: 627-640.

Gray C, Baird DJ, Baumgartner S, *et al.* 2014. FORUM: Ecological networks: The missing links in biomonitoring science. *Journal of Applied Ecology* 51: 1444–1449.

Griffin JL, Bauerle MJ, Stephenson DO, Miller DK, Boudreaux JM. 2013. Soybean response to dicamba applied at vegetative and reproductive growth stages. *Weed Technology* 27: 696– 703.

Guardiola JL, García-Luis A. 2000. Increasing fruit size in citrus. Thinning and stimulation of fruit growth. *Plant Growth Regulation 2000 31:1* 31: 121–132.

Grossmann K. 2010. Auxin herbicides: Current status of mechanism and mode of action. *Pest Management Science* 66: 113–120.

Grover R, Maybank J, Yoshida K. 1972. Droplet and vapor drift from butyl ester and dimethylamine salt of 2,4-D. *Weed Science* 20: 320–324.

Hald AB. 1999. Weed vegetation (wild flora) of long established organic versus conventional cereal fields in Denmark. *Annals of Applied Biology* 134: 307–314.

Hilty J. 2019. https://illinoiswildflowers.info

Holzschuh, A, Steffan-Dewenter, I, Tscharntke, T. 2008. Agricultural landscapes with organic crops support higher pollinator diversity. *Oikos*, *117*: 354–361.

Iriart V, Baucom RS, Ashman TL. 2021. Herbicides as anthropogenic drivers of eco-evo feedbacks in plant communities at the agro-ecological interface. *Molecular Ecology* 30: 5406–5421.

Jacobsen DJ, Raguso RA. 2018. Lingering effects of herbivory and plant defenses on pollinators. *Current Biology* 28: R1164–R1169.

Jin Z, Li J, Li Y. 2015. Interactive effects of arbuscular mycorrhizal fungi and copper stress on flowering phenology and reproduction of *Elsholtzia splendens*. *PLOS ONE* 10: e0145793.

Johnson N, and Baucom RS. *In press*. Dicamba drift alters plant-herbivore interactions at the agro-ecological interface. *Ecosphere.*

Karamaouna F, Kati V, Volakakis N, *et al.* 2019. Ground cover management with mixtures of flowering plants to enhance insect pollinators and natural enemies of pests in olive groves. *Agriculture, Ecosystems & Environment* 274: 76–89.

Kassambara, A. 2019. *ggpubr: 'ggplot2' Based publication ready plots*. R package version 0.2.2. https://CRAN.R-project.org/package=ggpubr.

Kati V, Karamaouna F, Economou L, *et al.* 2021. Sown wildflowers enhance habitats of pollinators and beneficial arthropods in a tomato field margin. *Plants* 10: 1003. doi: 10.3390/plants10051003

Katti SK, Rao AV. 1968. Handbook of the Poisson distribution. *Technometrics* 10: 412.

Kelley KB, Riechers DE. 2007. Recent developments in auxin biology and new opportunities for auxinic herbicide research. *Pesticide Biochemistry and Physiology* 89: 1–11.

Kent M, Coker P, 1992. Vegetation description end analysis: A practical approach. London, UK: Belhaven press.

Klein AM, Vaissière BE, Cane JH, *et al.* 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274: 303–313.

Kluser S, Peduzzi P. 2007. *Global pollinator decline: A literature review*. Geneva: UNEP 2007.

Knezevic SZ, Osipitan OA, Scott JE. 2018. Sensitivity of grape and tomato to micro-rates of dicamba-based herbicides. *Journal of Horticulture* 5: 1–5.

Kudo G, Ida TY. 2013. Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology* 94: 2311–2320.

Liao K, Gituru RW, Guo Y-H, Wang Q-F. 2011. The presence of co-flowering species facilitates reproductive success of *Pedicularis monbeigiana* (Orobanchaceae) through variation in bumble-bee foraging behaviour. *Annals of Botany*: 877–884.

Larson DL, Rabie PA, Droege S, Larson JL, Haar M. 2016. Exotic plant infestation is associated with decreased modularity and increased numbers of connectors in mixed-grass prairie pollination networks. *PLOS ONE* 11: e0155068.

Leite DCA, Salles JF, Calderon EN, *et al.* 2018. Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. *Frontiers in Microbiology* 0: 833.

Lenth R. 2020. *emmeans: Estimated marginal means, aka least-squares means*. R package version 1.4.6. https://CRAN.R-project.org/package=emmeans.

Liscum E, Askinosie SK, Leuchtman DL, Morrow J, Willenburg KT, Coats DR. 2014. Phototropism: growing towards an understanding of plant movement. *The Plant Cell*, 26: 38–55.

Marshall EJP, Brown VK, Boatman ND, Lutman PJW, Squire GR, Ward LK. 2003. The role of weeds in supporting biological diversity within crop fields. *Weed Research* 43: 77–89.

Martín González AM, Dalsgaard B, Olesen JM. 2010. Centrality measures and the importance of generalist species in pollination networks. *Ecological Complexity* 7: 36–43.

Mayerová M, Mikulka J, Soukup J. 2018. Effects of selective herbicide treatment on weed community in cereal crop rotation. *Plant, Soil and Environment* 64: 413–420.

Mikaberidze A, McDonald BA. 2020. A tradeoff between tolerance and resistance to a major fungal pathogen in elite wheat cultivars. *New Phytologist* 226: 879–890. doi: 10.1111/nph.16418

Miller JED, Ives AR, Harrison SP, Damschen EI. 2018. Early- and late-flowering guilds respond differently to landscape spatial structure. *Journal of Ecology* 106: 1033–1045.

Mitchell RJ, Flanagan RJ, Brown BJ, Waser NM, Karron JD. 2009. New frontiers in competition for pollination. *Annals of Botany* 103: 1403–1413.

Mithila J, Hall JC, Johnson WG, Kelley KB, Riechers DE. 2011. Evolution of resistance to auxinic herbicides: Historical perspectives, mechanisms of resistance, and implications for broadleaf weed management in agronomic crops. *Weed Science* 59: 445–457.

Moeller DA. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85: 3289–3301.

Mothershead K, Marquis RJ. 2000. Fitness impacts of herbivory through indirect effects on pollinator interactions in *Oenothera macrocarpa*. *Ecology* 81: 30–40.

Mulligan, G. 1979. *The biology of Canadian weeds*, Vol. 1-32. Agriculture Canada.

Neve P, Powles S. 2005. High survival frequencies at low herbicide use rates in populations of *Lolium rigidum* result in rapid evolution of herbicide resistance. *Heredity* 95: 485–492.

O'Gorman EJ, Fitch JE, Crowe TP. 2012. Multiple anthropogenic stressors and the structural properties of food webs. *Ecology* 93: 441–448.

Olesen JM, Bascompte J, Dupont YL, Jordano P. 2007. The modularity of pollination networks. *Proceedings of the National Academy of Sciences* 104: 19891–19896.

Olszyk D, Pfleeger T, Lee EH, Plocher M. 2015. Glyphosate and dicamba herbicide tank mixture effects on native plant and non-genetically engineered soybean seedlings. *Ecotoxicology* 24: 1014–1027.

Olszyk D, Pfleeger T, Shiroyama T, Blakeley-Smith M, Lee EH, Plocher M. 2017. Plant reproduction is altered by simulated herbicide drift to constructed plant communities. *Environmental Toxicology and Chemistry* 36: 2799–2813.

Ouvrard P, Jacquemart AL. 2018. Agri-environment schemes targeting farmland bird populations also provide food for pollinating insects. *Agricultural and Forest Entomology* 20: 558–574.

Parra-Tabla V, Vargas CF. 2004. Phenology and Phenotypic Natural Selection on the Flowering Time of a Deceit-pollinated Tropical Orchid, *Myrmecophila christinae*. *Annals of Botany*. 94(2): 243-250.

Poole RW, Rathcke BJ. 1979. Regularlity, randomness, and aggregation in flowering phenologies. *Science* 203: 470-471.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE. 2010. Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution* 25: 345–353.

Qi Y, Li J, Guan X, *et al.* 2020. Effects of herbicides on non-target plant species diversity and the community composition of fallow fields in northern China. *Scientific Reports 10:1* 10: 1–10.

R Core Team. 2019. *R: A language and environment for statistical computing.* https://www.R-project.org/

Ramos SE, Rzodkiewicz LD, Turcotte MM, Ashman TL. 2021. Damage and recovery from drift of synthetic-auxin herbicide dicamba depends on concentration and varies among floral, vegetative, and lifetime traits in rapid cycling *Brassica rapa*. *Science of The Total Environment* 801: 149732.

Rathcke B. 1988. Flowering phenologies in a shrub community: Competition and constraints. *Journal of Ecology* 76: 975-994.

Requier F, Odoux JF, Tamic T, *et al.* 2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications* 25: 881–890.

Saunders ME. 2018. Insect pollinators collect pollen from wind-pollinated plants: Implications for pollination ecology and sustainable agriculture. *Insect Conservation and Diversity* 11: 13–31.

Schoener TW. 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* 51: 408-418.

Schütte G, Eckerstorfer M, Rastelli V, *et al.* 2017. Herbicide resistance and biodiversity: Agronomic and environmental aspects of genetically modified herbicide-resistant plants. *Environmental Sciences Europe* 29: 5.

Searle SR, Speed FM, Milliken GA. 1980. Population marginal means in the linear model: An alternative to least squares means. *American Statistician* 34: 216–221.

Sharma, AK. 2005. Correlation Analysis. In: *Text Book of Correlations and Regression*. New Delhi, India: Discovery Publishing House, 1-42.

Strandberg B, Sørensen PB, Bruus M, *et al.* 2021. Effects of glyphosate spray-drift on plant flowering. *Environmental Pollution* 280: 116953.

Stuart A. 1955. A test for homogeneity of the marginal distributions in a two-way classification. *Biometrika* 42: 412-416.

Timberlake TP, Vaughan IP, Memmott J. 2019. Phenology of farmland floral resources reveals seasonal gaps in nectar availability for bumblebees. *Journal of Applied Ecology* 56: 1585–1596.

USGS (United States Geological Survey). 2021a. Estimated use in million pounds by year and crop. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2017&map=DICAMBA&hilo=L&disp=Dicamba. Accessed June 26, 2022.

USGS (United States Geological Survey). 2021b. Estimated agricultural use for dicamba, 2018 (Preliminary). https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2018&map=DICAMBA&hilo=L. Accessed June 26, 2022.

US EPA (United States Environmental Protection Agency). 2021. *Status of over-the-top dicamba: summary of 2021 usage, incidents and consequences of off target movement, and impacts of stakeholder-suggested mitigations (DP # 464173: PC Code 128931)*. https://www.regulations.gov/document/EPA-HQ-OPP-2020-0492-0021.

Vieira BC, Luck JD, Amundsen KL, Werle R, Gaines TA, Kruger GR. 2020. Herbicide drift exposure leads to reduced herbicide sensitivity in *Amaranthus* spp. *Scientific Reports* 10: 1–11.

Vila-Aiub MM, Neve P, Powles SB. 2009. Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytologist* 184: 751–767. doi: 10.1111/j.1469-8137.2009.03055.x

Vitt P, Havens K, Jolls CL, Knight TM. 2020. Temporal variation in the roles of exotic and native plant species in plant–pollinator networks. *Ecosphere* 11: e02981.

Waser NM, Real LA. 1979. Effective mutualism between sequentially flowering plant species. *Nature* 281: 670–672.

Watts S, Dormann CF, Martín González AM, *et al*. 2016. The influence of floral traits on specialization and modularity of plant–pollinator networks in a biodiversity hotspot in the Peruvian Andes. *Annals of Botany* 118: 415–429.

Wickham H. 2016. *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.

Xiong Y, Jiao Y. 2019. The Diverse Roles of Auxin in Regulating Leaf Development. *Plants* 8: 243.

Younginger BS, Sirová D, Cruzan MB, Ballhorn, DJ. 2017. Is biomass a reliable estimate of plant fitness? *Applications in Plant Sciences* 5: 1600094.

FIGURE CAPTIONS

Figure 1. Conceptual framework for assessing the impact of herbicide drift on coflowering interaction networks. A: Hypothetical flowering phenologies for four plant species (different colored lines) in an herbicide drift unexposed (left) and exposed (right) community over a growing season. **B**: Corresponding coflowering interaction networks for the four hypothetical plant species (different colored flower icons) based on flowering deployment shown in **A**. Links between species represent coflowering interactions (flowering overlap between species). The thickness of the lines reflects the strength of interactions (duration and intensity of flowering overlap). Different colored filled circles represent different modules (groups of species that interact more strongly, i.e. are more likely to coflower, with each other than with other species); different colored lines indicate when species within modules (green or pink) or from different modules (grey) are interacting. The size of the circles reflects species betweenness centrality (the average percentage of shortest paths in the coflowering network that must go through a species, i.e. the relative importance of a species to network stability).

Figure 2. Plant species vary in resistance and tolerance to dicamba drift and in how drift affects floral traits. A: Estimated marginal means $\pm$ 95% confidence intervals show the proportion of undamaged leaves 48 hours after dicamba drift treatment by species, i.e. resistance scores. The vertical dashed line at 1 is a reference for no damage. **B-F**: Contrast estimates $\pm$ 95% confidence intervals show the difference between dicamba drift-treated plants and control plants, relative to control plants, in short-term tolerance (i.e., plant size at 21 days post-treatment; **B**), long-term tolerance (i.e., final biomass at 145 days post-treatment; **C**), day of first flower (**D**), flowering duration (**E**), and biomass per flower (**F**). Red denotes species that (A-C) were significantly negatively impacted by dicamba drift, (D) dicamba drift delayed the day of first flower, (E) shortened flowering duration, or (F) decreased biomass per flower. Light blue shows significant effects in the opposite

direction and black indicates no significant change. See Supplementary data Table S2, 5-9 for results of tests of significance. Species are designated by four-letter codes as in Table 2. Values plotted are back-transformed (see Supplementary data Fig. S4 for transformed data used in statistical models).

Figure 3. Coflowering networks of control and dicamba drift exposed synthetic glasshouse communities. A-B: Full networks when all flowering species (n = 22) are represented in the control (**A**) and drift (**B**) synthetic plant community. Each plant species is represented as a circle, and links between them represent coflowering interactions. The thickness of the lines reflects the strength of coflowering overlap (duration and intensity), and circle size reflects species betweenness centrality (the relative importance of species for network stability). **C-D**: Betweenness centrality for each species according to the full networks in rank order for the control (**C**) and drift (**D**) community. High values reflect higher relative importance in the network. **A-D:** Different colors represent different modules (groups of species that coflower more strongly with each other than with other species). See Table 2 for species codes noted in circles (**A-B**) and on y-axes (**C-D**), and Supplementary data Fig S11 for results of subset networks that only show species that flowered in both communities.

Figure 4. Species-level tolerance is correlated with a change in coflowering interactions between dicamba drift- exposed and control synthetic communities. Species (blue points labeled with four-letter codes; Table 2) and long-term tolerance scores (Table 1; Fig. 2C) correlated with the change in (drift subtracted by control) weighted degree (**A**; Table 3) and log-transformed betweenness centrality (**B**) between the dicamba drift and control glasshouse communities.

TABLES

Table 1. Key terms for defining plant responses to dicamba drift across the 25 species in this study.

Term	General Definition	Functional Definition
Resistance	The ability to inhibit or rapidly reduce immediate damage caused by a stressor.	The estimated marginal mean of the proportion of undamaged leaves (1- the number of damaged leaves divided by the total number of leaves) at 48 hours post-treatment of dicamba drift is not significantly different than 1.
Tolerance	The ability to minimize damage caused by a stressor on fitness.	The contrast estimate of the difference in either short-term growth (plant size 21 days post-treatment) and/or final biomass (dry shoot biomass 145 days post-treatment) between dicamba drift- and control-treated plants is not significantly different than 0, i.e., $\text{growth/biomass}_{drift} - \text{growth/biomass}_{control} = 0$.
Overcompensation	The ability to utilize dicamba drift exposure to enhance fitness in the short-term or long-term.	The contrast estimate of the difference in either short-term growth or final biomass between dicamba drift- and control-treated plants is significantly greater than 0, i.e., $\text{growth/biomass}_{drift} - \text{growth/biomass}_{control} > 0$.

Under 'Functional Definition', see 'statistical analysis' for information about how estimated marginal means, contrast estimates, and significance were determined.

Table 2. Twenty-five agro-eco species used in the glasshouse experiment.

Species	**Code**	**Family**	**Category**	**Life Cycle**	**Pollination**	**Traits not analyzed**
Amaranthus palmeri	AMPA	Amaranthaceae	Eudicot	A	Wind	-
Daucus carota	DACA	Apiaceae	Eudicot	B	Insect	PF
Asclepias syriaca	ASSY	Apocynaceae	Eudicot	P	Insect	FT, FD, PF, BF, FN, SN
Erigeron annuus	ERAN	Asteraceae	Eudicot	A	Insect	FT, FD, PF, BF, SN
Eupatorium serotinum	EUSE	Asteraceae	Eudicot	P	Insect	-
Solidago canadensis	SOCN	Asteraceae	Eudicot	P	Insect	FT, FD, PF, BF, SN
Taraxacum officinale	TAOF	Asteraceae	Eudicot	P	Insect	-
Lepidium virginicum	LEVI	Brassicaceae	Eudicot	A, B, P	Insect	FT, FD, PF, BF
Commelina virginica	COVI	Commelinaceae	Monocot	P	Insect	-
Ipomoea hederacea	IPHE	Convolvulaceae	Eudicot	A	Insect	-
Ipomoea lacunosa	IPLA	Convolvulaceae	Eudicot	A	Insect	-

Desmanthus illinoensis	DEIL	Fabaceae	Eudicot	P	Insect	PF
Senna obtusifolia	SEOB	Fabaceae	Eudicot	A, P	Insect	-
Trifolium pratense	TRPR	Fabaceae	Eudicot	P	Insect	-
Abutilon theophrasti	ABTH	Malvaceae	Eudicot	A	Insect	-
Sida spinosa	SISP	Malvaceae	Eudicot	A	Insect	-
Oxalis stricta	OXST	Oxalidaceae	Eudicot	P	Insect	-
Plantago lanceolata	PLLA	Plantaginaceae	Eudicot	A, B, P	Wind	BF, PF,
Plantago virginica	PLVI	Plantaginaceae	Eudicot	A, B	Wind	FT, FD, PF, BF, SN
Persicaria pensylvanica	PEPE	Polygonaceae	Eudicot	A	Insect	-
Rumex crispus	RUCR	Polygonaceae	Eudicot	P	Wind	FT, FD, PF, BF, FN, SN
Geum canadense	GECA	Rosaceae	Eudicot	P	Insect	FT, FD, PF, BF, FN, SN
Cardiospermum halicacabum	CAHA	Sapindaceae	Eudicot	A, B, P	Insect	-

Physalis philadelphica	PHPH	Solanaceae	Eudicot	A	Insect	-
Solanum carolinense	SOCA	Solanaceae	Eudicot	P	Insect	-

'Life cycle' relates to the United States Department of Agriculture official PLANTS database characterization as annual (A), biennial (B), and/or perennial (P; USDA 2018). 'Pollination' (insect or wind) is based on Mulligan (1979) and Hilty (2019). 'Traits not analyzed' identifies species that were excluded from analyses on flowering time (i.e. day of first flower and flowering duration; FT), floral display (FD), probability of flowering (PF), biomass per flower (BF), 'full' (FN) and/or 'subset' (SN) coflowering networks or none of the above (indicated by a dash). In 'Category', eudicots are considered susceptible to synthetic auxin herbicides such as 2,4-D or dicamba whereas monocots are not. Taxonomic source for species names: Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew (http://www.plantsoftheworldonline.org, accessed 26 September 2022).

Table 3. Estimated values for each coflowering network metric in the control and dicamba drift synthetic communities when all species that produced at least one flower in both communities were included in the analysis. *W* and *P*-values were obtained from Wilcoxon Mann-Whitney Tests for significant differences between the control and drift communities, except for connectance and modularity where *P*-values were obtained by comparing observed data against null models.

Network Metric	**Community**		***W***	***P***
	Control	**Dicamba Drift**		
Degree	16.55	12.73	367	<.01
Strength	0.22	0.15	32700	<.001
Weighted Degree	4.56	3.20	327	<.05
Connectance	0.788	0.606	-	<.01
Modularity	0.109	0.212	-	<.01

Figure 1

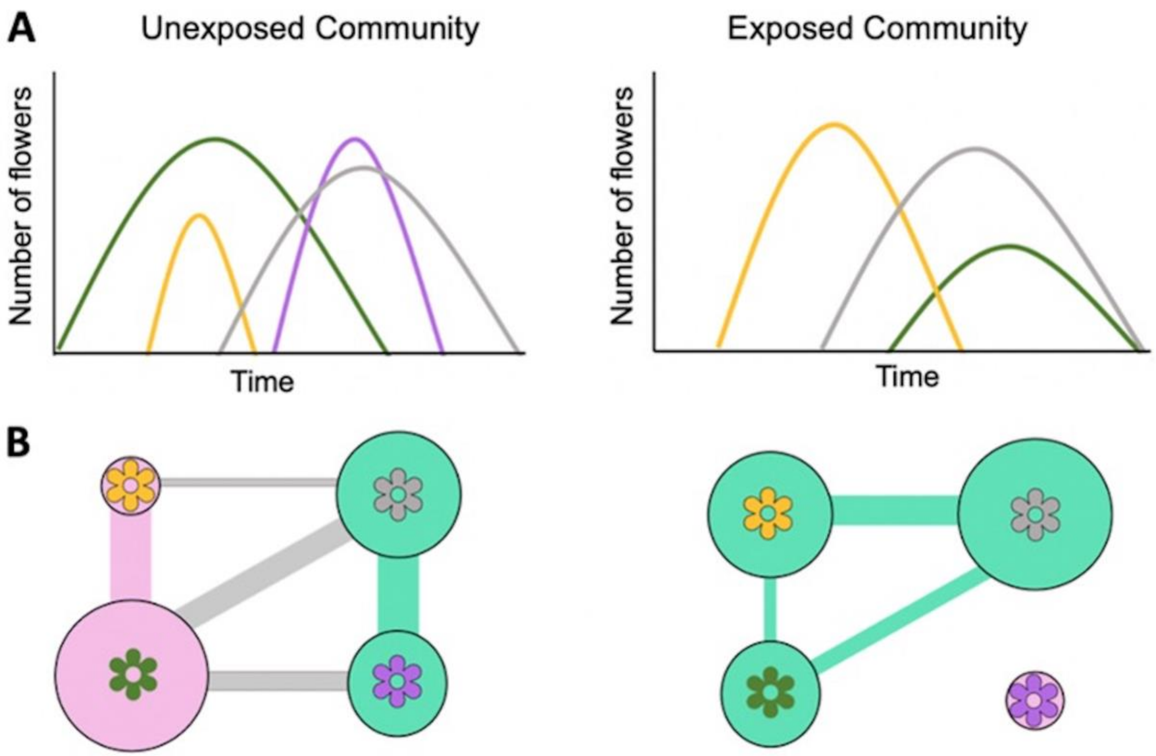
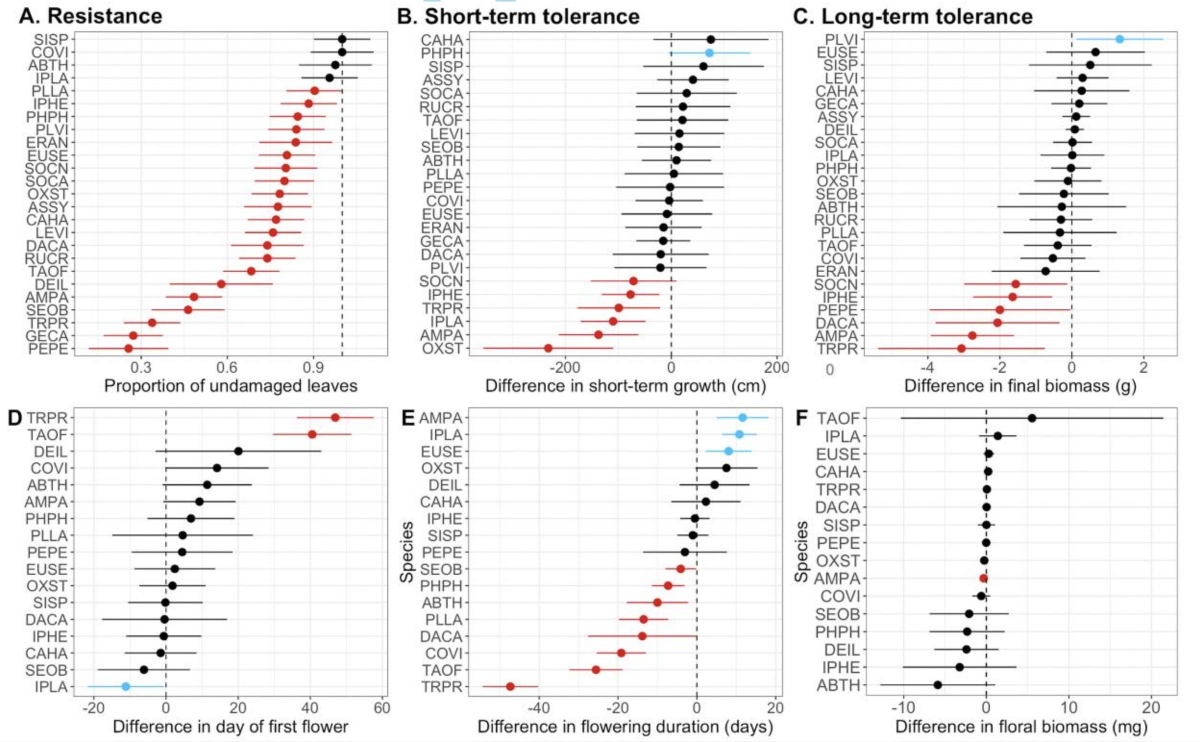
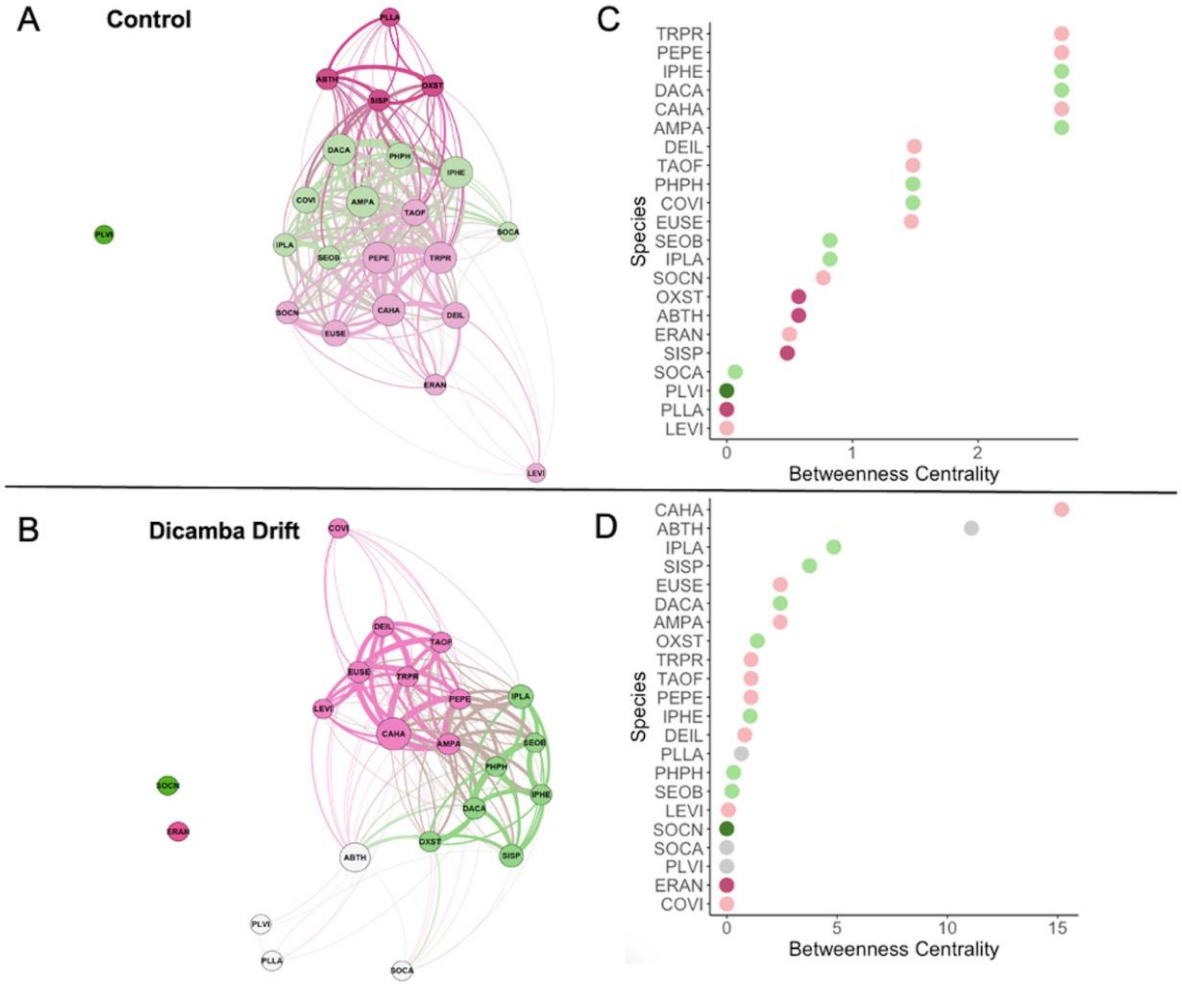

A
Unexposed Community
Number of flowers
Time
Exposed Community
Number of flowers
Time
B

Figure 2



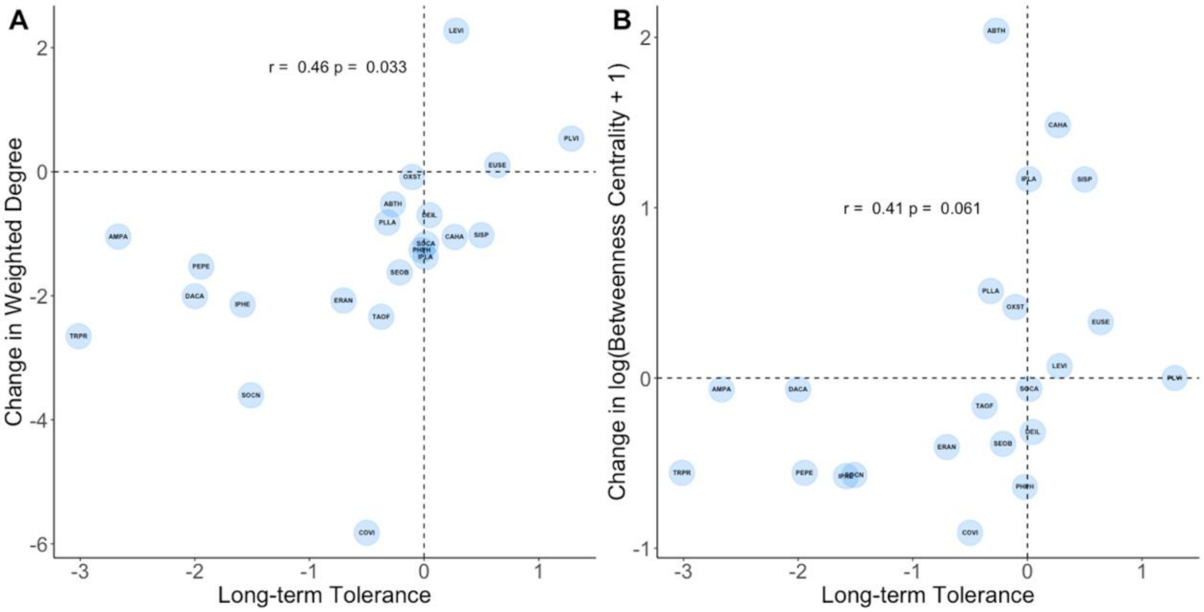
A. Resistance
B. Short-term tolerance
C. Long-term tolerance
Proportion of undamaged leaves
Difference in short-term growth (cm)
Difference in final biomass (g)
D
E
F
Difference in day of first flower
Difference in flowering duration (days)
Difference in floral biomass (mg)
Species

Figure 3



A
Control
B
Dicamba Drift
C
D
PLLA
ABTH
OXST
SISP
DACA
PHPH
IPHE
COVI
AMPA
TAOF
SOCA
PLVI
IPLA
SEOB
PEPE
TRPR
SOCN
CAHA
DEIL
EUSE
ERAN
LEVI
TRPR
PEPE
IPHE
DACA
CAHA
AMPA
DEIL
TAOF
PHPH
COVI
EUSE
SEOB
IPLA
SOCN
OXST
ABTH
ERAN
SISP
SOCA
PLVI
PLLA
LEVI
Species
0
1
2
Betweenness Centrality
CAHA
ABTH
IPLA
SISP
EUSE
DACA
AMPA
OXST
TRPR
TAOF
PEPE
IPHE
DEIL
PLLA
PHPH
SEOB
LEVI
SOCN
SOCA
PLVI
ERAN
COVI
Species
0
5
10
15
Betweenness Centrality

Figure 4


A
B
r = 0.46 p = 0.033
r = 0.41 p = 0.061
Change in Weighted Degree
Change in log(Betweenness Centrality + 1)
Long-term Tolerance
Long-term Tolerance
LEVI
PLVI
EUSE
OXST
ABTH
PLLA
DEIL
CAHA
SISP
SOCA
PHRH
IPLA
SEOB
ERAN
TAOF
AMPA
PEPE
DACA
IPHE
TRPR
SOCN
COVI