Contents lists available at ScienceDirect





journal homepage: www.elsevier.com/locate/scitotenv

Dicamba drift

0.5%, 1%, 10%

Vegetative stage

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*



Sergio E. Ramos^{*,1}, Lacey D. Rzodkiewicz¹, Martin M. Turcotte, Tia-Lynn Ashman

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

HIGHLIGHTS

GRAPHICAL ABSTRACT

- Herbicide drift is a major threat to biodiversity and ecosystem services.
- Damage by synthetic-auxin herbicide drift varies among functional plant traits.
- Floral traits are more sensitive to drift, but modularity may allow full recovery.
- Flowering time and female fitness but no other lifetime traits were affected by drift.
- Variation in damage and recovery, and fitness could cascade to ecological interactions.

ARTICLE INFO

Article history: Received 23 June 2021 Received in revised form 4 August 2021 Accepted 13 August 2021 Available online 18 August 2021

Editor: Yolanda Picó

Keywords: Phenotypic plasticity Short-term effects Stress response variation Stress recovery Ecological impacts Anthropogenic stressors



ABSTRACT

Herbicides can drift from intended plants onto non-target species. It remains unclear how drift impacts plant functional traits that are important for fitness. To address this gap, we conducted an experiment where fast cycling Brassica rapa plants were exposed to one of three drift concentrations (0.5%, 1%, 10%) of synthetic-auxin dicamba. We evaluated damage to and capacity of floral and vegetative traits to recover as well as lifetime fitness by comparing treated plants to controls. Response to dicamba exposure was concentration-dependent across all traits but varied with trait type. At 0.5% dicamba, three out of five floral traits were affected, while at 1% dicamba, four floral traits and one out of two vegetative traits were negatively impacted. At 10% dicamba all floral and vegetative traits were stunted. Overall, floral traits were more responsive to all dicamba drift concentrations than vegetative traits and displayed a wide range of variation ranging from no response (e.g., pistil length) to up to 84% reduction (ovule number). However, despite floral traits were more affected across the dicamba drift concentrations they were also more likely to recover than the vegetative traits. There was also variation among lifetime traits; the onset of flowering was delayed, and reproductive fitness was negatively affected in a concentration-dependent manner, but the final biomass and total flower production were not affected. Altogether, we show substantial variation across plant traits in their response to dicamba and conclude that accounting for this variation is essential to understand the full impact of herbicide drift on plants and the ecological interactions these traits mediate.

Early flowering damage

Control

Drift

Recoverv

Control

Drift

© 2021 Published by Elsevier B.V.

* Corresponding author.

E-mail address: s.ramos.castro@gmail.com (S.E. Ramos). ¹ Equal contribution.

https://doi.org/10.1016/j.scitotenv.2021.149732 0048-9697/© 2021 Published by Elsevier B.V.

1. Introduction

The use of agrochemicals (e.g. herbicides and insecticides) is one of the major threats to biodiversity and ecosystem services like pollination. Pesticide use has increased worldwide in the last twenty years (SCBD report, 2020), and in the United States alone, the use intensity of herbicides and their associated toxicity to target plants has increased substantially (Kniss, 2017; Schulz et al., 2021).

Since the development of synthetic herbicides in the 1950s, one of the first documented side effects was drift from the intended target plant reaching non-target crops and natural plant communities (Akesson and Yates, 1964). Herbicide drift occurs due to a combination factors including the large-scale aerial or ground-based spraying, the volatilization of the herbicide's chemical formulation, and weather conditions (Akesson and Yates, 1964; Felsot et al., 2011; Zimmerman et al., 1953). Herbicide particles can drift up to several kilometers before alighting on non-target plant foliage at levels up to 10% of the field application rates (Felsot et al., 2011; Gove et al., 2007; Prosser et al., 2016). Such drift concentrations are generally sublethal, and can provoke a wide range of responses that alter plant growth, metabolism, life history and reproductive fitness (reviewed in Iriart et al., 2020). Furthermore, these herbicide-induced plastic changes in non-target plants have been implicated in indirect effects on higher trophic levels, affecting invertebrate and vertebrate communities alike (Gutiérrez et al., 2020; Iriart et al., 2020; Prosser et al., 2016; Wang et al., 2021).

The problem of herbicide drift has gained more attention in the last two decades owing to the increasing reports of damage in non-target sensitive crops and wild plant species (Carpenter et al., 2020; Felsot et al., 2011; Russo et al., 2020). The rapid and extensive adoption of glyphosate and synthetic-auxin herbicide resistant crop technologies worldwide (e.g., canola, corn, cotton and soybean), contribute to the increased likelihood of off-target drift damage (Korres et al., 2019; Powles and Yu, 2010). Synthetic-auxin herbicides (e.g., 2,4-D, dicamba) act by mimicking an overdose of the phytohormone indole-3-acetic acid (IAA) which is an essential auxin for growth and development, provoking whole-plant phytohormonal unbalances, resulting in death at field application doses (Benjamins and Scheres, 2008; Grossmann, 2003). However, our understanding of the extent of damage across floral, vegetative traits and lifetime fitness under various drift scenarios in agroecosystem landscape, especially on wild plants is only beginning to develop (Bohnenblust et al., 2013, 2016; Egan et al., 2014; Olszyk et al., 2015, 2017). There is evidence that dicamba drift concentrations as little as 0.001% of the field application rates can result in significant phenotypic damage in the plants, with negative ecological consequences (Bohnenblust et al., 2013; Prosser et al., 2016). Moreover, these negative effects typically worsen with increasing drift concentrations (Iriart et al., 2020).

Furthermore, a wide range of variation in sensitivity (i.e., extent of response in a trait) to drift concentrations have been observed across plant species (Iriart et al. in prep.), but some common responses include delay in flowering, and reduction in plant height, biomass and flower production (reviewed in Iriart et al., 2020). However, the complex mechanisms in which endogenous auxin levels are regulated makes it hard to predict the full impact of synthetic-auxin herbicide drift based on only a few traits. Natural endogenous auxin levels are delicately regulated temporally and spatially in a tissue-specific way (Zhao, 2010), thus producing an uneven and dynamic distribution across tissues (Parízková et al., 2021). For instance, in wild tobacco plants (Nicotiana attenuata), the natural auxin levels in pistils are up to 6.5-fold higher than that of leaves and inflorescences (Li et al., 2018). Also, a study with ¹⁴C-labelled 2,4-D sprayed at drift concentrations on wild radish (Raphanus raphanistrum) showed that plants accumulated it more in the stem than in leaves and roots (Goggin et al., 2018). With this background, we hypothesized that there might be variation across plant tissues in response to dicamba drift, for instance between floral and vegetative traits, and even within these trait categories. If natural auxin levels are higher in floral tissues, then floral traits may be more sensitive to dicamba than vegetative ones. The presence of variation in the phenotypic trait responses thus would indicate that syntheticauxin herbicides produce tissue-specific perturbations of auxin balances resulting in differential damage across tissues.

For these reasons, organ-specific variation in sensitivity to syntheticauxin herbicides has the potential to change the ecology of plants in different directions. For instance, if ovule and pollen production are affected in different ways then the functional gender of a hermaphroditic plant would be impacted (e.g., less pollen = reduced male function; fewer ovules = reduced female function). Changes in vegetative traits can also affect ecological interactions, especially with insect herbivores. For instance, variation in stem diameter can determine susceptibility to stem borers (Niveyro and Salvo, 2017), and plant height or size can increase the appearance and therefore the damage by herbivores (Turcotte et al., 2014). Thus, documenting the variation across floral and vegetative traits in their sensitivity to damage is a key step toward advancing understanding of the extent of herbicide drift impacts on higher trophic levels.

Plants, however, can have the ability to recover from early, acute herbicide exposure (Follak and Hurle, 2004). Recovery is a plastic developmental response wherein organs of affected plants regain the phenotype of non-exposed (control) plants and/or express tolerance in terms of seed production (Carpenter et al., 2013). For instance above-ground plant biomass, height, and seed production in drift-treated plants have been shown to recover partially or fully despite acute damage (Al-Khatib and Peterson, 1999; Carpenter et al., 2013; Carpenter and Boutin, 2010; Riemens et al., 2009; Rotchés-Ribalta et al., 2015). Higher drift concentrations usually limit the recovery ability, as observed for plant height and biomass in several plant species (Carpenter et al., 2013; Follak and Hurle, 2004). For instance, soybean plants remained stunted under higher dicamba drift concentrations but fully recovered their height under lower doses (Al-Khatib and Peterson, 1999). Because herbicides are most commonly applied during seedling emergence or juvenile stage of non-target plants in crops (Korres et al., 2019), reversibility of drift effects may be advantageous for plants if it lead to maintenance of reproductive fitness (Carpenter et al., 2013; Olszyk et al., 2017). Interestingly, due to the tissue-specific regulation endogenous auxin balances, it is likely that recovery from synthetic-auxin herbicide drift also vary across plant tissues, however this aspect has not yet been explored. Recovery could vary among traits because plants are modular organisms and some tissues are constantly produced throughout maturity while others not (e.g., flowers and vegetative traits) (Herrera, 2009). Previous studies have mainly focused on variation in the recovery at the level of species (e.g., Carpenter and Boutin, 2010; Carpenter et al., 2013; Rotchés-Ribalta et al., 2015), but not across different functional traits within a plant. Thus, we are limited in predicting the extent of the differences in recovery across floral and vegetative traits. We hypothesized that if floral and vegetative traits vary in their sensitivity to short-term responses, then they should also vary in their recovery, too. Overall, we do not know yet whether the short-term damage and recovery from herbicide drift vary across floral, vegetative, and lifetime traits, so we focused on addressing these gaps in our knowledge.

Here we evaluated the plastic responses to drift concentrations of the synthetic-auxin herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) on rapid-cycling *Brassica rapa* (Brassicaceae), a model system for studying stress responses because of its fast lifetime (Franks et al., 2018; Olszyk et al., 2010; Steinbrenner et al., 2012), and relationship to many crops and wild species of the agroecological landscape (Olszyk et al., 2010; Rotchés-Ribalta et al., 2015). We exposed greenhouse-grown *B. rapa* to three drift concentrations equivalent to 0.5%, 1% and 10% of field application rates, and investigated the plastic response (damage and the recovery) to dicamba drift. To evaluate whether responses differed between floral and vegetative traits, we scored floral (flower size, stamen and pistil length, ovule number and pollen production), vegetative (plant height and stem diameter), and lifetime traits (onset of flowering, biomass, flower number and reproductive fitness). We asked the following questions:

- 1) Do floral and vegetative traits show different immediate response to dicamba exposure and does it depend on drift concentration?
- 2) Does the ability to recover vary between or within floral and vegetative traits, and is recovery limited at higher dicamba drift concentration?
- 3) Does the effect of dicamba exposure on lifetime traits or fitness depend on drift concentration?

2. Materials and methods

2.1. Study system and experimental design

We used fast cycling *B. rapa* plants from the base population seed stock obtained from Wisconsin Fast Plants® (Carolina Biological Supply Co., Burlington, USA). Despite their history of breeding for fast cycle, these plants exhibit substantial genetic and phenotypic variation. The species is mostly self-incompatible and completes a lifecycle in approximately two months (Williams and Hill, 1986). As most Brassicaceae, *B. rapa* features a distinctive cruciform corolla shape, with four petals, two short, and four long stamens relative to the pistil, the latter being longer than the stamens (Nikolov, 2019).

B. rapa plants (N = 144) were sown singly in pots ($7 \times 7 \times 8 \text{ cm}^3$) with standardized peat/bark mix soil (Old Castle, BFG Supply, Burton, OH, USA), 15 g of Osmocote® fertilizer (ICL Fertilizers Europe, NLD). Plants were placed in a growth chamber (PGR15, Conviron, USA) under 24 h fluorescent lights, 21 °C, and 60% humidity until each plant had 6-10 open flowers and was phenotyped (1st phenotyping, see details in next subsection). After each plant was phenotyped for the first time (between day 15 to 25), it was transferred to a greenhouse under 18 h light, 22-23 °C, and 54% for further growth (Fig. 1a, c); the second phenotyping was done in the greenhouse (see next subsection).

For application of the herbicide we followed the guidelines for toxicity testing at the juvenile stage of development, which also mimic the timing of field application (cf. Carpenter and Boutin, 2010). On day 11 after sowing out, when plants had two true leaves they were sprayed with low-volatility formulation of dicamba (Clarity® herbicide, BASF, USA) (Fig. 1a). Thus, all scored traits during the phenotyping periods (see next subsection) reflect indirect effects of dicamba because the new tissues were not in direct contact with the herbicide. A total of 36 plants were haphazardly assigned to each dicamba drift level regarding field application rates: Control 0% dicamba (only surfactant and water), 0.5%, 1%, and 10% of dicamba (Felsot et al., 2011; Prosser et al., 2016). Five hours after spraying the droplets dried out, and the plants were put back in the growth chamber in a random order to continue development. The final number of plants that reach flowering stage and were phenotyped was N = 123; more specifically, control (0% dicamba) = 26 plants; 0.5% dicamba = 33 plants; 1% dicamba = 36 plants; 10% dicamba = 28 plants. Some plants remained in vegetative stage and did not flower, even in the control 0% dicamba plants and were removed; this is a common observation in rapid cycling B. rapa and it is not related to dicamba treatment (S. Ramos pers. obs.).

2.2. Plant phenotyping

We recorded floral and vegetative phenotype at two time periods (Fig. 1a). The first phenotyping period, referred to as "early flowering", was conducted in the growth chamber when the plants reached 6 to 10 open flowers, spanning days 15 to 25. A few plants (N = 5) however, were phenotyped after day 25, mostly from the 1% and 10% dicamba treatments. Fully open flowers in *B. rapa* plants are recognized when petals are fully expanded and anthers dehiscent. The second phenotyping period referred to as "late flowering" was conducted in the greenhouse, when plants were 35-36 days old. Plant height and diameter of the stem at the base were measured with a 1000 mm long ruler and electronic caliper of the nearest 0.001 mm (Mitutoyo, Japan) respectively. We collected and dissected organs from the three most recently

opened flowers per plant from the main inflorescence and took pictures from above at a parallel angle with a slide placed over petals with mobile devices, including a ruler adjacent to flowers in the image for scale. With these pictures we measured petal area (flower size from now on), pistil length, and the length of one long stamen using image J software. The three pistils were preserved in 70% ethanol for further dissecting. We counted the number of ovules per pistil using a stereoscopic microscope (Stemi SV6, Zeiss, Germany) from which an average number of ovules per flower was estimated per plant. One fully developed flower bud (i.e. yellow color and with the stigma protruding) in the same inflorescence was used for pollen counting. Pollen from dried floral buds was obtained following a standard protocol via acetolysis (Cullen et al., 2021; Jones, 2014). Pollen counting was done using a compound microscope (ICC50 W, Leica, Switzerland). Samples were placed on a hemocytometer, and a minimum of three large grid cells (1 mm² each, 100 nL) were counted; if a threshold value of 100 pollen grains was not reached, additional squares were counted and recorded to avoid underestimation. Afterwards, the pollen grains counted per cell (pollen grains/ (number of cells x 100 nL)) was extrapolated to the total volume of pollen preserved (~250 mL). Pictures of the pollen under a 400× magnification were taken per sample and processed with the software LAS-EZ (v.3.3.0, Leica Application Suite, Switzerland).

For late flowering phenotyping, we chose a random subsample of plants in each of the four dicamba treatments and scored the same set of traits. The total sample size was N = 65; more specifically, control = 15 plants; 0.5% dicamba = 17 plants; 1% dicamba = 15 plants; 10% dicamba = 18 plants. The second phenotyping was conducted in the greenhouse after the plants were manually outcrossed (see below), their position remained within treatment groups. While this means that at this late stage in the experiment treatment and location are confounded, microenvironmental conditions across greenhouse benches differ in temperature by an average of 0.87 °C (one-way ANOVA, location effect; SS = 4.672, $F_{3,44} = 5.504$, P = 0.002, N = 48) but not in light intensity (SS = 8434, $F_{3,44} = 0.309$, P = 0.819, N = 48), suggesting the confounds were not great.

To determine reproductive fitness, we performed manual outcrosses when all plants had been transferred to the greenhouse, from day 26 to day 32 after sowing out (Fig. 1a). We spread out the plants through the greenhouse with a distance of 30 cm between them. Plants were grouped by treatment rather than randomized to avoid accidental pollination among groups and remained there until senescence (36 more days after the second phenotyping). We outcrossed 15 to 20 flowers per plant from random pairs within each treatment. We marked these flowers with paint so that only painted fruits were collected when plants dried out. We counted the number of seeds per fruit and calculated the weight per seed by dividing the total weight of seeds produced per plant by their seed number (electronic balance of the nearest 0.0001 mg, AE200, Mettler Toledo, USA).

Lifetime fitness correlates were evaluated only once per plant and included onset of flowering, final above-ground biomass, final number of flowers and seed production. Onset of flowering was recorded when plants had their first open flowers for the first time based on daily observations. On day 72, the final above-ground dry biomass was collected and weighed in a balance of the nearest 0.01 mg (PL602E, Mettler Toledo, USA), as well as the final number of flowers counted. Female reproductive fitness was estimated as a composite variable per plant by multiplying the final number of flowers, the number of fruits/flowers produced from manual outcrossings (see next subsection), the number of seeds/fruit, and the average seed weight.

2.3. Statistical analyses

To account for possible correlative responses between traits we first conducted MANOVAs separately by trait category, i.e., floral (petal area, long stamen length, pistil length, ovule and pollen number) and vegetative (plant height and stem diameter), as well as separately by



Fig. 1. Timeline of our study showing the timeframe of activities and data collection (A), a hypothetical figure showing examples of the types of treatment comparisons performed in our study (B), and a panoramic image of the plants on day 25 (C). In B), under Early flowering are shown between treatment comparisons of short-term effects of dicamba drift. Under Late flowering, hypothetical recovery effects are shown (see Methods section for full explanation). Photo credit in panel C: Lacey Rzodkiewicz).

flowering period (early and late). In addition, Pearson partial correlation coefficients among all traits combined were calculated using the pcor function from ppcor package in R (Kim, 2015). The MANOVAs included dicamba treatment (factor), day of phenotyping (numeric) and the interaction between dicamba treatment*day of phenotyping (T*D afterwards). We then obtained the univariate ANOVAs per single trait and flowering period from the MANOVAs using the summary.aov function in R (R Core Team, 2021). The ANOVAs from the early flowering period were used to test for short-term trait responses, and included dicamba treatment, day of phenotyping, and T*D as predictors. In these analyses, day of phenotyping accounted for the variation produced by the day span in which plants were phenotyped in each flowering period. Thus, a significant T*D interaction indicates that the differences between treatments are not only explained by a direct effect of dicamba, but

also an indirect effect triggered by a delay in flowering time. We conducted additional ANOVAs where we excluded the few observations (N = 3) of the 10% dicamba treatment to avoid biased results in some traits during early flowering (petal area, long stamen, and ovule number; see Fig. 3 Early flowering, and Table S3). Finally, we conducted Bonferroni corrected pairwise contrasts for between treatment comparisons from all 'early flowering' ANOVAS.

Recovery for the same traits was evaluated with univariate ANOVAs and repeated measures ANOVAs using "late flowering" phenotypes. The univariate ANOVAs followed the same structure described above for the early flowering. Between treatment comparisons were also tested via pairwise contrasts with Bonferroni correction. Repeated measures included: dicamba treatment (factor), flowering period (a factor with two levels: early and late flowering), and the interaction of dicamba treatment and flowering period as predictors (T*Fp). A significant effect of the T*Fp interaction indicates that at least one dicamba treatment at the within treatment level was different between flowering periods, thus implying recovery. This was further tested via pairwise contrasts with Bonferroni correction. For interpretation of recovery, the dicamba treatments were compared to the control plants (0% dicamba) during the late flowering together with the within treatment comparisons across flowering periods.

Three recovery outcomes may be expected; 1) Full recovery, indicated by a within treatment difference between early and late flowering, and lack of difference against control plants in the late flowering period (green color figures and text in Fig. 1b). 2) Partial recovery, indicated by a within treatment difference between early and late flowering, and a difference from the control plants during late flowering (light blue color figures and text in Fig. 1b). 3) No recovery would be indicated by a lack of within treatment difference between early and late flowering, and a difference from control plants during late flowering (light green color figures, text and dotted line in Fig. 1b).

For lifetime traits (onset of flowering, final above-ground biomass, final number of flowers and female reproductive fitness) we first performed a MANOVA with the four traits combined to account for correlative effects. With the univariate ANOVAs obtained with summary.aov we tested the effect of dicamba treatment (factor). The onset of flowering, final number of flowers and female reproductive fitness were log transformed to achieve normality.

All ANOVAs were performed with the anova function of the stats package embedded in R (sum squares type two were used; Langsrud, 2003). Pairwise contrasts with Bonferroni correction were done with the emmeans function of the emmeans package in R. Plots were done with the ggplot2 package (Wickham et al., 2016). We used R version 3.6.3 (R Core Team, 2021).

3. Results

Dicamba had a strong impact in the floral and vegetative traits in each flowering period (MANOVA; Table S1). In the early flowering period, we found a general pattern of concentration dependent effects of dicamba across floral and vegetative traits (ANOVA and pairwise contrasts; Table S2, Fig. 2a-g 'early flowering'). More importantly, we found variation across traits in their sensitivity and response to the dicamba drift treatments, not only between floral and vegetative traits, but also within these trait categories. For instance, out of the total of seven traits, only three floral traits were strongly reduced up to half the values of control plants in the lowest level of dicamba of 0.5% (Fig. 2a, c and d, Table 1). On the other hand, 1% dicamba affected four floral traits and one vegetative, with only the pistil length and stem diameter not being affected in each trait category. There was a broad range of variation in the sensitivity within floral traits, ranging from no response to up to a dramatic 76% reduction (Table 1). Finally, 10% dicamba triggered responses in all floral and vegetative traits, with the exception of the pistil length (Table 1, Fig. S1). Also interesting was that while most traits are negatively affected by dicamba drift, one vegetative trait (stem diameter) showed an increase compared to control plants (Fig. 2f 'early flowering'). A similar pattern was observed in the pistil length (Fig. S1 'early flowering'). Altogether, these results indicated substantial variation in the short-term effects of dicamba drift across and within floral and vegetative traits.

Moreover, the recovery ability from damage depended on the dicamba concentration. The recovery of traits declined with higher dicamba concentrations in regard of the number of traits affected during early flowering (Table 1). Furthermore, we found variation in the recovery ability across traits and dicamba drift concentrations. For instance, even traits that were strongly reduced in the lowest level of 0.5% dicamba like flower size and ovule number showed a full recovery by the late flowering period (Table 1, Fig. 2a, c 'late flowering'). On the other hand, four out of five affected traits in the 1% dicamba during early flowering were able to fully recover, and all these were floral traits (Table 1); only plant height did not recover from the early stunting, even though it was equally or less sensitive than floral traits (e.g., 30% reduction; Table 1, Fig. 2e 'late flowering'). Finally, in the highest dicamba level of 10%, only some floral traits were able to recover from short-term effects, while none of the vegetative traits did so (Table 1, floral traits, Fig. 2a-d; vegetative traits, Fig. 2e, f). Altogether, the results suggest that although floral traits are generally more sensitive across dicamba drift concentrations than the vegetative traits, they also harbor higher recovery ability.

Dicamba also had effects on lifetime fitness correlates (MANOVA; Table S1). When inspecting the individual responses of each variable we found that not all lifetime traits were equally sensitive to the dicamba drift concentrations tested (ANOVA and pairwise contrasts; Table S2, Fig. 3). For instance, the onset of flowering and composite reproductive fitness showed concentration dependent effects (Fig. 3a, b), while the above-ground biomass and final number of flowers were not affected by dicamba (Fig. 3c, d). Compared to control plants, the onset of flowering was delayed by 1.9 and 7.6 days in 1% and 10% dicamba respectively (days to first open flower per treatment, mean days \pm sd; control plants, 17.57 \pm 1.65; 0.5% dicamba, 17.78 \pm 2.08; 1% dicamba, 19.52 \pm 2.61; 10% dicamba, 25.25 \pm 6.06; Fig. 3a). Furthermore, the composite reproductive fitness was reduced by 57% under 1% of dicamba, and by 88% under 10% dicamba (Fig. 3b). These patterns did not change when the few 10% dicamba observations were omitted in a separate analysis (Table S3).

4. Discussion

In this study, we found a broad range of variation in the sensitivity and response to three dicamba drift concentrations across floral and vegetative traits, as well as within each of these trait categories. The majority of traits were negatively affected by dicamba, but stem diameter was positively affected. We found that floral traits were most sensitive to dicamba drift but also more likely to fully recover than vegetative traits, even in the highest (10%) dicamba concentration tested. Overall, most floral, vegetative, and lifetime traits showed concentrationdependent effects, with higher dicamba levels triggering stronger responses, and limiting the recovery. Below, we discuss our findings in the context of their mechanisms and their potential ecological and evolutionary implications.

4.1. Damage varies across plant traits and drift concentrations

Previous studies on the response to drift concentrations of syntheticauxin herbicides have mostly focused on a few traits like above-ground biomass, plant height and root length in crops and wild species (Al-Khatib and Peterson, 1999; Bohnenblust et al., 2016; Carpenter and Boutin, 2010; Leclere et al., 2018; Olszyk et al., 2015; Riemens et al., 2009). Consistent with these previous studies, we also found concentration dependent effects across floral and vegetative traits.

In contrast, the variation in the responses among different traits has received much less attention. We found that not all traits were equally sensitive to the dicamba drift concentrations tested. Although we evaluated more floral than vegetative traits, our results suggest that floral traits were more sensitive. For instance, no vegetative trait was affected by 0.5% dicamba, while two floral traits –flower size and ovule numberwere reduced in average by around half compared to control plants. Under 1% dicamba, half of the traits within the floral and vegetative categories were sensitive. Finally, only the 10% dicamba level triggered a plastic response of all traits, with mostly negative responses. Because we sprayed our plants at the juvenile-vegetative stage, the differences in sensitivity across traits, particularly flower traits cannot be attributed to a direct contact of the herbicide with the different plant tissues. Instead, different plant tissues might vary in their sensitivity to drift concentrations of dicamba because auxins are temporally and spatially



Fig. 2. Between treatment comparisons of the dicamba drift effects during early flowering, recovery based on within treatment (early versus late), and between treatment comparisons in the late flowering period. See Tables S1–S3 for statistics. In the boxplots, treatments are connected by lines based on their means (empty symbols). Lines connecting same treatments indicate full or partial recovery (solid line), or no recovery (dotted line, but note exception in B). (a-d) flower traits, (e, f) vegetative traits. Different letters on top of the boxplots indicates significant differences based on Bonferroni corrected pairwise contrasts.

regulated in the plant (Zhao, 2010). For instance, flowers and leaves at their different developmental stages can biosynthesize their own auxins, hence auxin homeostasis naturally differ by tissue (Benjamins and Scheres, 2008). Thus, as we hypothesized, the differential sensitivity across our studied plant traits seems to mirror tissue-specific alterations of auxin balances, which apparently differ even at a finer scale like within a flower (e.g., ovule and pollen numbers). These findings highlight that conclusions drawn from examining only a subset of traits

could underestimate the negative effects of drift concentrations of dicamba (but see Rotchés-Ribalta et al., 2015). A next step to better understand the mechanisms behind the variation in response to auxin herbicide drift would be to quantify the endogenous levels of auxins and other phytohormones per tissue upon exposure.

While these short-term effects might be temporary, they can still have important impacts on ecological interactions that occur during the vegetative growth and early flowering of plants in natural

Table 1

Summary of short-term effect sizes and recovery ability from dicamba drift in seven plant traits. Percentages in bold indicate a significant effect of dicamba drift during the early flowering, with either an increase (arrow up) or a decrease (arrow down) compared to control plants. A superscript on these bold percentage values indicate whether the trait recovered by the late flowering period (R, full recovery), or not (NR, no recovery). Also shown are, the total number of traits affected (i.e., sensitive traits) per drift level out of seven traits (e.g., $P \le 0.05$), and the number of traits recovered out of the traits affected. Percentage values of traits during the late flowering period are not shown.

Trait	Effect and recovery from dicamba drift		
	0.5%	1%	10%
Flower			
Flower size	↓ 43% ^R	↓ 51% ^R	↓ 58% ^R
Long stamen	↓7%	↓15% ^R	↓27% ^R
Pistil length	0%	↓2%	↑44%
Ovule number	↓ 56% ^R	↓ 76% ^R	↓ 84 % ^{NR}
Pollen number	↓27% ^R	↓ 30% ^R	↓ 56% ^R
Vegetative			
Plant height	↓17%	↓ 30% ^{NR}	↓ 80 % ^{NR}
Stem diameter	↓10%	↓13%	↑ 44%^{NR}
Traits affected	3	5	6
Traits recovered	3	4	3

conditions. For instance, we might erroneously conclude that drift concentrations of dicamba of 0.5% are negligible if we only focus on the effects on plant height or the onset of flowering, as most studies do. However, flower size, pollen and ovule number were severely reduced under 0.5% dicamba. A reduction of flower size could reduce pollinator visitation with consequences for the plants and insects alike. For instance, in Raphanus sativus (Brassicaceae), the experimental reduction of flower size by 29% compared to control flowers was enough to decrease pollinator visitation significantly (Conner and Rush, 1996). Likewise, reductions of pollen production by half of that produced by control plants experiencing high dicamba drift concentrations (≥10%) could have strong consequences for the beneficial insect community. Indeed, both pollinators and beneficial insect predators strongly rely on pollen of non-agricultural plants even in crop dominated landscapes (Bertrand et al., 2019). For the plants themselves, the combined reduction of flower size and pollinator attraction, and changes in the functional gender (via reduced pollen and ovule production) might severely impair plant's sexual reproduction by increasing the degree of pollen limitation and affecting the mating system and seed production (Ashman et al., 2004). On the other hand, synthetic-auxin herbicide drift commonly result in stunted plants (Olszyk et al., 2015; Rotchés-Ribalta et al., 2015), as we observed with $\geq 1\%$ dicamba (Fig. 1c). Since



Fig. 3. Between treatment comparisons of the four lifetime traits. (a) onset of flowering, (b) composite female reproductive fitness, (c) final above-ground biomass, and (d) final number of flowers. See Tables S1–S3 for statistical results. Different letters on top of the boxplots indicates significant differences based on Bonferroni corrected pairwise contrasts. Empty symbols indicate the mean values.

plant size is important to herbivores for host location (Strauss et al., 2015; Turcotte et al., 2014), stunted plants could be less apparent and provide less plant biomass to butterfly larvae, thus limiting development to reach adult stage (Bohnenblust et al., 2013). Herbivores like butterflies that usually seek for their host plant during the vegetativegrowing stage of plants are especially vulnerable to reduced plant sizes (Pleasants and Oberhauser, 2013). On the other hand, the increased stem diameter can also make plants more or less susceptible to damage by insect stem borers. For instance, Niveyro and Salvo (2017) found higher damage by beetle stem borers in Amaranthus plants with thicker stems than in thin stems. Ishii and Hirano (1963) found that larvae of a moth stem borer grew better in rice plants sprayed with the synthetic-auxin 2,4-D as a result of higher nitrogen content in the plants. For the plants, a higher risk of stem borers can also compromise the structural strength of plants (Niveyro and Salvo, 2017). Altogether, we suggest that the wide variation in short-term responses across floral and vegetative traits can have cascading effects on the ecological interactions, particularly the insect communities that rely on plant resources early in the season.

4.2. Variation in recovery from dicamba damage

Previous studies have reported variation in the potential of and rate of recovery by comparing non-target plant species under different drift concentrations of herbicide and formulations (Carpenter et al., 2013; Carpenter and Boutin, 2010; Follak and Hurle, 2004; Rotchés-Ribalta et al., 2015). What remains less clear is whether there is variation in the potential of recovery across traits within plants. Yet, some studies indicate that there can be substantial variation in recovery between plant traits. For instance, studies that have evaluated recovery to dicamba in several non-target species solely in terms of biomass and reproduction found that some species could recover one but not both traits (Carpenter et al., 2013; Carpenter and Boutin, 2010; Rotchés-Ribalta et al., 2015). Similarly, across our studied plant traits, we found variation in their ability to recover, but floral traits were more likely to recover than vegetative traits. For instance, across dicamba drift concentrations, none of the vegetative traits that were sensitive could recover, while floral traits recovered well even under the highest dicamba level of 10%. To our knowledge, this is the first study that shows variation in recovery from herbicide drift exposure across different functional plant traits. Also, although we hypothesized that recovery could be either full or partial we found no evidence of partial recovery, as traits either fully recovered or not. Contrary to previous anecdotal reports (e.g., Al-Khatib and Peterson, 1999; Follak and Hurle, 2004; Carpenter and Boutin, 2010), our measure of partial recovery was quantitatively characterized and statistically evaluated (via repeated measures and univariate ANOVAs) for which we found no support. As a caveat, the observed patterns of recovery might include any effect of bench position due to the non-random location of the plants in the greenhouse bench during the late flowering. However, it is unlikely that bench location has a strong influence in the patterns and variation in recovery given that only temperature, but no light varied slightly between bench locations by an average of 0.87 °C.

How can plants recover from dramatic effects of drift concentrations of dicamba like those observed in flower size and ovule number? Early reports of the ability of non-target plants to recover from dicamba suggested that the recovery was dependent of the rates at which plants could metabolize the synthetic auxin (e.g., detoxification) (Chang and Vanden Born, 1971; Grossmann et al., 2002). For instance, recovery from synthetic-auxin herbicides could be facilitated by the high metabolic rates of plants during early plant development and vegetative growth (Ljung et al., 2001), which implies that recovery could be facilitated during early ontogenetic changes (Farnsworth, 2004). Ontogeny can influence the patterns of trait responses mediated by any source of stress. For instance, plants respond differently to herbivory damage when they are seedling, juvenile of reproductive as a result of changes in phytohormone balances and resource allocation (Boege and Marguis, 2005). Thus, further studies should explicitly explore plant responses to herbicide drift at different ontogenetic stages. In Arabidopsis and Nicotiana seedlings, the rate of leaf cell division is rapid and it correlates negatively with auxin concentration (Ljung et al., 2001), thus, lower auxin concentrations at later ontogenetic stages could influence the extent of damage by dicamba. In our study, despite the perturbation of auxin homeostasis provoked by dicamba, the rapid plant's metabolism and auxin transportation dynamics at the vegetative stage could facilitate recovery. Nevertheless, as our results suggest, higher dicamba drift concentrations seem to slow down the breakdown rate of auxin excess, thus likely explaining concentration-dependent effects and limited recovery at higher dicamba levels. Gleason et al. (2011) showed that dicamba up-regulated several stress responsive genes over 3-fold in Arabidopsis thaliana. Among these genes was the GH3 family of genes, which have a role in maintaining auxin homeostasis, hence, their upregulation suggest that the plant's response to dicamba is to remove the excess of auxins (Gleason et al., 2011). Although the abovementioned studies help to understand the potential mechanisms involved in the recovery ability, it is still unclear why there is substantial variation in recovery between and within floral and vegetative traits. As mentioned previously, floral traits were more prone to recover than vegetative traits. Thus, given that auxin regulation is tissue specific (Benjamins and Scheres, 2008; Zhao, 2010), a likely explanation for variation in recovery might be that the rates of auxin detoxification are also tissue-specific. In this case, our results suggest that the rates of auxin detoxification are higher in floral than in vegetative tissues. The variation in recovery across traits could also be a consequence of the body plan of plants consisting in repeated structures performing the same function like flowers and leaves (i.e., modularity) (Herrera, 2009). Thus, floral traits might fully recover due to their constant replacement during the flowering period. Indeed, the idea that plants are "integrated physiological units" is considered one of the main mechanisms generating within-plant variation (Watson, 1986).

Finally, the recovery ability of plants might have evolutionary consequences. The "plasticity-first" hypothesis states that plastic changes induced by environmental changes can jump-start phenotypic change in an adaptive direction, thus preceding and promoting evolution (Levis and Pfennig, 2016). Thus, if detoxification of drift herbicide doses starts as a plastic response, selection can then refine this mechanism, making it more efficient in response to repeated exposure (Iriart et al., 2020). Eventually, this process could result in the evolution of non-target site resistance, either due to genetic accommodation (Levis and Pfennig, 2016), or by a de novo mutation (Kreiner et al., 2018).

4.3. Lifetime fitness and dicamba drift

In line with previous studies, we found a delay in the onset of flowering and the reproductive fitness as dicamba concentration increased (Bohnenblust et al., 2016; Rotchés-Ribalta et al., 2015). Delays in the onset of flowering in natural plant populations could shift the availability of floral resources to the pollinators (Arceo-Gómez et al., 2018; Bohnenblust et al., 2016), and the beneficial insect-predator communities (e.g., parasitoids; Fuchs et al., 2021) that depend on these resources early in the season. For instance, solitary bees could be strongly affected by flowering delays, as their emergence time from diapause can be tightly synchronized with early floral resources needed for nest construction and offspring provisioning (Crone, 2013; Wcislo and Cane, 1996). On the other hand, reductions in reproductive fitness with increasing herbicide drift concentrations have been observed on other studies including brassicaceous plants (Olszyk et al., 2010; Carpenter et al., 2013; Olszyk et al., 2017; Yu et al., 2017). Indeed, a previous study showed that a variety of herbicides including dicamba usually provoke both flowering delays and reduced reproductive fitness in non-target plants in the field when spraying occurs at the seedling stage (Boutin et al., 2014). Also, compared to the other lifetime traits of final above-ground biomass and final number of flowers, the reproductive fitness was more sensitive, as noted previously by Boutin et al. (2014). This variation in the effects of dicamba drift to lifetime traits highlight that vegetative and floral-related endpoint traits do not necessarily predict effects on reproductive fitness.

A next step would be to investigate the potential extended effects of dicamba on seed germination. In grasses, different synthetic-auxin herbicides including dicamba reduced gemination in seeds from sprayed plants (Rinella et al., 2010), suggesting that this might be another fitness component sensitive to dicamba worth to evaluate. While our results of reproductive fitness fit with these patterns, it is possible that our procedure of performing hand-crosses only once in the middle of the experiment might have bias our results. Given that seed production from handcrosses depends on the ovules and pollen available at a given moment, our design might have not captured the true drift effects on reproductive fitness. Thus, future studies quantifying herbicide drift effects on reproductive fitness must consider the influence of recovery and design manual or pollinator-mediated crossings at different time points throughout the plants' life (but see; Olszyk et al., 2010; Rotchés-Ribalta et al., 2015). Likewise, pollen traits (not quantified here) such as pollen tube growth and chemistry could alter male fitness directly and female fitness indirectly, thus these traits deserve consideration in further studies.

5. Conclusion

We followed a trait-based approach to study the variation in herbicide-mediated stress responses and recovery in plants and demonstrated substantial variation both between and within ecologically and physiologically linked functional traits. These responses, however, vary with herbicide drift concentration. Our results clearly highlight the modular nature of plants with tissue-specific physiological dynamics, resulting in complex trait responses. Thus, because trait-specific responses can affect trophic interactions in unanticipated ways, a more complete understanding of the effects of herbicide drift will only be achieved by recognizing such a variation. We advocate for future work on herbicide-mediated stress in plants to adopt a similar trait-based approach.

Funding

MMT was supported by an NSF DEB grant (#1935410); TLA by DEB (1834496) and USDA (2017- 09529/1016564); SER was supported by a PEEP postdoctoral fellowship from the Dietrich School of Arts and Sciences at the University of Pittsburgh.

CRediT authorship contribution statement

Conceptualization: SER, MTT, TLA Performed the experiments: SER, LR Data curation and analysis: SER, LR Writing- original draft: SER Writing- review and editing: SER, MTT, TLA, LR

Data availability

The data and code is publicly available at Mendeley Data under doi: https://data.mendeley.com/datasets/wbsbzxvxwc/1.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

We thank the help of Laurie Follweiler and the green team for taking care of the plants. Liz O'Neill provided us with lab and logistic support. We also thank Steffani O'Neill, Kayla Downs, Madison Jerome and Santiago Barratt-Boyes for their help during phenotyping. We thank the Ashman and Turcotte lab members for their input during discussions and three reviewers for comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2021.149732.

References

- Akesson, N.B., Yates, W.E., 1964. Problems relating to application of agricultural chemicals and resulting drift residues. Annu. Rev. Entomol. 9, 285–318. https://doi.org/10.1146/ annurev.en.09.010164.001441.
- Al-Khatib, K., Peterson, D., 1999. Soybean (Glycine max) response to simulated drift from selected sulfonylurea herbicides, dicamba, glyphosate, and glufosinate. Weed Technol. 13, 264–270.
- Arceo-Gómez, G., Kaczorowski, R.L., Ashman, T.-L., 2018. A network approach to understanding patterns of coflowering in diverse communities. Int. J. Plant Sci. 179, 569–582. https://doi.org/10.1086/698712.
- Ashman, T.L., Cole, D.H., Bradburn, M., 2004. Sex-differential resistance and tolerance to herbivory in a gynodioecious wild strawberry. Ecology 85, 2550–2559. https://doi. org/10.1890/03-0495.
- Benjamins, R., Scheres, B., 2008. Auxin: the looping star in plant development. Annu. Rev. Plant Biol. 59, 443–465. https://doi.org/10.1146/annurev.arplant.58.032806.103805.
- Bertrand, C., Eckerter, P.W., Ammann, L., Entling, M.H., Gobet, E., Herzog, F., Mestre, L., Tinner, W., Albrecht, M., 2019. Seasonal shifts and complementary use of pollen sources by two bees, a lacewing and a ladybeetle species in european agricultural landscapes. J. Appl. Ecol. 56, 2431–2442. https://doi.org/10.1111/1365-2664.13483.
- Boege, K., Marquis, R.J., 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol. Evol. 20, 441–448. https://doi.org/10.1016/j.tree.2005.05.001.
- Bohnenblust, E., Egan, J.F., Mortensen, D., Tooker, J., 2013. Direct and indirect effects of the synthetic-auxin herbicide dicamba on two lepidopteran species. Environ. Entomol. 42, 586–594. https://doi.org/10.1603/EN13021.
- Bohnenblust, E.W., Vaudo, A.D., Egan, J.F., Mortensen, D.A., Tooker, J.F., 2016. Effects of the herbicide dicamba on nontarget plants and pollinator visitation. Environ. Toxicol. Chem. 35, 144–151. https://doi.org/10.1002/etc.3169.
- Boutin, C., Strandberg, B., Carpenter, D., Mathiassen, S.K., Thomas, P.J., 2014. Herbicide impact on non-target plant reproduction: what are the toxicological and ecological implications? Environ. Pollut. 185, 295–306. https://doi.org/10.1016/j.envpol.2013.10.009.
- 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. https://doi.org/10.1007/s10646-010-0519-7.
- Carpenter, D., Boutin, C., Allison, J.E., 2013. Effects of chlorimuron ethyl on terrestrial and wetland plants: levels of, and time to recovery following sublethal exposure. Environ. Pollut. 172, 275–282. https://doi.org/10.1016/j.envpol.2012.09.007.
- Carpenter, D.J., Mathiassen, S.K., Boutin, C., Strandberg, B., Casey, C.S., Damgaard, C., 2020. Effects of herbicides on flowering. Environ. Toxicol. Chem. 39, 1244–1256. https://doi. org/10.1002/etc.4712.
- Chang, F.Y., Vanden Born, W.H., 1971. Dicamba uptake, translocation, metabolism, and selectivity. Weed Sci. 19, 113–117. https://www.jstor.org/stable/4041832.
- Conner, J.K., Rush, S., 1996. Effects of flower size and number on pollinator visitation to wild radish, Raphanus raphanistrum. Oecologia 105, 509–516.
- Crone, E.E., 2013. Responses of social and solitary bees to pulsed floral resources. Am. Nat. 182, 465–473. https://doi.org/10.1086/671999.
- Cullen, N., Xia, J., Wei, N., Kaczorowski, R., Arceo-Gómez, G., O'Neill, E., Hayes, R., Ashman, T.L., 2021. Diversity and composition of pollen loads carried by pollinators are primarily driven by insect traits, not floral community characteristics. Oecologia 196, 131–143. https://doi.org/10.1007/s00442-021-04911-0.
- Egan, J.F., Bohnenblust, E., Goslee, S., Mortensen, D., Tooker, J., 2014. Herbicide drift can affect plant and arthropod communities. Agric. Ecosyst. Environ. 185, 77–87. https:// doi.org/10.1016/j.agee.2013.12.017.
- Farnsworth, E., 2004. Hormones and shifting ecology throughout plant development. Ecology 85, 5–15. https://doi.org/10.1890/02-655.
- Felsot, A.S., Unsworth, J.B., Linders, J.B., Roberts, G., Rautman, D., Harris, C., Carazo, E., 2011. Agrochemical spray drift; assessment and mitigation- a review. J. Environ. Sci. Health B 46, 1–23. https://doi.org/10.1080/03601234.2010.515161.
- Follak, S., Hurle, K., 2004. Recovery of non-target plants affected by airborne bromoxyniloctanoate and metribuzin. Weed Res. 44, 142–147. https://doi.org/10.1111/j.1365-3180.2004.00383.x.
- Franks, S.J., Genovese, N., Stockdale, M., Weber, J.J., Ansaldi, B.H., van Wilgenburg, E., 2018. The effects of artificial selection for rapid cycling in Brassica rapa on herbivore preference and performance. Int. J. Plant Sci. 179, 175–181. https://doi.org/10.1086/ 696220.
- Fuchs, B., Saikkonen, K., Helander, M., 2021. Glyphosate-modulated biosynthesis driving plant defense and species interactions. Trends Plant Sci. 26, 312–323. https://doi. org/10.1016/j.tplants.2020.11.004.
- Gleason, C., Foley, R.C., Singh, K.B., 2011. Mutant analysis in arabidopsis provides insight into the molecular mode of action of the auxinic herbicide dicamba. PLoS One 6. https://doi.org/10.1371/journal.pone.0017245.

- Goggin, D.E., Kaur, P., Owen, M.J., Powles, S.B., 2018. 2,4-D and dicamba resistance mechanisms in wild radish: subtle, complex and population specific? Ann. Bot. 122, 627–640. https://doi.org/10.1093/aob/mcv097.
- Gove, B., Power, S.A., Buckley, G.P., Ghazoul, J., 2007. Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys. J. Appl. Ecol. 44, 374–384. https://doi.org/10.1111/j.1365-2664.2007.01261.x.
- Grossmann, K., 2003. Mediation of herbicide effects by hormone interactions. J. Plant Growth Regul. 22, 109–122. https://doi.org/10.1007/s00344-003-0020-0.
- Grossmann, K., Caspar, G., Kwiatkowski, J., Bowe, S.J., 2002. On the mechanism of selectivity of the corn herbicide BAS 662H: a combination of the novel auxin transport inhibitor diflufenzopyr and the auxin herbicide dicamba. Pest Manag. Sci. 58, 1002–1014. https://doi.org/10.1002/ps.549.
- Gutiérrez, Y., Ott, D., Scherber, C., 2020. Direct and indirect effects of plant diversity and phenoxy herbicide application on the development and reproduction of a polyphagous herbivore. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-64252-5.
- Herrera, C., 2009. Multiplicity in Unity: Plant Subindividual Variation and Interactions With Animals. The University of Chicago Press, Chicago.
- Iriart, V., Baucom, R.S., Ashman, T.-L., 2020. Herbicides as anthropogenic drivers of ecoevo feedbacks in plant communities at the agro-ecological interface. Mol. Ecol. 1–16. https://doi.org/10.1111/mec.15510.
- Ishii, S., Hirano, C., 1963. Growth responses of larvae of the rice stem borer to rice plants treated with 2,4-D. Entomol. Exp. Appl. 6, 257–262.
- Jones, G.D., 2014. Pollen analyses for pollination research, acetolysis. J. Pollinat. Ecol. 13, 203–217. https://doi.org/10.26786/1920-7603(2014)19.
- Kim, S., 2015. Ppcor: an R package for a fast calculation to semi-partial correlation coefficients. Commun. Stat. Appl. Methods 22, 665–674. https://doi.org/10.5351/CSAM. 2015.22.6.665.
- Kniss, A.R., 2017. Long-term trends in the intensity and relative toxicity of herbicide use. Nat. Commun. 8. https://doi.org/10.1038/ncomms14865.
- Korres, N.E., Burgos, N.R., Duke, S.O., 2019. Weed Control: Sustainability, Hazards and Risks.
- Kreiner, J.M., Stinchcombe, J.R., Wright, S.I., 2018. Population genomics of herbicide resistance: adaptation via evolutionary rescue. Annu. Rev. Plant Biol. 69, 611–635. https:// doi.org/10.1146/annurev-arplant-042817-040038.
- Langsrud, Ø., 2003. ANOVA for unbalanced data: use type II instead of type III sums of squares. Stat. Comput. 13, 163–167.
- Leclere, S., Wu, C., Westra, P., Sammons, D., 2018. Cross-resistance to dicamba, 2,4-D, and fluroxypyr in Kochia scoparia is endowed by a mutation in an AUX/IAA gene. PNAS 115, E6668. https://doi.org/10.1073/pnas.1809836115.
- Levis, N.A., Pfennig, D.W., 2016. Evaluating "plasticity-first" evolution in nature: key criteria and empirical approaches. Trends Ecol. Evol. 31, 563–574. https://doi.org/ 10.1016/j.tree.2016.03.012.
- Li, J., Schuman, M.C., Halitschke, R., Li, X., Guo, H., Grabe, V., Hammer, A., Baldwin, I.T., 2018. The decoration of specialized metabolites influences stylar development. elife 7, 1–24. https://doi.org/10.7554/eLife.38611.
- Ljung, K., Bhalerao, R.P., Sandberg, G., 2001. Sites and homeostatic control of auxin biosynthesis in arabidopsis during vegetative growth. Plant J. 28, 465–474. https://doi.org/ 10.1046/j.1365-313X.2001.01173.x.
- Nikolov, L.A., 2019. Brassicaceae flowers: diversity amid uniformity. J. Exp. Bot. 70, 2623–2635. https://doi.org/10.1093/jxb/erz079.
- Niveyro, S., Salvo, A., 2017. Cultural practices to reduce damage by borer insects in commercial cultivars of amaranthus. Crop Prot. 100, 138–149. https://doi.org/10.1016/j. cropro.2017.06.020.
- Olszyk, D., Pfleeger, T., Henry Lee, E., Plocher, M., 2010. Phytotoxicity assay for seed production using Brassica rapa L. Integr. Environ. Assess. Manag. 6, 725–734. https:// doi.org/10.1002/ieam.89.
- Olszyk, D., Pfleeger, T., Lee, E.H., Plocher, M., 2015. Glyphosate and dicamba herbicide tank mixture effects on native plant and non-genetically engineered soybean seedlings. Ecotoxicology 24, 1014–1027. https://doi.org/10.1007/s10646-015-1442-8.
- Olszyk, D., Pfleeger, T., Shiroyama, T., Blakeley-Smith, M., Lee, E.H., Plocher, M., 2017. Plant reproduction is altered by simulated herbicide drift to constructed plant communities. Environ. Toxicol. Chem. 36, 2799–2813. https://doi.org/10.1002/etc.3839.
- Parízková, B., Žukauskaite, A., Vain, T., Grones, P., Raggi, S., Kubeš, M.F., Kieffer, M., Doyle, S.M., Strnad, M., Kepinski, S., Napier, R., Doležal, K., Robert, S., Novák, O., 2021. New

fluorescent auxin probes visualise tissue-specific and subcellular distributions of auxin in arabidopsis, New Phytol, 230, 535–549. https://doi.org/10.1111/nph.17183.

- Pleasants, J.M., Oberhauser, K.S., 2013. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. Insect Conserv. Divers. 6, 135–144. https://doi.org/10.1111/j.1752-4598.2012.00196.x.
- Powles, S.B., Yu, Q., 2010. Evolution in action: plants resistant to herbicides. Annu. Rev. Plant Biol. https://doi.org/10.1146/annurev-arplant-042809-112119.
- Prosser, R.S., Anderson, J.C., Hanson, M.L., Solomon, K.R., Sibley, P.K., 2016. Indirect effects of herbicides on biota in terrestrial edge-of-field habitats: a critical review of the literature. Agric. Ecosyst. Environ. 232, 59–72. https://doi.org/10.1016/j.agee.2016.07.009.
- R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.
- Riemens, M.M., Dueck, T., Kempenaar, C., Lotz, L.A., Kropff, M.J., 2009. Sublethal effects of herbicides on the biomass and seed production of terrestrial non-crop plant species, influenced by environment, development stage and assessment date. Environ. Pollut. 157, 2306–2313. https://doi.org/10.1016/j.envpol.2009.03.037.
- Rinella, M.J., Masters, R.A., Bellows, S.E., 2010. Growth regulator herbicides prevent invasive annual grass seed production under field conditions. Rangel. Ecol. Manag. 63, 487–490. https://doi.org/10.2111/REM-D-09-00141.1.
- Rotchés-Ribalta, R., Boutin, C., Blanco-Moreno, J.M., Carpenter, D., Sans, F.X., 2015. Herbicide impact on the growth and reproduction of characteristic and rare arable weeds of winter cereal fields. Ecotoxicology 24, 991–1003. https://doi.org/10.1007/s10646-015-1440-x.
- Russo, L., Buckley, Y.M., Hamilton, H., Kavanagh, M., Stout, J.C., 2020. Low concentrations of fertilizer and herbicide alter plant growth and interactions with flower-visiting insects. Agric. Ecosyst. Environ. 304, 107141. https://doi.org/10.1016/j.agee.2020.107141.
- Schulz, R., Bub, S., Petschick, L.L., Stehle, S., Wolfram, J., 2021. Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. Science 372, 81–84. https://doi.org/10.1126/science.abe1148.
- Secretariat of the Convention on Biological Diversity, 2020. Global Biodiversity Outlook 5. CBD, Montreal, Canada www.cbd.int/GB05.
- Steinbrenner, A.D., Agerbirk, N., Orians, C.M., Chew, F.S., 2012. Transient abiotic stresses lead to latent defense and reproductive responses over the Brassica rapa life cycle. Chemoecology 22, 239–250. https://doi.org/10.1007/s00049-012-0113-y.
- Strauss, S.Y., Cacho, N.I., Schwartz, M.W., Schwartz, A.C., Burns, K.C., 2015. Apparency revisited. Entomol. Exp. Appl. 157, 74–85. https://doi.org/10.1111/eea.12347.
- Turcotte, M.M., Davies, T.J., Thomsen, C.J.M., Johnson, M.T.J., B, P.R.S., 2014. Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. Proc. R. Soc. B 281, 20140555.
- Wang, Z., Yu, S., Zhang, L., Liu, R., Deng, Y., Nie, Y., Zhou, Z., Diao, J., 2021. Effects of simazine herbicide on a plant-arthropod-lizard tritrophic community in territorial indoor microcosms: beyond the toxicity. Sci. Total Environ. 781, 146723. https://doi.org/10. 1016/j.scitotenv.2021.146723.
- Watson, M.A., 1986. Integrated physiological units in plants. Trends Ecol. Evol. 1, 119–123. https://doi.org/10.1016/0169-5347(86)90005-4.
- Wcislo, W.T., Cane, J.H., 1996. Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. Annu. Rev. Entomol. 41, 257–286. https://doi.org/10.1146/annurev.en.41.010196.001353.
- Wickham, H., et al., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
- Williams, P.H., Hill, C.B., 1986. Rapid-cycling populations of brassica. Science 232, 1385–1389. https://doi.org/10.1126/science.232.4756.1385.
- Yu, C.Y., Dong, J.G., Hu, S.W., Xu, A.X., 2017. Exposure to trace amounts of sulfonylurea herbicide tribenuron-methyl causes male sterility in 17 species or subspecies of cruciferous plants. BMC Plant Biol. 17, 1–11. https://doi.org/10.1186/s12870-017-1019-1.
- Zhao, Y., 2010. Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol. 61, 49–64. https://doi.org/10.1146/annurev-arplant-042809-112308.
- Zimmerman, P., Hitchcock, A., Kirkpatrick Jr., H., 1953. Methods for determining relative volatility of esters of 2,4-D and other growth regulants based on response of tomato plants. Weeds 2, 254–261.