Clonal versus non-clonal milkweeds (Asclepias spp.) respond differently to stem damage, affecting oviposition by monarch butterflies

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ABSTRACT

Background: Oviposition decisions are critical to the fitness of herbivorous insects and are often impacted by the availability and condition of host plants. Monarch butterflies (Danaus plexippus) rely on milkweeds (Asclepias spp.) for egg-laying and as food for larvae. Previous work has shown that monarchs prefer to oviposit on recently regrown plant tissues (after removal of above-ground biomass) while larvae grow poorly on plants previously damaged by insects. We hypothesized that these effects may depend on the life-history strategy of plants, as clonal and non-clonal milkweed species differ in resource allocation and defense strategies.

Methodology/Principal Findings: We first confirmed butterfly preference for regrown tissue in a field survey of paired mowed and unmowed plots of the common milkweed A. syriaca. We then experimentally studied the effects of plant damage (comparing undamaged controls to plants clipped and regrown, or damaged by insects) on oviposition choice, larval performance, and leaf quality of two closely related clonal and non-clonal species pairs: (1) A. syriaca and A. tuberosa, and (2) A. verticillata and A. incarnata. Clonal and non-clonal species displayed different responses to plant damage, impacting the proportions of eggs laid on plants. Clonal species had similar mean proportions of eggs on regrown and control plants (≈35–40% each), but fewer on insect-damaged plants (≈20%). Meanwhile non-clonal species had similar oviposition on insect-damaged and control plants (20–30% each) but more eggs on regrown plants (40–60%). Trait analyses showed reduced defenses in regrown plants and we found some evidence, although variable, for negative effects of insect damage on subsequent larval performance.

Conclusions/Significance: Overall, non-clonal species are more susceptible and preferred by monarch butterflies following clipping, while clonal species show tolerance to clipping and induced defense to insect herbivory. These results have implications for monarch conservation strategies that involve milkweed habitat management by mowing. More generally, plant life-history may mediate growth and defense strategies, explaining species-level variation in responses to different types of damage.

Keywords Butterfly conservation, Clonal plants, Plant-insect interactions

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INTRODUCTION

Oviposition choices by herbivorous insects strongly impact fitness. Specifically, the suitability of an oviposition site influences hatching success, larval performance, and susceptibility to enemies (Resetarits, 1996). Consequently, as suggested by optimal oviposition theory and the preference-performance hypothesis, the most suitable plants for offspring survival and performance should be preferred for oviposition if they are available (Jaenike, 1978; Gritenberg et al., 2010). Like many lepidopterans, monarch butterflies (Danaus plexippus) are host specific—they lay eggs exclusively on one group of plants (primarily milkweeds in the genus Asclepias) and their larvae exclusively feed on these plants. Following recent reports of declining monarch populations in Eastern North America (Agrawal & Inamine, 2018; Brower et al., 2012; Vidal & Rendón-Salinas, 2014), some researchers have suggested strategically removing above-ground portions of milkweed to induce regrowth as a management strategy to increase monarch numbers because regrowth foliage is highly preferred by monarchs. Specifically, Fischer et al. (2015), Knight et al. (2019), and Haan & Landis (2019b) experimentally showed that mowing Asclepias syriaca plots at appropriate times in the summer can increase densities of eggs naturally laid by monarchs. Similarly, Baum & Sharber (2012) found that summer-prescribed fire on A. viridis increased egg and larval densities in late summer and early fall. Besides extending the growing season of milkweed by providing fresh regrowth when plants would otherwise be senescing (Alcock, Brower & Williams, 2016) and reducing predator numbers during the weeks needed to recolonize regrowing milkweed (Haan & Landis, 2019a, 2019b), removing above-ground tissue including apical buds of milkweed may also alter leaf quality traits in ways that are favorable for monarchs.

Many previous studies have found that plant damage can influence leaf quality for herbivorous insects (Karban & Baldwin, 1997). Generally, insect damage has been found to increase defense (Chen, 2008; Green & Ryan, 1972; Van Zandt & Agrawal, 2004), while damaging plants in a way that removes apical buds often increases leaf quality for herbivorous insects and grazers (Agrawal & Spiller, 2004; Danell & Huss-Danell, 1985; Nykänen & Koricheva, 2004). For example, leaf damage and defoliation of mountain birches induced phenolic compounds (Tuomi et al., 1988) and reduced insect performance (Haukioja, 1982), while removing apical buds on the same species induced ramets to produce larger leaves with higher water content, improving insect performance (Senn & Haukioja, 1994). Opposing plant responses to leaf feeding vs. damage that removes apical meristems (including mowing) may be driven by differential physiological responses and may be quite general (Karban & Niño, 1995; Karban & Baldwin, 1997). For monarchs on milkweed, insect herbivory typically increases latex production and stronger resistance to larvae (Agrawal et al., 2015; Van Zandt & Agrawal, 2004), while regrown tissues after apical bud or shoot removal are preferred for monarch oviposition (Zalucki & Kitching, 1982), suggesting higher leaf quality or lower defenses in those plants (Agrawal, 2017).

There are over 140 species of Asclepias of varying life-history strategies, including clonal and non-clonal species, that inhabit diverse habitats across North America (Agrawal et al., 2015). Non-clonal species primarily regrow from shoot removal by reallocating energy...
and activating dormant buds on existing stems. Clonal plants have additional meristems stored underground and thus have additional strategies including translocation and reallocation of resources in rhizomes along with activation of new stems, increasing their ability to regrow after disturbance. This makes clonal plants potentially more tolerant to shoot removal events such as grazing (Schmid et al., 1988; Liu et al., 2007) and abiotic disturbances (Fischer et al., 2015; Schmid et al., 1988). Theory on plant growth and defense predicts that resource allocation to growth trades off with defense, suggesting that clonal plants, which have higher regrowth capacities, may have lower defenses and higher leaf quality for herbivores (Herms & Mattson, 1992; Jong & Meijden, 2000). The resource availability hypothesis predicts that although growth-dominated species may be less constitutively defended, they may show higher levels of plasticity, including induced defense (Coley, Bryant & Chapin, 1985). Previous studies found that clonal reproduction repeatedly evolved in Asclepias species associated with greater investment in root biomass and lower leaf toxin concentrations, suggesting this trade-off may impact plant-herbivore interactions (Pellissier et al., 2016). Given that monarch butterflies have oviposition preferences for specific milkweed species (Haribal & Renwick, 1998; Jones & Agrawal, 2019; Ladner & Altizer, 2005; Pocius et al., 2018; Zalucki, Oyeyele & Vowles, 1989), we predicted that oviposition decisions may be impacted by plant life-history and previous damage, both which alter resource allocation within the plant.

Here we first confirmed monarch preference for clipped and regrown tissue in a field survey of mowed and unmowed patches of common milkweed, A. syriaca. We then tested the effect of two types of plant damage (stem clipping vs. leaf herbivory by monarch caterpillars) on adult female monarch oviposition preferences by conducting choice experiments in field cages. We conducted this work on two phylogenetic pairs of species, each with a clonal and non-clonal representative, respectively: A. syriaca and A. tuberosa, and A. verticillata and A. incarnata. Other plant traits may accompany the differences in clonality we observed, and we consider these part of the life-history effects we sought to test. We predicted that (1) insect herbivory would induce plant resistance, (2) clipping to mimic mowing would induce susceptibility, and (3) the strength of these effects would be dependent on the extent of clonality of the species. In particular, non-clonal species were predicted to induce stronger resistance to insect damage and greater susceptibility to clipping compared to the same treatments in clonal species. These results were also expected to be reflected in assays of plant traits and larval growth.

**MATERIALS AND METHODS**

**Field survey**

In 2019, we surveyed seven paired mowed and unmowed patches (blocks) of A. syriaca at Cornell University’s Dunlop Meadow Natural Area (Brooktondale, NY, USA: 42.386, −76.395). Within each pair, one block of milkweeds had been mowed regularly prior to the survey, ~25 cm off the ground twice annually during the growing season, and the other was not mowed. In 2019, mowing occurred on July 29. We surveyed five milkweed ramets randomly, each separated by at least two meters, within each block on two dates: once before mowing on July 23 and once after mowing on August 17 when mowed plants had
regrown. On July 23, milkweed plants in blocks that were regularly mowed were 36% shorter than milkweeds in blocks that were not mowed during the growing season. After plants had regrown on August 17, milkweeds in mowed blocks were 82% shorter than milkweeds in unmowed blocks. On each survey date, we measured the height in cm, number of leaves, number of monarch eggs, and number of monarch feeding circles (circular holes on leaves that are indicative of initial chewing by first instar larvae) on each plant. We analyzed the number of monarch eggs and monarch feeding circles across treatments separately for each sampling date, as different plants were randomly sampled each time, using generalized linear mixed models with negative binomial distributions in the lme4 package in R. For July 23 (before mowing), mowing treatment, height, and number of leaves were included as fixed effects. For August 17 (after mowing), only mowing was included as a predictor as no other factors were predictive. Pair and block nested within pair were included as random effects. We tested for significance of fixed effects using likelihood ratio chi-square tests.

### Monarch oviposition choice

We studied two pairs of closely related clonal and non-clonal milkweed species—(1) *A. syriaca* and *A. tuberosa*, and (2) *A. verticillata* and *A. incarnata* (Table 1). *A. syriaca* and *A. tuberosa* are closely related in the Temperate North American clade and *A. verticillata* and *A. incarnata* are closely related in the Incarnatae clade (Fishbein et al., 2011). Seeds of all plant species were washed in 10% bleach, scarified, cold stratified for 4 days at 4 °C, then incubated for 3 days at 28 °C. Seedlings were planted in 10 cm diameter pots with a peat-based soil (Lamberts LM1) and grown in a growth chamber at 14 h day: light cycle, 28 °C during the day and 26 °C at night (400 microeinsteins of light). Plants were watered when the soil was dry, approximately every 2–3 days, and fertilized once per week (N:P:K 21:5:20, 150 ppm N (μg/g)). A total of 5 weeks after planting, we transplanted the plants into 15 cm diameter pots. For each species, three treatments—clipped and regrown, insect-damage, and undamaged control—were randomly assigned. Regrowth treatments were imposed by clipping just above the cotyledons near soil level to simulate mowing 5 weeks before the oviposition trials. We specifically cut above cotyledons to allow non-clonal species to activate dormant buds and regrow. At the time of oviposition trials, the majority of clipped plants had regrown to their previous heights or taller. For the insect-damage treatments, we placed newly-hatched monarch caterpillars on each plant 5 days before the oviposition trials and allowed them to feed until removal

| Species     | Clade                      | Habitat                  | Clonal potential | Clonality     |
|-------------|----------------------------|--------------------------|------------------|---------------|
| *A. syriaca*| Temperate North American   | North eastern open fields| 31               | Clonal        |
| *A. tuberosa*| Temperate North American | Dry fields and open woodlands| 0.3             | Non-clonal    |
| *A. verticillata*| Incarnatae | Midwestern prairie     | 1.4              | Clonal        |
| *A. incarnata*| Incarnatae                 | Eastern wetlands         | 0.4              | Non-clonal    |

Note: Shown are each species’ clade (Fishbein et al., 2011), natural habitats (Woodson, 1954), and clonal potential defined as root buds per plant after ~45 days of growth (Pellissier et al., 2016).
right before the trials. We employed this scheme because previous work demonstrated the strongest induced responses to monarch herbivory occur 5 days after initial feeding (Agrawal, Patrick & Hastings, 2014), while effects of clipping require more time for new tissue growth. When experiments were conducted 8 weeks after initial planting, insect-damaged and control plants were ~1.7–3.2 times taller than clipped and regrown plants, varying depending on species.

To conduct oviposition choice experiments, we transported the plants to a field site and used a paired-cage design. We had 16 paired trials for A. syriaca and A. tuberosa and 13 for A. verticillata and A. incarnata. In each trial, a single mated female monarch reared from a laboratory colony was placed in a 1 m³ cage in a plowed field in Dryden, NY, USA. In each cage, the monarch was first exposed to a set of three plants of a single species (clipped and regrown, insect-damaged, and control). After a sufficient amount of time for at least 10 eggs to be laid in the cage, the set of plants was removed, and the monarch was then exposed to a new set of plants (the three treatments, but of the paired species). The lengths of trials varied as sets were replaced when at least 10 eggs were laid or after 3 h if fewer than 10 eggs were laid. At the end of each trial, the eggs on each plant were counted and removed.

We analyzed differences in oviposition using a generalized linear mixed effects model in the lme4 package in R for each species pair separately. The proportion of eggs laid was the response variable, and species, treatment, and the interaction between species and treatment were included as fixed effects. Plant height was included as a covariate. Species within cage was included as a random effect. Significance of fixed effects was determined using Type III analysis of variance with Satterthwaite’s method. Pairwise comparisons between treatments were conducted using Tukey’s HSD using the emmeans package.

To evaluate larval performance, we placed one newly hatched caterpillar on each plant after the oviposition trial and caterpillars were allowed to feed for 5 days. Caterpillars were then removed, frozen, and weighed (N ≥ 8 per treatment per host species), and masses were compared using a linear model in R (with species, treatment, and height as fixed effects). The number of lost caterpillars (plant abandonment or mortality) at the time of removal was counted and examined using a general linear model with a binomial distribution using the lme4 package. The number of lost caterpillars was the response variable and species, treatment, height, and the interaction between species and treatment were included as fixed effects. Analysis was done using Type II Wald Chi-square tests using the Anova function in the car package.

On a subset of each species by treatment combination (N = 5), we measured and analyzed leaf quality traits by evaluating latex exudation and trichome density. To analyze latex exudation, the tip of the youngest fully expanded leaf was cut and the latex exuded was absorbed onto a pre-weighed 1 cm diameter filter paper. To measure trichome density, we took circular leaf disks using a 0.25 in diameter puncher on the same youngest fully expanded leaf next to where it had been cut for latex exudation. Images of fresh leaf punches were taken using a camera attached to a microscope and we counted the number of trichomes on each punch using ImageJ. These leaf quality measurements across treatments were analyzed using one-way ANOVA and Tukey’s HSD post-hoc tests.
RESULTS

Field survey

To confirm monarch preference for regrown tissue, a field survey was conducted on mowed and unmowed plots with abundant *A. syriaca*. Before mowing on July 23, there was no difference in number of monarch eggs (Table S1, LRT = 0.122, \( p = 0.727 \)). Because very few eggs were observed (three in total), we also sampled numbers of initial monarch feeding circles; similarly, we found no difference in their numbers (mean ± SE circles per plant, mowed 3.60 ± 0.92, unmowed 3.57 ± 0.94, LRT = 0.184, \( p = 0.667 \)). A total of 19 days after mowing, mowed plants that resprouted had >3 times more monarch eggs than unmowed plants (Table S1, LRT = 5.429, \( p = 0.020 \), Fig. 1).

Monarch oviposition choice

Oviposition choice experiments were conducted on single plant species, but were paired such that members of the clonal and non-clonal pairs were sequentially tested with the same butterfly. For *A. syriaca* and *A. tuberosa*, the proportion of eggs laid on each plant in the set was impacted by treatment, and differentially between the two species (Fig. 2; Table S2, species by treatment interaction \( F_{2,90} = 4.323, p = 0.016 \)). When clonal *A. syriaca* was analyzed separately, regrown and control plants had the same proportion of eggs on average (Fig. 2, ~38%), while insect-damaged plants had substantially fewer (24%); nonetheless, the treatment effect was not significant in this one-way analysis (\( F_{2,43} = 1.5783, p = 0.218 \)). Treatment had a significant effect for non-clonal *A. tuberosa*, with clipped and regrown plants having about three times more eggs than either control or insect-damaged plants (Fig. 2, \( F_{2,44} = 4.209, p = 0.021 \)). The difference was significant between regrown plants and control plants (Tukey HSD, \( p = 0.023 \)) and between regrown plants and insect-damaged plants (Tukey HSD, \( p = 0.035 \)).

The patterns of differential monarch oviposition for the second clonal—non-clonal milkweed pair, *A. verticillata* and *A. incarnata*, were remarkably similar to that of the first
pair (Fig. 2). We again found evidence for a treatment by species interaction predicting egg laying, although the difference was marginal (Fig. 2; Table S2, species × treatment: $F_{2,72} = 3.014, p = 0.057$). In single species analyses, the effect of treatment was significant only for clonal A. verticillata ($F_{2,34} = 1.578, p = 0.014$), with insect-damaged plants having 47% fewer eggs than clipped and regrown plants and 59% fewer eggs than control plants on average (control vs. insect-damaged plants, Tukey HSD, $p = 0.024$). Treatment had no significant effect on the proportion of eggs on non-clonal A. incarnata plants alone (there was a significant effect of plant height, $F_{2,34} = 7.858, p = 0.008$), although clipped and regrown plants had ~38% more eggs than either insect-damaged or control plants.

Larval performance

While species and treatment affected oviposition, there were no overall effects on larval mass in either clonal and non-clonal species pair (Table S3). However, for both species of the A. syriaca and A. tuberosa pair, ~3 times more larvae disappeared (via plant abandonment or mortality) on insect-damaged plants than either regrown or control plants (Table S4, $p = 0.087$), causing 15–24% lower survival rates on insect-damaged plants (Fig. 3A). Within the second pair of species, treatment had a significant effect only on non-clonal A. incarnata, with larvae raised on regrown plants having two times the mass of larvae raised on insect-damaged plants (Fig. 3B, $F_{2,27} = 4.004, p = 0.030$).

Trait comparisons

Latex exudation was measured only for A. syriaca as exudation was negligible in the other three species. For A. syriaca, latex mass was significantly affected by treatment ($F_{2,12} = 4.359, p = 0.038$), increasing by >3-fold in insect-damaged compared to clipped and regrown plants (Tukey HSD, $p = 0.030$; insect-damage 7.12 ± 1.75, clipped and regrown 2.29 ± 0.42, undamaged controls 4.90 ± 0.88).
Overall, variation in trichome densities among treatments showed similar trends across species. For *A. syriaca*, treatment had a strong effect on trichome density ($F_{2,12} = 9.825$, $p = 0.003$). Specifically, clipping reduced trichome density by about half compared to controls (Tukey HSD, $p = 0.005$, Table S5) and insect-damaged plants (Tukey HSD, $p = 0.008$, Table S5). Clipped and regrown *A. tuberosa* plants similarly had fewer trichomes compared to control and insect-damaged plants, although the treatment effect was non-significant ($F_{2,12} = 3.112$, $p = 0.082$, Table S5). As with the milkweeds of the first pair, damage treatments significantly affected trichome densities of *A. incarnata* plants. Clipped and regrown plants had lower trichome densities compared to the other treatments ($F_{2,12} = 4.844$, $p = 0.029$, Table S5). As the only exception, there was no effect of treatments on trichome densities of *A. verticillata* ($F_{2,12} = 0.571$, $p = 0.579$, Table S5).

**DISCUSSION**

Across the field survey and experiments, monarchs showed an overall increased preference toward regrown tissue of clipped plants and decreased preference toward insect-damaged plants, supporting previous studies which found that shoot removal (including mowing,
clipping, and apical bud damage) induces susceptibility, while insect damage induces defenses (Green & Ryan, 1972; Karban & Niiho, 1995; Karban & Baldwin, 1997; Van Zandt & Agrawal, 2004; Chen, 2008). As predicted, the strength of these effects depended on the life-history strategy (clonality) of the species. On non-clonal milkweed species, the effect of clipping was strongly positive for oviposition choice. Meanwhile, on clonal milkweed species, insect damage treatment had the strongest (negative) effect on oviposition choice. This divergence may be caused by non-clonal species having lower capacities for regrowth after disturbance and are thus heavily affected by shoot and apical bud removal (Schmid et al., 1988; Liu et al., 2007). At the same time, non-clonal species were less impacted by insect damage, perhaps because of a slower growth rate and investment in constitutive defense. These effects may be general across Asclepias of varying life-histories, as tolerance (i.e., the ability to regrow) and resistance (i.e., having traits that reduce herbivore performance or preference) may be alternate defense strategies (Van der Meijden, Wijn & Verkaar, 1988; Karban & Baldwin, 1997; Strauss & Agrawal, 1999; Agrawal & Fishbein, 2008). Indeed, Agrawal et al. (2015) found that more phylogenetically derived Asclepias species tended to have enhanced clonal growth potential with increased tolerance to herbivory from the ability to sprout new ramets, while resistance traits such cardenolides and latex have declined with diversification. In our study, clonal species displayed not only strong tolerance but also inducibility, as oviposition decreased following insect attack. This may be because fast growing clonal species are overall less well defended, but have the capacity for plasticity (Coley, Bryant & Chapin, 1985; Mooney et al., 2010). In a study of clonal Trifolium repens, insect damage increased susceptibility in mature ramets while it induced defense in young ramets (Gómez et al., 2008). We used young plants with singular ramets, where tolerance strategies may not be strong enough for protection (Boege et al., 2007). Future work on clonal species should consider changes in allocation to growth and defense associated with plant ontogeny (Martínková et al., 2020).

While our field survey confirmed oviposition preference for regrown tissue, it also displayed a significant effect of clipping on a clonal species, A. syriaca, inconsistent with our experimental results. This may have been caused by increased apparency of regrown milkweed in mowed plots, as surrounding vegetation was also mowed (and very short). In unmowed plots, A. syriaca was surrounded by dense, tall vegetation. Another possible explanation is that mowing in field plots may have decreased total milkweed stem density, as higher mowing frequencies during the growing season are correlated with lower total number of stems (Dee & Baum, 2019). Indeed, some effects of clipping on oviposition have been shown to be dependent on plant density (Knight et al., 2019). It is also possible that in a field of established non-clonal milkweeds, the effect of clipping may be similarly magnified due to density reduction, although the lack of multiple ramets may increase priority and allocation to regrowth. Finally, in our experimental cage trials, all clipped plants were juvenile and cut above the cotyledons. In the field, mowing of mature plants may be a stronger inducer, activating new clonal stems, than clipping above the cotyledon (Paige, 1994).
Larval performance measured using caterpillar mass, unlike oviposition choice experiments, showed few differences between different species and treatments. Our analysis of larval performance suffered from low sample sizes, as many larvae had disappeared before they could be weighed. Nonetheless, previous studies of monarchs have also found a lack of support for the preference-performance hypothesis. Ladner & Altizer (2005) found that mean larval performance did not correspond to oviposition choices among four Asclepias species. Similarly, Jones & Agrawal (2019) found highest oviposition preference for a species associated with poor larval growth and low sequestration of plant toxins. In A. syriaca patches, Haan & Landis (2019b) found no significant changes in egg and larval survival after mowing despite increased oviposition. One possibility for the lack of a preference-performance link is that beyond leaf quality traits, oviposition stimulants such as flavonoid glycosides (Haribal & Renwick, 1996) and other plant volatiles (Bergström et al., 1994) may have an effect on oviposition. Here we measured latex exudation and trichome density, however a more comprehensive study with more plant trait measures is needed to clarify relationships between plant traits, oviposition choices and larval performance. Although trichomes are certainly deterrent to monarchs (caterpillars shave them on high density leaves), the responses we observed (reduction of trichomes on clipped plants) was consistent across species and likely associated with a growth enhancement (Agrawal, 2017). Furthermore, to fully assess larval performance, future work should assess effects of different types of plant damage on predation of monarch larvae. In a study of common milkweed in Michigan (USA), the abundance of monarch predators decreased following mowing, but direct links between mowing and the predator-prey interaction are not yet clear (Haan & Landis, 2019b).

Implications for monarch butterfly conservation

The results of our study have implications for habitat management and monarch conservation. Previous studies have indicated that A. syriaca (clonal) and A. viridis (non-clonal) milkweed patches can be mowed or burned to increase eggs densities (Baum & Sharber, 2012; Fischer et al., 2015; Knight et al., 2019). Our results suggest that it may be relevant to consider the life-history strategy of the plant species being disturbed. Specifically, positive effects of plant clipping for monarchs may be highest on non-clonal species, although such species tend to be at lower stem-densities than clonal species. Future comparative work in natural populations will be needed to address the effects of clipping on monarch populations feeding on clonal vs. non-clonal milkweeds.

Any management strategy should be considered in conjunction with species preferences, as many studies have shown monarchs prefer ovipositing on particular milkweed species (Haribal & Renwick, 1998; Jones & Agrawal, 2019; Ladner & Altizer, 2005; Pocius et al., 2018; Zalucki, Oyeyele & Vowles, 1989). As different milkweed species often co-occur in the same field, these factors can interact to influence oviposition choices, and the strength of these factors may be patch-dependent. For example, Knight et al. (2019) found that while mowing in the summer generally increases monarch egg laying on mowed plots of A. syriaca, there were no effects on oviposition preferences in low-density...
patches (≤11 milkweeds/m²). Additionally, if there is no preference-performance linkage, the effectiveness of mowing programs may need to be re-evaluated.

Lastly, while mowing programs may increase egg densities, it is unclear whether the larvae produced will be able to contribute to the overall population regardless of whether larval performance corresponds to oviposition choices. The mowing programs proposed by Fischer et al. (2015) and Knight et al. (2019) induce freshly regrown tissues preferred by monarchs and extend their breeding season, but it is uncertain whether the new generation of monarchs that emerge can successfully join the migration to Mexico. As expressed by Alcock, Brower & Williams (2016), problems with this strategy include the replacement of senescing milkweed late in summer, which is important for stimulating the southern migration (Bataldon & Oberhauser, 2015). Overall, the connection between increased egg densities and population-level effects needs substantial investigation.

**Conclusion: a general model for plant clonality, defense, and responsiveness to damage**

Clonality in plants is generally seen as an adaptive response to environmental conditions (Fischer & Van Kleunen, 2001; Van Groenendael et al., 1996), specifically to spatial and temporal variability as clonal traits allow rapid recovery from frequent disturbance and seasonal inactivity (Suzuki & Stuefer, 1999). Across herbaceous plants, clonality increases with higher latitudes and greater seasonality (Ye et al., 2014). Clonal milkweed species have been found to inhabit colder and drier regions, where there may be lower herbivore pressure but higher climatic variability and relatively higher abiotic stresses such as trampling or fire (Pellissier et al., 2016). Conversely, resistance traits are often associated with environments with high herbivory (Karban & Baldwin, 1997; Chen, 2008) or warmer, tropical climates, where there is higher herbivore pressure (Pellissier et al., 2016).

In our study, we found a weaker effect of abiotic disturbance (clipping) for clonal compared to non-clonal milkweed species. These effects may be general and apply to other systems—tolerance through clonality and regrowth protects plants against abiotic disturbances, where herbivore pressure may typically be low or unpredictable. Conversely, non-clonal plants may favor defenses overall, but respond to clipping by prioritizing growth at the cost of susceptibility to herbivores. A framework linking plant clonality and responses to damage therefore depends on the type of damage (abiotic disturbance or herbivory) that is most prevalent in the environment. We advocate future work that melds comparisons of closely related species with differing life-history strategies and incorporates both abiotic disturbance and insect attack.

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**ADDITIONAL INFORMATION AND DECLARATIONS**

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The authors declare that they have no competing interests.

**Author Contributions**
- Elise He conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Anurag A. Agrawal conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

**Data Availability**
The following information was supplied regarding data availability:
Raw data is available in the Supplemental Files.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.10296#supplemental-information.

**REFERENCES**

Agrawal A. 2017. Monarchs and milkweed: a migrating butterfly, a poisonous plant, and their remarkable story of coevolution. Princeton: Princeton University Press.

Agrawal A, Ali JG, Rasmann S, Fishbein M. 2015. Macroevolutionary trends in the defense of milkweeds against monarchs: latex, cardenolides, and tolerance of herbivory. In: Oberhauser K, Altizer S, Nail K, eds. Monarchs in a Changing World: Biology and Conservation of an Iconic Insect. Ithaca: Cornell University Press, 47–59.

Agrawal AA, Fishbein M. 2008. Phylogenetic escalation and decline of plant defense strategies. *Proceedings of the National Academy of Sciences* 105(29):10057–10060 DOI 10.1073/pnas.0802368105.

Agrawal AA, Inamine H. 2018. Mechanisms behind the monarch’s decline. *Science* 360(6395):1294–1296 DOI 10.1126/science.aat5066.

Agrawal AA, Patrick ET, Hastings AP. 2014. Tests of the coupled expression of latex and cardenolide plant defense in common milkweed (*Asclepias syriaca*). *Ecosphere* 5(10):art126 DOI 10.1890/ES14-00161.1.
Agrawal AA, Spiller DA. 2004. Polymorphic buttonwood: effects of disturbance on resistance to herbivores in green and silver morphs of a Bahamian shrub. *American Journal of Botany* 91(12):1990–1997 DOI 10.3732/ajb.91.12.1990.

Alcock J, Brower LP, Williams EH. 2016. Monarch butterflies use regenerating milkweeds for reproduction in mowed hayfields in Northern Virginia. *Journal of Lepidopterists' Society* 70(3):177–181 DOI 10.18473/107.070.0302.

Batalden RV, Oberhauser KS. 2015. Potential changes in eastern North American monarch migration in response to an introduced milkweed, *Asclepias curassavica*. In: Oberhauser K, Altizer S, Nail KR, eds. *Monarchs in a Changing World: Biology and Conservation of an Iconic Insect*. Ithaca: Cornell University Press, 215–224.

Baum KA, Sharber WV. 2012. Fire creates host plant patches for monarch butterflies. *Biology Letters* 8(6):968–971 DOI 10.1098/rsbl.2012.0550.

Bergström G, Rothschild M, Groth I, Crighton C. 1994. Oviposition by butterflies on young leaves: investigation of leaf volatiles. *Chemoeconomy* 5(3–4):147–158 DOI 10.1007/BF01240599.

Boege K, Dirzo R, Siemens D, Brown P. 2007. Ontogenetic switches from plant resistance to tolerance: minimizing costs with age? *Ecology Letters* 10(3):177–187 DOI 10.1111/j.1461-0248.2006.01012.x.

Brower LP, Taylor OR, Williams EH, Slayback DA, Zubieta RR, Ramírez MI. 2012. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? *Insect Conservation and Diversity* 5(2):95–100 DOI 10.1111/j.1752-4598.2011.00142.x.

Chen M-S. 2008. Inducible direct plant defense against insect herbivores: a review. *Insect Science* 15(2):101–114 DOI 10.1111/j.1744-7917.2008.00190.x.

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. *Science* 230(4728):895–899 DOI 10.1126/science.230.4728.895.

Danell K, Huss-Danell K. 1985. Feeding by insects and hares on birches earlier affected by moose browsing. *Oikos* 44(1):75–81 DOI 10.2307/3544046.

Dee JR, Baum KA. 2019. Mowing frequency influences number of flowering stems but not population age structure of *Asclepias viridis*, an important monarch host plant. *American Midland Naturalist* 182(1):27–35 DOI 10.1674/0003-0031-182.1.27.

Fischer M, Van Kleunen M. 2001. On the evolution of clonal plant life histories. *Evolutionary Ecology* 15(4–6):565–582 DOI 10.1023/A:1016013721469.

Fischer SJ, Williams EH, Brower LP, Palmiotto PA. 2015. Enhancing monarch butterfly reproduction by mowing fields of common milkweed. *American Midland Naturalist* 173(2):229–241 DOI 10.1674/amid-173-02-229-240.1.

Fishbein M, Chuba D, Ellison C, Mason-Gamer RJ, Lynch SP. 2011. Phylogenetic relationships of *Asclepias* (Apocynaceae) inferred from non-coding chloroplast DNA sequences. *Systematic Botany* 36(4):1008–1023 DOI 10.1600/036364411X605010.

Gómez S, Onoda Y, Ossipov V, Stuefer JF. 2008. Systemic induced resistance: a risk-spreading strategy in clonal plant networks? *New Phytologist* 179(4):1142–1153 DOI 10.1111/j.1469-8137.2008.02542.x.

Green TR, Ryan CA. 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* 175(4023):776–777 DOI 10.1126/science.175.4023.776.

Gripenberg S, Mayhew PJ, Parnell M, Roslin T. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters* 13(3):383–393 DOI 10.1111/j.1461-0248.2009.01433.x.

He and Agrawal (2020), *PeerJ*, DOI 10.7717/peerj.10296
Haan NL, Landis DA. 2019a. The importance of shifting disturbance regimes in monarch butterfly decline and recovery. *Frontiers in Ecology and Evolution* 7:893 DOI 10.3389/fevo.2019.00191.

Haan NL, Landis DA. 2019b. Grassland disturbance increases monarch butterfly oviposition and decreases arthropod predator abundance. *Biological Conservation* 233:185–192 DOI 10.1016/j.biocon.2019.03.007.

Haribal M, Renwick JAA. 1996. Oviposition stimulants for the monarch butterfly: flavonol glycosides from *Asclepias curassavica*. *Phytochemistry* 41(1):139–144 DOI 10.1016/0031-9422(95)00511-0.

Haribal M, Renwick JAA. 1998. Differential postalightment oviposition behavior of monarch butterflies on *Asclepias* species. *Journal of Insect Behavior* 11(4):507–538 DOI 10.1023/A:1022363329446.

Haukioja E. 1982. Inducible defences of white birch to a geometrid defoliator, *Epirrita autumnata*. In: *Proceedings of the 5th International Symposium on Insect-Plant Relationships*, Wageningen: Centre for Agricultural Publishing and Documentation, 199–203.

Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* 67(3):283–335 DOI 10.1086/417659.

Jaenike J. 1978. On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology* 14(3):350–356 DOI 10.1016/0040-5809(78)90012-6.

Jones PL, Agrawal AA. 2019. Beyond preference and performance: host plant selection by monarch butterflies, *Danaus plexippus*. *Oikos* 128(8):1092–1102 DOI 10.1111/oik.06001.

Jong TJD, Meijden EVD. 2000. On the correlation between allocation to defence and regrowth in plants. *Oikos* 88(3):503–508 DOI 10.1111/j.1600-0706.2000.880305.x.

Karban R, Baldwin IT. 1997. *Induced responses to herbivory*. Chicago: University of Chicago Press.

Karban R, Niiho C. 1995. Induced resistance and susceptibility to herbivory: plant memory and altered plant development. *Ecology* 76(4):1220–1225 DOI 10.2307/1940928.

Knight SM, Norris DR, Derbyshire R, Flockhart DTT. 2019. Strategic mowing of roadside milkweeds increases monarch butterfly oviposition. *Global Ecology and Conservation* 19:e00678 DOI 10.1016/j.gecco.2019.e00678.

Ladner DT, Altizer S. 2005. Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. *Entomologia Experimentalis et Applicata* 116(1):9–20 DOI 10.1111/j.1570-7458.2005.00308.x.

Liu H-D, Yu F-H, He W-M, Chu Y, Dong M. 2007. Are clonal plants more tolerant to grazing than co-occurring non-clonal plants in inland dunes? *Ecological Research* 22(3):502–506 DOI 10.1007/s11284-007-0332-9.

Martínková J, Klimeš A, Puj J, Klimešová J. 2020. Response of clonal versus non-clonal herbs to disturbance: different strategies revealed. *Perspectives in Plant Ecology, Evolution and Systematics* 44:125529 DOI 10.1016/j.ppees.2020.125529.

Mooney KA, Halitschke R, Kessler A, Agrawal AA. 2010. Evolutionary trade-offs in plants mediate the strength of trophic cascades. *Science* 327(5973):1642–1644 DOI 10.1126/science.1184814.

Nykänen H, Koricheva J. 2004. Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* 104(2):247–268 DOI 10.1111/j.0030-1299.2004.12768.x.

Paige KN. 1994. Herbivory and ipomopsis aggregata: differences in response, differences in experimental protocol: a reply to Bergelson and Crawley. *American Naturalist* 143(4):739–749 DOI 10.1086/285630.
Pellissier L, Litsios G, Fishbein M, Salamin N, Agrawal AA, Rasmann S. 2016. Different rates of defense evolution and niche preferences in clonal and nonclonal milkweeds (Asclepias spp.). *New Phytologist* **209**(3):1230–1239 DOI 10.1111/nph.13649.

Pocius VM, Debinski DM, Pleasants JM, Bidne KG, Hellmich RL. 2018. Monarch butterflies do not place all of their eggs in one basket: oviposition on nine Midwestern milkweed species. *Ecosphere* **9**(1):e02064 DOI 10.1002/ecs2.2064.

Resetarits WJ. 1996. Oviposition site choice and life history evolution. *Integrative and Comparative Biology* **36**(2):205–215 DOI 10.1093/icb/36.2.205.

Schmid B, Puttick GM, Burgess KH, Bazzaz FA. 1988. Clonal integration and effects of simulated herbivory in old-field perennials. *Oecologia* **75**(3):465–471 DOI 10.1007/BF00376953.

Senn J, Haukojoa E. 1994. Reactions of the mountain birch to bud removal: effects of severity and timing, and implications for herbivores. *Functional Ecology* **8**(4):494–501 DOI 10.2307/2390074.

Suzuki J-I, Stuefer J. 1999. On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biology* **14**(1):11–17 DOI 10.1046/j.1442-1984.1999.00002.x.

Van der Meijden E, Wijn M, Verkaar HJ. 1988. Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* **51**(3):355–363 DOI 10.2307/3565318.

Van Groenendael JM, Klimeš J, Klimešová J, Hendriks RJJ. 1996. Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **351**(1345):1331–1339 DOI 10.1098/rstb.1996.0116.

Van Zandt PA, Agrawal AA. 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed Asclepias syriaca. *Oikos* **104**(2):401–409 DOI 10.1111/j.0030-1299.2004.12964.x.

Vidal O, Rendón-Salinas E. 2014. Dynamics and trends of overwintering colonies of the monarch butterfly in Mexico. *Biological Conservation* **180**:165–175 DOI 10.1016/j.biocon.2014.09.041.

Woodson RE. 1954. The North American species of Asclepias L. *Annals of the Missouri Botanical Garden* **41**(1):1–211 DOI 10.2307/2394652.

Ye D, Hu Y, Song M, Pan X, Xie X, Liu G, Ye X, Dong M. 2014. Clonality-climate relationships along latitudinal gradient across China: adaptation of clonality to environments. *PLOS ONE* **9**(4):e94009 DOI 10.1371/journal.pone.0094009.

Zalucki MP, Kitching RL. 1982. Dynamics of oviposition in Danaus plexippus (Insecta: Lepidoptera) on milkweed, Asclepias spp. *Journal of Zoology* **198**(1):103–116 DOI 10.1111/j.1469-7998.1982.tb02063.x.

Zalucki MP, Oyeyele S, Vowles P. 1989. Selective oviposition by Danaus Plexippus (L.) (Lepidoptera: Nymphalidae) in a mixed stand of Asclepias fruticosa and A. curassavica in Southeast Queensland. *Australian Journal of Entomology* **28**(2):141–146 DOI 10.1111/j.1440-6055.1989.tb01211.x.