Herbivory and jasmonate treatment affect reproductive traits in wild Lima bean, but without transgenerational effects

Carlos Bustos-Segura | Johnattan Hernández-Cumplido | Juan Traine | Betty Benrey

Abstract

Premise: Plant responses to herbivores and their elicitors include changes in traits associated with phenology, defense, and reproduction. Induced responses by chewing herbivores are known to be hormonally mediated by the jasmonate pathway and can cascade and affect late-season seed predators and pollinators. Moreover, herbivore-induced plant responses can be transmitted to the next generation. Whether herbivore-induced transgenerational effects also apply to phenological traits is less well understood.

Methods: Here, we explored responses of wild Lima bean plants (Phaseolus lunatus) to herbivory and jasmonate treatment and possible transgenerational effects of herbivore-induced early flowering. In a controlled field experiment, we exposed Lima bean plants to herbivory by leaf beetles or methyl jasmonate sprays (MJ). We then compared plant development, phenology, reproductive fitness and seed traits among these treatments and undamaged, untreated control plants.

Results: We found that MJ and leaf herbivory induced similar responses, with treated plants growing less, flowering earlier, and producing fewer seeds than undamaged plants. However, seed size, phenolics and cyanogenic glycosides concentrations did not differ among treatments. Seed germination rates and flowering time of the offspring were similar among maternal treatments.

Conclusions: Overall, the results confirm that responses of Lima bean to herbivory by leaf beetles are mediated by jasmonate; however, effects on phenological traits are not transmitted to the next generation. We discuss why transgenerational effects of herbivory might be restricted to traits that directly target herbivores.

Keywords

early flowering, Fabaceae, herbivore induction, jasmonate, leaf beetles, Phaseolus lunatus, plant phenology, seed traits, transgenerational effects

Induced responses to herbivory can be triggered by leaf damage and elicitors produced by herbivores (Alborn et al., 1997; Agrawal, 1998). Induced defenses in plants include production of trichomes, secondary metabolites, extrafloral nectar, plant volatiles, and tolerance responses (Turlings et al., 1995; Agrawal, 2000; Dicke and Hilker, 2003; Heil, 2004; Dicke and Baldwin, 2010; Fornoni, 2011). However, plant responses to herbivory also include traits that are not directly associated with plant defenses. Herbivory can influence plant phenology by changing plant architecture and altering development (Karban and Baldwin, 1997; Ohgushi, 2005). The induction of plant responses is the result of signaling pathways that activate the synthesis of proteins contributing to the expression of defensive traits. Two main defensive signaling pathways are the jasmonic acid pathway as a response to chewing damage and the salicylic acid pathway as a response to sucking insects and pathogen infection.

© 2021 The Authors. American Journal of Botany published by Wiley Periodicals LLC on behalf of Botanical Society of America.
In addition, jasmonic acid (JA) also activates induced responses in phenology. For example, in wild radish, both damage by herbivores and application of JA without damage increase the concentration of defensive indole glucosinolates in leaves and, at the same time, delay flowering and reduce the number of seeds and pollen grains, revealing a cost of the induction (Agrawal et al., 1999b). In inflorescences of *Brassica nigra*, herbivory is also associated with an increase of JA, which is involved in defense against florivory (Chrétien et al., 2018). However, changes in JA in floral tissue can potentially modify flower development, an important component of plant fitness (Yuan and Zhang, 2015). Thus, an herbivore influence on plant defenses can be linked to changes in flower development mediated by JA. Yet, the effects of JA on flowering vary and depend on the type of defense induction (physical or biotic) and the plant species (Pak et al., 2009; Chehab et al., 2012; Yuan and Zhang, 2015; Niwa et al., 2018).

Antagonistic interactions between plants and animals continue with pollination and seed predation after flowering. For example, induction by leaf herbivores can have indirect consequences for future plant–animal interactions mediated by changes in phenology and not direct defenses. In lima bean, previous studies have shown that leaf herbivory by chrysomelid beetles and generalist caterpillars alters seed traits that later influence seed predation by seed beetles (Hernández-Cumplido et al., 2016b; Bustos-Segura et al., 2020). Similarly, in common evening primrose, herbivory by the invasive Japanese beetle induces JA in flowers, which in turn reduces seed predation by native Lepidoptera (McArt et al., 2013). Given that flowering time is important for many associated organisms, mutualists and antagonists might exert opposing selective pressures on flowering phenology. In general, pollinators select for earlier flowering, while pre-dispersal seed predators select for delayed flowering, although there is great variation among plant systems (Elzinga et al., 2007). Nevertheless, according to a meta-analysis, on average, earlier flowering time is favored in most of the studied plant species (Munguía-Rosas et al., 2011). In turn, herbivore-induced changes in floral morphology and physiology can change the behavior of pollinators, as observed in *Brassica nigra* (Rusman et al., 2019), and even pollinators can force earlier flowering by chewing on leaves when there is a lack of pollen resources (Pashalidou et al., 2020).

Induced plant responses to herbivory can be transmitted to the next plant generation through transgenerational effects (Holeski et al., 2012). The transgenerational effects of herbivores on plants have been observed mainly as an increase of antiherbivore defenses in plants whose parents had induced defenses against herbivory (Agrawal, 2001; Holeski et al., 2013; Aguirrebengoa et al., 2018). When jasmonate is directly applied to plants, it induces responses associated with herbivory and activates the plant defense pathway (Farmer and Ryan, 1990; Thaler, 1999; Alves et al., 2007), but it can also be associated with transgenerational effects of herbivory (Agrawal, 2002; Rasmann et al., 2012). It is possible that induced changes by herbivory in plant phenological traits, such as flowering time, can also be transmitted to the next generation, which could have important consequences for plant development, interactions with pollinators, and consequently affect fertilization and plant fitness. However, to date, studies on transgenerational effects of herbivory on plant traits have been largely limited to defensive traits.

These examples show that the effects of herbivory and JA induction on plant and seed traits are vast and can have important consequences on plant fitness. In lima bean (*Phaseolus lunatus*), JA also induces the release of volatiles and increases nectar production in extrafloral nectaries (Heil, 2004; Hernández-Cumplido et al., 2016a). Both of these indirect defenses attract natural enemies of herbivores such as parasitic wasps and ants that ultimately benefit lima bean plants (Kost and Heil, 2008). In addition, transgererational effects of herbivory and exogenous application of JA on lima bean have been found for cyanogenic glycosides (Ballhorn et al., 2016) and for the susceptibility and quality of seeds for late-season insects (J. Hernández-Cumplido et al., unpublished data). However, transgenerational effects on induced phenological changes such as flowering time have rarely been studied. Thus, in the present work, we investigated how herbivory by leaf beetles and exogeneous application of methyl jasmonate (MJ, an organic volatile derived from JA) on wild lima bean plants influence fitness components, including flowering time and seed traits which may play a role in future interactions. Because flowering time in lima bean is triggered by seasonal changes (Baudoin, 1988; Heil, 2004), we planted seeds at different times throughout the season to explore whether environmental variations affect the response of flowering time to herbivory. Finally, we tested whether herbivory and MJ application have transgenerational effects on flowering time.

**MATERIALS AND METHODS**

**Biological system**

*Phaseolus lunatus* L. (Fabaceae) is an annual plant that occurs from Mexico to South American and Andean America (Freytag and Debouck, 2002), with populations belonging to two main genetic pools, the Mesoamerican and Andean (Salgado-Gutiérrez et al., 1995). It is a climbing vine up to 8 m long with abundant branching, so it often covers trees and shrubs (Freytag and Debouck, 2002; Heil, 2004). Its compound leaves have three leaflets, and its purple/whitish flowers are arranged in panicle inflorescences (Freytag and Debouck, 2002). After fertilization, flowers develop dehiscent pods with ~3–4 seeds per pod (Freytag and Debouck, 2002). It also has extrafloral nectaries that can recruit ants for its defense (Hernández-Cumplido et al., 2010). In southern Mexico, where our field site is located, this species germinates in June to July and starts
flowering in October and producing seeds in November. Seeds are dispersed between January and February (Heil, 2004; Moreira et al., 2015).

At our field site, some of the most common herbivore species that attack *P. lunatus* are leaf-chewing beetles from the Chrysomelidae family, such as Diabrotica balteata and Cerotoma ruficornis (Hernández-Cumplido et al., 2016b; Moreira et al., 2016). *Cerotoma ruficornis* is particularly active from July to November, when adults feed on the underside of the leaves (Hernández-Cumplido et al., 2016b).

### Field experiment

Field experiments were done in 2015 at the experimental campus of the Universidad del Mar 15 km northwest of Puerto Escondido (Oaxaca, Mexico, 15°55’33.3”N, 97°09’03.0’’W). Seeds were obtained from 20–30 randomly selected plants next to our field site, then we sowed three seeds per pot (5-L) on four dates: 12, 19, and 26 October and 2 November (hereafter referred to as first, second, third, and fourth plant cohort, respectively). This planting scheme allowed us to separate the independent effects of flowering time on plant and seed traits from the induction effects of herbivory and MJ application and to determine whether induced responses are consistent throughout the season. Upon germination, seedlings were thinned to one plant per pot and were covered with nylon field tents before the start of the treatments (Bioquip, Outdoor Cage 3.66 × 1.83 × 1.83 m, Lumite mesh, Rancho Dominguez, CA, USA) to protect them from unwanted herbivory. Three treatments were randomly assigned to tents across all plant cohorts. Treatments were applied as soon as the plants were moved to the small tents (3 weeks after sowing). Three treatments were randomly distributed in smaller tents (Bioquip cages 1.83 × 1.83 × 1.83 m, Lumite mesh) with three plants per tent (*N* = 108 plants, a total of 36 tents across all plant cohorts). Treatments were applied as soon as the plants were moved to the small tents (3 weeks after sowing). Three treatments were randomly assigned to tents within a plant cohort (one treatment in each tent): H, herbivory treatment (by *Cerotoma ruficornis* Coleoptera: Chrysomelidae); MJ, methyl jasmonate treatment; C, control (no herbivores or MJ). In total, we had 12 tents per treatment for all the plant cohorts. Because MJ application could induce responses in the neighbor plants inside the same tent, we opted for this design and accounted for the non-independence between plants in the linear model specifications (see Statistical methods section).

Plants were 100 cm from each other, and the distance between tents was 2 m. For every plant cohort, three tents had plants infested with the foliar herbivore *C. ruficornis*, and three tents had plants sprayed with 1 mM MJ in H2O (Merck, Zug, Switzerland). In addition, three tents per plant cohort were used as a control without herbivores. In each tent of the herbivory treatment, we introduced 10 beetles collected from the field each week for 4 weeks, starting when plants were transplanted from the growing tents (i.e., 40 individuals in each tent at the end of the experiment). The number of beetles was based on methods from previous studies (Ballhorn et al., 2013; Hernández-Cumplido et al., 2016b). For the MJ treatment, the application was repeated once a week for 4 weeks but on different trifolia (10 trifolia each time), resulting in 40 treated trifolia per plant. When applying MJ on new trifolia, we sprayed the adaxial side of the 10 trifolia. For the herbivory treatment, here we also used the protocol from a previous study (Heil, 2004). Control plants were treated in a similar manner as the MJ-treated plants, except that distilled water was applied instead of a MJ dilution.

Every week, for 12 weeks, from 2 November 2015 to 18 January 2016, we counted the number of trifolium produced per plant as a proxy for plant size (plant mass could not be obtained without killing the plants). For each plant, we recorded the date that the first flower opened to estimate the flowering time as days from germination to the first flower. Flowering started during the first week of December and continued over 2 months. Pods were collected as soon as they matured and just before the plants were ready to shed seeds. Ten seeds per plant were measured at the longest diameter with a digital caliper to obtain the size in millimeters. Because seed mass is reduced by damage (M. A. C. Cuny et al., University of Neuchatel, unpublished manuscript), to avoid biasing the results, we measured seed size instead of seed mass.

### Chemical analyses

Twenty healthy undamaged seeds per plant were ground to a fine powder in a mortar. Following the method of Moreira et al. (2014), 200 mg were mixed in 2 mL of methanol (50%), held in an ultrasonic bath for 15 min, and centrifuged for 2 min (10,000 rpm). The supernatant was recovered for phenolics analyses, 300 µL were mixed with 100 µL of deionized water and then subjected to the Folin–Ciocalteu assay. After a 2-h reaction, absorbance was measured at 750 nm with a spectrophotometer (Ultrospec 3100, Amersham Biosciences, UK). We used a concentration curve for tannic acid as a standard and expressed concentration as mg tannic acid equivalent per g−1 dry seed mass. For cyanogenic glycosides (CNGs), 20 mg were added to 1 mL of methanol (70% v/v), the samples were heated at 90°C for 3 min, and placed in an ultrasonic bath for 4 min. Samples were then centrifuged at 10,000 rpm for 2 min. The supernatant was collected and diluted 1:50 v/v with 70% methanol and analyzed for linamarin and lotaustralin (the two main cyanogenic glycosides present in lima bean seeds) content with liquid chromatography as described by Shlichta et al. (2014).

### Transgenerational effects

Seeds from the first experiment were maintained at 4°C, and the following year they were used to test whether differences
in flowering time between treatments were transmitted to the next plant generation. Conditions in the local area between the years were consistent (Appendix S1). We used seeds randomly drawn from eight plants per treatment. We confirmed that the chosen maternal plants differed in flowering time ($P = 0.012$). We sowed 10 seeds per plant in each of two 5-L pots filled with local soil. Pots were kept inside nylon field tents (Bioquip, Outdoor Cage 3.66 × 1.83 × 1.83 m, Lumite mesh) to protect them from unwanted herbivory in the same field site. As with the parental plants, unwanted insects were removed from the plants every day. We recorded seed germination each day, and 1 week after the first seed germinated, seedlings were thinned to one plant per pot. Two weeks after germination, 48 plants were randomly distributed among 12 medium tents (1.83 × 1.83 × 1.83 m) in the same location as in the previous year. Insects that entered the cages were removed every day. Trifolia were counted on three different days, and the date of first flower was recorded. Seeds produced by the plants by the end of the season were collected and counted.

Statistical methods

All analyses were performed in the R environment (version 3.3.3; R Core Team, 2017). The number of trifolia per plant was analyzed with a repeated measurement structure using a generalized linear mixed model (GLMM) with the function glmer from the package lme4 (Bates et al., 2015). For this model, number of trifolia was the response variable, while the fixed explanatory variables were treatment, plant cohort (ordinal factor), and their interaction. Tent was included as a random factor; each tent had a different ID, allowing us to consider the non-independence among plants in the same tent. To account for the repeated measures design, time from germination (plant age) and its interaction with treatment were included as fixed factors and plant ID as a random factor.

Time to first flower among treatments was analyzed with a proportional hazards mixed model (coxme package), which compares the risk of an event through time between several grouping factors. Number of seeds and pods per plant were analyzed using a GLMM with a Poisson distribution for the error family. For these models, treatment, plant cohort, and their interaction were included as fixed factors, with tent as a random factor. For the Poisson GLMMs, an observation level factor was included as a random factor to control for any overdispersion of the data. Similarly, the effects of treatment on seed size and concentration of total phenolics and CNGs in seeds were analyzed with linear mixed models with a normal distribution using tent as a random effect.

To analyze the differences in leaf number and seeds among maternal treatments in the offspring generation, we used GLMMs. To analyze the effects of maternal treatment on germination and flowering time of offspring, we used proportional hazards models. Additionally, to explore the influence of maternal traits, we preformed models for germination and flowering time with maternal plant and seed size as covariates. Tent and plant genotype were included as random effects.

RESULTS

Plant traits

Plants of the last cohort (2 November), produced very few seeds and were excluded from the analyses (Appendix S2). The number of trifolia per plant was explained by an interaction between plant age (time from germination) and treatment ($\chi^2 = 78$, $P < 0.0001$). Control plants produced more trifolia than herbivory or MJ-treated plants, but this difference was stronger when the plants were older (i.e., after herbivory and MJ treatments took place; Figure 1A). Plant cohort or its interaction with treatment did not have an effect on number of trifolia ($\chi^2 = 0.13$, $P = 0.94$; $\chi^2 = 6.3$, $P = 0.18$; Figure 1B). The flowering time (days from germination to production of the first flower) was affected by the interaction between plant cohort and plant treatment ($\chi^2 = 12.45$, $P = 0.014$). Thus, control plants flowered later than herbivory and MJ-treated plants, but plants subjected to herbivore damage flowered sooner when the planting was done later in the season (Figure 2). Control plants produced on average more seeds than herbivory- and MJ-treated plants ($\chi^2 = 9.27$, $P = 0.0097$; Figure 3A). There was a significant interaction of the plant cohort and the treatment ($\chi^2 = 9.76$, $P = 0.045$), with a larger difference in seed number from control compared to treated plants in the second cohort and plants with herbivory producing fewer seeds in the second plant cohort than in the first and third cohorts. The effect of treatment on pod number followed the same trend ($\chi^2 = 6.46$, $P = 0.04$), but the interaction with plant cohort was marginally significant ($\chi^2 = 9.38$, $P = 0.052$; Figure 3B). The number of seeds per pod depended on an interaction between treatment and plant cohort ($\chi^2 = 12.37$, $P = 0.015$), control plants had more seeds per pod than herbivory- and MJ-treated plants, but only in the second plant cohort.

Seed traits

Seed size was not significantly different among treatments ($\chi^2 = 4.64$, $P = 0.098$), but plant cohort had an effect ($\chi^2 = 12.17$, $P = 0.0023$), with seeds from the third plant cohort being the smallest. Total phenolic concentration was also affected by the plant cohort ($\chi^2 = 15.17$, $P = 0.0005$), with seeds from the third plant cohort having the highest concentration. Herbivory and MJ treatment did not affect the phenolic concentration. The concentration of cyanogenic
compounds linamarin and lotaustralin in seeds was not different among treatments or plant cohorts ($P > 0.1$).

**Transgenerational effects**

There were no differences on germination among treatments of the maternal plants ($\chi^2 = 1.47, P = 0.48; \text{mean} = 0.65$).

Neither number of trifolia ($\chi^2 = 0.15, P = 0.93$) nor flowering time ($\chi^2 = 0.33, P = 0.85; \text{mean} = 59.8 \text{ days}$) differed among maternal treatments in plants of the next generation. For exploring the effects of maternal traits on offspring germination and flowering time, we included maternal plant and seed size as covariates of treatments; however, neither of those traits were significant (all tests $P > 0.05$; Appendix S3). Seeds output was not affected by the treatment of maternal plants ($\chi^2 = 0.68, P = 0.71; \text{mean} = 91.9 \text{ seeds}$).

**DISCUSSION**

In the present study, we confirmed that herbivory by leaf beetles reduces the number of seeds produced by lima bean plants and hastens flowering time. The hypothesis that the induced plant responses involve the jasmonate pathway was supported because the MJ application on undamaged plants produced similar responses to herbivory. However, we did not detect any transgenerational effects for the induced early flowering. By testing all treatments on plants that were planted at different times during the season, we could show that the effects of herbivory on flowering time are consistent regardless of the time of the season.

**Effects of MJ and herbivory are similar for fitness and flowering time**

The application of MJ to lima bean plants induced some of the responses observed with herbivory. In *Brassica rapa*
plants, the exogenous application of jasmonate delays flowering time, similarly to the effects of herbivory by leaf caterpillars (Agrawal et al., 1999b). Here, flowering time was similar between lima bean plants subjected to beetle herbivory and to MJ; however, time to flower was shortened in both sets of treated plants compared to undamaged plants. The effects of MJ on complex traits as flowering time depend on several factors, such as developmental stage of the reproductive tissue, floral part, and plant species. For example, in Brassica napus, the application of MJ induces early flowering (Pak et al., 2009), while in A. thaliana, the jasmonate receptor COI1 can mediate the induction of delayed flowering by regulating transcription factors associated with flower development (Zhai et al., 2015). Therefore, the specific conditions such as ontogenetic stage and frequency of treatment in which lima bean plants were treated might determine what effects herbivory and MJ application will have on flowering time. Interestingly, seed output was reduced in plants from the herbivore and MJ treatments. This result indicates that induction of plant defense responses by jasmonate, like the induction of cyanogenic glucosides, extrafloral nectar, and plant volatiles (Heil, 2004; Ballhorn et al., 2016; Hernández-Cumplido et al., 2016a), is costly to the plants in terms of reproductive fitness. The results support the hypothesis that when defenses are costly, they evolve toward induction whenever possible to reduce the cost of defense in the absence of natural antagonists (Agrawal, 2000).

Influence of flowering time on late-season insects

Even if seed phenolics and CNGs were not different among treatments, it is possible that changes in phenology like flowering time could affect interactions with late-season insects such as seed-associated insects. Plants experiencing herbivory that will flower and produce seeds earlier in the season may escape attack by specialist seed beetles whose phenology is synchronized with that of the plant (Hernández-Cumplido et al., 2016b). However, in a recent study also with lima bean, but with caterpillars instead of leaf beetles, damaged plants flowered later than undamaged plants (Bustos-Segura et al., 2020). It is possible that different herbivores will induce different plant responses. Indeed, in a previous study, Moreira et al. (2015) found that effects of herbivory on seed traits of lima bean were species-specific and varied according to which herbivore, a beetle or a caterpillar, was the first to damage the plant. Induced changes in flowering may also be context dependent, influenced by the herbivore species (beetles and caterpillars cause different type of damage and should have different elicitors), frequency and intensity of herbivory, and plant developmental stage at the time of herbivory. Thus, it would be interesting to test in the same experiment how a different leaf herbivore species affects flowering time and whether there are consequences for interactions with seed predators. Herbivory and JA might also induce changes in the chemical defense of reproductive tissues that could influence the
prefer ee and performance of late-season insects. When leaves of *Oenothera biennis* are eaten by invasive beetles, increases in JA and phenolics are induced in flowers (McArt et al., 2013). As a result, fewer buds and seeds are consumed by specialist herbivores in plants that experienced leaf herbivory. In *Brassica nigra*, leaf damage by caterpillars in flowering plants also increased the production of JA in flowers (Chrétien et al., 2018). Thus, plants could allocate induced defenses to more important tissues such as inflorescences. In lima bean, it would be of interest to measure defense chemicals induced in flower buds and flowers and explore whether changes in JA levels in flowers can affect seed beetle choices and performance.

### Transgenerational effects

Several studies to date have demonstrated that changes in phenotypical traits in plants caused by herbivory can be inherited by the next plant generation (Agrawal et al., 1999a; Agrawal, 2002; Holeski et al., 2012; Ballhorn et al., 2016; Aguirrebengoa et al., 2018; J. Hernández-Cumplido et al., unpublished manuscript). These transgenerational effects have the potential to improve plant performance in the presence of herbivores, mainly by priming the next generation to express more efficient defenses against herbivores via maternal effects or epigenetic mechanisms (Holeski et al., 2012; Rasmann et al., 2012). Transgenerational effects are expected to be common in plants; however, whether changes in phenological nondefensive traits (but ecologically relevant) are also transmitted to the next generation is still unclear. In the present study, we explored differences in reproductive traits in the progeny of plants subjected to herbivory, treated with MJ, or non-treated. We found that flowering time and seed traits were similar among progenies from different maternal treatments in field conditions. Kellenberger et al. (2018) looked for transgenerational effects of herbivory in flower and reproductive traits of *Brassica rapa* and found that changes in flower volatiles were not transmitted to the next generation, but some morphological changes were maintained in subsequent generations. Other studies have found transgenerational effects of herbivory on flower traits like proportion of flowering plants and number flowers (Steets and Ashman, 2010; Nihranz et al., 2020) and flower color (Sobral et al., 2021). Interestingly, these types of effects may depend on the reproductive mechanism (cleistogamy vs. chasmogamy, Steets and Ashman, 2010) or inbreeding (Nihranz et al., 2020). Nihranz et al. (2020) also found differences in time to flowering, with flowering delayed in the offspring of damaged maternal plants. However, this experiment was done in the greenhouse, which highlights the importance of analyzing the contribution of transgenerational effects in natural conditions.

In wild lima bean, defenses against herbivory are subject to transgenerational effects (Ballhorn et al., 2016; Hernández-Cumplido et al., 2010). Therefore, in this species, transgenerational effects of herbivory are actually possible, but it is likely that not all traits influenced by herbivory will show transgenerational effects. It could be possible that induced traits that are transmitted to the next generation are more associated with direct defenses against herbivores rather than traits that respond to changes in resource allocation due to herbivore attack. The absence of transgenerational effects in this study cannot be attributed to seed provisioning or maternal plant size, since using these traits as covariates did not help to explain the variation on germination and flowering time in the offspring generation. Whether phenological traits are subject to transgenerational effects of herbivory in the wild should be further explored in other systems.

### CONCLUSIONS

Overall, we found that leaf herbivory and jasmonate treatment modified several plant traits including flowering time; however, the induced effect on phenology was not transmitted to the next generation. Given that transgenerational effects of herbivory on cyanogenesis have been reported for lima bean, it seems possible that transgenerational effects are restricted to particular sets of traits directly related to plant defenses. Future studies should identify which seed traits are associated with induced defenses against seed beetles. In addition, it would be interesting to test whether any induced plant responses in defensive, developmental, and phenological traits are specific or restricted to some herbivore species, their type, frequency, and amount of damage.

### ACKNOWLEDGMENTS

We thank academic and technical staff of the Universidad del Mar, campus Puerto Escondido for providing the facilities and logistics for the field experiments. We also thank Alfredo Rojas, Maximilien Cuny, Charlyne Jaccard, and Mar Salas for their help in the field and Véronique Douet for guidance with the phenolics analyses. Gaëtan Glauser and staff at NPAC-UNINE performed the UHPLC analyses. We thank the Editor in Chief and two anonymous reviewers whose comments helped to improve the manuscript. This research was supported by the SNSF with the grant Project No. 3100AO-109239 awarded to B.B. Open access funding provided by Université de Neuchatel.

### AUTHOR CONTRIBUTIONS

B.B. and J.H.C. designed the experiments. J.H.C. performed the field experiments. J.T. and C.B.S. performed lab experiments. C.B.S. analyzed data and led the writing of the manuscript with input from all the coauthors.

### DATA AVAILABILITY STATEMENT

The data set and analyses associated with this article are stored in the Zenodo repository with https://doi.org/10.5281/zenodo.4725696 (Bustos-Segura et al., 2021).
REFERENCES

Agrawal, A. A. 2002. Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. Ecology 83: 3408–3415.

Alborn, H. T., T. C. J. Turlings, T. H. Jones, G. Stenhagen, J. H. Loughrin, Ballaré, C. L. 2011. Jasmonate induction of early flowering and transgenerational effects models using lme4. Biotechnology, and Biochemistry 82: 292–303.

Elzinga, J. A., A. Atlan, A. Biere, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. Trends in Ecology & Evolution 22: 432–439.

Farmer, E. E., and C. A. Ryan. 1990. Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proceedings of the National Academy of Sciences, USA 87: 7713–7716.

Freytag, G. F., and D. G. Debouch. 2002. Taxonomy, distribution, and ecology of the genus Phaseolus (Leguminosae—Papilionoideae) in North America, Mexico and Central America. Sida, Botanical Miscellany no. 23. Botanical Research Institute of Texas (BRIT), Fort Worth, TX, USA.

Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. Functional Ecology 25: 399–407.

Heil, M. 2004. Induction of two different defenses benefits lima bean (Phaseolus lunatus, Fabaceae) in nature. Journal of Ecology 92: 527–536.

Hernández-Cumplido, J., B. Benrey, and M. Heil. 2010. Attraction of flower visitors to plants that express indirect defense can minimize ecological costs of ant-pollinator conflicts. Journal of Tropical Ecology 26: 555–557.

Hernández-Cumplido, J., B. Porter, X. Moreira, M. Heil, and B. Benrey. 2016a. Induced floral and extraloral nectar production affect ant–pollinator interactions and plant fitness. Biotropica 48: 342–348.

Hernández-Cumplido, J., G. Glauser, and B. Benrey. 2016b. Cascading effects of early-season herbivory on late-season herbivores and their parasitoids. Ecology 97: 1283–1297.

Holeski, L. M., G. Jander, and A. A. Agrawal. 2012. Transgenerational defense induction and epigenetic inheritance in plants. Trends in Ecology & Evolution 27: 619–626.

Kellenberger, R. T., G. A. Desurmont, P. M. Schlüter, and F. P. Schiestl. 2018. Trans-generation inheritance of herbivory-induced phenotypic changes in Brassica rapa. Scientific Reports 8: 1–9.

Kost, C., and M. Heil. 2008. The defensive role of volatile emission and extraloral nectar secretion for lima bean in nature. Journal of Chemical Ecology 34: 2–13.

Munguía-Rosas, M. A., J. Ollerton, V. Parra-Tabla, and J. A. De-Novoa. 2011. Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. Ecology Letters 14: 511–521.

Niwa, T., T. Suzuki, Y. Takebayashi, R. Ishiguro, T. Higashiyama, H. Sakakibara, and S. Ishiguro. 2018. Jasmonic acid facilitates flower opening and floral organ development through the upregulated expression of SIMYB21 transcription factor in tomato. Bioscience, Biotechnology, and Biochemistry 82: 292–303.

Segura, C., J. Hernandez, M. S. Zinkgraf, J. J. Couture, T. G. Whitham, and R. L. Lindroth. 2013. Transgenerational effects of herbivory in a group of long-lived tree species: Maternal damage reduces offspring allocation to resistance traits, but not growth. Journal of Ecology 101: 1062–1073.

Cost, C., and M. Heil. 2008. The defensive role of volatile emission and extraloral nectar secretion for lima bean in nature. Journal of Chemical Ecology 34: 2–13.
Ohgushi, T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. Annual Review of Ecology, Evolution, and Systematics 36: 81–105.

Pak, H., Y. Guo, M. Chen, K. Chen, Y. Li, S. Hua, I. Shamsi, et al. 2009. The effect of exogenous methyl jasmonate on the flowering time, floral organ morphology, and transcript levels of a group of genes implicated in the development of oilseed rape flowers (Brassica napus L.). Planta 231: 79–91.

Pashalidou, F. G., H. Lambert, T. Peybernes, M. C. Mescher, and C. M. De Moraes. 2020. Bumble bees damage plant leaves and accelerate flower production when pollen is scarce. Science 368: 881–884.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website: https://www.R-project.org/

Rasmann, S., M. De Vos, C. L. Casteel, D. Tian, R. Halitschke, J. Y. Sun, A. A. Agrawal, et al. 2012. Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiology 158: 854–863.

Rusman, Q., E. H. Poelman, F. Nowrin, G. Polder, and D. Lucas-Barbosa. 2019. Floral plasticity: Herbivore-species-specific-induced changes in flower traits with contrasting effects on pollinator visitation. Plant Cell Environment 42: 1882–1896.

Salgado-Gutiérrez, A., P. Gepts, and D. G. Debouck. 1995. Evidence for two gene pools of the lima bean, Phaseolus lunatus L., in the Americas. Genetic Resources and Crop Evolution 42: 15–28.

Shlichta, J. G., G. Glauser, and B. Benrey. 2014. Variation in cyanogenic glycosides across populations of wild lima beans (Phaseolus lunatus) has no apparent effect on bruchid beetle performance. Journal of Chemical Ecology 40: 468–475.

Sobral, M., I. P. Neylan, E. Narbona, and R. Dirzo. 2021. Transgenerational plasticity in flower color induced by caterpillars. Frontiers in Plant Science 12: 1–7.

Steets, J. A., and T. L. Ashman. 2010. Maternal effects of herbivory in Impatiens capensis. International Journal of Plant Sciences 171: 509–518.

Thaler, J. S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399: 686–688.

Thaler, J. S., P. T. Humphrey, and N. K. Whittema. 2012. Evolution of jasmonate and salicylate signal crosstalk. Trends in Plant Science 17: 260–270.

Turlings, T. C. J., J. H. Loughrin, P. J. McCall, U. S. Röse, W. J. Lewis, and J. H. Tumlinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proceedings of the National Academy of Sciences, USA 92: 4169–4174.

van Loon, L. C. 2016. The intelligent behavior of plants. Trends in Plant Sciences 21: 286–294.

Yuan, Z., and D. Zhang. 2015. Roles of jasmonate signalling in plant inflorescence and flower development. Current Opinions in Plant Biology 27: 44–51.

Zhai, Q., X. Zhang, F. Wu, H. Feng, L. Deng, L. Xu, M. Zhang, et al. 2015. Transcriptional mechanism of jasmonate receptor COI1-mediated delay of flowering time in Arabidopsis. Plant Cell 27: 2814–2828.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

**Appendix S1.** Climatic conditions at the field site across 2 years.

**Appendix S2.** Seed number per plant from lima bean plants grown in field conditions.

**Appendix S3.** Summary of the results from models testing an effect of maternal plant treatment on offspring traits.

---

**How to cite this article:** Bustos-Segura, C., J. Hernández-Cumplido, J. Traine, and B. Benrey. 2021. Herbivory and jasmonate treatment affect reproductive traits in wild Lima bean, but without transgenerational effects. American Journal of Botany 108(10): 2096–2104. https://doi.org/10.1002/ajb2.1786