Extended Consequences of Plant-Herbivore Phenological Mismatch

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A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements

For the Degree of Master of Science (Biology) at

Concordia University

Montreal, Quebec, Canada

August 2023

CONCORDIA UNIVERSITY
School of Graduate Studies

This is to certify that the thesis prepared

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Entitled: Extended Consequences of Plant-Herbivore Phenological
                Mismatch

and submitted in partial fulfillment of the requirements for the degree of

Master of Science (Biology)

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Abstract

Extended Consequences of Plant-Herbivore Phenological Mismatch

Dana Martin

Phenological mismatches between plants and insects occur in response to climate change owing to differences in environmental sensitivity. Herbivory negatively affects plant development by altering the allocation of resources from growth to defense. Changes in the timing of herbivory can exacerbate these effects as young plants have limited resources to allocate towards regrowth. Early-onset and high-intensity herbivory can affect plant traits; however, the extended consequences on pollinator activity are largely unknown. Here, I conducted an experiment to investigate the effects of plant-herbivore phenological mismatches on swamp milkweed (*Asclepias incarnata)* and pollinator activity. I manipulated the onset date and percentage of herbivory on milkweed and measured the response of non-floral traits, floral traits, and pollinator activity. I found that the effect of onset of herbivory on non-floral and floral traits was dependent on the intensity. Specifically, early-onset high-intensity herbivory, or late-onset low-intensity herbivory, resulted in more leaves and open flowers. Although the onset and intensity of herbivory did not affect pollinator activity, there was a positive relationship between the frequency and diversity of visiting pollinators with the number of open flowers. Taken together, these results suggest that milkweed may exhibit enhanced growth when subjected to varying intensities of herbivory depending on the onset of damage. Changes in the phenology of insect herbivores may benefit plant and pollinator fitness by increasing the growth of floral traits and pollinator activity. Understanding herbivore-plant-pollinator interactions in the face of climate change provides insight into how ecosystem dynamics, such as pollination, may shift in the future.

Keywords

Finally, I want to acknowledge my family, whose love and understanding provided the motivation to overcome the challenges of graduate school. To my parents, who have been my pillars of strength, thank you for your constant encouragement, sacrifices, and for instilling in me the values of perseverance and determination. To my chosen family: Dana, Darcey, Kyle, Marc, Noah, and Noah, thank you for standing by me, even during the most stressful times. Your presence, laughter, and shared experiences have made this journey more enjoyable and meaningful. Finally, thank you to my partner Sidney for cheering me on, keeping me grounded and focused, and sharing in both my highs and lows. Thank you for your patience and embracing the late nights and weekends I dedicated to research and writing. I am truly grateful to have you all by my side and could not have done this without you.

Although it is impossible to name everyone, please know that your support, no matter how big or small, has left a lasting impact on my academic pursuits. Thank you for being part of this journey and contributing to my growth, both as a researcher and individual.

Contribution of Authors

I contributed to the experimental design, data collection, data analysis, data interpretation and drafting of the manuscript. Dr. Jean-Philippe Lessard contributed to the experimental design, data analysis, data interpretation and editing of the manuscript. Dr. Nate Sanders and Dr. Mariano Rodriguez-Cabal contributed to the experimental design and editing of the manuscript.

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INTRODUCTION

Ongoing climatic changes are transforming interactions among organisms in complex ways, including interactions between plants and their insect associates (DeLucia et al. 2012; Inouye et al. 2022). Changes in temperature and precipitation can affect plants and insects differently owing to differences in physiology, such as thermal tolerance or drought resistance (Jamieson et al. 2012). Specifically, the phenology of herbivores is advancing much faster than that of plants due to the greater sensitivity of insects to abiotic factors (Visser and Both 2005; Menéndez 2007; Körner and Basler 2010). These differential responses could lead to asynchronous shifts in phenology, resulting in mismatches between the timing of life history events, which are increasingly documented as a major consequence of climate change (Parmesan and Yohe 2003; Thackeray et al. 2016). Phenological mismatches between plants and insect herbivores alter the timing of herbivory, which could have further consequences on growth and fitness (Dewar and Watt 1992; DeLucia et al. 2012). Plants respond to herbivory by investing in defensive traits and removing energy from plant growth (Züst et al. 2015). Although the effects of herbivory on floral traits and pollinator visitation have been explored, the extended consequences of phenological mismatches on floral traits and plant mutualists, such as insect pollinators, remain largely unexplored (Jacobsen and Raguso 2018).

Herbivory can induce higher investments in plant chemical defenses that can alter floral traits via various physiological pathways (Paré and Tumlinson 1999). First, herbivory can directly affect plant growth by decreasing available photosynthetic area, carbohydrate reserves, and stored nutrients (Bi and Felton 1995; Mutikainen and Delph 1996). Herbivory can also indirectly affect plant growth when plants invest limited resources in defensive strategies, for example, by increasing secondary metabolite production instead of investing in growth (Bi and Felton 1995;

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Mutikainen and Delph 1996). Secondary metabolites are organic compounds created by plants that are used as a defense against herbivory; they can have toxic, repellent, or anti-nutritional effects on herbivores (Usha Rani and Jyothsna 2010; War et al. 2011a; War et al. 2011b). The production of secondary metabolites requires energy and resources to be taken from primary metabolites, which are organic compounds used for plant growth, reproduction, and storage (Erb and Kliebenstein 2020). Second, energy and resources that are reallocated away from plant growth may reduce non-floral and floral plant traits (Züst et al. 2015; Struckman et al. 2019; Brys et al. 2011). Others have suggested that reductions in floral display size and nectar reward are due to increasing investments in chemical defenses (Mothershead and Marquis 2000). These trade-offs between growth and defense explain why plants that invest more in defensive traits in response to herbivory can become less attractive to pollinators (Whitney and Glover 2007; Lucas-Barbosa 2016; Burkle and Runyon 2016; Lehtilä and Strauss 1997; Poveda et al. 2003).

The indirect effects of insect herbivory on floral traits could have consequences for pollinator activity, but to my knowledge, no study has linked changes in the onset and intensity of herbivory to changes in non-floral traits, floral traits, and pollinator activity. Understanding these relationships will provide a novel and more mechanistic perspective than previous studies (Brys et al. 2011; Lucas-Barbosa 2016). Variation in floral traits can alter the visitation frequency by pollinators and the diversity of pollinators visiting the plant (Mothershead and Marquis 2000; Gustafson et al. 2023; Fornoff et al. 2017; de Brito et al. 2017; Johnson et al. 1995; Strauss et al. 1996; Galen and Plowright 1985; Kessler et al. 2011). It is well-documented that flowering plants use several strategies to attract pollinators, but they can generally be broken down into three categories: 1) visual cues, 2) rewards, and 3) odour (Kumar et al. 2020). A bigger floral display size, which includes both flower size and number of flowers, acts as a visual cue by increasing

visibility, offers greater reward by holding more nectar, and increases floral odour by omitting more volatile organic compounds (VOCs) (Makino and Sakai 2007; Farré-Armengol et al. 2013). Flowers containing larger volumes of nectar, and higher concentrations of sugar or other rewards, are more attractive due to their ability to provide nutrients (Cnaani et al. 2006; Watt et al. 1974, Finkelstein et al. 2022). Conversely, nectar with a high concentration of amino acids can lead to a lower visitation frequency and richness (Fornoff et al. 2017). This may be caused by amino acids altering the taste profile of nectar to be less appealing (Gardener and Gilman 2002). However, butterflies may prefer nectar with a high concentration of amino acids, which has been shown to increase their fecundity (Mevi-Schütz and Erhardt 2003; Mevi-Schütz and Erhardt 2005). If herbivory negatively affects floral traits, there may be simultaneous negative effects on pollinator activity (Fornoff et al. 2017; La Rosa and Conner 2017; Rafferty and Ives 2011).

The ways in which timing and intensity of herbivory affect plants and pollinators have been studied independently; however, how these factors might interact to affect plants and pollinators remains unexplored (Mercader and Isaacs 2003; García and Ehrlén 2002; Knight 2007; Botto-Mahan et al. 2011; Mothershead and Marquis 2000; Fabina et al. 2010; but see Whigham and Chapa. 1999). The timing of herbivory, whether early-, mid- or late-season, can influence the magnitude of physiological responses in plants (Marshall et al. 2005; Rasmussen and Yang 2023; Mercader and Isaacs 2003, García and Ehrlén 2002; Knight 2007). Under climate warming, insects generally emerge earlier, indicating that herbivores are active earlier in plant development (Diamond et al. 2011; Bell et al. 2015; Forrest et al. 2016; Visser and Holleman 2001). Therefore, the onset and peak intensity of herbivory are expected to occur earlier (Dewar and Watt 1992; DeLucia et al. 2012; Abarca and Lill 2015; Ren et al. 2020). The effects of early-onset herbivory on overall plant fitness might be limited if there is enough time to recover before reproduction;

however, consequences could be more pronounced later in the season when the allocation of resources shifts from growth to reproduction (García and Ehrlén 2002). If herbivory takes place right before the flowering period, herbivores may deplete the plant's resources or damage the structures necessary for reproduction, leaving insufficient time for the plant to recover before the optimal reproductive period has passed (García and Ehrlén 2002). Early-onset herbivory can reduce floral display size, nectar reward, and delay flowering, as young plants have limited energy reserves; depleting these reserves further reduces the resources plants can allocate to these traits during development (Knight et al. 2007; Marshall et al. 2005; Rasmussen 2023; Boege and Marquis 2005).

Induced defense mechanisms in milkweed exemplify the chemistry and physiology employed by many species to ward off herbivores. Milkweed is known to invest in induced defensive traits such as cardenolides (cardiac glycosides), latex production, and carbon to nitrogen (C/N) ratio (Rasmann et al. 2009; Malcolm and Zalucki 1996; Konno et al. 2004; Behmer 2009). These induced defenses have also been shown to reduce photosynthesis in milkweed which may further reduce growth potential (Delaney et al. 2008). Cardenolides are present in all species of milkweed but vary in concentration depending on species and plant tissue (Agrawal & Konno 2009; Rasmann & Agrawal 2011). Cardenolides impede the activity of sodium-potassium pumps, crucial for upholding the membrane potential in the majority of animal cells (Rasmann et al. 2009). The highest concentrations have been found in latex, which serves as both a physical barrier and a toxin against herbivores (López-Goldar et al. 2021; Agrawal and Konno 2009). After herbivory, plants can allocate resources, consisting of mainly carbon and nitrogen, away from sites of attack (Orians et al. 2011; Tao and Hunter 2011). Milkweed has been shown to allocate more nitrogen to stems after the damage of other tissues such as leaves and roots (Tao and Hunter 2013). The C/N

ratio in plant tissues is a significant regulator of susceptibility to herbivores, making it an additional type of defense again herbivores (Rasmann et al. 2009; Behmer 2009).

In this experimental study, I investigate how the onset and intensity of herbivory influence floral traits and pollinator activity. Specifically, I asked a series of inter-related questions: 1) Will plants with early-onset and high-intensity herbivory be shorter, have fewer leaves, smaller flowers, fewer flowers, delayed onset of flowering, less nectar and lower nectar sugar concentration (Mothershead and Marquis 2000; Strauss 1997; Theis et al. 2009; Poveda et al. 2003; Strauss et al. 1996; Krupnick et al. 1999; Blue et al. 2015; Narbona and Dirzo 2010; Marshall et al. 2005; Rasmussen and Yang 2023; Poveda et al. 2005)? 2) Will plants with early-onset and high-intensity herbivory experience decreased pollinator visitation frequency and richness (Fornoff et al. 2017; La Rosa and Conner 2017; Rafferty and Ives 2011; Cnaani et al. 2006; Williams et al. 2015; de Brito et al. 2017; Petanidou et al. 2014)? 3) Will plants with delayed onset flowering also experience decreased pollinator visitation frequency, but increased richness (Petanidou et al. 2014)?

METHODS

Study site

The experiment was conducted at the University of Michigan Biological Station (UMBS) in Pellston, Michigan (45.556°N, -84.679°W) from May 9th to August 14th, 2022. UMBS is located in the transitional zone between mixed hardwood and boreal forests in the northern lower peninsula of Michigan (Karl et al. 2004). The forests near UMBS represent a diverse array of ages and disturbance histories, and consist mainly of bigtooth aspen (*Populus grandidentata* Michx.) and trembling aspen (*Populus tremuloides* Michx.), but also contain northern red oak (*Quercus rubra* L.), paper birch (*Betula papyrifera* Marsh.), American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), and white pine (*Pinus strobes* L.) (Gough et al. 2008). Common flowering plants in the field include common milkweed (*A. syriaca*), purple crown vetch (*Securigera varia*), staghorn sumac (*Rhus typhina*), and the common St. John's wort (*Hypericum perforatum*). The area is also dominated by the common bracken fern (*Pteridium aquilinum*). The soils of UMBS are acidic, sandy, excessively drained, mixed frigid Entic Haplorthods with little relief (Hardiman et al. 2011). The area receives an average of 817 mm of precipitation per year and has an average annual temperature of 5.5°C, but the climate varies greatly from year to year (Gough et al. 2008; Gough et al. 2013).

Study system

Asclepias incarnata, commonly known as swamp milkweed, is an herbaceous perennial plant that occurs in wetlands and uplands across North America (Woodson 1954). This species is primarily found in wet habitats, such as swamps and marshes, but can also grow in drier conditions (Kirk and Belt 2011). Individuals can grow 90 to 180 centimeters tall and produce clusters of small, pink

flowers from late spring to early fall (Kirk and Belt 2011; Borders and Lee-Mäder 2014). I chose this study system as it is highly attractive to a wide variety of pollinators, including bees, butterflies, and hummingbirds, and is an important host plant for the larvae of the monarch butterfly (*Danaus plexippus*) ((Borders and Lee-Mäder 2014; Kirk and Belt 2011). In addition to its ecological importance, *A. incarnata* has medicinal properties and has been used by Indigenous peoples to treat a variety of ailments (Kirk and Belt 2011). Despite that only specialized herbivores with adaptations to sequester secondary metabolites, like cardenolides, can tolerate milkweed, it can still receive large amounts of herbivory (Borders and Lee-Mäder 2014).

Experimental design

To examine how the onset and intensity of herbivory affect the quality and quantity of floral traits, and whether this cascades to affect pollinator activity, I artificially manipulated the onset date and percentage of foliar herbivory on *A. incarnata*. I obtained overwintered, second year plants from East Michigan Native Plants, LLC (Durand, MI, USA) as they are more likely to flower than plants in their first year of growth. I grew the plants in the UMBS greenhouse starting on May 9th, 2022, where they grew for about a month. Then, I transported them to the study site on June 2nd, 2022, after the risk of frost had passed. I placed them in large 26.5-L pots filled with potting soil to prevent the roots from being overly confined and to avoid stunting growth (A. Nelson, personal communication, April 9, 2022).

The experimental site was fenced and measured approximately 30 m × 18 m. It was exposed to full sun from morning to late afternoon. The pots were arranged in a randomized complete block design (RCBD) with 15 blocks from June 2nd to July 21st (Figure S1). This design was chosen as it limits any confounding variables such as exposure to sun and wind. I placed the

blocks five meters apart, and the treatments one meter apart within each block to ensure independence and avoid perturbations of pollinators during surveys (Brown et al. 2002; Pfunder and Roy 2000). Each block contained six plants, and each plant was exposed to one of the following six treatments: 1) early and low herbivory, 2) late and low herbivory, 3) early and high herbivory, 4) late and high herbivory, 5) no herbivory (control), and 6) natural herbivory. I covered treatments 1 to 5 with mosquito netting until the plants were flowering to exclude natural herbivores. On July 22nd, before herbivory treatments were applied, plants were rearranged in a new block design such that plants at similar stages of development were placed in the same block. This was done so that the netting could be removed from all treatments at the same time and pollinator surveys could begin. Hereafter, block one refers to the first design, and block two refers to the second design.

Onset of herbivory

To test how the onset of herbivory affects non-floral traits, floral traits, and pollinator activity, I artificially damage the milkweed by removing leaf tissue at two different times during the study period. I damaged all leaves by hole punching and ripping non-midrib leaf tissue; I used both methods to simulate the various patterns of natural herbivory I observed in the field (Baldwin 1990). I chose leaves at random to decide which method of tissue removal they would receive. Main leaf-chewing herbivores in this system include weevils, monarch caterpillars, milkweed beetles, milkweed bugs, many of which emerge and peak at different times from early spring to late summer (Van Zandt and Agrawal 2004; Betz et al. 2000). In the region, weevils are active beginning in May, monarchs, common milkweed beetles, and leaf beetles beginning in June, and milkweed bugs beginning in July (Van Zandt and Agrawal 2004; Betz et al. 2000). I damaged the

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early-onset treatments once on June 22nd (after block rearrangement) to reflect damage from early active herbivores. Two weeks later, on July 6th, I damaged the late-onset treatments to reflect damage from later occurring herbivores. Treatments were two weeks apart as phenological shifts between insects and plants are expected to shift anywhere from a few days to a few weeks (Forrest et al. 2016; Kharouba et al. 2014; Rafferty and Ives 2011). The plants received either early-onset or late-onset herbivory, not both. The new leaves that emerged after the treatments were not damaged as I was only interested in onset, not continuous herbivory. I conducted visual surveys of the surrounding populations of *A. syriaca* before applying each treatment to estimate the level of herbivory at that point in the season.

Intensity of herbivory

To test how the intensity of herbivory affects non-floral traits, floral traits, and pollinator activity, I removed different percentages of leaf tissue. Intensity of herbivory can vary between years and locations; therefore, I chose percentages based on previous studies (Theis et al. 2009; Strauss 1997; Agrawal et al. 1999), personal observations, and recorded observations of natural levels of herbivory on common milkweed at UMBS from 2008 to 2017 (Figure S2; Mark D. Hunter, unpublished data). I manually removed 5% of each leaf for plants in the low herbivory treatments, and 50% of each leaf for plants in the high herbivory treatments. I visually estimated the percentage of leaf removal. The plants grew at different rates; therefore, I removed a percentage of leaf tissue rather than a standardized amount of biomass to avoid potentially inflicting major damage to the smaller plants but insignificant damage to the larger plants, as several studies have done (Mercader and Isaacs 2003; Strauss 1997; Quesada et al. 1995).

Non-floral trait response to herbivory

I measured plant height, number of leaves and total leaf area because non-floral traits can provide a mechanistic basis to understand floral trait response in *A. incarnata* (Roach and Smith 2020; Bolmgren and Cowan 2008; Hochwender et al. 2000; Brys et al. 2011). These measurements also help to account for the differences caused by greater biomass removal from larger plants. The plant height was measured from the point at which the plant emerged from the soil to the uppermost leaf. I counted the number of leaves, including both damaged and undamaged leaves. I estimated the area of the largest leaf on each plant by multiplying the length and width of the leaf; I multiplied this value by the number of leaves per plant to get a total leaf area index (Bowles et al. 2015). I took these measurements once per week as these traits did not change drastically from day to day. I recorded the percentage of damage and number of damaged leaves for plants in the natural treatment to track the progression of herbivory throughout the season. I counted the number of damaged leaves every few days as new signs of herbivory did not occur every day but were noticeable on the plants for several weeks.

Floral trait response to herbivory

I measured flower size, the number of open flowers, onset flowering date, nectar volume, and nectar sugar concentration because those traits were expected to respond to herbivory and influence pollinator activity. The day prior to each sampling day, I chose five flowers per plant at random to receive both flower size and nectar measurements (Hazlehurst and Karubian 2016; Keaser et al. 2008; Manetas and Petropoulou 2000; Cavalcante et al. 2018). I covered the chosen flowers with small mesh bags overnight to prevent pollinators from removing any nectar. The following day, I removed the bags and measured the onset of budding and flowering date, flower

size, number of open flowers, nectar volume and nectar sugar concentration (three blocks did not flower on time and were therefore excluded from all analyses). Flower size (i.e., hood length, hood height, gynostegium width and petal length) was measured using a digital caliper (ADORIC, 0.1 mm resolution, 0.2 mm accuracy). I counted the number of open flowers, excluding wilted or partially opened flowers. I collected nectar by inserting glass capillary tubes (5μL and 10μL Drummond MicroCaps, accuracy 1%) into the flower hoods and measuring the length of the nectar column (Power et al. 2017). I measured sugar concentration in degrees Brix, a measure of dissolved solids in a liquid, as a proxy of nectar quality. One degree Brix is equal to one gram of sucrose in 100 g of the solution. Measurements were taken using either a 0-50° or 45-90° Eclipse low-volume refractometer (resolution 0.2). The nectar was expelled from the glass capillary tubes onto the refractometer prism.

I did not measure pollen as it is inaccessible to pollinators (only pollinia is accessible), making nectar the only available reward (Borders and Lee-Mäder 2014).

Pollinator activity

To test how the onset and intensity of herbivory affect pollinator visitation frequency and richness, I performed standardized visual surveys. Surveys occurred between 09:00 and 17:00, with light winds (<29 km/h), low cloud cover (< 50%), and air temperatures above 13°C, as described by O'Connor et al. (2019). Pollinators are generally more active when it is sunny, warm, and with low wind speeds (McCall and Primack 1992; Vicens and Bosch 2000).

Since the flowering time of all plants were not aligned, I conducted pollinator surveys when each plant in a block had at least one inflorescence fully bloomed. This prevented collecting nectar samples from most open flowers which would result in a lack of reward for pollinators. I conducted

surveys prior to taking measurements or daily watering to avoid disturbing the environment. I observed an individual block for ten minutes in random order, during which time I counted and identified each pollinator that visited each plant (Primack and Inouye 1993). I observed 5503 visits, which were characterized by contact between the pollinator's body and the anthers or stigma of a flower to differentiate pollinators from flower visitors (Primack and Inouye 1993). If a pollinator visited multiple plants in a block, I counted those observations as separate visits because they could result in unique pollination events (Lundin et al. 2019; Krupnick et al. 1999).

I identified pollinators in the field after a substantial training period from June 10th to July 23rd. First, I compiled a list of species observed in the surrounding area using multiple sources including the Global Biodiversity Information Facility (GBIF.org; including iNaturalist observations) and "The Bees of Michigan" (Gibbs et al. 2017). Then I created a reference collection of morphotypes by collecting specimens in the field and identifying them using several taxonomic keys (Brothers et al. 1993; Packer et al. 2007), and field guides (Nielsen et al. 1999; Williams et al. 2014; Skevington et al. 2019; Carril et al. 2021). Once the list of morphotypes was complete, I compiled a visual key to aid with visual identification in the field (Table S1). Individuals that could not be identified in the field were captured with nets, placed into a killing jar, and identified in the lab.

Statistical analyses

Non-floral traits

The non-floral traits were measured once per week. Using the pairs.panels function in the *psych* package (Revelle 2023) in R (R Core Team 2023; version 4.2.2), I assessed if there was any multicollinearity among traits (Figure S3). The total leaf area index was highly correlated with the

number of leaves. To avoid redundancy, the total leaf area index was excluded from further analyses. Using the lmer function from the *lme4* package (Bates et al. 2015) in R, I constructed univariate linear mixed effect models (LMM) to assess how the onset and intensity of herbivory, and their interaction, affects plant height and the number of leaves. This uses residual maximum likelihood (REML) for variance parameter estimation. Random effects included block one and block two to account for growth conditions and phenology, week to account for temporal variation in average trait values across plants, and plant ID as plants received only one measurement per week. To satisfy the assumptions of normality and homoscedasticity, I 3rd power transformed plant height. All transformations were chosen via trial and error, starting with the weakest transformations. I assessed model assumptions via the simulateResiduals function from the *DHARMa* package in R (Hartig 2022). I visualized relationships between all herbivory treatments and non-floral traits using violin plots (Figure S4-S5). Plants in the natural treatment were not included in the main analyses; however, were visualized alongside the other treatments (Figure S6).

I conducted type III ANOVAs with Satterthwaite's method to test the main and interactive effects of herbivory treatments on all non-floral traits. If a relationship was significant, I conducted *post hoc* Tukey's HSD tests using the emmeans and contrast functions from the *emmeans* package (Lenth 2023) in R to compare specific treatment combinations. To allow for easier interpretation, model estimates were backtransformed to original units using the regrid function from the *emmeans* package before *post hoc* tests.

Floral traits

Floral traits that were measured daily were averaged per week to match the non-floral data. I assessed if there was any multicollinearity among traits (Figure S3). The number of inflorescences was highly correlated with the number of flowers, and the onset budding date was highly correlated with the onset flowering date. To avoid redundancy, the number of inflorescences and onset budding date were excluded from further analyses. Next, using the PCA function in the *FactoMineR* package (Le et al. 2008) in R, I ran a Principal Component Analysis (PCA) for the four metrics of flower size: hood height, hood length, petal length, and gynostegium width. I extracted the scores from the first axis to represent flower size. I constructed univariate LMMs to assess how the onset and intensity of herbivory, and their interaction, affects onset flowering date, flower size, the number of open flowers, nectar volume, and nectar sugar concentration. Block one and block two were included as random effects in all models, week was included as a random effect for all models, except for onset flowering date, and time of day was included in the nectar volume and sugar concentration models as a fixed effect to account for evaporation as the temperature increased later in the day. To satisfy the assumptions of normality and homoscedasticity, I square root transformed the number of open flowers and nectar volume, and 3rd power transformed nectar sugar concentration. I visualized relationships between all herbivory treatments and floral traits using violin plots (Figure S4-S5).

I conducted type III ANOVAs with Satterthwaite's method to test the main and interactive effects of herbivory treatments on all floral traits. If a relationship was significant, I conducted *post hoc* Tukey's HSD tests to compare specific treatment combinations. Model estimates were backtransformed to original units before *post hoc* tests.

Pollinator activity

The number of visits and the richness of morphotypes per observation period were averaged per plant per week. I constructed univariate LMMs to assess how the onset and intensity of herbivory, and their interaction, affects pollinator visitation frequency and richness per week. Block one, block two, and week were included as random effects. Time of day was included as a fixed effect to account for differences in pollinator activity due to temperature (Primack and Inouye 1993). I conducted type III ANOVAs with Satterthwaite's method to test the main and interactive effects of herbivory treatment on pollinator activity.

I constructed univariate LMMs to assess how flower size, onset flowering date, the number of open flowers, nectar volume, nectar sugar concentration and plant height and number of leaves affects pollinator visitation frequency and richness. Block one, block two, week, and plant ID (specified for plant height and number of leaves) were included as random effects, and time of time was included as a fixed effect in both models. To satisfy the assumptions of normality and homoscedasticity, I square root transformed visitation frequency and richness. In any model, random effects that resulted in zero variance were removed. I visualized relationships between all plant traits with pollinator visitation frequency and richness (Figure S7-S8) and how standardized values for plant traits, pollinator visitation frequency and richness changed over time (Figure S9).

I calculated Bray-Curtis dissimilarity among all samples and conducted permutational multivariate analysis of variances (PerMANOVA) to test for differences in community composition among treatments of onset and intensity of herbivory, and non-floral and floral traits. Looking into community composition will provide more information about the relative abundances of different pollinator morphotypes. I tallied the observations per morphotype for each block, treatment, and week, then calculated their relative abundances (Table S4).

RESULTS

Effects of early-onset herbivory on non-floral traits

The number of leaves, but not plant height, was significantly affected by the herbivory treatments. The effect of onset of herbivory on the number of leaves was dependent on the intensity of herbivory (Table 1). Early-onset herbivory at high-intensity resulted in 53 and 70.4 more leaves compared to late-onset herbivory at high-intensity and the control group, respectively (Figure 1A, Table S2). Similarly, late-onset herbivory at low-intensity resulted in 57.4 and 74.8 more leaves compared to late-onset herbivory at high-intensity and the control group, respectively (Figure 1A, Table S2).

Effects of early-onset herbivory on floral traits

Onset flowering, flower size, the number of open flowers, and nectar volume, but not nectar sugar concentration, were significantly affected by the herbivory treatments. Intensity of herbivory was a significant predictor for onset flowering and flower size (Table 1). The effect of onset of herbivory on the number of flowers and nectar volume was dependent on the intensity of herbivory (Table 1). Plants with low-intensity herbivory bloomed 1.7 and 2.1 days later than those with high-intensity herbivory when onset was early and late, respectively (Figure 1B, Table S2). Plants with early-onset herbivory at low-intensity bloomed 2.5 days later than those with late-onset herbivory at high-intensity (Figure 1B, Table S2). Plants exposed to low-intensity herbivory had significantly smaller flowers than those exposed to high-intensity herbivory and to the control group (Figure 1C and S10, Table S2). Plants with early-onset herbivory at high-intensity resulted in 60.7, 78.4 and 65.7 more open flowers compared to those with early-onset herbivory at low-intensity, late-onset herbivory at high-intensity, and the control group, respectively (Figure 1D, Table S2). Plants with

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late-onset herbivory at low-intensity resulted in 65.3 more open flowers than those with late-onset herbivory at high-intensity (Figure 1D, Table S2). Early-onset herbivory at high-intensity resulted in 1.4 and 1.1 more microliters of nectar compared to early-onset herbivory at low-intensity and late-onset herbivory at high-intensity, respectively (Figure 1E, Table S2).

Effects of early-onset herbivory on pollinator activity

Pollinator visitation frequency, richness, and community composition did not significantly differ among herbivory treatments (Table 1 and 2). However, visitation frequency and richness were positively correlated with plant height and the number of open flowers (Figure 2). A ten-cm increase in plant height and the number of open flowers was associated with an increase of 0.8 and 3.4 square root visits per observation period, respectively (Table S3). A ten-cm increase in plant height and the number of open flowers was associated with an increase of 0.5 and 0.9 square root morphotypes per observation period, respectively (Table S3).

DISCUSSION

I artificially manipulated herbivory in milkweed to investigate the extended consequences of plant-herbivore phenological mismatch on floral traits and flower visits by pollinators. I highlight that early herbivory can trigger milkweed to produce more flowers, but only when early-onset herbivory is intense. However, pollinators were not more abundant or diverse on plants experiencing early-onset high-intensity herbivory. Nevertheless, when examining the relationship between pollinator visits and the number of open flowers across all experimental plants, regardless of the treatments they experienced, there was a positive relationship. Taken together, these results suggest that plants may respond to early herbivory by allocating more resources to flower production. Plants may perceive herbivory as a threat to its survival and by producing more flowers, the plant increases its chances of reproductive success (Bauer et al. 2017). Moreover, changes in herbivorous insect phenology could have direct and indirect benefits for plant and pollinator fitness (Cuny et al. 2018).

Effects of early-onset herbivory on non-floral traits

I found no overall effect of onset or intensity of herbivory on plant height or the number of leaves; however, there was an interaction for the number of leaves. This is not consistent with my predictions that plants with early-onset and high-intensity herbivory would be shorter and have fewer leaves due to trade-offs between growth and defense (Züst et al. 2015; Brys et al. 2011). A previous study found that mean leaf lifespan, but not the number of leaves, decreased after subjecting mangroves to increasing levels of insect herbivory ranging from 20% to 80% leaf area removal (Lee 1991). They suggest that this pattern is due to a cost-benefit analysis and regulation of resource allocation (Lee 1991). I indicate that early-onset high-intensity herbivory and late-

onset low-intensity herbivory resulted in increased leaf production which could be explained by overcompensation. Part of my results mirror that of Boege (2005) who found that *Casearia nitida* compensates best for high levels of defoliation at the sapling stage by producing more leaves. Similarly, Cuny et al. (2018) found evidence of overcompensation when plants exposed to a single bout of herbivory produced more leaves than undamaged plants. In this case, *A. incarnata* may be able to overcompensate for different intensities of herbivory depending on when the herbivory event takes place. As insects continue to emerge earlier in the year, and if they inflict increasing amounts of damage to plants, leaf production may increase correspondingly.

Effects of early-onset herbivory on floral traits

Intensity of herbivory influenced onset flowering date and flower size, but not the number of open flowers, nectar volume or nectar sugar concentration. I found that plants exposed to low-intensity herbivory experienced delayed blooming compared to high-intensity herbivory but did not differ from the control. Plants with low-intensity herbivory also had smaller flowers, contrary to my predictions. Several studies show that higher levels of herbivory delay flowering (Agren and Schemske 1993; Traw 2002; Schiestl et al. 2014; Kettenring et al. 2009), while some show no relationship at all (Erneberg 1999). To my knowledge, no study has reported delayed flowering in low-intensity, but not high-intensity, herbivory treatments. These conclusions may also be made for onset budding date, as it was positively correlated with onset flowering date. Additionally, many studies show that flower size decreases with increasing levels of herbivory (Lehtilä and Strauss 1999; Mothershead and Marquis 2000; Strauss 1997), but none to my knowledge have shown plants to compensate for high-intensity, but not low-intensity herbivory. Lower levels of herbivory may not exert strong selective pressure on plants to invest into producing larger flowers

as a reproductive strategy (Garcia and Eubanks 2019). Smaller flowers may still attract enough pollinators for successful reproduction, while the plant conserves resources for other essential functions. A meta-analysis on overcompensation for insect herbivory found that damage intensity had no effect on plant response, despite the strong evidence for plants being able to best compensate for low-intensity herbivory (Garcia and Eubanks 2019). However, they discussed one study by Tito et al. (2016) who showed that *Actinocephalus polyanthus* produced more seeds when completely defoliated, compared to undamaged trees.

There was no overall effect of onset of herbivory on any floral traits; however, there was an interaction between onset and intensity of herbivory for the number of open flowers and nectar volume. Sharing a similar pattern as leaf production, I show that early-onset high-intensity herbivory resulted in increased flower production, which may also be explained by overcompensation. When onset of herbivory was late, plants with low-intensity herbivory resulted in more open flowers than those with early-onset herbivory but did not differ from the control. A recent study by Peschuitta et al. (2020) found similar results using sawfly leaf herbivory on cherry trees; trees with more than 50% leaf damage produced more flowers than those with essential no herbivory. Another study found that *Ipomopsis aggregata* individuals with simulated herbivory produced almost twice as many flowers than those with no herbivory which increased their fitness (Paige and Whitham 1987). However, both studies do not take into account the timing of herbivory. These conclusions may also be made for the number of inflorescences, as it was positively correlated with the number of flowers. As for nectar volume, no treatment differed from the control; however, when intensity of herbivory was high, plants in the early-onset treatment yielded more nectar than those in the late-onset treatment. Two studies found that extrafloral nectar volume was higher in plants exposed to herbivory, although no reference to timing or intensity was made

(Wäckers et al. 2001; Koptur 1990). Conversely, Smith et al. (1990) found no relationship between nectar volume and varying levels of herbivory, ranging from 25% to 75% leaf area removal. These results may provide evidence for differential overcompensation, specifically, the ability to compensate for high, but not low levels of herbivory. With further climate warming and earlier insect emergence, we may witness an increase in the number of open flowers and nectar volume.

Effects of early-onset herbivory on pollinator activity

Pollinator visitation frequency and richness were both positively correlated with plant height and the number of open flowers, but no other traits. These results confirm previous findings that pollinator visitation frequency and richness increases with the number of open flowers, which contributes to floral display size (Fornoff et al. 2017; Conner and Rush 1996; Klinkhamer et al. 1989; Cohen et al. 2021). These results are also consistent with the idea that plants that are more easily detected receive more visits from a larger diversity of pollinators (Williams et al. 2015; Hegland and Totland 2005; Mustajärvi et al. 2001). More open flowers increase floral display size and emit more VOCs, increasing visibility, scent, and visitation frequency (Makino and Sakai 2007; Farré-Armengol et al. 2013). A larger floral display size was observed to support more individuals at a given time. It is possible that a larger diversity of pollinators can be found on a single plant.

There was no effect of herbivory treatments on pollinator visitation frequency, richness, and community composition. This information is particularly interesting as the herbivory treatments affected the number of open flowers, which increased pollinator visitation frequency and richness. The lack of effect of herbivory treatments on pollinator activity suggests that the mediating effect of number of open flowers was not very strong. Overall, there is no strong

evidence that mismatches can cascade down to affect pollinators; however, results suggest a link exists through the number of open flowers. Several studies have shown that herbivory indirectly affects pollinators through reduced visitation, time spent per flowers, and pollinator survival (Jacobsen and Raguso 2018). This trend likely exists but may not have been picked up in our data due to seasonal variation or certain aspects of the experimental design such as sample size.

Implications

These results attest to the importance of the onset and intensity of herbivory in understanding ecosystem dynamics such as community structure, species interactions and ecosystem services (Agrawal and Maron 2022; Maguire et al. 2015). I observed changes in pollinator community structure, plant-pollinator interactions, and pollination as changes in patterns of herbivory altered milkweed traits. Some traits were then linked to pollinator visitation frequency and diversity. Studying these patterns can provide insight into the mechanisms driving changes in ecosystem dynamics under climate change. For example, changes in the timing of herbivory may be driven by plant phenology, changes in herbivore behaviour, or interactions with the environment (Ekholm et al. 2019; Rasmussen et al. 2023; Meineke et al. 2021). These results may also be taken into account when considering ecosystem management strategies as they relate to agricultural systems. This information could benefit these systems which are reliant on provisioning, regulating and cultural services (Maguire et al. 2015). If climate change leads to an earlier onset of herbivory, it may be necessary to adjust planting or harvesting schedules to minimize crop damage or conserve vulnerable plant species (Horgan 2020). The onset of herbivory can coincide with critical stages of plant growth and development, leading to reduced productivity and economic losses (Tscharntke et al. 2005). It is important to note that the effects of herbivory can be context-

dependent and vary across ecosystems; factors such as the onset and intensity of herbivory, and the resilience and adaptive capacity of plant species can all influence potential outcomes (Poelman et al. 2008; Stam et al. 2014). By investigating these mechanisms, we gain a better understanding of the underlying processes shaping patterns of herbivory and can make more accurate predictions about future changes.

Conclusions

By assessing the impacts of potential phenological mismatches between plants and insects, I contributed to bridging the gap between herbivory, plants traits, and pollinator activity. I expected plants with early-onset and high-intensity herbivory to have reduced non-floral and floral traits, and experience decreased pollinator visitation frequency and richness. Although there was no overall effect of onset of herbivory, there was an overall effect of intensity of herbivory and an interactive effect on some non-floral and floral traits. Additionally, I linked pollinator visitation frequency and diversity with the number of open flowers. My work highlights that changes in the timing of herbivory due to phenological mismatch may have benefits or consequences for milkweed traits, depending on its onset and intensity. However, we do not have conclusive findings for the indirect effects of herbivory on pollinator attraction. The findings of this study should be taken into account to help predict and mitigate ecological or agricultural losses in the face of climate change (Tscharntke et al. 2005). The future of phenological mismatch between milkweed and its herbivores is uncertain. Nevertheless, given the threat of climate change disrupting the synchronization of milkweed flowering and monarch butterflies' life cycles, there is a growing concern that rising temperatures could exacerbate this mismatch, posing challenges for the already vulnerable population (Howard 2018; Yang and Cenzer 2020). Future work should explore: 1)

how phenological mismatch affects specific pollinator taxa, 2) different types of herbivory including belowground herbivory, and 3) the long-term consequences of plant-herbivore mismatch on plant-pollinator interactions. While we looked at the effects of mismatch on an entire pollinator community, species-specific effects likely exist and could allow for more relevant applications (Lucas-Barbosa 2016; Jacobsen and Raguso 2018). Looking into belowground herbivory, especially for perennials like milkweed which develop complex root systems and can invest in longer term defense strategies compared to annuals, could provide a more complete picture (Jacobsen 2022; Staley et al. 2008). Finally, long-term consequences can be studied through community science which involves collecting data using broad networks like iNaturalist, Nature's Notebook and iSpot, or specific networks like eBird. Recently, there has been discourse surrounding community data collection, focusing on enhancing both accuracy and precision, while also considering the integration of best practices to mitigate potential bias (Feldman et al. 2018; Primack et al. 2023; Di Cecco 2022). Understanding how changes in herbivory affect pollination success, reproduction and plant fitness in the following years may provide more reliable predictions.

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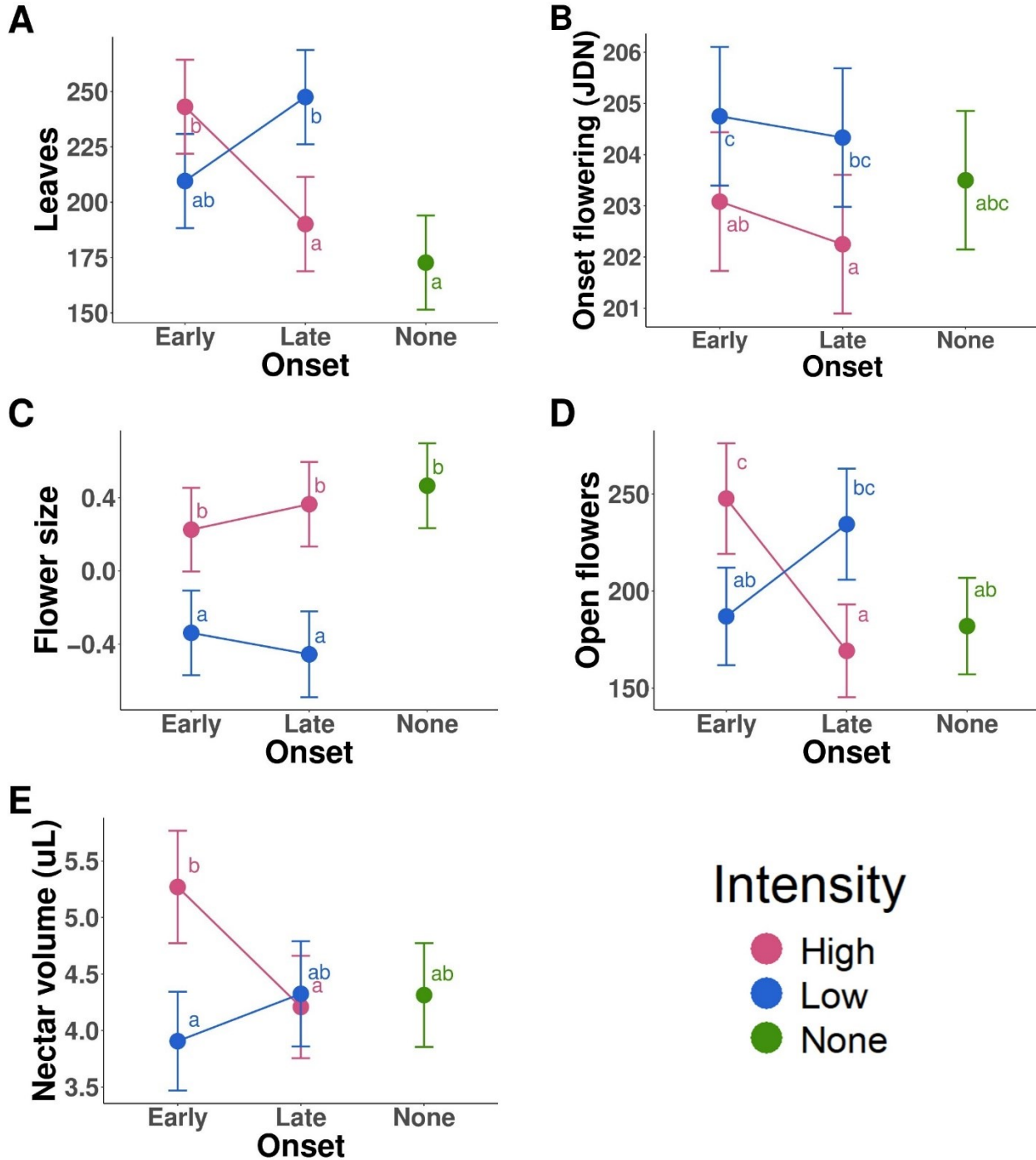
Figure 1. Pairwise comparisons of (A) number of leaves, B) onset flowering date, (C) flower size, (D) number of open flowers, and (E) nectar volume per combination of onset and intensity of herbivory (n=184 for panels A, C-E; n=60 for panel B). Comparisons are between onset (early or late) and intensity (high or low) of herbivory, and no herbivory (none). Estimated marginal means (EMM) are shown as dots with standard error bars. Means not sharing any letter are significantly different from each other.
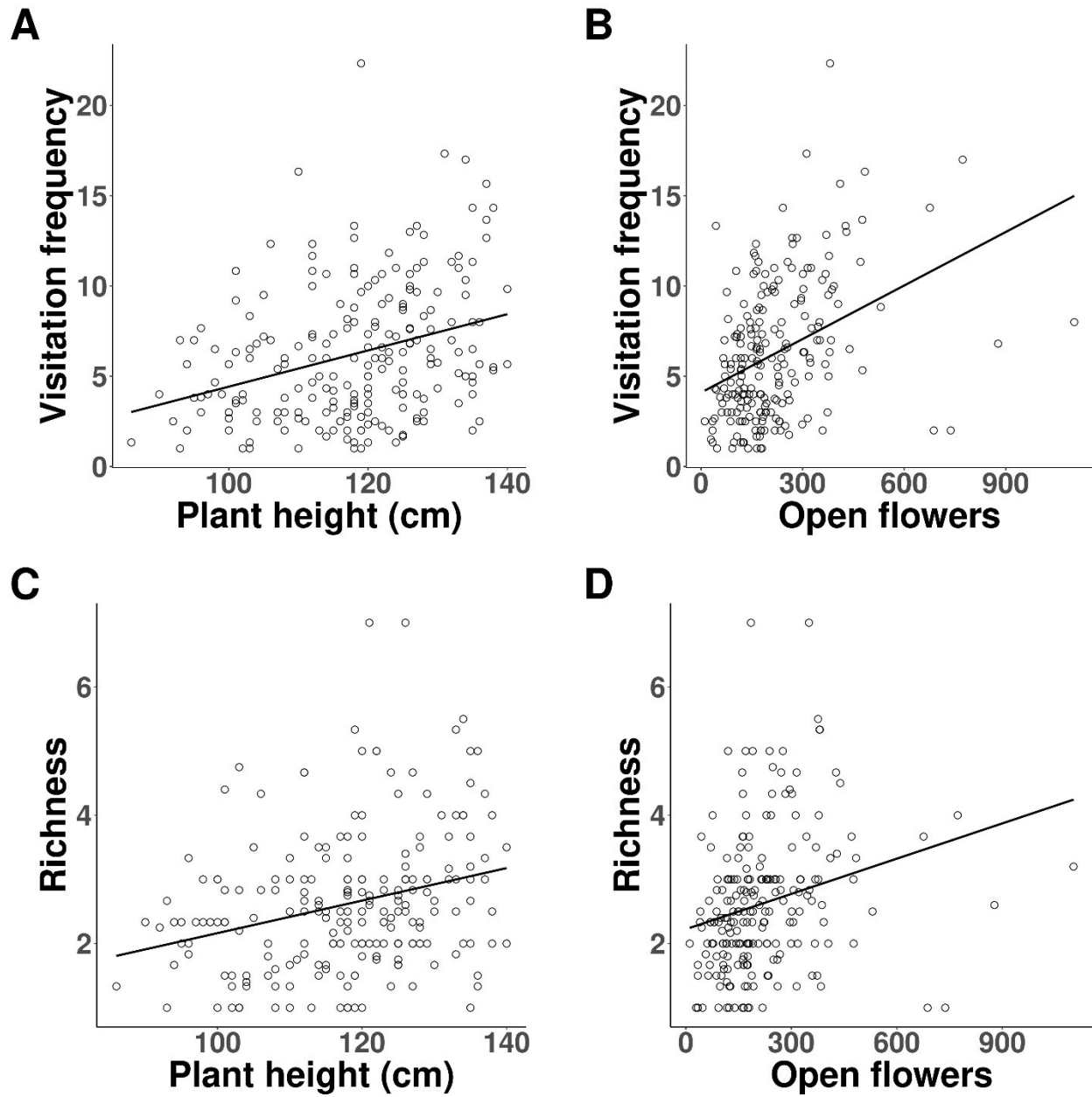
Figure 2. Scatterplots showing the relationship between pollinator visitation frequency with (A) plant height and (B) number of open flowers, and richness with (C) plant height and (D) number of open flowers (n=214). All plots are statistically significant with *p* values < 0.001.

TABLES

Table 1. Type III Analysis of Variance Table with Satterthwaite's method for the linear mixed-effects model of non-floral, floral and pollinator traits. Interactions between factors are represented as "×". Bold values denote statistical significance ($p < 0.05$).

Dependent variable	Predictor	Sum of squares	Mean square	df	F	p
Non-floral traits						
Plant height	Onset	2.62E+9	1.31E+9	2	0.08	0.928
	Intensity	2.40E+10	2.40E+10	1	1.38	0.246
	Onset × Intensity	7.29E+9	7.29E+9	1	0.42	0.521
Leaves	Onset	3933.87	1966.94	2	2.35	0.107
	Intensity	416.54	416.54	1	0.50	0.484
	Onset × Intensity	**6050.47**	**6050.47**	**1**	**7.24**	**0.010**
Floral traits						
Onset flowering	Onset	10.24	5.12	2	1.44	0.249
	Intensity	**42.19**	**42.19**	**1**	**11.83**	**0.001**
	Onset × Intensity	0.52	0.52	1	0.15	0.704
Flower Size	Onset	0.64	0.32	2	0.51	0.602
	Intensity	**15.27**	**15.27**	**1**	**24.16**	**0.000**
	Onset × Intensity	0.58	0.58	1	0.92	0.340
Open flowers	Onset	28.16	14.08	2	0.83	0.438
	Intensity	0.48	0.48	1	0.03	0.866
	Onset × Intensity	**170.97**	**170.97**	**1**	**10.08**	**0.002**
Nectar volume	Onset	0.39	0.19	2	0.71	0.492
	Intensity	0.71	0.71	1	2.62	0.108
	Onset × Intensity	**1.08**	**1.08**	**1**	**3.99**	**0.048**
Nectar sugar	Onset	9.32E-4	4.66E-4	2	0.17	0.848
	Intensity	4.20E-4	4.20E-4	1	0.15	0.700
	Onset × Intensity	0.01	0.01	1	3.43	0.066
Pollinator activity						
Visitation frequency	Onset	11.70	5.85	2	0.63	0.534
	Intensity	19.16	19.16	1	2.07	0.153
	Onset × Intensity	9.83	9.83	1	1.06	0.305
Richness	Onset	0.03	0.01	2	0.24	0.787
	Intensity	0.08	0.08	1	1.25	0.265
	Onset × Intensity	3.68E-4	3.68E-4	1	0.01	0.939

Table 2. PerMANOVA results for the effects of herbivory treatments, non-floral and floral traits on pollinator community composition. Community composition is estimated using the relative abundance per morphotype for each block, treatment, and week.

Predictor	Sum of squares	df	R^2	F	p
Herbivory treatment					
Onset	0.29	2	9.40E-3	0.84	0.549
Intensity	0.03	1	8.65E-4	0.16	0.977
Onset × Intensity	0.12	1	3.88E-3	0.70	0.588
Non-floral traits					
Plant height	0.09	1	2.99E-3	0.54	0.725
Leaves	0.10	1	3.13E-3	0.56	0.711
Floral traits					
Flower size	0.18	1	5.97E-3	1.07	0.373
Open flowers	0.06	1	2.00E-3	0.36	0.853
Nectar volume	0.17	1	5.47E-3	0.98	0.403
Nectar sugar	0.20	1	6.47E-3	1.16	0.292
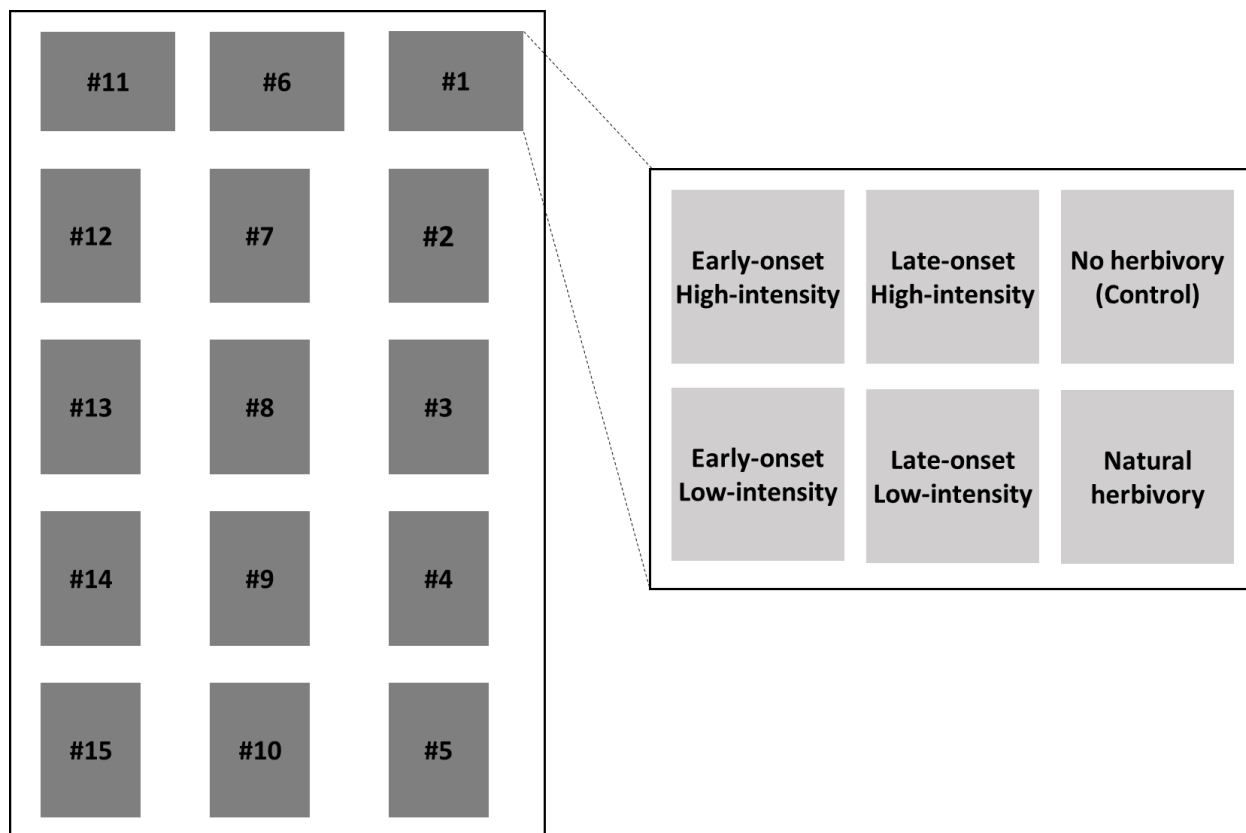
Figure S1. Randomized complete block design (RCBD) showing 15 blocks (left) and 6 treatments (right). Treatments are randomized within each block.
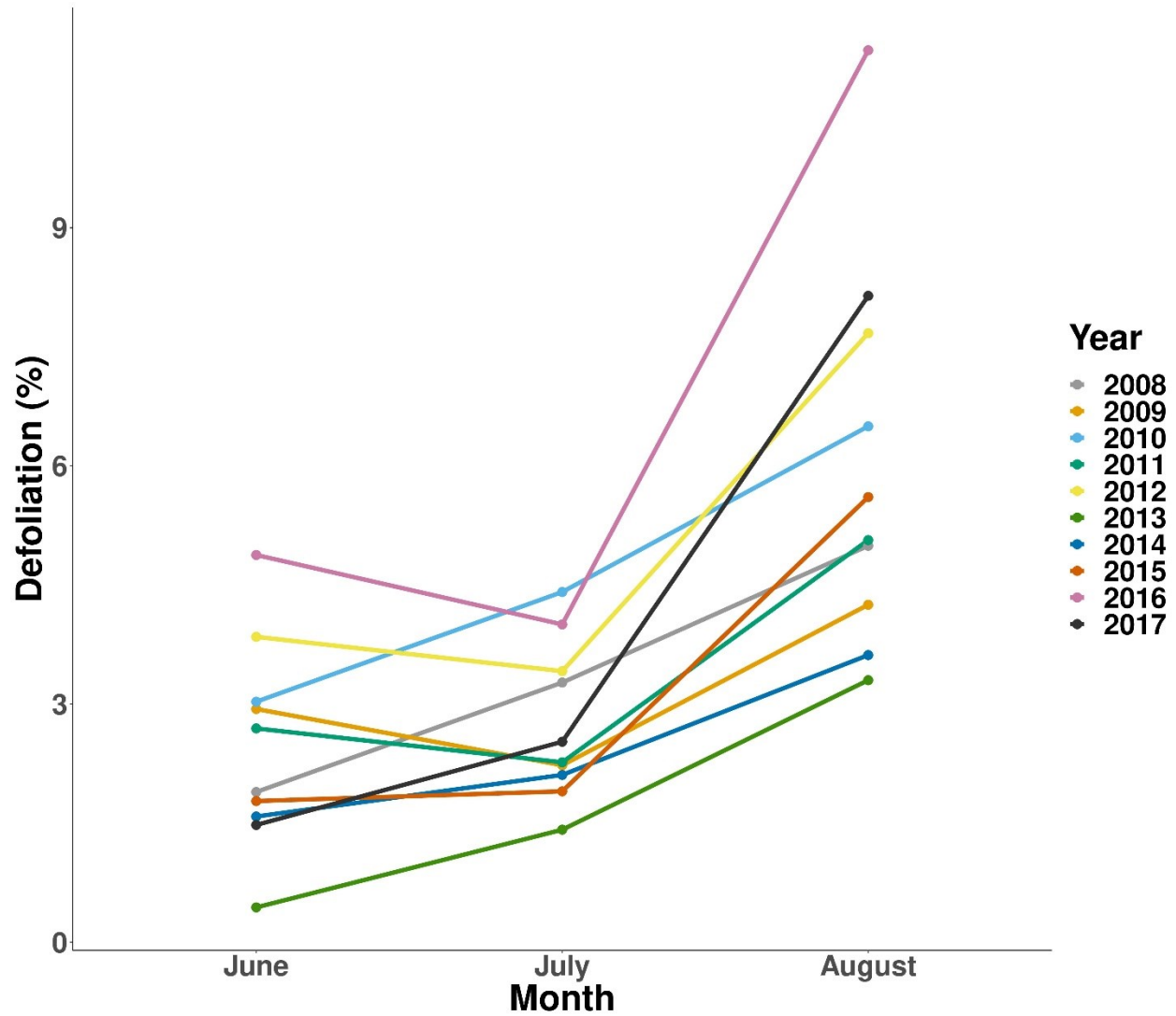
Figure S2. Average monthly percentage of defoliation on natural populations of *Asclepias syriaca* at the University of Michigan Biological Station from 2008 to 2017 (n=30). Unpublished data provided by Mark D. Hunter.
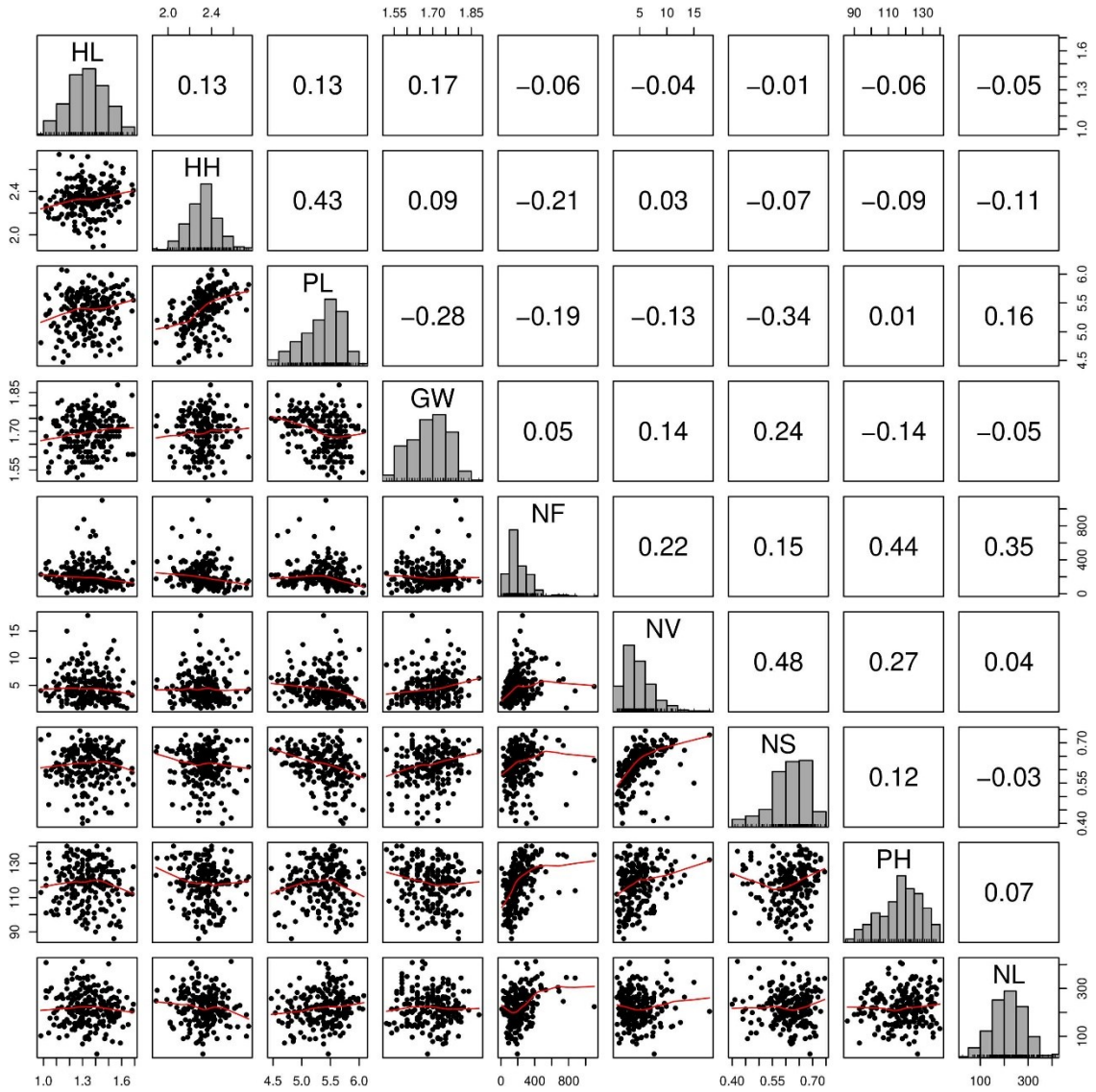
Figure S3. Pearson correlation matrix among plant traits (HL=hood length, HH=hood height, PL=petal length, GW=gynostegium width, NF=number of flowers, NV=nectar volume, NS=nectar sugar concentration, PH=plant height, NL=number of leaves).
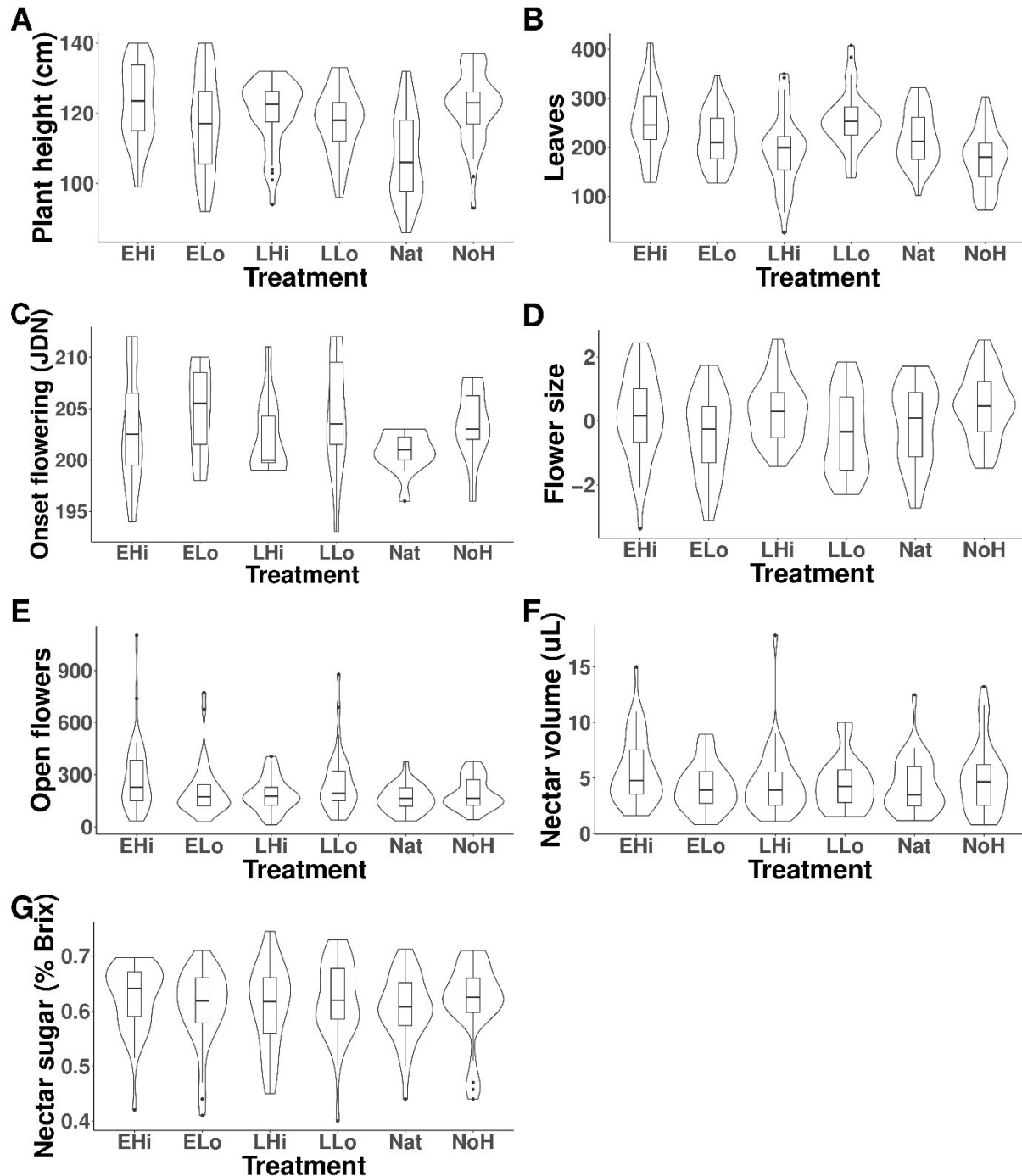
Figure S4. Violin plots showing (A) plant height, (B) number of leaves, (C) onset flowering (Julian date), (D) flower size, (E) number of open flowers, (F) nectar volume (uL), and (G) nectar sugar concentration (% Brix), per combination of onset and intensity of herbivory. Onset and intensity treatment abbreviations are EHi (early high), ELo (early low), LHi (late high), LLo (late low), NoH (no herbivory).
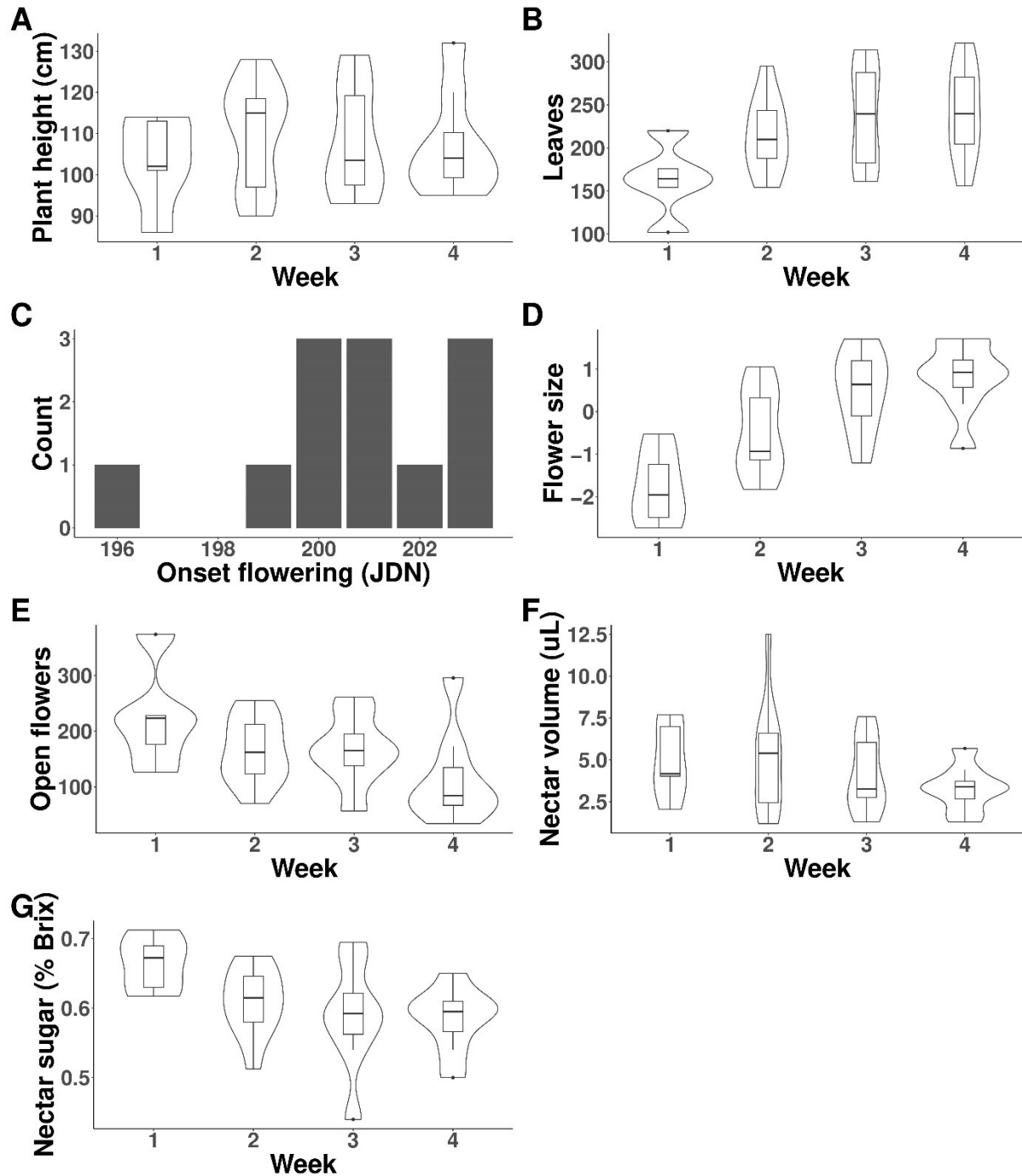
Figure S5. Violin plots showing weekly values of (A) plant height, (B) number of leaves, (D) flower size, (E) number of open flowers, (F) nectar volume (uL), and (G) nectar sugar concentration (% Brix) for plants in the natural treatment. Panel (C) shows the count of natural plants that flowered on each day (Julian date).
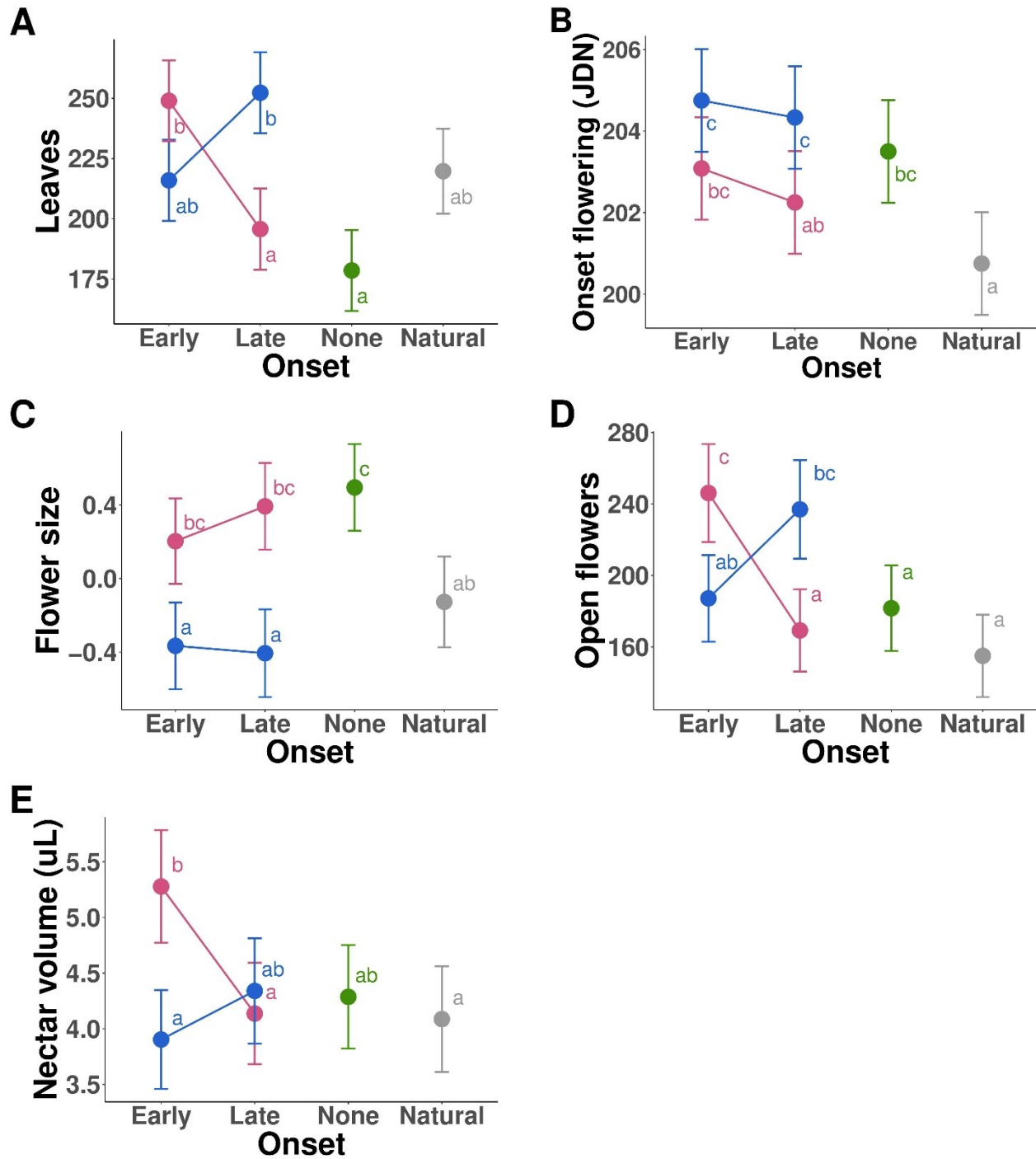
Figure S6. Pairwise comparisons of (A) number of leaves, B) onset flowering date, (C) flower size, (D) number of open flowers, and (E) nectar volume per combination of onset and intensity of herbivory (n=216 for panels A, C-E; n=72 for panel B). Comparisons are between onset (early or late) and intensity (high or low) of herbivory, no herbivory (none), and natural herbivory. Estimated marginal means (EMM) are shown as dots with standard error bars. Means not sharing any letter are significantly different from each other.
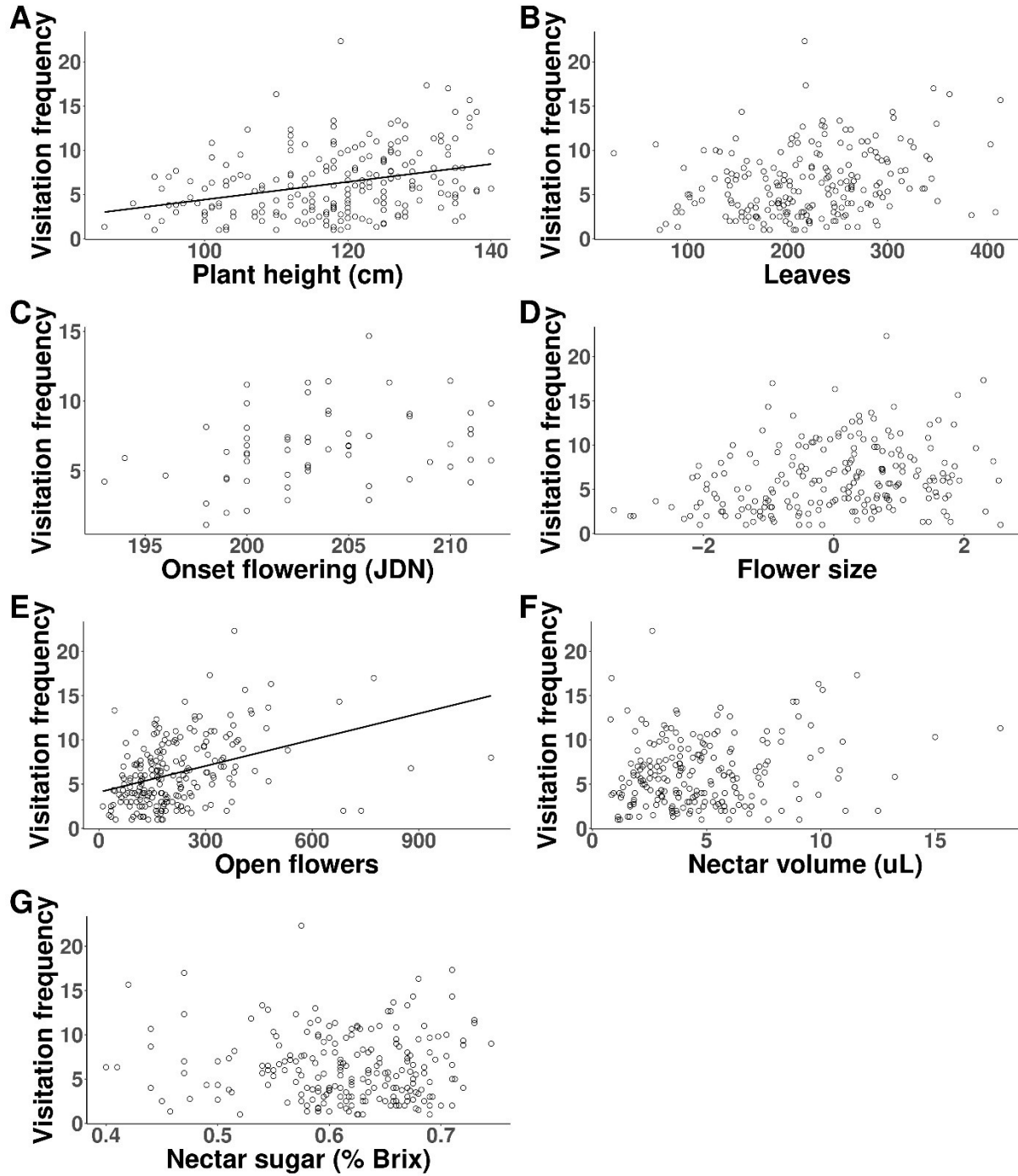
Figure S7. Scatterplots showing the relationship between (A) plant height, (B) number of leaves, (C) onset flowering (Julian date), (D) flower size, (E) number of open flowers, (F) nectar volume (uL), and (G) nectar sugar concentration (% Brix) with pollinator visitation frequency. Significant relationships are shown with a trendline.
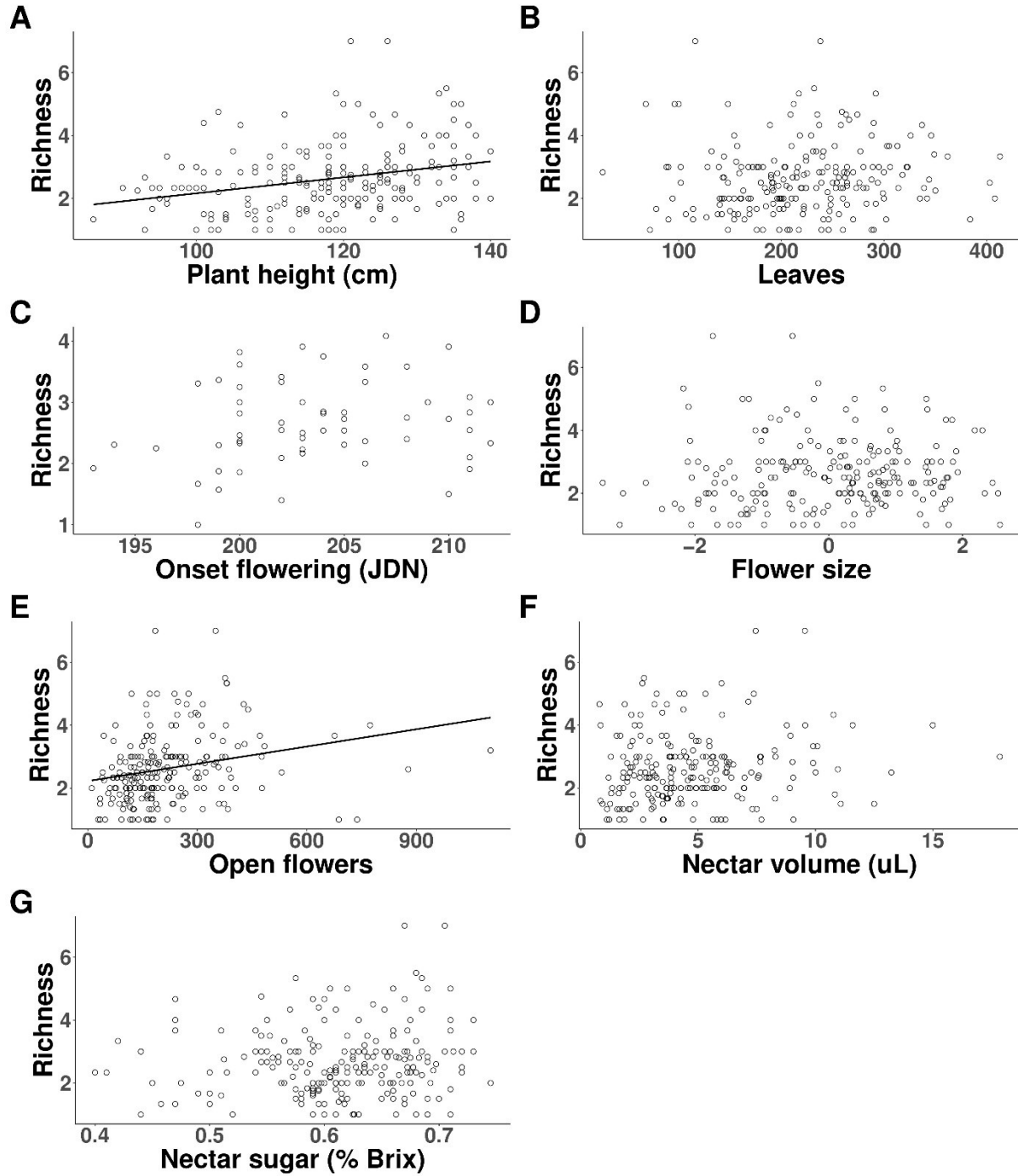
Figure S8. Scatterplots showing the relationship between (A) plant height, (B) number of leaves, (C) onset flowering (Julian date), (D) flower size, (E) number of open flowers, (F) nectar volume (uL), and (G) nectar sugar concentration (% Brix) with pollinator richness. Significant relationships are shown with a trendline.
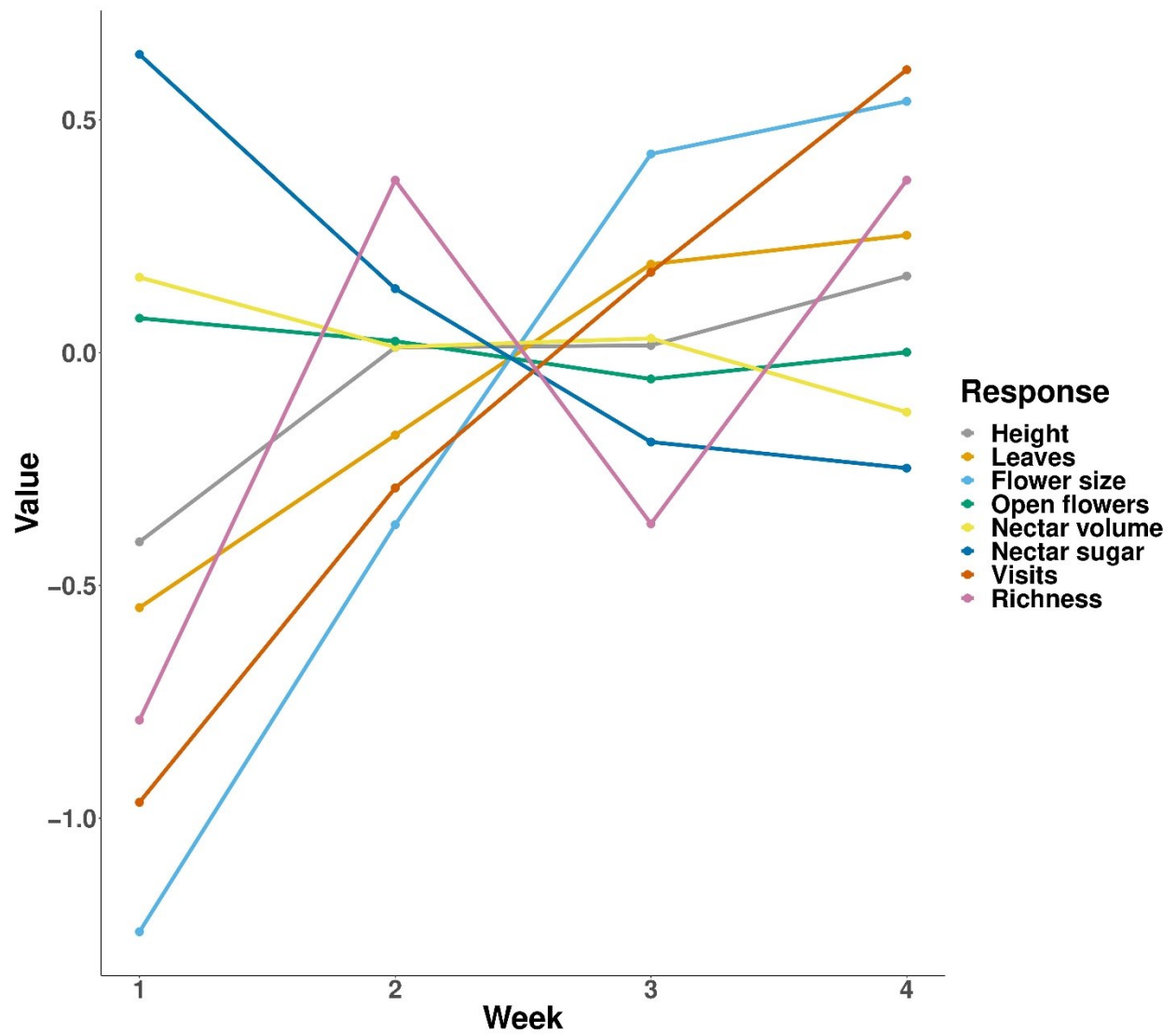
Figure S9. Average weekly values of all plant traits, pollinator visits and richness per observation period. All values are standardized between -1 and 1.
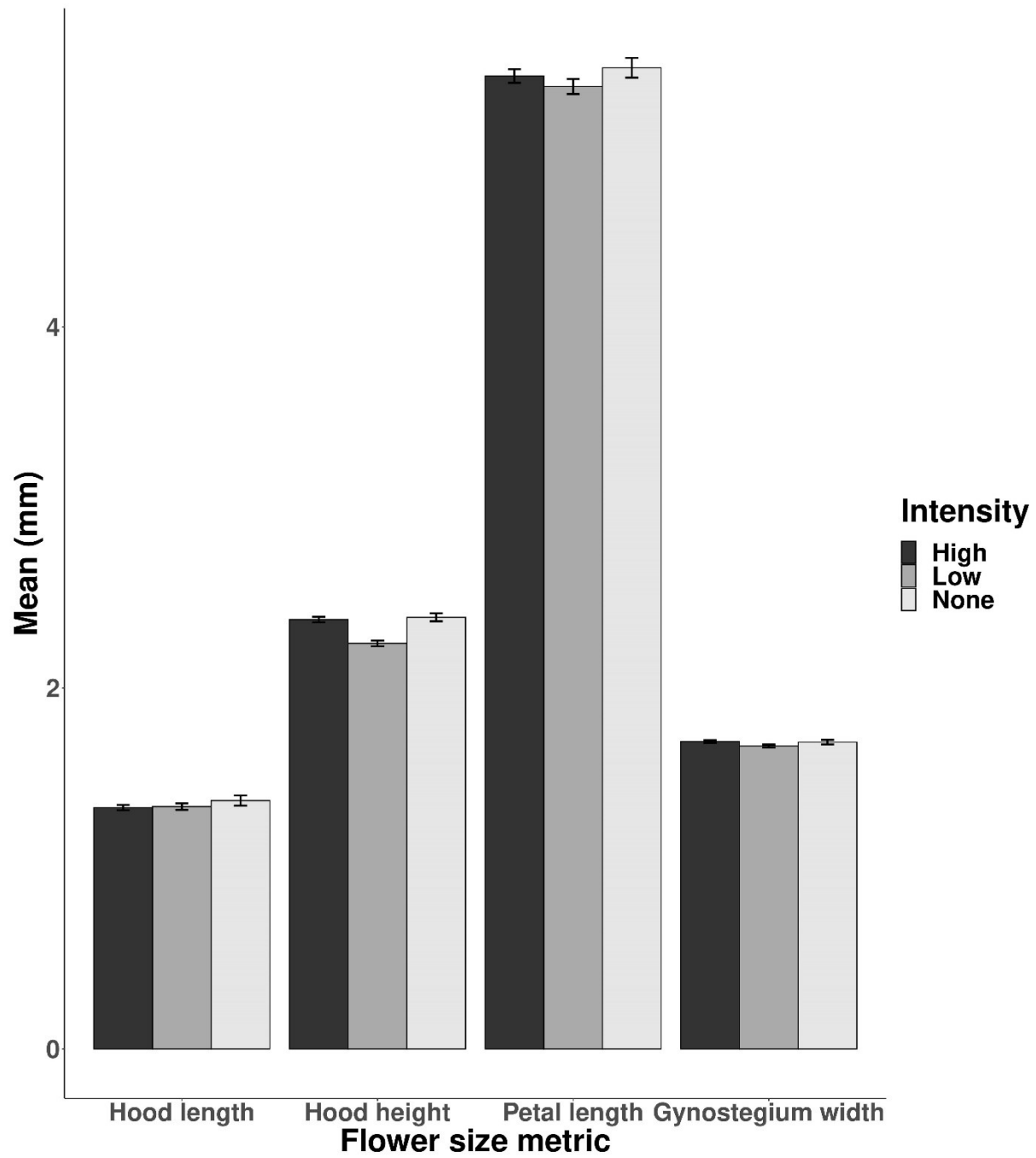
Figure S10. Bar plot showing the raw mean and standard error bars of four flower size metrics (mm) among different levels of intensity of herbivory.

Table S1. Visual key for 26 observed morphotypes.

Morphotype	Included Species	Visual Key
Apis mellifera	*Apis mellifera*	
Bombus	*Bombus* spp.	
Crabronidae (Black)	*Tachysphex* spp.	
Crabronidae (Sand)	*Bembix* spp.	
Crabronidae (Yellow)	*Philanthus* spp., *Cerceris* spp.	
Danaus plexippus	*Danaus plexippus*	
Halictidae (Black)	*Halictus* spp., *Lasioglossum* spp.	

Halictidae (Dull metallic)	*Lasioglossum* spp.	
Halictidae (Metallic green)	*Augochlorella* spp.	
Halictidae (Red)	*Sphecodes* spp.	
Hesperiidae (Brown)	N/A	
Ichneumonidae	N/A	
Megachilidae (Banded)	*Megachile* spp.	
Megachilidae (Black)	*Megachile* spp.	

Megachilidae (Metallic)	*Osmia* spp.	
Melissodes	*Melissodes* spp.	
Pompilidae	*Tachypompilus* spp.	
Sphecidae	*Sphex* spp.	
Symphyta	*Aneugmenus* spp.	
Syrphidae (Hairless yellow)	*Eristalis* spp., *Syritta* spp., *Eupeodes* spp., *Sphaerophoria* spp.	
Thynnidae	N/A	

Tiphiidae	N/A	
Vespidae (Black)	*Ancistrocerus* spp.	
Vespidae (Dark)	*Polistes* spp.	
Vespidae (Yellow)	*Polistes* spp.	
Vespula	*Vespula* spp.	

Table S2. Multiple pairwise comparisons (Tukey's HSD test) for the number of leaves, onset flowering, flower size, number of open flowers, and nectar volume. Contrasts are between onset (early or late) and intensity (high or low) of herbivory, and no herbivory (none). Bold values denote statistical significance ($p < 0.05$).

Dependent variable	Contrast	Estimate	Standard error	df	p
Leaves					
	Early High - Late High	**52.98**	**23.87**	**43.94**	**0.032**
	Early High - Early Low	33.52	23.87	43.94	0.167
	Early High - Late Low	-4.38	23.86	43.86	0.855
	Early High - None	**70.37**	**23.85**	**43.80**	**0.005**
	Late High - Early Low	-19.46	23.90	44.16	0.420
	Late High - Late Low	**-57.37**	**23.89**	**44.09**	**0.021**
	Late High - None	17.39	23.88	44.02	0.470
	Early Low - Late Low	-37.91	23.89	44.08	0.120
	Early Low - None	36.85	23.88	44.01	0.130
	Late Low - None	**74.76**	**23.87**	**43.94**	**0.003**
Onset flowering					
	Early High - Late High	0.83	0.77	44.00	0.286
	Early High - Early Low	**-1.67**	**0.77**	**44.00**	**0.036**
	Early High - Late Low	-1.25	0.77	44.00	0.112
	Early High - None	-0.42	0.77	44.00	0.592
	Late High - Early Low	**-2.50**	**0.77**	**44.00**	**0.002**
	Late High - Late Low	**-2.08**	**0.77**	**44.00**	**0.010**
	Late High - None	-1.25	0.77	44.00	0.112
	Early Low - Late Low	0.42	0.77	44.00	0.592
	Early Low - None	1.25	0.77	44.00	0.112
	Late Low - None	0.83	0.77	44.00	0.286
Flower size					
	Early High - Late High	-0.14	0.19	132.83	0.465
	Early High - Early Low	**0.56**	**0.19**	**134.71**	**0.004**
	Early High - Late Low	**0.68**	**0.20**	**142.89**	**0.001**
	Early High - None	-0.24	0.19	138.02	0.216

Dependent variable	Contrast	Estimate	Standard error	df	p
	Late High - Early Low	**0.70**	**0.19**	**135.22**	**0.000**
	Late High - Late Low	**0.82**	**0.20**	**140.15**	**0.000**
	Late High - None	-0.10	0.20	138.29	0.609
	Early Low - Late Low	0.12	0.20	138.18	0.553
	Early Low - None	**-0.81**	**0.20**	**135.78**	**0.000**
	Late Low - None	**-0.92**	**0.20**	**138.37**	**0.000**
Open flowers					
	Early High - Late High	**78.44**	**28.30**	**46.21**	**0.006**
	Early High - Early Low	**60.70**	**28.98**	**46.21**	**0.037**
	Early High - Late Low	13.19	31.81	46.21	0.679
	Early High - None	**65.70**	**29.00**	**46.21**	**0.024**
	Late High - Early Low	-17.75	26.54	48.98	0.504
	Late High - Late Low	**-65.25**	**28.93**	**48.86**	**0.024**
	Late High - None	-12.75	26.65	47.17	0.633
	Early Low - Late Low	-47.50	29.21	48.86	0.104
	Early Low - None	5.00	27.08	47.17	0.854
	Late Low - None	52.50	29.36	47.17	0.074
Nectar volume					
	Early High - Late High	**1.06**	**0.54**	**70.43**	**0.049**
	Early High - Early Low	**1.36**	**0.53**	**70.43**	**0.011**
	Early High - Late Low	0.95	0.57	70.43	0.097
	Early High - None	0.96	0.55	68.92	0.081
	Late High - Early Low	0.30	0.51	74.91	0.551
	Late High - Late Low	-0.12	0.53	74.19	0.826
	Late High - None	-0.11	0.52	68.92	0.841
	Early Low - Late Low	-0.42	0.51	74.19	0.416
	Early Low - None	-0.41	0.51	68.92	0.426
	Late Low - None	0.01	0.53	68.92	0.984

Table S3. Summary of results from the linear mixed-effects models of pollinator visitation frequency and richness. Bold values denote statistical significance ($p < 0.05$).

Dependent variable	Predictor	Estimate	SE	*df*	*t*	*p*
Visitation frequency						
	Plant height	**0.08**	**0.04**	**136.22**	**2.16**	**0.033**
	Leaves	-4.29E-4	0.04	180.59	-0.01	0.991
	Onset flowering[a]	0.13	0.09	15.24	1.52	0.150
	Flower size	0.04	0.04	180.31	0.94	0.348
	Open flowers	**0.34**	**0.05**	**186.73**	**7.55**	**0.000**
	Nectar volume	0.06	0.04	189.99	1.28	0.201
	Nectar sugar	0.04	0.05	198.38	0.82	0.412
Richness						
	Plant height	**0.05**	**0.02**	**148.49**	**2.64**	**0.009**
	Leaves	0.01	0.02	187.80	0.31	0.757
	Onset flowering[a]	0.01	0.02	21.05	0.57	0.577
	Flower size	0.01	0.02	187.37	0.67	0.505
	Open flowers	**0.09**	**0.02**	**193.74**	**4.05**	**0.000**
	Nectar volume	0.01	0.02	194.94	0.30	0.765
	Nectar sugar	0.04	0.03	198.98	1.67	0.096

[a] Models for onset flowering were conducted separately from the other response variables.

Table S4. Total number of visits per morphotype for each plant and week of observation. Onset and intensity of herbivory treatment abbreviations are EHi (early high), ELo (early low), LHi (late high), LLo (late low), NoH (no herbivory).

| Block	Treatment	Week	Apis mellifera	Bombus	Braconidae	Crabronidae (black)	Crabronidae (sand)	Crabronidae (yellow)	Halictidae (black)	Halictidae (dull metallic)	Halictidae (metallic green)	Halictidae (red)	Hesperiidae	Ichneumonidae	Megachilidae (black)	Megachilidae (banded)	Megachilidae (metallic)	Melissodes	Monarch	Pompilidae	Sphecidae	Symphyta	Syrphidae (hairless yellow)	Thynnidae	Tiphiidae	Vespidae (black)	Vespidae (dark)	Vespidae (yellow)	Vespula
1	EHi	4	1						11																			1	4
1	EHi	1	3											1															
1	EHi	3	1				1		3																				11
1	EHi	2	3	1				2	1	1														4			2		26
1	ELo	4		1					2																				3
1	ELo	3																											8
1	ELo	2	3																					1					6
1	LHi	4	3	1					3																			1	11
1	LHi	1	1																										
1	LHi	3					1																						8
1	LHi	2										1															2		4
1	LLo	4	1						3																				5
1	LLo	1	3																						1				
1	LLo	3		1																									7
1	LLo	2	3	1					1						1						1			2			1		24
1	NoH	4	3	1					6																		1		3
1	NoH	1	2	1					1																				
1	NoH	3	2						1																				12
1	NoH	2	3	1																				2			2		15
2	EHi	4	1				1		7											1							2		22
2	EHi	1	1	3																									2
2	EHi	3	3						7											1				2					36
2	EHi	2	1	1														1		1			2	1					1
2	ELo	4	1						1																				2
2	ELo	1	5	3											2														2
2	ELo	3	1				1		7																				18
2	ELo	2		2				2	1											1									3
2	LHi	4	1						12															1					15
2	LHi	1	2	2				3	2		1																		1
2	LHi	3	5				1		4	3														1	1				23
2	LHi	2	1	2				3	1									1		1									1
2	LLo	4	1						11																		2		17
2	LLo	1	6	1				1																					1
2	LLo	3	4				1		5	1														2					17
2	LLo	2	2																										2
2	NoH	4	1						7																				1
2	NoH	1	3	4			1																						1
2	NoH	3	3						4												1								25
2	NoH	2	1	1			1		1																1				
3	EHi	1	1																										

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3  EHi  3 |   1                                              1              2
3  EHi  2 | 1 1                                                             1
3  ELo  1 |   1
3  ELo  3 |                                                                 3
3  ELo  2 | 2 2           1                        1              1       2  2
3  LHi  1 | 3 1
3  LHi  3 |   1       2  15     1                                     4  1 34
3  LHi  2 | 3     1     1                          1                       1
3  LLo  1 | 2 1
3  LLo  3 | 6     1  1  1   9     3                1           1    5  1 27
3  LLo  2 | 6 3   3     1      1                   1                 1     3
3  NoH  1 | 2
3  NoH  3 |           2   8  1     1                  1        1          12
3  NoH  2 | 4 2   3  1  1   1                                     2
4  EHi  1 | 2                                                            1
4  EHi  3 | 5     1     2                         2        2  2  2       66
4  EHi  2 | 4 4   1  2  5        1                1        1  2  2  5     1
4  ELo  1 | 2
4  ELo  3 | 2     1        1                      1        3  3  2      29
4  ELo  2 | 4 2   1  3  3   1        1             1        2  1     2   5
4  LHi  3 |                1                                             2
4  LHi  2 | 4           2       1                                 1     3
4  LLo  1 | 2 1                                                         1
4  LLo  3 | 1 1     2                             1        2  1  3     42
4  LLo  2 | 3 5        5                   3              2        5    7
4  NoH  1 | 1
4  NoH  3 | 1 1     1                                                 21
4  NoH  2 | 2                             1                            1
5  EHi  4 | 1 1       4      7  1              1              2       14
5  EHi  1 |   2        2  2  1                                         1
5  EHi  3 |       1          3                       2               27
5  EHi  2 | 2 2   1  3  1      1                  3                    4
5  ELo  4 | 2 1       2  13                            1        2     3
5  ELo  1 |          2                                                 2
5  ELo  3 |       2          1                     1  1  1  5        32
5  ELo  2 | 2 1   2  2  4           1     1     1  1              1    3
5  LHi  4 | 2                1                     1                   1
5  LHi  1 | 1 1        1  2                    1                       1
5  LHi  3 |                6  1                    1                   8
5  LHi  2 | 2 1   4  3  2   1           1        1        1        1   2
5  LLo  4 | 2        3  12  2                                    1    2
5  LLo  1 |          2   1  1                                          1
5  LLo  3 | 1                3                     1                 25
5  LLo  2 | 3     2     2                                              3
5  NoH  4 | 2           12                                            3
5  NoH  1 |          1   2                                             2
5  NoH  3 |              3                                            11
5  NoH  2 | 2 2   5     1   2                     1        2     1    8
6  EHi  4 | 14 3           7                                         25
6  EHi  3 | 1     2  1  1                              4           1  54
6  EHi  2 |   1   5  1  1                              2           1  12
6  ELo  4 | 2           4
6  ELo  3 | 4           1                             1              13
6  ELo  2 | 3 1                      1                             1  4
6  LHi  4 | 12 1        5                                            16
6  LHi  3 | 3           3                                            33
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 6   LHi  2 | 2        3 1  2                                          7
 6   LLo  4 | 3                9                1              6       16
 6   LLo  3 | 2                3                        1 1           24
 6   LLo  2 | 2 1     2      2                                3        4
 6   NoH  4 | 11 3           1      1                                 27
 6   NoH  3 | 3 1       2 2                           3               39
 6   NoH  2 | 2 1     3 4                             1                5
 7   EHi  4 | 5          1 1  6    1                          2       17
 7   EHi  3 | 3            1  6                 1             1       35
 7   EHi  2 | 5 4       4 2        2      1           1 2              5
 7   ELo  4 | 1 2            13 1    1                        2       21
 7   ELo  3 | 1              2                  1                     15
 7   ELo  2 | 2 1       1 2                                   3        4
 7   LHi  4 | 5             13                                1        9
 7   LHi  3 | 3             2                                         8
 7   LHi  2 | 1         1                                             1
 7   LLo  4 | 1           1  1      1                        2 3     28
 7   LLo  3 | 3             2                                        14
 7   LLo  2 | 1 1       2 2                                           3
 7   NoH  4 | 6 3       2 1 14    1                   1      6 2      4
 7   NoH  3 | 5             4                                1       22
 7   NoH  2 | 5 3 1 3 1 1               1          1                  4
 8   EHi  4 | 1 8            5                  1            1 4
 8   EHi  3 | 13            1                                        26
 8   EHi  2 |          1                              3      3       11
 8   ELo  4 | 13 6         12                                2        1
 8   ELo  3 | 5          1  9            1            2      1        3
 8   ELo  2 | 4 3 3 2                    1            3      3       15
 8   LHi  4 | 13 1      2   15           1            1      2 1     22
 8   LHi  3 | 1              5 1         1                   2       57
 8   LHi  2 | 4 3       3 2              1                            2
 8   LLo  4 | 11 5      2   13           1                            5
 8   LLo  3 | 9          1  24                        2      1       28
 8   LLo  2 | 1                     1    1            1      1        4
 8   NoH  4 | 12 4          7                         1        2 7
 8   NoH  3 | 4             7 1              1        1      3       18
 8   NoH  2 | 2 2       2                             2      1       13
 9   EHi  4 | 3 1           2                                5       11
 9   EHi  3 | 3          1  4 1                       1      5       25
 9   EHi  2 | 3 2                                     2      1       22
 9   ELo  4 | 3 5       3 2 14                               2       11
 9   ELo  3 | 1          1 2 12                              2       34
 9   ELo  2 | 2 2       1            1                1 1    1        6
 9   LHi  4 | 5 7           4            1                   5       12
 9   LHi  3 | 2          1  1     1                          4       44
 9   LHi  2 | 4 2 1 2                                 3 1    1       18
 9   LLo  4 | 3             6                                3        6
 9   LLo  3 | 3   1         5 1                       2      2        2
 9   LLo  2 | 1         2                             1      1        5
 9   NoH  4 | 3 6       2   11                               3       12
 9   NoH  3 | 1          1  11 1                      2    2 6        4
 9   NoH  2 | 4 1       5                             3 1    1        2
11   EHi  4 | 1            22                                2        5
11   EHi  3 |              2                                         2
11   EHi  2 | 1       4 1         1                                  5
11   ELo  4 | 7 3 1       28                                 6       14
```

11	ELo	3						1										4
11	ELo	2			4	1		1										6
11	LHi	4	4	2			1	15									5		11
11	LHi	3	1					1											11
11	LHi	2	3		4	1		1						2					1
11	LLo	4	8					15									9		12
11	LLo	3	1																8
11	LLo	2												1					4
11	NoH	4	3	1				15					1			3			11
11	NoH	3	2																3
11	NoH	2	1		2	1			1										
12	EHi	4	26					14								1			14
12	EHi	3						1											2
12	EHi	2	1	2	2								1	1					13
12	ELo	4	8	1				1											2
12	ELo	3						2								1			6
12	ELo	2	1		1			1						2	1	1			6
12	LHi	4	6					8											3
12	LHi	3	1					3								2			21
12	LHi	2	2	1	3						1		1	2		1			2
12	LLo	4	32	1				21				1				2	1		7
12	LLo	3	1					5								1			21
12	LLo	2	4		1								1	3		1			18
12	NoH	4	16	1				16									1		2
12	NoH	3						7								1			6
12	NoH	2	1					1	1					1		3			6
13	EHi	4	7	2				5								2			1
13	EHi	3	1					3					1	1					37
13	ELo	4	1	4				7											7
13	ELo	3					1									1			32
13	LHi	4	9	2				5							1				13
13	LHi	3			1	2		2	1					3					45
13	LLo	4	17	11		2		15											32
13	LLo	3	1					7	1	1			1			1			29
13	NoH	4	16	7			1	12								2			27
13	NoH	3	1		1			5											37

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