Article

ASSESSMENT OF POLLINATION ECOLOGY OF TWO UNDERSTUDIED NATIVE MUSTARDS AND THE POTENTIAL FOR INTERFERENCE FROM INVASIVE GARLIC MUSTARD

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Abstract—The family Brassicaceae is agriculturally and economically important. However, ecological knowledge gaps persist for some wild species, even in parts of the world that have broadly well-studied flora, like eastern North America. Knowledge gaps for two eastern North American species, *Cardamine concatenata* and *C. diphylla*, are concerning for two reasons. First, these species have known associations with rare, specialist insects. Second, the threat posed by the introduction and spread of noxious invasive confamilial garlic mustard (*Alliaria petiolata*) within their shared native range. Because *C. concatenata* and *C. diphylla* have a short phenological window for seasonal development and reproduction they are at increased risk for mutualism disruption via global change pressures. We aim to evaluate the potential for pollination interference by *A. petiolata*. We compared pollinator visitation and floral phenology in sympatric wild populations of *C. concatenata, C. diphylla*, and *A. petiolata* in northeast Ohio. Pollinator counts and morphotype identifications were recorded during *in situ* pollinator observations. To supplement morphotype identifications, we collected pollinator specimens and identified them to genus or family in the lab. We found overlap in flowering phenology between *A. petiolata* and *C. diphylla*. Fourteen pollinator taxa were observed visiting at least one of the focal plant species; of these, seven taxa were recorded visiting all three plant species. *Alliaria petiolata* is likely disrupting pollination for the native *C. diphylla* in our sites because flowering phenology overlaps and pollinator taxa are shared. Future studies should focus on range-wide phenological observation and modelling for all three plant species, and pollen load analysis of native plant stigmas in invaded habitats.

Keywords—Pollination ecology, pollinator behaviour, species invasion

INTRODUCTION

Mutualism disruptions due to stressors such as climate change, land use change, biodiversity loss, and/or the presence of competitive invasive species have been widely documented in the literature and pose significant threats to native communities (Schweiger et al. 2010). Even in cases where focal species are directly unimpacted by anthropogenic change, decreases in fitness can arise from disruptions to critical interspecific interactions, such as loss of or phenological misalignment with mutualistic partners (Aslan et al. 2013). For example, Kudo and Cooper (2019) demonstrated that early snowmelt under natural conditions increased the risk of phenological mismatch between a spring ephemeral (*Corydalis ambigua*) and its pollinator (overwintered bumblebees) in a deciduous forest of northern Japan, potentially leading to reduced fitness for both partners in the form of seed set for the plant and reduced colony success for the bees.

Mutualism disruptions have been recorded in plant-pollinator systems across North America, particularly where native plants share both floral morphology and reproductive phenology with highly competitive invasive plants. In general, when native pollinators are attracted to morphologically similar invasive species, overall visitation rates to native plant species are reduced, and the potential for heterospecific pollen transfer

(HPT) is increased, ultimately decreasing the overall reproductive fitness of native plant species. Reduced reproductive fitness in native plant species results from either the inability of conspecific pollen tubes to fertilize ovules (i.e., invasive pollen blocks the stigma of the native) and/or by increased pollen limitation (i.e., native pollen is deposited on the invasive stigma), both of which characterize HPT (Waser 1978). For example, Brown et al. (2002) found that invasive purple loosestrife (*Lythrum salicaria*) in Ottawa County, Ohio, reduced pollinator visitation to native winged loosestrife (*Lythrum alatum*), with pollinators often moving between the flowers of these species, likely leading to HPT, and ultimately resulting in reduced seed set for *L. alatum*. Similarly, Powell et al. (2011) found that the presence of a highly competitive invasive species, *Cirsium vulgare*, which produced more flowering heads, drew more pollinator visits than any of a suite of five native *Cirsium* species in their Marin County, California study sites. In these instances and others, ecological communities resulting from mutualism disruptions often behave as novel assemblages (Schweiger et al. 2010).

Such mutualism disruptions have been recorded in regions of North America invaded by the noxious herb *Alliaria petiolata*. For example, Stinson et al. (2006) found that *A. petiolata* allelopathy was capable of eliminating mycorrhizal associations in maple (*Acer*) and ash (*Fraxinus*) seedlings almost entirely, and Hale et al. (2011) followed this up with the subsequent discovery that the elimination of these associations decreased stomatal conductance and photosynthetic rate in the native spring ephemeral herb *Maianthemum racemosum*. In addition to allelopathy, *A. petiolata* is widely known to create dense monocultures that can exclude native understory herbs. For example, in a container experiment, Davis (2015) demonstrated that *A. petiolata* decreased the growth of a native spring ephemeral mustard, *Cardamine diphylla*; when grown alongside flowering *A. petiolata,* as compared to a conspecific individual, *C. diphylla* produced significantly lower root and shoot biomass (Davis 2015). However, studies considering whether *A. petiolata* is capable of disrupting pollination in native plant assemblages are absent from the literature, even for closely related taxa with similar phenology and morphology, and for which negative competitive effects have already been documented, such as species in the genus *Cardamine* (see Fig. 1).

Two eastern North America *Cardamine* species with widespread geographic distributions are *Cardamine concatenata* and *C. diphylla*. These inhabit mature mesic forest understories, where they are considered to be species of low conservation concern. *C. concatenata* and *C. diphylla* are likely insect-pollinated (Spooner 1984; Montaut & Bleeker 2013; Wilhelm pers. obs.) spring ephemeral species (Kudo et al. 2008; Yancy et al. 2024), meaning they are adapted to take advantage of the cool (but not freezing) temperatures and short periods of intense light in early spring before

Figure 1. From left to right, the inflorescences of *Cardamine concatenata*, *C. diphylla*, and *Alliaria petiolata*. All species are members of the family Brassicaceae and exhibit the same floral anatomy. Images were taken in spring 2024 at the Holden Arboretum, Kirtland, OH, U.S.

the forest canopy closes. *C. concatenata,* listed by NatureServe as G5 ("Secure"), occurs in more than a dozen states and two Canadian provinces (NatureServe Explorer 2025). Its congener, *C. diphylla,* shares a similar global and state-level conservation status (G5, Unranked-Apparently Secure through most of its range; NatureServe Explorer 2025). While *C. diphylla* is found in fewer total states and provinces than *C. concatenata*, it still has a large regional distribution throughout eastern North America (NatureServe Explorer 2025). *Alliaria petiolata* is a species of biennial mustard native to Eurasia. In its invaded range, *A. petiolata* often inhabits the same mature, mesic understory habitat as *Cardamine*. Since its introduction to Long Island, New York in 1868, *A. petiolata* has established in 38 states (Rodgers et al. 2008 and 2022). Its ability to proliferate, in addition to its unique allelopathic properties (among other traits), have been key to its success (Prati & Bossdorf 2004).

It is likely that the flowering phenology of North American *Cardamine* species and *A. petiolata* overlap throughout parts of their shared range (Wilhelm unpublished data). If floral overlap occurs, and if pollinators are shared in both space and time, the potential for shifts in species interactions between native plants and their respective pollinators exists; mutualism disruptions of this nature have been documented in similarly structured systems (Brown et al. 2002; Wang et al. 2023). Hymenopterans that emerge abundantly in early spring are thought to be the most frequent floral visitors for *C. concatenata*, particularly those of the families Andrenidae, Megachilidae, Halictidae, and Apidae (Wilhelm & Rericha 2017). Flies of the families Bombyliidae and Syrphidae also pollinate *C. concatenata* (Hassall et al. 2017; Parker et al. 2018; Doyle et al. 2020). Pollinators of the same bee families (Halictidae, Andrenidae, Megachilidae, and Apidae) have been documented pollinating *A. petiolata* in its invaded range (Wilhelm & Rericha 2017), lending further evidence to the potential for mutualism disruption. Pollinators for *C. diphylla* remain undocumented in the literature.

Though not of conservation concern themselves, both *C. concatenata* and *C. diphylla* support specialist, spring-flying insects that are largely of unraked conservation status and that have not been subject to much ecological study. For example, *Andrena arabis,* the North American endemic mustard miner bee, specializes on members of Brassicaceae in North America, including both *C. concatenata* and *C. diphylla.* This bee is vulnerable through much of its range (NatureServe Explorer 2025), though it remains GNR ("Unranked"), and is largely absent from the literature (see Robertson 1929; Ribble 1974). *C. diphylla* and *C. concatenata* (the latter to a lesser extent) are also among the native larval hosts for the rare univoltine West Virginia white butterfly (*Pieris virginiensis*; Fig. 2). This butterfly is listed as G4 ("Apparently secure"), though it is considered vulnerable or imperilled across much of its range (Davis 2015; NatureServe Explorer 2025). In recent years, *P. virginiensis* has garnered the attention of conservation organizations, namely Lake Erie Allegheny Partnership for Biodiversity (LEAP). Flight time for adult *P. virginiensis* begins in April and ends in May in northern portions of its range, sometimes lasting as little as a week (Wilhelm unpublished data). While survival rate of larvae in uninvaded habitat is only 10-15%, larval survival declines to 3-5% in habitats invaded by *A. petiolata* (Davis 2015). *C. diphylla* is also the main food source for the woodland flea beetle, *Phyllotreta bipustulata* (Hicks & Tahvanainen 1974). This beetle

Figure 2. A West Virginia white butterfly, *Pieris virginiensis*. Image taken in spring 2024 at the Holden Arboretum, Kirtland, OH, U.S.

is listed as G5 ("Secure"), though it is largely unranked across its range (NatureServe Explorer 2025), nor is it referenced in the literature outside of the single 1974 publication by Hicks and Tahvanainen. Such examples demonstrate potentially concerning knowledge gaps surrounding aspects of the ecology of *C. concatenata* and *C. diphylla*. Their status as spring ephemerals, which are at increased risk of deleterious climate change-induced fitness effects (Kudo & Cooper 2019; Heberling et al. 2019), and their known (but under-explored) relationships with threatened, specialist insects, underscores the need for more thorough examination.

In this study, we investigated the potential for invasive *Alliaria petiolata* to disrupt pollination in two native mustards, *Cardamine concatenata* and *C. diphylla*, which serve as host plants for specialist insects, as well as pollen and nectar sources for early-emerging spring pollinators. To do so, we tracked flowering phenology for all three plants (*C. concatenata*, *C. diphylla*, and *A. petiolata*) where they co-occur in sites in northeastern Ohio, U.S., to first determine whether native *Cardamine* flowering phenology overlaps with *A. petiolata*. We then performed standardized pollinator observations and collected pollinator specimens for all three species. We hypothesized that all three plant species would experience flowering phenology overlap and share pollinator taxa.

MATERIALS AND METHODS

INTRODUCTION TO *CARDAMINE*

The genus *Cardamine* is estimated to contain between 100 and 200 species worldwide, comprising approximately two-thirds of the species in the large Brassicaceae Tribe Cardamineae (Franzke et al. 2011). Species in the genus *Cardamine* are ubiquitous members of spring understory assemblages in North America, with approximately 50 species found throughout the U.S. and Canada (Carlsen et al. 2009). Twenty-one of these occur in eastern North America (Brandenburg 2010; Flora of North America 2025). Of these 21 species, six are exotic (*C. flexuosa*, *C. hirsuta*, *C. impatiens*, *C. pratensis*, *C. bulbifera*, and *C. nymanii*) and 15 are native (*C. angustata, C. bulbosa, C. concatenata, C. diphylla, C. dentata, C. dissecta, C. douglassii, C. maxima, C. pensylvanica, C. rotundifolia, C. clematitis, C. flagellifera, C. longii, C. micranthera,* and *C. beldifolia*; Flora of North America 2025; Biota of North America Program 2025). Although not discussed here, numerous hybrids likely exist within this geographic region (Sweeney & Price 2000; 2001; Carlsen et al. 2009; Zozomova-Lihova & Marhold 2006).

STUDY SITES

Our study sites, located at the Holden Arboretum in Kirtland, Ohio, U.S., are predominantly mesic woodlands, dominated by oak, beech, and maple, with a thick layer of leaf litter. Four areas (Stebbins Natural Area, Pierson Valley, Baldwin Woods, and the Working Woods) were selected using target species abundance data derived from the Holden Arboretum Conservation and Research Departments. Study sites were 0.4-1.6 km apart, separated by 2-lane roadways and/or the East Branch of the Chagrin River. Study sites ranged from 20-100 hectares. All sites boasted a variety of native spring ephemerals. *C. concatenata* was present at all sites, while *C. diphylla* and *A. petiolata* were present at three sites.

FLOWERING PHENOLOGY

In northeast Ohio, *C. concatenata* flowers from late March to late April and fruits from May until early June. *C. diphylla* blooms slightly later, from mid-April to mid-May, and sets fruit beginning in early May (Wilhelm unpublished data). To assess the precise timing of spring reproductive events in the context of pollination (and ultimately, mutualism disruption), we tracked flowering phenology in spring 2024 by recording the number of open flowers for *C. concatenata, C. diphylla,* and *A. petiolata* associated with pollinator observations and collections (see *Pollinator assemblages* below). From these data, we calculated mean flowering duration for each study site by adding the number of days from the first observed flower to the last observed flower at each site and dividing by the number of sites (four in total; Stebbins Natural Area, Baldwin Woods, Pierson Valley, and the Working Woods).

POLLINATOR ASSEMBLAGES

We documented pollinator visitation and the composition of pollinator communities for *C. concatentata, C. diphylla* and *A. petiolata* in our study sites via *in situ* pollinator observations. Observations involved recording the number of pollinator visitors and assigning morphotype identifications to each visitor. All pollinator observations were conducted between March 25 and May 7, 2024 (43 days). To supplement coarse field identifications, we also collected and identified pollinator specimens in the laboratory for each plant species at each survey site.

Within each study site, we identified areas where focal plants were present prior to flowering. Upon flowering (i.e., flowers were observed to be present and open), 1 m² observational quadrats were placed to capture blooms of all study species in each site (18 quadrats for Stebbins Natural Area across eight unique sampling dates; 27 for Baldwin Woods across eight unique sampling dates; 34 for Pierson Valley across six unique sampling dates; and 12 for the Working Woods across four unique sampling dates). This resulted in a total of 91 20-minute observations for the entire study (a total of 1,820 minutes of observations); 49 total 20-min observations (980 total minutes of observation) for *C. concatenata*, 24 total 20-min observations (480 total minutes of observation) for *C. diphylla*, and 18 total 20-min observations (360 total minutes of observation) for *A. petiolata.*

Observational quadrats remained free from the pressure of insect sampling (i.e., observational quadrats were placed in different locations than the quadrats used for specimen collection, described below). Observations took place between 10 am and 5 pm, when temperatures were above 40°F (4.4°C) and wind speed was low (i.e., under 5 mph). Similar parameters have been employed in other studies (Masters & Emery 2015). We recorded the start and end time for each observation and accessed temperature data for those timeframes from Weather Underground (Weather Underground 2024). Pollinator activity was monitored for 20 minutes per quadrat, and the number and morphotype of observed pollinators was recorded for all open flowers of focal plant species within the quadrat. We did not mark individual plants within quadrats because individuals are not easily discerned in *Cardamine* due to their clonal growth habit and because our interest was in understanding trends in pollinator visitation among populations of native and invasive plants as opposed to individual-level effects.

Morphotype identifications, which were accomplished using high-level visual markers (i.e. coloration, shape, number of wings, etc.), allowed us to sort observed pollinators into broad taxonomic categories (Ustinova & Lysenkov 2020). The following pollinator morphotypes were used: butterflies (Lepidoptera), beetles (Coleoptera), bee flies (Bombyliidae), hoverflies (Syrphidae), house flies (Muscoidea), small black bees (*Andrena*, *Osmia*, *Halictus*), small black bees with red abdomens (*Nomada*), small metallic bees (*Augochlora pura*, *Lasioglossum*), and bumblebees (*Bombus*). Ustinova and Lysenkov (2020) observed unique pollinator assemblies for two invasive goldenrods in Russia using similar pollinator groupings.

To supplement morphotype identifications during observations, we also collected pollinator specimens in separate 1 m² quadrats designated for sampling (i.e., collection quadrats were separate from observation quadrats, described above, to ensure pollinator observations were unbiased). Pollinator sampling was conducted by placing quadrats in the same manner as observation quadrats, again between 10 am and 5 pm. Within 20-minute sampling windows (used to mimic duration of pollinator observations), we collected all insect pollinators observed interacting with open flowers of any of the three study plants. Collections occurred from April 8 to May 2 (31 days) across the study sites.

Following collection (accomplished by catching insects in aerial nets and then placing captured specimens in sealed jars with ethyl acetate), we returned specimens to the laboratory, where they were processed, identified to genus, and counted. When it was not possible to identify a specimen to genus, we identified it to family or order. Formal taxonomic identifications not only enabled us to confirm that the same insect morphotypes were visiting all study plants, but that the same taxa were visiting all three focal plants—a result that would not have been possible strictly through pollinator observations.

STATISTICAL ANALYSES

All statistical analyses for this study were performed in RStudio (R Core Team 2020). From our observational quadrats, we calculated visitation as the number of visits per flower per hour. Visits per flower per hour was a non-normal, continuous dataset including positive values and true zeroes (i.e., where no pollinator visits were observed during the observational period). Zero-inflated gamma generalized models (Z-GGMs) implement two separate processes to handle zero and non-zero positive values. The first process models the probability of observing a true zero as opposed to a non-zero. Results from this portion of model indicate the effect that predictor variables have on the probability of observing a true zero. The second process models the influence of non-zero responses using a gamma distribution (Lee et al. 2010; de Freitas Costa et al. 2021). Results from this portion of the model indicate the effect that the predictor variable has on observing higher or lower values in the response variable.

We used Z-GGMs (package glmmTMB; Brooks et al. 2017) to evaluate differences in visitation rates among the three focal plant species. We also ran Z-GGMs, accounting for the effect of temperature where needed, to test whether visitation rate by pollinator morphotype was significantly different between plant species. For significant models, we used post hoc tests (Tukey Contrasts, package multcomp; Hothorn et al. 2008) to evaluate pairwise differences among plant species. These models, which included fixed (i.e., temperature, day of year, plant species) and random effects (i.e., site), were compared to a null model accounting for only the random effect of site using likelihood ratio tests. Four taxa (*Bombus* [bumblebees], Coleoptera [beetles], Lepidoptera [butterflies], and Muscoidea [house flies]) were not included for individual morphotype analysis because they were infrequent floral visitors and counts from observational quadrats were insufficient (Table 1). Finally, we used Z-GGMs to test whether plant species, temperature, day of the year, and different combinations of these were significant predictors of visitation rate. Temperature and day of year were incorporated into models as fixed effects as a proxy for seasonality and because we expected that their relationship to our response variable, visits per flower per hour, was constant. Statistical frameworks like this have been employed in similar studies (Kehrberger & Holzschuh 2019). We used Akaike Information Criterion (AIC) to select models that best explained variation in visitation rates. All models described included study site as a random effect.

RESULTS

FLOWERING PHENOLOGY

Cardamine concatenata was prevalent at all study sites and flowered from day 85 (March 25 in 2024, a leap year) to day 110 (April 19, 2024), with a mean duration of 18.5 days across the four sites (*SD* = 7.3 days, *N* = 4 sites). *Alliaria petiolata* was present at three of the four study sites and flowered from day 109 (April 18, 2024) to day 128 (May 7, 2024), with a mean duration of 9.33 days across the three sites (*SD* = 7.2 days, *N* = 3 sites). *Cardamine diphylla* was also present at three of the four study sites and flowered from day 107 (April 16, 2024) to day 120 (April 29, 2024), with a mean duration of 3.66 days (*SD* = 2.1 days, *N* = 3 sites).

Table 1. Visitation by morphotype for each plant species. Percent of total visits is given and in parentheses the number of visits from that morphotype. For *C. concatenata* we observed 428 unique visits across 49 observational quadrats (980 minutes). For *C. diphylla* we observed 181 unique visits across 24 observational quadrats (480 minutes). For *A. petiolata* observed 256 unique visits across 18 observational quadrats (360 minutes). See Table 2 for taxonomic identifications.

	C. concatenata	*C. diphylla*	*A. petiolata*
Black bees	75.7% (324)	29.8% (54)	41% (105)
Black bees with red abdomen	0.9% (4)	25.4% (46)	12.9% (33)
Metallic bees	15.6% (67)	10.5% (19)	33.6% (86)
Bee flies	2.8% (12)	2.2% (4)	3.9% (10)
hoverflies	2.6% (11)	21% (38)	1.6% (4)
Houseflies	0.5% (2)	2.2% (4)	0.4% (1)
Beetles	1.9% (8)	3.9% (7)	0% (0)
Butterflies	0% (0)	5% (9)	6.2% (16)
Bumblebees	0% (0)	0% (0)	0.4% (1)

Table 2. Number of individual specimens collected visiting flowers of the study species, belonging to identifiable functional and taxonomic groups. In total, 48 specimens were collected. The following morphotypes were observed but not collected: bumblebees (*Bombus*), butterflies (Lepidoptera), and bee flies (Bombyliidae). Quantities in this table are useful as a means of tracking specimen identity but should not be used to compare floral visitation rates across plant species.

Morphotype	Taxonomic group	Number of specimens collected		
		Cardamine concatenata	*Cardamine diphylla*	*Alliaria petiolata*
Small black bees	*Andrena*	5	3	1
	Osmia	9	0	0
	Halictus	1	0	1
Black bees with red abdomen	*Nomada*	2	1	0
Small metallic bees	*Lasioglossum*	0	1	5
	Augochlora pura	1	1	4
	Ceratina	5	0	1
Beetles	Meloidae	0	2	0
	Eristalinae	1	0	0
Flies	Syrphinae	0	2	0
	Muscoidea	0	2	0

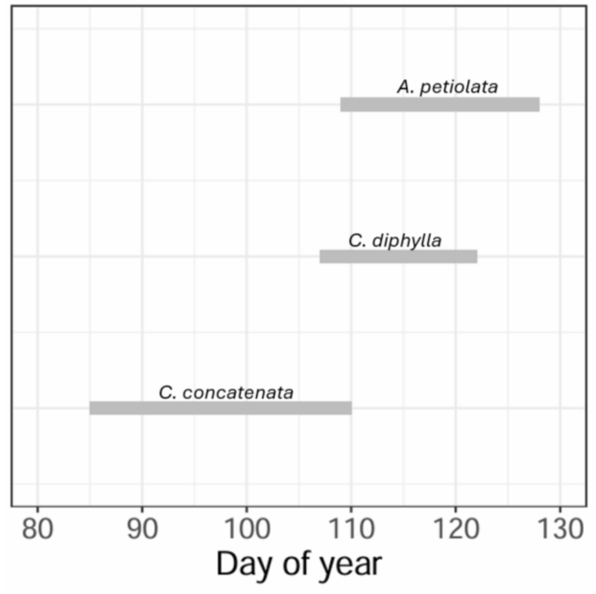

Figure 3. Duration of flowering for each plant species across study sites. See Supplemental Table 1 for dates of first and last recorded bloom at each site.

The flowering window of *C. concatenata* overlapped briefly with *C. diphylla,* from April 16 to April 19 at the Pierson Valley site (Fig. 3; Supplemental Table 1). No instances of flowering overlap were recorded between *C. concatenata* and *A. petiolata*; *A. petiolata* began flowering at least one day after the last *C. concatenata* blooms were observed at all sites (Fig. 3; Supplemental Table 1). *Cardamine diphylla* flowering overlapped partially with *A. petiolata* at the Pierson Valley site and overlapped entirely with *A. petiolata* at the Baldwin Woods and Stebbins Natural Area sites (Fig. 3; Supplemental Table 1).

POLLINATOR ASSEMBLAGES

In total, we observed 865 individual pollinator visits across 91 observation quadrats (a total of 1,820 minutes) across all study sites and all plants. For *C. concatenata,* we observed 428 floral visits; for *C. diphylla,* we observed 181 floral visits; and for *A. petiolata*, we observed 256 floral visits (Table 1).

In total, we conducted 26 pollinator sampling events (five at Stebbins Natural Area; eight at Baldwin Woods; eight at Pierson Valley, and five at Working Woods) across 26 quadrats and identified 48 specimens in the lab (24 for *C. concatenata*; 12 for *C. diphylla*; 12 for *A. petiolata*). Fourteen pollinator taxa were identified across all focal plant species through a combination of *in situ* observation and specimen collection. From both identified specimens and *in situ* observation, we identified seven taxa visiting all three species (*Osmia*, *Ceratina*, *Nomada*, Bombyliidae, Syrphinae, and Muscoidea; Table 1 & 2).

In all models in this study, between site variation was influential in explaining patterns of visitation rates. For total pollinator visitation rate (all taxa pooled), AIC indicated that variation was best explained by the Z-GGMs accounting for the interactive or individual effects of plant species and temperature (Supplemental Table 2). These models yielded a marginally significant effect on total pollinator visitation rate (Supplemental Table 3; Fig. 4). A post hoc test revealed that the visitation rate for *C. concatenata* was significantly higher than that of *A. petiolata*, while all other pairings, including between the co-flowering *A. petiolata* and *C. diphylla*, were not significantly different (Fig. 4). It should be noted that this dataset included influential outliers (identified using the IQR rule; Tukey 1970), but because of the small sample size (i.e., number of quadrats observed), we chose not to remove the outliers.

For visitation rates parsed by individual pollinator morphotypes, Z-GGMs found that visitation rate did not differ significantly between co-flowering *A. petiolata* and *C. diphylla* for four pollinator morphotypes (black bees [*Andrena, Osmia, Halictus*], metallic bees [*Augochlora pura, Lasioglossum*], bee flies [Bombyliidae], and hoverflies [Syrphidae]; Suppplemental Table 4). For the morphotypic grouping "black bees" (including *Andrena, Osmia, Halictus*), the interaction between temperature and plant species was a significant predictor of visitation rate (Fig. 5A), and post hoc tests revealed that the early-blooming *C. concatenata* had a significantly higher rate of visitation rate by black bees than the later-blooming *C. diphylla* and *A. petiolata*. For the "metallic bees" morphotype (including *Augochlora pura, Ceratina,* and *Lasioglossum),* the individual effects of plant species and temperature were significant predictors of visitation rate; post hoc tests revealed that *A. petiolata* had a significantly higher visitation rate than *C. concatenata*, while all other pairings were not significant (Fig. 5B). Visitation rates by bee flies (Bombyliidae) did not vary significantly by plant species, temperature, or their interaction (Fig. 5C). Hoverfly (Syrphidae) visitation rate was marginally significantly different for plant species, with *C. diphylla* experiencing a significantly higher visitation rate than *C. concatenata*, while all other pairings were not significant (Fig. 5D). Visitation rate by only one pollinator morphotype (genus *Nomada*) differed significantly between co-flowering *A. petiolata* and *C. diphylla*, with *C. diphylla* experiencing significantly higher visitation rate by *Nomada* than *A. petiolata* (Fig. 6). All other pairings were not significant (Supplemental Table 4).

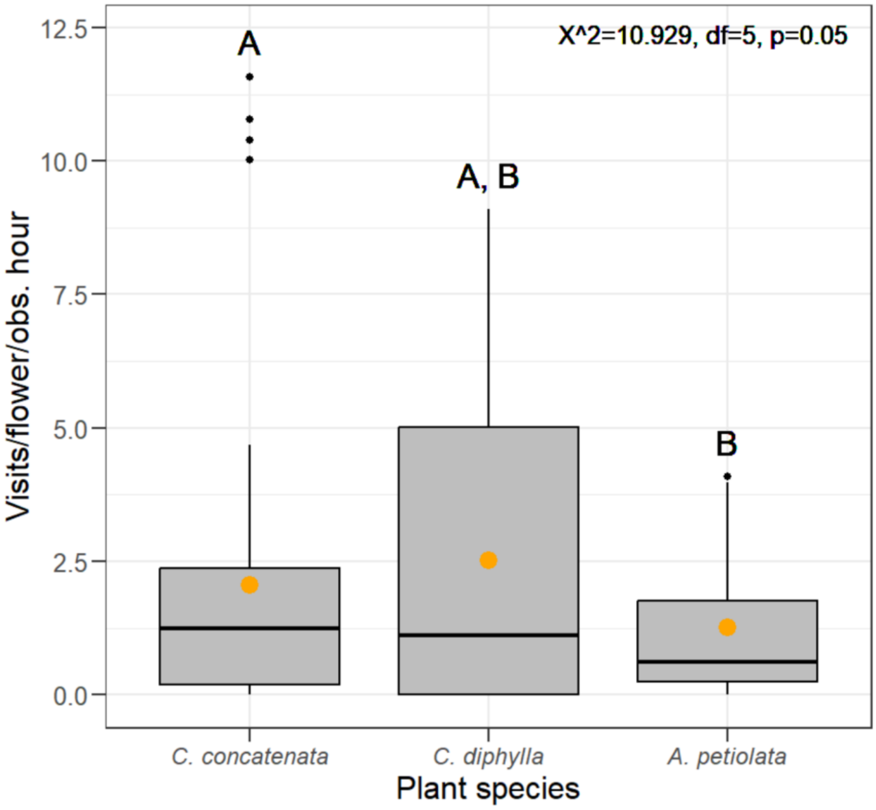

Figure 4. Total visits per flower per hour by plant species across study sites over the duration of the 2024 season. Bolded centre lines represent the median, orange points represent the mean, and lower and upper sides of the boxes represent the 1st and 3rd quartile respectively. Small black points represent outliers. Results of Z-GGM indicated in the top right. Letters indicate results of Tukey's post hoc comparisons with a significance of α = 0.05.

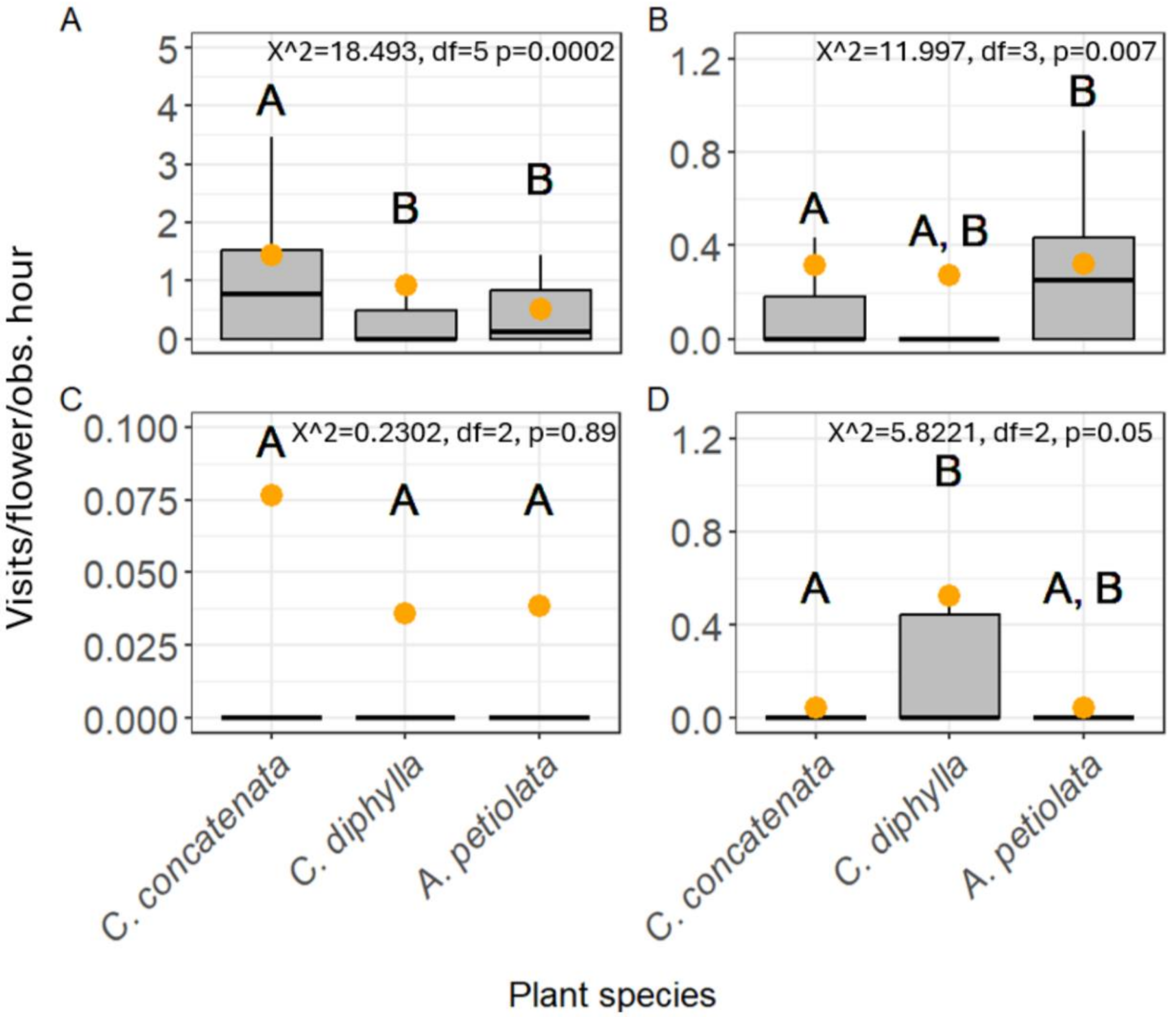

Figure 5. Visitation rate for each focal plant species by morphotype of indiscriminate foragers (i.e. morphotypes with no significant difference in visitation between co-blooming mustards) across all sites over the duration of the 2024 field season. A) Visitation by *Andrena*, *Osmia*, and *Halictus* (i.e. black bee morphotype). B) Visitation by *Augochlora pura*, *Ceratina*, and *Lasioglossum* (i.e. metallic bee morphotype). C) Visitation by Bombyliidae (i.e. bee flies). D) Visitation by Syrphidae (i.e. hoverflies). Outliers were included in all analyses but omitted from boxplots for the purposes of visualization. Results of morphotype Z-GGMs indicated in the top right of each plot. Bolded centre lines indicate the median, orange points represent the mean, and lower and upper edges of the boxes represent the 1st and 3rd quartile respectively. Letters indicate results of Tukey's post hoc comparisons with a significance of α = 0.05. See Table 2 for taxonomic identification of visitors discovered on each plant.

DISCUSSION

The objective of this study was to examine the possibility of pollination disruption for two native mustards, *Cardamine concatenata* and *C. diphylla,* by the noxious confamilial invasive herb, *Alliaria petiolata*. We hypothesized that flowering phenology of the native *Cardamine* species would overlap with that of *A. petiolata*, as all three species are spring-flowering, understory herbs with limited phenological windows for reproductive development. We also hypothesized that because of shared morphology, phylogeny, and phenology, the three focal plants would share at least some pollinator taxa, thus enabling the possibility for mutualism disruption by *A. petiolata*.

In contrast to our hypothesis, *C. concatenata* and *A. petiolata* did not have overlapping flowering phenology at our study sites in spring 2024. This suggests that, at least in northeast Ohio, the potential for *A. petiolata* to disrupt pollination for *C. concatenata* is very low, because the two plants do not flower at the same time of year. However, flowering phenology of *C. diphylla* did overlap

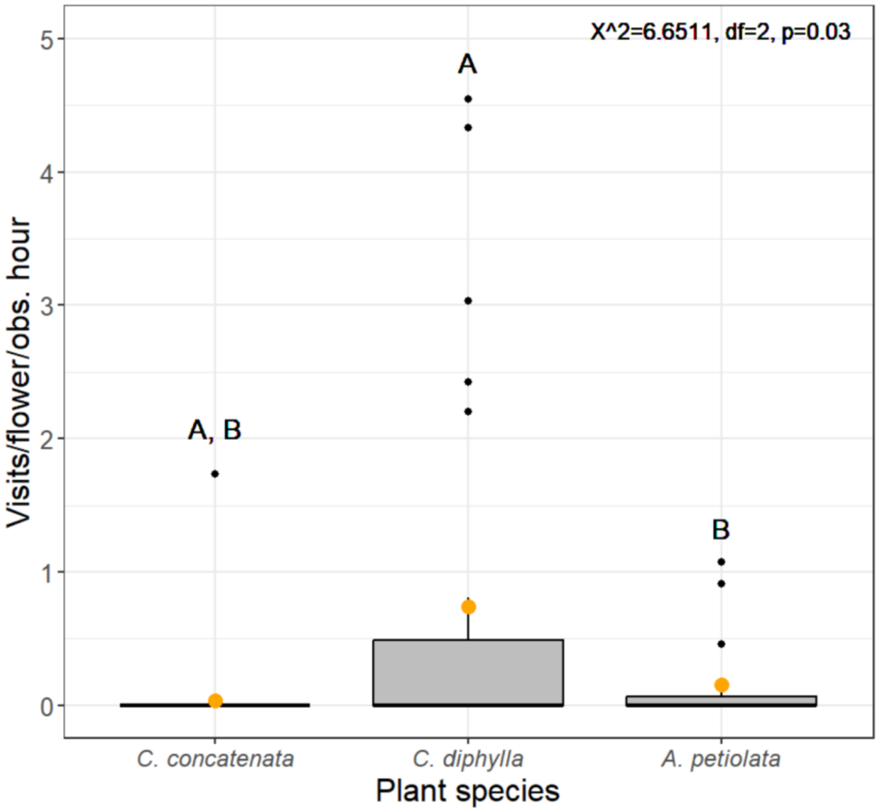

Figure 6. Visitation by *Nomada* (i.e. black bee with red abdomen morphotype) across all sites over the duration of the 2024 field season. Bolded centre lines on each of the three boxes indicate the median, orange points represent the mean, and lower and upper edges of the boxes represent the 1st and 3rd quartile respectively. Small black points represent outliers. Results of Z-GGM accounting for temperature and plant species indicated in the top right. Letters indicate results of Tukey's post hoc comparisons with a significance of α = 0.05. See Table 2 for taxonomic identification of members of this morphotype.

with that of *A. petiolata,* suggesting that *C. diphylla* may be at risk of pollination disruption stemming from invasive *A. petiolata*. To our knowledge, these results comprise the first official record of overlapping phenology between *C. diphylla* and *A. petiolata*, and thus constitute a starting point for future phenological studies, which should focus on establishing range-wide phenological windows.

We found evidence of both differential (i.e. significantly different) and non-differential (i.e. not significantly different) pollinator visitation rates among co-flowering *C. diphylla* and *A. petiolata*. The visitation rates of *Nomada* were particularly noteworthy, as they are the only recorded instances of differential visitation rates in this study; *Nomada* showed preference for *C. diphylla* over *A. petiolata*. However, it is unlikely that *Nomada* is contributing to pollination of the focal plant species because this taxon is comprised of solitary kleptoparasites that forage only on nectar. Of the taxa that foraged non-differentially, *Augochlora pura*, *Lasioglossum*, *Halictus*, *Ceratina*, *Andrena*, and *Osmia* forage on both nectar and pollen. Although both are largely foraging for nectar, Bombyliidae and Syrphidae have been known to act as pollinators as well (Campbell 1985; Doyle et al. 2020). These findings support our hypotheses that species composition of floral visitors is similar between the native and invasive mustards. For these reasons, we conclude that *A. petiolata* does not constitute an outlier in this group, and that pollination disruption could be occurring in the co-flowering *A. petiolata*-*C. diphylla* system. We acknowledge, however, that the shared taxa identified in our study likely contribute differentially to potential increases in heterospecific pollen transfer (HPT), as variation in pollination efficacy among and between pollinator taxa, as well as across plant taxa has been recorded (Zych et al. 2013; Gallagher & Campbell 2020). Regardless, these results allow us to infer that increases in HPT are likely occurring in this system and have the potential to negatively impact reproductive output in the native mustards, particularly *C. diphylla*. Such detrimental effects on reproductive output have been documented in other systems in which native cruciferous taxa are found in close proximity to invasive plants and experience the addition of novel heterospecific pollen loads (Masters & Emery 2015).

For example, Masters and Emery (2015) investigated the potential for HPT in a Louisville, Kentucky *C. concatenata* population exposed to the co-flowering, invasive *Ranunculus ficaria*. In invaded habitats, microscopic analysis of pollen grains on *C. concatenata* stigmas indicated a 280.3% increase in conspecific pollen deposition and the presence of only 6.8 *R. ficaria* pollen grains on average. Even though conspecific pollen deposition in invaded habitats is far greater than in uninvaded habitats and HPT occurs at seemingly small scales, *C. concatenata* in uninvaded habitats produced 104.6% more mature fruits when compared to *C. concatenata* in invaded habitats (Masters & Emery 2015). Reductions in fruit set exceeding those recorded by Masters and Emery (2015) could be expected in sympatric, co-blooming *Cardamine-A. petiolata* systems due both to their close phylogenetic relatedness, the invasive status of *A. petiolata* (Arceo-Gómez and Ashman 2016).

However, it is not entirely clear how to view potential HPT in species that also reproduce prolifically through clonal propagation, as do *Cardamine* (Montgomery 1955; Spooner 1984; Sweeney & Price 2000; Wilhelm pers. obs.). Rhizomes, parts of the root system that spread laterally from parent plants, can generate new stems above ground. For *C. diphylla* specifically, several sources suggest that clonal growth through rhizomes is the predominant mode of reproduction (Montgomery 1955; Spooner 1984; Sweeney & Price 2000), and some authors suggest that where seeds are produced, they rarely reach maturity (Montgomery 1955; Montaut & Bleeker 2013). Because asexual reproduction is not dependent on pollination, this feature of *C. diphylla* life history may mitigate the impact of HPT on population persistence. Although the predominant mode of reproduction appears to be asexual, Sweeny and Price (2001) note that *C. diphylla* is capable of infrequent sexual reproduction, as well as hybridization. Additionally, sporadic fruit production was observed in our study sites in 2025, leading us to believe that successful sexual reproduction is possible (Wilhelm personal obs.). If *C. diphylla* possesses the capacity for sexual reproduction, shared floral phenology with *A. petiolata* will likely be detrimental to fruit production, and thus the creation of novel genotypes that could safeguard against environmental stressors under global change. In contrast to *C. diphylla, C. concatenata* is thought to be entomophilous, reproducing primarily or entirely by outcrossing (Spooner 1984; Masters & Emery 2015). Currently, floral phenologies of *C. concatenata* and *A. petiolata* do not overlap within our study sites, buffering *C. concatentata* from potential negative effects from HPT with *A. petiolata*. Although outcrossing events may be an important feature of *Cardamine* reproductive biology in general, we believe that research concerning the frequency of sexual reproduction in both *C. concatenata* and *C. diphylla* is needed to meaningfully evaluate the effects of pollination disruption and the potential for HPT from *A. petiolata* on *Cardamine* reproductive success.

Global change drivers such as climate change are projected to have differential effects on different taxa (Renner & Zohner 2018), and perhaps even differential effects on geographically distinct populations of the same taxa (Rodgers et al. 2022). For example, climate change is expected to have differing effects on *A. petiolata* invasion success depending upon location: in eastern portions of its invaded range, *A. petiolata* is expected to experience range contraction, whereas range expansion is predicted for western edges of the invaded range (Rodgers et al. 2022). However, knowledge gaps hinder the evaluation of possible range expansions and/or contractions for native species like those in the genus *Cardamine*. Research by Petrauski et al. (2019) has suggested that *Cardamine* phenology is responsive to climate change, so it is possible that ranges will be altered in addition to, or even in conjunction with, shifts in phenology. Thus, future research should identify and characterize global change drivers relevant to possible phenological and range shifts for native mustards, with the goal of identifying possible areas increased overlap with *A. petiolata* to ensure that proper mitigation measures may be taken for the native mustards and the specialist insects that rely on them.

Future research should consider the likelihood that any or all of these species may shift their phenology, as well as the magnitude and direction of such shifts, in response to ongoing climate change. If *A. petiolata* is poised become more common across woodlands of eastern North

America and is able to shift flowering phenology to take advantage of early spring light exposure, it may constitute a phenological "winner". At the same time, natives such as plants in the genus *Cardamine* may be poised to decline in abundance and face intense pressure from pollination disruption engendered by the invasive confamilial due to a spatial and/or temporal increase in co-flowering, constituting phenological "losers" (see Dawson-Glass et al. 2025). Future research should also focus on establishing the occurrence and quantifying the magnitude of HPT and its effects on *Cardamine* reproduction where it coexists and phenology overlaps with *A. petiolata*. Finally, more work is needed to determine the relative contribution of asexual versus sexual reproduction in these species, as even infrequent seed production may introduce novel genotypes that enhance population resilience to environmental stressors. Investigations into the frequency and viability of seed production in North American *Cardamine* as well as implementation of repeated demographic surveys of established populations may yield information on this front. These explorations would contribute greatly to our ability to characterize *Cardamine* reproductive strategies and to assess the risk posed to these native ephemerals by *A. petiolata*.

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AUTHOR CONTRIBUTION

Concept and design SMW & CNM, data collection SMW, data analysis SMW & CNM, edits and approval for publication SMW & CNM.

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The authors have declared no conflict of interest.

GENERATIVE AI DISCLOSURE STATEMENT

No generative AI in any way was used to produce this article.

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The data used to write this article are available at https://doi.org/10.5061/dryad.66t1g1kfr

APPENDICES

Additional supporting information may be found in the online version of this article:

Supplemental Table 1. Dates of first and last recorded bloom for each plant species at each study site.

Supplemental Table 2. The models including either the interactive effects or individual effects of plant and temperature.

Supplemental Table 3. Results of models used for AIC.

Supplemental Table 4. Zero-inflated gamma model outputs & Tukey's post hoc tests

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