




## RESEARCH ARTICLE

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# Induced phenological avoidance: A neglected defense mechanism against seed predation in plants

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## Abstract

1. Flowering phenology is an important life-history trait affecting plant reproductive performance and is influenced by various abiotic and biotic factors. Pre-dispersal seed predation and pollination are expected to impose counteracting selection pressure on flowering phenology, with pre-dispersal seed predation expected to favour off-peak flowering and pollination to favour synchronous flowering.
2. Here we studied the effect of pre-dispersal seed predation by the beetle *Byturus ochraceus*, a specialist seed herbivore, on the flowering phenology of *Geum urbanum*. This forest understorey plant species is self-pollinating, so that the influence of seed predation can be studied independent from pollination. We measured in detail the timing and predation rate of individual flowers during two consecutive years in more than 60 individuals. We tested the hypotheses that pre-dispersal seed predation exerts selection for within-season compensatory flowering as well as for induced phenological avoidance in the following season.
3. We found no indication for compensatory flowering within a growing season, but plants that experienced predation shifted their flowers to the end of the flowering season the subsequent year. This induced phenological avoidance points to a plastic response to pre-dispersal seed predation that may be adaptive. Importantly, the delay in flower production came at a cost, since flowers later in the season had a reduced seed output, presumably because of increasing light limitation following forest canopy closure.
4. *Synthesis.* Herbivory by specialist enemies can cause serious fitness decline in hosts. We here show that induced shifts in phenology can form an important defense strategy against pre-dispersal seed predation. The induced mismatches between herbivore and host phenology are anticipated to be adaptive when herbivory is predictable across successive flowering periods.

## KEYWORDS

flowering phenology, phenotypic plasticity, plant defense, plant development and life-history traits, plant–herbivore interactions, selection, timing

## 1 | INTRODUCTION

Once established, individual plants cannot move in space to find optimal conditions for growth and reproduction, but they can move in time by adapting the phenology of their life-history. The timing of flowering is important in this context as it directly affects reproductive success. Plant flowering phenology is determined by a multitude of biotic and abiotic factors. The impact of abiotic factors is relatively well studied: temperature, precipitation and photoperiod are constraints as well as cues for the phenology of plants (Forrest & Miller-Rushing, 2010). However, not only abiotic forces play a role in shaping a plant's flowering phenology. Selection on flowering phenology can also be modulated by biotic interactions (Elzinga et al., 2007). In general, selection for early flowering is found to be consistent and strong across different plant species (Geber & Griffen, 2003; Munguia-Rosas, Ollerton, Parra-Tabla, & De-Nova, 2011).

Pre-dispersal seed predators and pollinators are the main biotic selective agents acting on flowering phenology (Elzinga et al., 2007; Munguia-Rosas et al., 2011). In a review study, Kolb, Ehrlén, and Eriksson (2007) showed that pre-dispersal seed predators act as selective agents on flowering phenology in more than 80% of all tested species. Hence, plants that shift their timing of reproduction and flower off-peak in order to avoid the moment of predation could experience a fitness advantage (Elzinga et al., 2007). On the other hand, plants that depend on pollinators often maximize successful fertilization when they flower during the peak of flower availability in the population. Hence, pollination and pre-dispersal seed predators are often two counteracting selective agents (Atlan, Barat, Legionnet, Parize, & Tarayre, 2010).

Off-peak flowering is a commonly observed response to pre-dispersal seed predation (Elzinga et al., 2007; Kolb et al., 2007), in addition to other possible responses such as mast seeding (Kelly & Sork, 2002; Kolb et al., 2007). However, different defense mechanisms and adaptive responses could lead to off-peak flowering. First, pre-dispersal seed predation could impose disruptive selection on the date of first flowering, and/or directional selection for longer flowering periods (Elzinga et al., 2007), resulting in changes in the mean and variance of flower phenology at the population-level. The defense mechanism deployed in this scenario can be classified as phenological avoidance. Secondly, pre-dispersal seed predation might also select for adaptive phenotypic plasticity (Grenier, Barre, & Litrico, 2016), which is classified as an induced defense in the plant defense literature.

On the one hand, seed predation early in the season can induce the growth of extra flowers within the same growing season, i.e. compensatory flowering, a tolerance trait. Compensatory flowering has been observed as a response to herbivory of inflorescences early in the season, before flowering, but not yet as a response to seed-predation (Eriksson, 1995; Pettersson, 1994). For instance, *Ipomopsis aggregata* plants that experienced damage to the apical meristem early in the season produced significantly more inflorescences, but there was a delay in flowering time and fewer flowers were produced in total (Brody & Irwin, 2012; Freeman, Brody, & Neefus, 2003). Yet the

delay in flowering still reduced the rate of seed predation because the overlap in time between the flowers and seed predators was smaller. Similarly, *Lythrum salicaria* plants produced more inflorescences and more flowers after clipping and this had no effect on the rate of pollen limitation (Thomsen & Sargent, 2017).

On the other hand, pre-dispersal seed predation might lead to phenotypic plasticity in the form of an induced response in the growing season following the predation event. It has been shown that perennial plants can plastically adapt in response to environmental pressures they experienced the year before (Karban, 2008; Stam et al., 2014). The timing of growth and flower development are traits expressed over an entire growing season and the onset of flowering cannot be adjusted within one season. But if the risk of seed predation is predictable based on the predation experienced the previous year, we can expect that plants respond to seed predation by delaying flowering phenology in the next growing season. We refer to this defense mechanism as an induced phenological avoidance. The two defense strategies 'compensatory flowering' and 'induced phenological avoidance' are not necessarily mutually exclusive.

Here we studied the effect of pre-dispersal seed predation on flowering phenology during three consecutive years using *Geum urbanum* L. as a study system. As this forest understorey plant species is mainly self-pollinating and reproduction is equally successful in self-pollinated as in cross-pollinated flowers (Ruhsam, Hollingsworth, Squirrell, & Ennos, 2010; Vandepitte, Honnay, Jacquemyn, & Roldan-Ruiz, 2010), the selective pressure of pre-dispersal seed predation is not counteracted by pollinators. Furthermore, *G. urbanum* has a long flowering period from the end of May until October and seed predation only occurs on the seeds of the early flowers, because adult predators are only active from the end of June until the end of July. The pre-dispersal seed predator is the larvae of the widespread and specialized beetle *Byturus ochraceus*, which exclusively feed on *G. urbanum* (Taylor, 1997). We collected detailed data on the flowering phenology of individual flowers to test the following hypotheses related to the consequences of predation for timing of flowering and plant fitness (here reproductive output):

1. In the absence of seed predation, the reproductive output of individual flowers within a plant is higher in early flowers than in later flowers, which is likely due to abiotic restrictions later in the growing season.
2. In plants that experience pre-dispersal seed predation, there is a substantial reduction of reproductive output in the early flowers, so that, on average, late flowers have the highest seed mass.
3. Pre-dispersal seed predation changes the selection gradient between plant-level flowering phenology and total seed mass from directional selection for early flowering in unpredated plants to directional selection for later flowering in predated plants.
4. Pre-dispersal seed predation induces compensatory flowering within a growing season and/or induced phenological avoidance, with the phenological response in the year after the predation event.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and species

The research was conducted in the Aelmoeseneie forest, located south of Ghent (Gontrode, Belgium). This c. 30 ha forested area entails a mix of ancient and post-agricultural forest and has been managed as high forest since 1950 (Vanhellemont & Verheyen, 2011). The soil consists of sand and sandy loam with alluvial ash forest on the more humid parts and acidophilous beech forest on the drier sandy parts. Within the forest, we selected ten groups of *Geum urbanum* plants in different locations spread over the forest. A group of individuals located close to each other, within a radius of 15 m, was considered a 'plot'. In 2015, 59 plants were marked for detailed monitoring ( $N = 10$  plots, 4–6 plants per plot). In 2016, the set of monitored plants was expanded with another 50 plants to obtain a larger sample size ( $N = 12$  plots, 4–15 plants per plot). In 2017 only the plants from 2016 were monitored. Differences in abiotic conditions, i.e. canopy openness, canopy phenology and chemical soil conditions were measured and controlled for but none of these variables were correlated with flowering phenology, reproductive output or presence of seed predators and could therefore be ignored in this study (unpubl. data).

*Geum urbanum* is a perennial semi-rosette hemicryptophyte; basal rosettes overwinter in the vegetative state. Photosynthetic capacity of leaves is the highest in winter and early spring, when the overwintering rosette leaves make up the bulk of the leaf area. The species is an early summer bloomer (Kudo, Ida, & Tani, 2008) that can take advantage of high levels of irradiance just prior to tree canopy closure through a combination of high photosynthetic capacity, the ability to maintain photosynthesis at relatively low temperatures and the presence of overwintering leaves. Rhizomes, rosette leaves and leaves from seedlings are moderately frost resistant (Graves, 1990; Taylor, 1997). *Geum urbanum* has a short rhizome which is little developed but supports production of flowering stems in March–April and new rosette leaves in spring and autumn. Plants flower every year and flowering takes place from the end of May to October. Inflorescences consist of 2–5 flowers on long stalks in an open cyme (Taylor, 1997). Flowers are mainly self-pollinated and populations show no inbreeding depression (Ruhsam et al., 2010; Vandepitte et al., 2010). In his description of the biology and ecology of *G. urbanum*, Taylor (1997) points to the following characteristics of the species relevant for the present study: a single flower produces ca 100 achenes; seeds are hairy and have a 5–7 mm long hook, facilitating exozoochorous seed dispersal; seed is set from July to September, germinates in spring; the species does not form a persistent seed bank; plants possess little capacity for vegetative spread so that reproduction by means of seeds germinating is far more important for regeneration.

*Byturus ochraceus* (Byturidae, Scriba 1790) is a small beetle which emerges about three weeks before the flowering of *Geum urbanum* (Pellmyr, 1985). During this interim period, the beetles are found feeding on pollen and nectar in the flowers of at least 30 different

plant species such as *Taraxacum officinale* and *Ranunculus acris*, and the forest understorey species *Anemona nemerosa*, *Geranium sylvaticum* and *Fragaria vesca* (Bohem, 2015; Pellmyr, 1985). The beetles will start to feed and mate exclusively on flowers of their host plant, *Geum urbanum*, as soon as it starts flowering. Oviposition takes place from mid-June to mid-July. After 14 days, the first larvae emerge and begin feeding on the fruit, leaves and seeds according to Springer and Goodrich (1986). In our study, leaf and fruit herbivory was negligible, and *Byturus ochraceus* predated almost exclusively on seeds. The larvae are present from the end of June until the end of August (Springer & Goodrich, 1986).

### 2.2 | Plant phenology and predation metrics

Plants were monitored once a week in 2015 from May until October. Timing of flower emergence was recorded as the day of the year and all open unmarked flowers were marked with a unique tag to enable identification of individual flowers. Total number of flowers per plant could be derived from these data at the end of the season. Based on the data of 2015, flowers were monitored on fewer occasions in 2016 (July 4, 12, 20, 27, August 12 and September 7; day of year 186, 194, 202, 209, 225, 251) and 2017 (July 13, September 19, October 18; day of year 194, 262, 291). We observed two flowering peaks per season during the three consecutive years of observations and during a preliminary study in 2014: high number of flowers in June (around day of year 150) and in August (around day of year 225) and a period with fewer flowers in between (around day of year 194) (Supporting Information S1). This period of fewer flowers coincides with the end of predator infection. Because of the bimodality of the flowering phenology of *G. urbanum*, off-peak flowering cannot be straightforwardly quantified. We therefore defined off-peak flowering relative to the activity period of the seed predators, that is, flowering away from the peak in seed predator activity. During the first flowering peak, most flowers emerged, and seed predation took place. We hence calculated, for each plant, the proportion of flowers that appeared in the second flowering peak relative to the total number of flowers produced on each plant as a measure of 'off-peak' flowering.

In 2015 and 2016, flowers were collected throughout the season when seeds were ripe, that is, for each plant, we collected flowers on different occasions. In 2015, few flowers contained beetle larvae, which means that seed herbivory was still ongoing. In 2016, flowers were collected slightly later in order to ensure that seed herbivory was completed and no larvae were found in the flowers anymore. No seeds were collected in 2017. Only flowering phenology data were collected to test for lagged effects of seed predation on flowering phenology. The seeds from all flowers were checked for herbivory by *Byturus ochraceus*. The number of seeds and seed mass was determined separately for predated and unpredated seeds for each flower. From these data, we calculated the mean mass per seed for unpredated seeds. We used seed mass as a proxy for reproductive output instead of the number of seeds,

since many flowers contained small seeds that were weakly developed. Moreover, we found a strong correlation between seed mass and germination probability (unpublished results). As a measure of reproductive output per flower, total seed mass per flower was calculated as the sum of the mass of predated and unpredated seeds. We included the weight of the predated seeds since predation rate could vary among seeds from completely eaten (with negligible weight) to minor damage, leaving most of the seed intact. Because we have no information on the germination probability of damaged seeds, the most conservative approach, that does not exaggerate the effect of predation on reproductive output, is to add the weight of predated seeds. As a measure of reproductive output per plant, total seed mass per plant was calculated as the sum of the seed mass of all flowers on that plant. For the analysis, we used a categorical variable for predation occurrence at flower and plant level with two categories: predated or unpredated ('predation' hereafter). A flower was considered predated if at least one seed showed signs of predation and a plant was considered predated if at least one flower was predated.

## 2.3 | Analysis

### 2.3.1 | Reproductive output per flower over time in predated and unpredated plants

To test the first two hypotheses, we quantified the change in reproductive output per flower with the timing of flowering in predated and unpredated plants. More specifically, we analysed whether, within a plant, seed mass per flower decreased linearly over time or was best predicted by a quadratic function and whether this pattern was different in plants that experienced predation compared with unpredated plants. This analysis was limited to the most detailed dataset in terms of sampling occasions that was collected in 2015. The first model regressed seed mass per flower as response variable against flowering date, plant-level predation (Y/N) and the interaction between both as explanatory variables. Plot identity and plant identity nested within plot were added as random effects. The second model was an expansion of the first model with the second-order polynomial of flowering date and the interaction with the plant-level predation as an extra explanatory variable. To ensure that the estimate of the intercept from the model is an estimation of the seed mass for the earliest possible flower we rescaled the flowering date. Instead of using the day of the year, which would estimate an intercept for the 1st of January, we set the date of the earliest flower in the population to zero. We compared the first and the second model using their AIC values and considered the model with the lowest AIC to be most consistent with the data and represented these in the result section. If the difference between AIC values was lower than two, the simplest model was preferred (Burnham & Anderson, 2004). We fitted the models with the 'lmer' function from the LME4 package in R.

### 2.3.2 | Selection gradients of flowering phenology in predated and unpredated plants

To quantify the effect of predation on the selection gradient of plant flowering phenology on plant-level fitness, that is, testing the third hypothesis, we fitted linear and quadratic selection gradients, (Lande & Arnold, 1983). Since predation was only scored in 2015 and 2016 (not 2017), this analysis is based on those two years of data. In the first model, the linear selection gradient, the logarithm of total seed mass per plant was used as response variable and the total number of flowers and the first polynomial of the proportion of flowers in the second peak were used as explanatory variables. The model included a random intercept for year and for plot identity. The second model, the quadratic selection gradient, was an expansion of the first model including the second order polynomial of the proportion of flowers in the second peak as an extra explanatory variable. To more easily infer how predation influences the fitness gradients, we created a model based on data from the plants that experienced no predation and a model based on data from the plants where at least one flower was predated. We used the logarithm of total seed mass per plant as response variable since the seed mass per plant showed a non-normal distribution without transformation. We selected the model that was most consistent with the data via the AIC values as described above. To support the split in two separate models, analysis of predated and unpredated plants separately, we made a model including 'predation occurrence' as a categorical fixed effect and the interaction with the first (and second in the quadratic selection gradient) polynomials of the proportion of flowers in the second peak. Both interaction effects were significant (Table S2; Figure S2), supporting the analysis of predated and unpredated plants separately.

### 2.3.3 | Compensatory flowering and induced phenological avoidance

According to the fourth hypothesis, we expected plants to use tolerance as a defense mechanism and show compensatory flowering, that is, plants that experienced predation early in the growing season compensated the loss in reproductive output by increasing the number of flowers later in the growing season in the same year. To test this expectation, we regressed the proportion of flowers per plant in the second peak against the percentage of flowers per plant in the first peak that experienced predation. Year and plot identity were included as a random effects. This analysis was done for the data of 2015 and 2016; predation was not recorded in 2017. We used a zero inflated binomial distribution to account for the high number of zero's for plants that did not have flowers in the second peak. We therefore used a Bayesian approach and fitted the models with the package 'brms' (Bürkner, 2016). In Supporting Information S4 we present an additional analysis to test for compensatory flowering with total number of flowers per plant as a response variable.

The fourth hypothesis also led to the expectation that plants use induced phenological avoidance as a defense mechanism. We analysed the effect of predation events on the flowering phenology the year after the predation event. As a measure of off-peak flowering, the proportion of flowers per plant in the second peak compared to the total number of flowers per plant was used as response variable. The explanatory variable was the proportion of flowers from the first peak that were predated the previous year. Year and plot identity were included as random effects. The model was fitted for a dataset including two subsequent time periods: the effect of predation in 2015 on the temporal distribution of flowers in 2016 and the effect of predation in 2016 on the temporal distribution of flowers in 2017. Models were fitted again with *BRMS* using a zero inflated binomial distribution after checking the posterior predictions. All analysis were performed in *R* (R Core Team, 2016).

### 3 | RESULTS

#### 3.1 | Introductory results

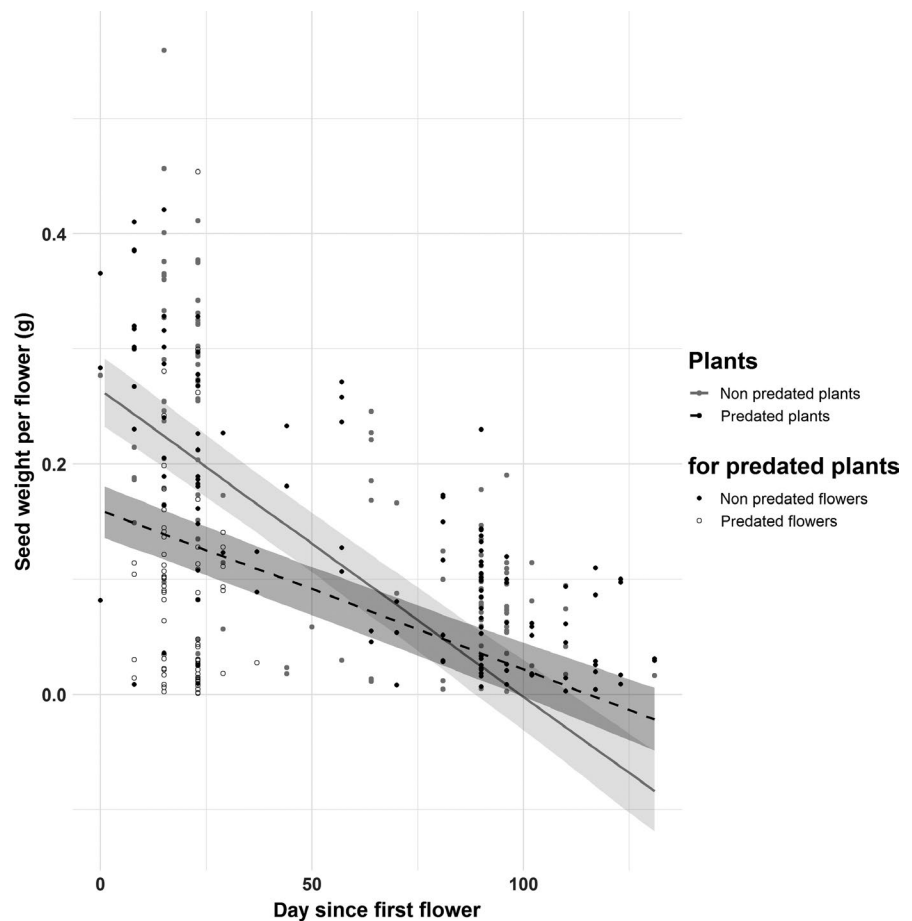
*Geum urbanum* has an elongated flowering period that stretches from June to October with two distinct peaks during the end of June and mid-August. Predation took place almost exclusively

during the first flowering peak in June and the beginning of July (Figure S1a–c). More than 50% of the plants experienced predation (2015: 65%, 2016: 56%, 2017: 56%). Both large and small plants were predated (Table S1) and the severity of predation differed from plant to plant, between 2% and 100% of all flowers of a plant were predated (Figure S1d–f; Table S1). From the flowers that were predated, on average about half of the seeds were eaten (2015: 60%, 2016: 65%).

#### 3.2 | Reproductive output per flower over time in predated and unpredated plants

The reproductive output of flowers, measured as total seed mass per flower, decreased throughout the growing season of 2015 within a plant (first hypothesis). The first model with only the first order polynomial was most consistent with the data (AIC of the linear model = –581; AIC of the quadratic model = –541) and had an intercept of 0.27 g ( $SE = 0.023$ ,  $p < .001$ ), i.e. seed mass of earliest flowers for an average-sized unpredated plant, and a significant slope of –0.0027 ( $SE = 0.0002$ ,  $p < .001$ ), i.e. the decrease in seed mass per flower per day for an average unpredated plant. The estimate for the categorical plant-level predation variable was –0.11 ( $SE = 0.025$ ,  $p < .001$ ), which means that the seed mass of the earliest flowers for an average-sized predated plant was 0.11 g lower than in an unpredated plant (second

**FIGURE 1** Change in seed mass per flower within *Geum urbanum* plants over time for the flowering season of 2015. The seed mass per flower of predated and unpredated plants was regressed against the flowering date (day of the year) and predation occurrence at plant level. The lines show the predicted values for the linear model  $\pm 95\%$  confidence intervals accounting for the fixed effects. For both predated (dashed, black) and unpredated plants (solid, grey), the mean seed mass per flower decreases linearly over time. For predated plants, the mean seed mass of early flowers is lower than in unpredated plants and, therefore, late flowers are relatively more important for reproductive output in predated plants than in unpredated plants



hypothesis). Moreover, the interaction between predation occurrence and the date of flowering was positive and significant (0.0013,  $SE = 0.00027$ ,  $p < .001$ ): the decrease in seed weight per flower in an average-sized predated plant over time was lower compared to unpredated plants. Predation was not so severe that the sign of the slope changed, that is, the average seed mass of early flowers was still higher than that of late flowers in predated plants. (Figure 1).

### 3.3 | Selection gradients of flowering phenology in predated and unpredated plants

The total plant fitness, measured as total seed mass per plant, was mostly determined by the total number of flowers of plants, since it was significant both for predated and unpredated plants (see Table S3 for model parameters). The more flowers, the higher the total plant-level seed mass. The effect of flower phenology on total seed mass per plant varied according to whether plants experienced seed predation or not. For unpredated plants, the difference in AIC values between the linear and quadratic model were small, therefore we considered the linear model to be most parsimonious (AIC of the linear model = 156.1; AIC of the quadratic model = 154.7), but no significant relationship between proportion of flowers in the second peak and total seed mass per plant was found. For predated plants, the quadratic model was most parsimonious (AIC of the linear model = 201.0; AIC of the quadratic model = 194.9). The total seed mass of predated plants showed a quadratic relationship, and estimates for all

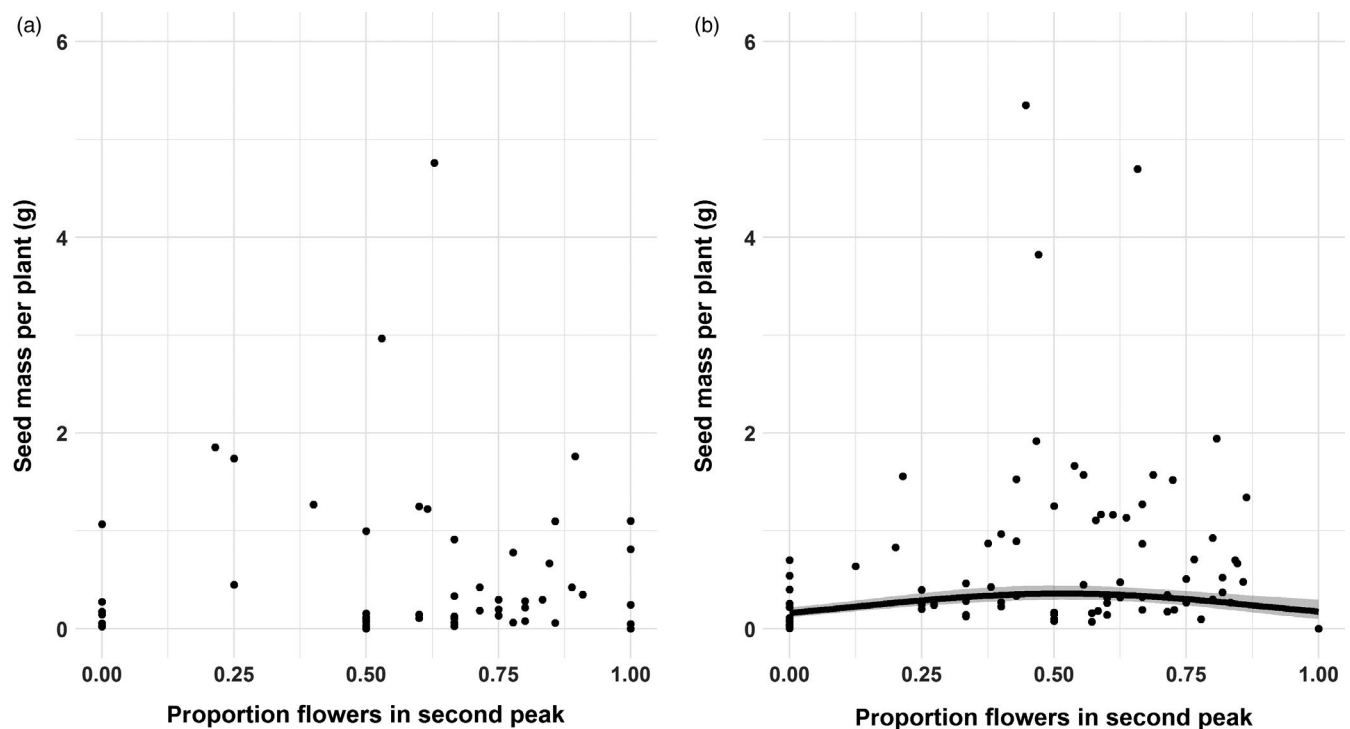
parameters were significant, so that plants with an intermediate degree of off-peak flowering (36% of flowers in the second peak) had the highest seed mass per plant (Figure 2) (third hypothesis).

### 3.4 | Compensatory flowering and induced phenological avoidance

We found no indication for compensatory flowering within a single year. The slope of the relationship between plant-level predation and the proportion of flowers per plant that flowered in the second peak in the same year did not differ significantly from zero (95% credible interval (CI) of slope:  $-0.0045$ – $0.0046$ ; Figure 3a). In an additional analysis (Supporting Information S4), we found that the total number of flowers per plant tended to be lower with higher predation rate, which is the opposite of what we expect in the case of compensatory flowering. Plants did show induced phenological avoidance, that is, plants that experienced high predation rates in year  $t$  had a higher proportion of their flowers in the second peak in year  $t + 1$  (95% CI:  $0.00043$ – $0.01526$ ; Figure 3b).

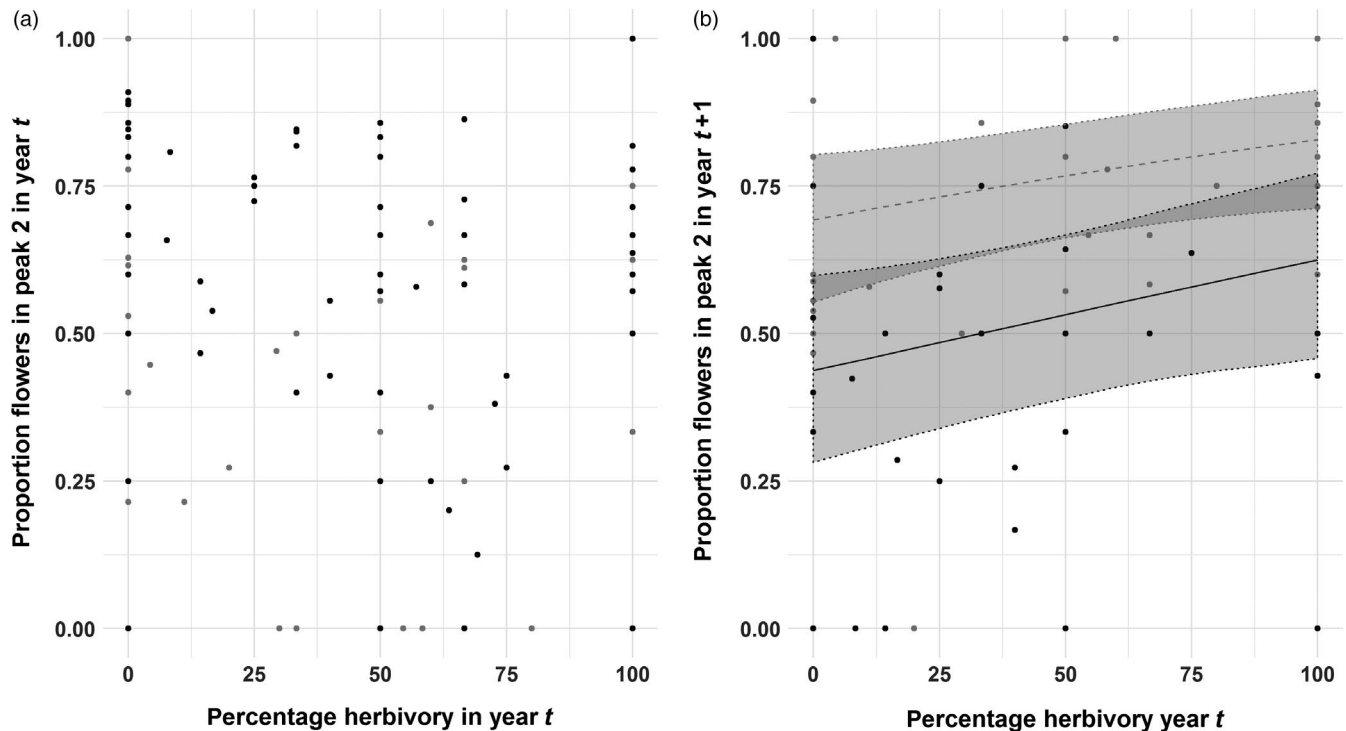
## 4 | DISCUSSION

Pre-dispersal seed predation can exert a selection pressure on the flowering phenology of plants, favouring off-peak flowering. However, in many plants, this selection pressure is counteracted by



**FIGURE 2** Selection gradients for total seed mass per plant (reproductive output) against the proportion of flowers in the second peak. Points represent data of (a) unpredated and (b) predated plants. The line ( $\pm 95\%$  credible interval) shows the fitted relationship for predated plants, which was derived for plants with an average number of flowers. No significant relationship was found for unpredated plants





**FIGURE 3** Tests for evidence of (a) compensatory flowering and (b) induced phenological avoidance. (a) The relationship between the plant-level proportion of flowers from the first peak that were predated (x-axis) versus the plant-level proportion of flowers flowering in the second peak within the same year (y-axis), that is, compensatory flowering. (b) The relationship between the plant-level proportion of flowers from the first peak that were predated (x-axis) versus the proportion of flowers flowering in the second peak the year after the predation event (y-axis), that is, induced phenological avoidance. Points represent plant-level observations of 2015 (grey) and 2016 (black). The lines ( $\pm$  95% credible interval) show the fitted relationships for the two years separately (dashed grey: year  $t$  = 2015; black solid: year  $t$  = 2016). No significant relationship was found for compensatory flowering in (a)

the selection pressure for flowering in synchrony with the population to attract pollinators (Atlan et al., 2010). The choice of *G. urbanum*, a selfing understory forest plant, as study species enabled us to study the effect of pre-dispersal seed predation without the counteracting effect of pollinators. We found that the species shows a long flowering duration and two distinct flowering peaks. In the absence of predation, most of the reproductive output of the species is realized in the first flowering peak and seed mass per flower declines in later flowers. Seed predation by the specialized seed herbivore *Byturus ochraceus* is also restricted to this first flowering peak and seed predation varied from 0 up to 100% of all flowers from the first flowering peak per plant and significantly reduced the average reproductive output of early flowers. Looking at plant fitness measured as total seed mass per plant, there was no indication for selection for early flowering in unpredated plants, but there was evidence that the pre-dispersal seed predation imposes selection for moderate off-peak flowering. This indicates that predation might exert a selective pressure on flowering phenology. We found no indication for compensatory flowering within the same year, but pre-dispersal seed predation induced phenological avoidance in the following year: a high predation rate led to a shift in flowering phenology to the second flowering peak the year after the predation event.

Selective pressures can only impose evolutionary changes in trait means and variances when they have a high heritability,

i.e. a high amount of additive genetic variation. Organisms can, however, also adapt to environmental changes by plastic responses, i.e. by adjusting trait means and variances during development, or across generations through induced maternal effects (Donohue, 2005). While genetic adaptation works over several generations, phenotypic plasticity can take place within one or two generations and promotes rapid adjustment to the environment (Grenier et al., 2016). We found pre-dispersal seed predation in *G. urbanum* to be unrelated to spatial variation in a set of environmental variables (soil pH, soil nitrogen, carbon and phosphorus content, light availability) and to plant characteristics (plant size, number of flowers) (Moenclaey et al. unpubl. data). In the context of our study system, where pre-dispersal seed predation is variable in space and likely equally subjected to variation on longer time frames, phenotypic plasticity is anticipated to be more adaptive than a genetically fixed phenology (Grenier et al., 2016; Karban, 2011). On a short timescale, the level of predation in one year is a good predictor for the level of predation the year after, as larvae hibernate and pupate in the forest floor litter close to the locations of the infected plants in which they developed (Burgess & Marshall, 2014). Furthermore, a prerequisite for the expression of a plastic response to predation is that plants can detect one way or another that they suffer predation. Plants are known to possess sophisticated perceptual

abilities that allow them to monitor and respond to a wide range of changing biotic and abiotic conditions. This sensory perception system can play a role in plant–animal interactions including the perception of herbivory and even oviposition (Mescher & De Moraes, 2014).

Plants that experienced pre-dispersal seed predation did not compensate the fitness loss via the production of a higher proportion of flowers in the second flowering peak within the same growing season. The lack of compensatory flowering might be the consequence of resource limitation (B. Sercu et al. unpubl. data), restrictive architectural and developmental pathways of the plant (Diggle, 1999; Prusinkiewicz, Erasmus, Lane, Harder, & Coen, 2007) or a combination of both. In the few studies that found compensatory flowering after predation, predation or clipping happened before bud formation or flowering. All three studies found a higher number of inflorescences after the apical meristem had been damaged, but also a delayed flowering phenology (Brody & Irwin, 2012; Freeman et al., 2003; Thomsen & Sargent, 2017). However, the time frame to plastically respond to herbivory is much smaller in the case of pre-dispersal seed predation compared with vegetative damage to the inflorescences before bud formation. From an architectural or developmental point of view, *G. urbanum* plants could compensate for damage in the first peak since the flowers from the second peak mostly originate from branches that grow on the main inflorescence branch and form extra flowers (pers. observ.). However, the elongation of lateral branches and the formation of new buds consumes time and resources leaving little opportunity for compensatory flowering later in the season. We can conclude that *G. urbanum* does not use tolerance traits as a defense strategy against pre-dispersal seed predation. Such tolerance strategies were also not found in other species with a similar bimodal flowering pattern that suffered predation in the first peak (Eriksson, 1995; Pettersson, 1994).

We found evidence that enemy attack is inducing phenological changes in *G. urbanum* to escape anticipated pre-dispersal seed predation. Plants that experienced seed predation partly shifted their flowering to the second flowering peak the year after the predation event. While phenological avoidance of pre-dispersal seed predation might lead to lower herbivory damage, it entails a fitness cost because flowering and seed ripening might happen at moments that are sub-optimal due to unfavorable abiotic conditions, especially limited light availability. To our knowledge, induced phenological avoidance in a plant species has not been reported before. Such adaptive shifts in timing need to be acknowledged as a so far neglected strategy of induced resistance. This adds to the more frequently reported morphological and biochemical mechanisms, such as increased production of spines (Alves-Silva & Del-Claro, 2016) or defense metabolites (Kaitaniemi, Ruohomäki, Ossipov, Haukioja, & Pihlaja, 1998; Korndörfer & Del-Claro, 2006; Martemyanov et al., 2012; Zvereva, Kozlov, Niemelä, & Haukioja, 1997) during seasons following substantial herbivory damage. Testing the degree

to which similar shifts in phenology induced by seed herbivory are more widely represented across species is challenging, as it requires detailed data collected on individual plants for several consecutive years. Beyond a field-based monitoring approach used here, experimental work in controlled environments (e.g. excluding pollinators) can further extend the range of species being subjected to pre-dispersal seed predation treatments to gain further insights into the types of species capable of showing induced phenological avoidance. We hypothesize that induced phenological avoidance is most likely in species with successful self-pollination that do not require animal or wind pollination to reproduce. Then there is less or no selective pressure to flower on-peak to increase the chance of pollination.

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## AUTHORS' CONTRIBUTIONS

D.B., L.B. and B.K.S. designed the study and methodology. B.K.S. and I.M. collected the data. B.K.S. analysed the data. B.K.S. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. D.B. and L.B. are supported by the FWO research community EVENET 'Eco-evolutionary network on biotic interactions' W0.003.16N.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.x3ffbg7dz> (Sercu, Moeneclaey, Bonte, & Baeten, 2019).

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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