RESEARCH ARTICLE

The effect of *Eurosta solidaginis* parasitism on pollinator preference in *Solidago canadensis*

Adam Meyer[1]*, Natasha Salter[1], Stephanie Shooner[1]

All authors contributed equally to this work
[1] Department of Biology, McGill University, Montreal, QC

*Email Correspondence:
gregory.meyer2@mail.mcgill.ca

Abstract

Solidago canadensis is a self-incompatible perennial species indigenous to North America that reproduces asexually via rhizomes and sexually via seeds. It is the favoured host of the gall fly, *Eurosta solidaginis*. Sexual reproduction leads to faster rates of adaptation in stressful environments and may be advantageous in the maintenance of host-parasite coevolution. The effect of infection by the gall fly on pollinator visitations at the patch and at the ramet level was assessed as a proxy for the ability to sexually reproduce. The study was conducted by analyzing pollinator preference at both the patch level and individual ramet level through successive observations of pollinator visitations. Though the variation in the number of pollinator visitations could be accounted for by time of day and median bloom stage, the percentage of infected ramets in a patch was not a significant explanatory variable. This suggests that gall formation does not affect pollinator preference and that the capacity to sexually reproduce is likely not reduced because of the host-parasite interaction. Broadly, this study served as an example of how pollinator preference may be utilized as a measure of fitness, and to further understand how selective pressures affect plant populations that reproduce both sexually and asexually.

Introduction

Solidago canadensis, the Canada Goldenrod, is a perennial species indigenous to North America that reproduces asexually via rhizomes and sexually via seeds. It colonizes abandoned fields and after one year of seedling growth it begins to reproduce vegetatively, extending out from the centre to form a circular clone. The ability to reproduce both sexually and asexually raises interesting questions as to how the plant responds to environmental stresses. For example, there is evidence that goldenrod clonal colonies (genets) can share resources among ramets (any stem belonging to the genet), but it is unclear whether a given stress to an individual ramet is indeed detrimental to the entire genet (1). One such stress is parasitization by the gall fly *Eurosta solidaginis*, which is responsible for the formation of stem ball-galls on the plant.

In the early summer, female gall flies oviposit their eggs in the developing leaves of *S. canadensis*; when the larvae hatch, they migrate to the meristem tissue and induce the formation of a gall. The stem continues to grow above the gall (2). As McCrea and Abrahamson (1985) demonstrate, there may be significant physiological and reproductive detriments to individual ramets with little to no perceived damage to the genet as a whole (1). For example, ramets infected by gallmaking parasitic insects have been found to allocate less energy resources towards both seed production and rhizome extensions (2), and devote an appreciable amount of energy to producing the gall, to the detriment of the ramet (3). Though effects to genet fitness remains apparently low, it has been suggested that if a significant number of damaged ramets were present within the genet, this may be sufficient to decrease the fitness of the whole (1). Furthermore, the fact that gall infections affect sexual reproduction in *S. canadensis* more than asexual reproduction (1), creates the possibility of selection for resistance to gallmaking insects.

Since *S. canadensis* is self-incompatible, outcrossing mediated by pollinators such as honeybees, bumblebees, soldier beetles and syrphid flies is obligatory for sexual reproduction (2). If differential resource allocation due to infection decreases the attractiveness of the clone to pollinators, thereby reducing its sexual reproductive success, then this genotype may be excluded from the population in favour of a parasite-resistant genotype. Through the sexual reproductive success of more resistant genotypes, *S. Canadensis* is both more likely to evolve resistance against parasites, and evolve resistance at faster rates. On the other hand, if the rate of cross-pollination is not affected by the presence of the parasite then population resistance may evolve more slowly compared to the rate of increased virulence of the parasite.

In order to better understand the effects of *E. solidaginis* on goldenrod evolution we focused on the plant's ability to attract pollinators.

This is an important aspect of the capacity to sexually reproduce in plants, an aspect that is absent in *Solidago* literature. There exists a rich literature describing pollinator (mainly bee) preference as being non-random with respect to floral colour, nectar concentration and other obvious indicators of fitness (4), as well as demonstrating an ability to respond to varying favourability of foraging patches (5). The importance of pollinators to sexual reproduction and to their ability to distinguish between favourable and unfavourable foraging patches demonstrates that pollinator preference should be considered among other measures of fitness already present in the literature.

Since studies have shown many instances in which the fitness of individual ramets was detrimentally affected by gall fly infection (1, 6, 7), and given the apparent ability for many pollinators, mainly bees, to make non-random decisions in choosing foraging patches, this study sought to test two hypotheses. Firstly, on the level of the genet, patches with a higher percentage of ball-gall infected ramets will receive overall less pollinator visitations and second, that on the level of individual ramets, pollinators will preferentially visit uninfected ramets over infected ramets.

Materials and Methods

Field Methods

Study Site

Our study population of *S. canadensis* grows on an east facing slope of Mont-Saint-Hilaire, an igneous montane part of the Monteregian Hills in Quebec, Canada. *S.canadensis* is one of several species that colonized an abandoned apple orchard on the Gault Nature Reserve, the private sector owned by McGill University of the Mont-Saint-Hilaire Biosphere Reserve. The orchard has been abandoned for approximately five years; *Solidago canadensis* could have been present in the field prior to the orchard being abandoned, however, its growth would have been inhibited by continual mowing. Therefore, we proceeded on the assumption that each of our study patches represented at most five years of growth. Data was collected on August 29th and 30th, 2012.

Patch Choice

Twenty-three patches were chosen. We were unable to determine if selected patches represented a single genetic individual as the use of genetic techniques was beyond the scope of this study. In order to obtain a sufficiently large sample size, the size range varied from 1.46 m^2 to 9.45 m^2 and the bloom stages varied from two to five with a median bloom stage across patches of four. Each patch was assigned a bloom stage value by randomly selecting 10 ramets per patch and identifying their bloom stage based on a predetermined number system (Table 1). In our analysis, we chose to consider the median bloom stage for each patch in order to avoid any outliers in our random ramet sample.

Patch Composition

The level of parasitization of each patch by the ball gallmaking *E. solidaginis* was determined. Each ramet was assessed for the presence of ball-galls. A total ramet count and a count of the number of infected ramets was tabulated so that the percentage of ramets parasitized by E. solidaginis could be obtained.

Flower Counts

A ramet infected with one gall and an uninfected ramet from each patch were removed at the end of the two days of fieldwork. Ramets were taken from the centre of the patch to ensure that they were of similar age and were adjacent to each other to maximize the chance of obtaining ramets from the same genetic individual. Ramets were also similar diameters just below the gall implying that they were ramets of similar size at the time of gall formation. Comparisons can only be made within a clone to ensure that differences between single gall ramets and uninfected ramets are not the consequence of genotype, but of parasitization. The number of open and closed flowers per ramet were counted to obtain the percentage of open flowers per ramet.

Pollinator Observations

To determine the number of pollinator visitations per patch and pollinator preference, visual snapshots (hereafter referred to as rounds) of each patch were taken. Assuming that bees are actively choosing patches, and ramets within patches, the probability of finding them on a given patch or ramet at any moment in time will be greater on favourable patches or ramets. Three rounds were performed consecutively with three rounds making up one trial; each patch was observed for three or four trials. Before beginning a trial, we waited 30 seconds to allow the pollinators to acclimatize to our presence. The patch was then visually assessed for the presence of pollinators. Pollinators were divided into three groups: bumblebees, honeybees, and other. Each round consisted of examining the patch for only the time necessary to document which pollinators were present and the

Patch Composition	**Bloom Stage Number**
Pre-bloom only	1
Mixed pre-bloom and full bloom	2
Full Bloom only	3
Mixed Full bloom and post-bloom	4
Post-bloom only	5

Table 1: Bloom stage classification system: Bloom stage numbers were assigned to 10 random flowers in each patch to determine the overall patch bloom stage.

type of ramet (infected versus uninfected) that they were visiting. Each ramet was only screened once per round for the presence of pollinators. Therefore, if a pollinator moved from one ramet to another, the pollinator was counted again if it landed on a ramet that had not yet been screened. To avoid the bias of time of day, the time of trials were staggered throughout the day for a given patch. The time when each trial was performed was recorded.

Statistical Methods

Patch-level Pollinator Preference

The hypothesis that if patches have higher levels of infection (percentage of ramets infected by *E.solidaginis*) then they will receive less pollinator visitations was evaluated using linear mixed-effects models with nested random factors. The process of building a model that best explained the variation in the response variable and the number of visitations by pollinators involved systematically establishing the best random structure and the significant fixed effects by comparing models using ANOVA. When models were not significantly different the most parsimonious model was chosen. The model was created using R statistical software and the package nlme (8). The percentage of infected ramets within a patch was arcsine transformed because non-normality is assumed for ratios. A graphical representation of the model was created using gamm4 in R statistical software (9).

Ramet-level Pollinator Preference

The hypothesis that pollinators would preferentially visit uninfected ramets over infected ramets was tested by taking the difference between the proportion of total visitations per patch to infected plants and the proportion of infected ramets in the patch. This difference was arcsine transformed because non-normality is assumed for ratios. A one sample t-test was then conducted to see if the mean difference across patches differed significantly from the null expectation of zero.

Flower Counts of Infected versus Uninfected Ramets

To test whether the proportion of open flowers in infected ramets differed significantly from the proportion of open flowers in uninfected ramets a two sample paired t-test was conducted. The proportion of open flowers was arcsine transformed because non-normality is assumed for ratios.

Results

Patch-level Pollinator Preference

In the 23 patches, the percentage of ramets infected by *E. solidaginis* ranged from 0% to 61.3%. The two models with the lowest Akaike information criterion (AIC) were determined after systematically isolating the linear mixed-effects model with nested random factors that best predict the response variable-number of visitations by all pollinators to a patch. The AIC is a measure of how well the model fits the data. Both models have trials nested within patch as random factors, but differ in their fixed effects. The fixed effects of model 1 are the percentage of infected ramets per patch (arcsine transformed), bloom stage median of the patch and time of day when the patch was visited (Table 2).

Random Effects	Standard Deviation		**AIC**	1327.44	
	Intercept	Residual			
Patch	4.096				
Trial	2.183				
Fixed Effects	Value	Standard Error	Degrees of Freedom	t-value	p-value
% of Infected Ramets (arcsine transformed)	-1.212	5.454	20	-0.2223	0.8263
Bloom Stage Median	-2.759	1.071	20	-2.575	0.0181
Time (min)	0.008692	0.002258	66	3.850	0.0003

Table 2:
Model 1: a linear mixed-effects model with nested random structure. This model was not the best fit because it is not statistically different from model 2 (ANOVA: Log likelihood ratio = 0.0566144 , p-value = 0.8119) which is the most parsimonious explanation for the variation in pollinator visitations between patches.

Random Effects	Standard Deviation		**AIC**	1327.44	
	Intercept	Residual			
Patch	3.993				
Trial	2.183				
Fixed Effects	Value	Standard Error	Degrees of Freedom	t-value	p-value
Bloom Stage Median	-2.755	1.047	21	-2.632	0.0156
Time (min)	0.008672	0.002258	66	3.842	0.0003

Table 3:
Model 2: a linear mixed-effects model with nested random structure. This model is the best fit for the data providing the most parsimonious explanation for the variation in pollinator visitations between patches.

The percentage of infected ramets per patch does not significantly affect the number of visitations by pollinators (P=0.8263) whereas bloom stage median of the patch and time of day do significantly affect the number of visitations (P=0.0181 and P=0.003, respectively) (Table 2). The fixed effects of model 2 are the median bloom stage of the patch and the time of day when the patch was visited (Table 3).

Bloom stage median and time of day both significantly affect the number of visitations (P=0.0156 and P=0.003, respectively) (Table 3). Model 1 and model 2 are not significantly different, thus model 2 is the best fit for the data because it is the most parsimonious predictor of pollinator visitations (ANOVA: Log likelihood ratio=0.0566144, P=0.8119). The model is a good predictor of the number of visitations by pollinators (pseudo R-squared=0.91) with little spread around the one-to-one line (Fig. 1)

Figure 2 demonstrates the relationship between the two fixed effects of model 2 and the number of visitations by pollinators. The time of day during which patches were observed for pollinator visitations ranges from 10:09 (609 minutes past midnight) to 17:35 (1055 minutes past midnight).

Number of visitations by pollinators fluctuates throughout the day, but consistently decreases as the bloom stage median increases (higher bloom stage medians are closer to the end of the bloom cycle of the plant). The number of pollinator visitations reached the highest peak near the end of the day with the most visitations occurring at this time on patches with lower bloom stage medians (Fig. 2).

Ramet-level Pollinator Preference

There was no significant difference between the number of visitations by pollinators to ramets with galls and the number of visitations that would be expected given the percentage of infected ramets within a patch (one sample t-test: t=1.18 , df=22, P=0.2497).

Flower Counts of Infected vs. Uninfected Ramets

The difference between the proportion of open flowers (mean=0.2114 (arcsine transformed)) on infected ramets and the proportion on uninfected ramets (mean=0.1936) is not statistically significant (paired t-test: t=0.2628, df=42.12, P=0.794).

Discussions

Contrary to our prediction that increased parasitization would negatively impact pollinator visitations, we found no relationship between the proportion of infected ramets within a patch and the number of pollinator visitations. However, despite an apparent apathy towards the degree of patch infection, the pollinator visitations can still be reliably predicted by the median bloom stage of the patch and the time of day (Model 2, Fig.1 and 2). The greatest number of pollinator visitations occurred in the late afternoon on patches with low median bloom stages. It has been previously demonstrated that bumblebee activity increases as temperature increases (10). It is also known that honeybees are able to remember the location of high quality plants and the time of day when the greatest reward can be obtained (5). .

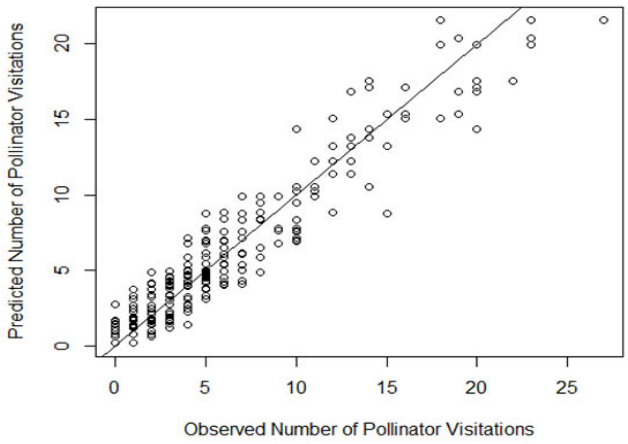

Fig. 1
The number of pollinator visitations predicted by model 2 compared to the observed. Linear regression represents a 1:1 relationship between observed and predicted number of pollinator visitations. The pseudo R-squared for model 2 = 0.91

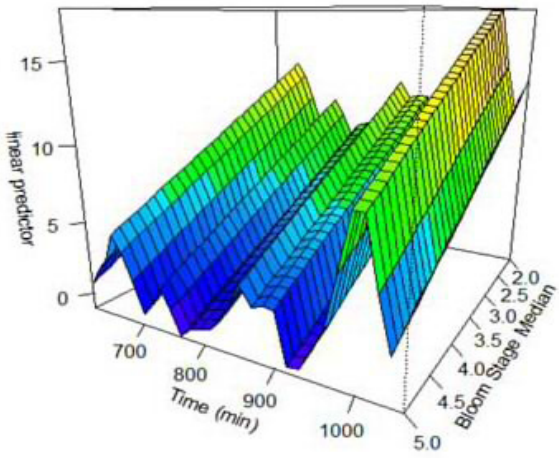

Fig. 2
Graphical representation of linear mixed-effects model with nested random structure: Model 2. Linear predictor is the number of visitations by pollinators. The warmer colours represent higher numbers of pollinator visitations.

The fluctuations throughout the day likely result from differences in peak reward time (greatest nectar production) and preferred foraging time (hottest time of day) though data is not available to confirm this prediction. Our data demonstrates that the pollinators in this study were responsive to differences in flower quality, further suggesting that galls do not affect flower attractiveness in a manner detectable by pollinators. Most importantly, this ability to make non-random foraging decisions serves to reinforce the notion that infection damage by *E. solidaginsis* at the genet level is negligible.

There may be several reasons why infection damage was apparently negligible at the genet level. Hartnett and Bazzaz (1983) find that selective pressures may be mitigated at the patch (genet) level through the physiological integration of connected ramets (11). Furthermore, they find that this may contribute to maintaining genetic diversity in the field by preventing genet death (11). Our study supports this notion by demonstrating how an environmental pressure such as a parasitic fly, which is draining to individual ramet, may be insignificant as a genet selection pressure.
This study also provides some evidence in contrast to the prediction put forth by McCrea and Abrahamson (1985), that the genet may be negatively influenced by *E. solidaginsis* infection, should the level of infection be sufficiently severe (1). Our study found that even when infection was high (roughly 50% for many patches, reaching a maximum of 61.3% galled ramets), detriment to the genet was not sufficient enough to influence pollinators. Thus, even if a patch is quite infected, an important aspect of sexual reproduction (i.e. pollinator attraction) may remain unchanged. This indicates that the damage done by *E. solidaginsis* is not significant enough to select for resistance to infection.

Additionally, the lower biomass allocated to the inflorescence in gall fly parasitized *S. canadensis* ramets (6) likely reduces the total volume of nectar available per inflorescence. Bumblebees are more sensitive to rewards based on sugar concentration, consistently choosing the reward with a higher sugar concentration even when the overall reward is equal. Therefore, if the decrease in total biomass allocated to the inflorescence resulted in flowers with nectar concentrations equivalent to those found in uninfected ramets, then it is possible that the presence of ball galls would have no effect on pollinator visitations at either the patch or the ramet-level (5).

With regards to pollinator preference within a patch, contrary to what was expected there was no non-random pollinator preference pattern with respect to infected or uninfected ramets. This could mean that individual ramets within the patches were not damaged enough by the parasite to (a) influence pollinator preference, or (b) lose out on randomly foraging pollinators. Both of these possibilities are supported by our data which demonstrates no significant difference between the proportion of open flowers on galled and ungalled ramets across patches. Additionally, while the literature puts forth evidence of many detrimental effects to galled ramets including reduced rhizome extension, seed production and biomass in general (1), it is possible that these effects were either not present in our sample size (supported by our comparison of open flower proportion) or simply not significant enough to influence pollinator behavior. Another possibility is that once pollinators have made patch-level decisions, they sample randomly within the patch.

Conclusions

The most significant findings of this study were that contrary to our prediction there was no detectable pollinator preference at the level of the genet or the ramet. While this result is negative, it remains interesting because of the larger implications it has on the understanding of selective pressures to plants as they can reproduce both sexually and asexually. For example, it is possible that in this case energy allocations between interconnected asexual ramets may provide a net benefit to the fitness of the genet (11) as it assists in keeping infected ramets healthy enough to not affect pollinator preference an important component of sexual reproduction in S. canadensis. Moreover, this study demonstrates how asexual reproduction in plants may contribute to stronger genet fitness by influencing sexual processes like pollination. The implications of this study call for further research into the effectiveness of using pollinator preference as a measure of plant fitness as well as further research towards understanding how selection pressures of varying severity act upon species, which can utilize both sexual and asexual reproduction.

Acknowledgements

We'd like to thank Felipe Perez, Simone Reader, Zophia Tanura, Andrew Hendry, Martin Lechowicz and Etienne Low-Decarie for all of the extra help and guidance.

References

[1] McCrea, K. D., Abrahamson, W. G. *Evolutionary impacts of the goldenrod ball gall maker on Solidago altissima clones.* Oecologia. 1985; 68: **20-22**.
[2] Werner PA, Gross RS, Bradbury IK. *The Biology of Canadian Weeds.* 45. Solidago canadensis L. Can. J. Plant Sci. 1980; 60: **1393-1409**.
[3] Stinner, B.R., Abrahamson, W.G., 1979. *Energetics of the Solidago canadensis–stem gall insect–parasitoid guild interaction.* Ecology 60(5), **918-926**.
[4] Nuttman, C.V., Seminda, F.M., 2006. *Visual cues and foraging choices: bee visits to floral colour phases in Alkanna orientalis (Boraginaceae).* Biological Journal of Linnean Society, (87), **427-435**.
[5] Cnaani J, Thomson JD, Papaj DR. *Flower Choice and Learning in Foraging Bumblebees: Effects of Variation in Nectar Volume and Concentration.* Ethol. 2006; 112: **278-285**.
[6] Hartnett DC, Abrahamson WG. *The Effects of Stem Gall Insects on Life History Patterns in Solidago canadensis.* Ecol. 1979; 60: **910-917**.
[7] Wise, M.J., Coffey, L.E., Abrahamson, W.G., 2008. *Nutrient Stress and Gall Flies interaction to Affect Floral-Sex ration In Gymnomonoecious Solidago Altissima.* American Journal of Botany. 95(10), **1233-1239**. 1 - 814 - 4514 - 888
[8] Pinheiro J, Bates D, DebRoy S, Sarkar D, et al. nlme: *Linear and Nonlinear Mixed Effects Models.* 2012; R package version 3.1-104.
[9] Wood S. gamm4: *Generalized additive mixed models using mgcv and lme4.* R package version 0.1-6. 2012.
[10] Comba L. *Patch use by bumblebees (Hymenoptera apidae): temperature, wind, flower density and traplining.* Ethol. Ecol. Evol. 1999; 11: **243-264**.
[11] Hartnett DC, Bazzaz FA. *Physiological Integration among Interclonal Ramets in Solidago canadensis.* Ecol. 1983; 63(4): **779-788**.