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Induced Defense and Varied Gall Density of *Rhopalomyia californica* on *Baccharis pilularis*

Abstract Induced defense can be described as a physical or chemical change to a plant in response to some form of herbivory, preventing further attack. In general, the herbivore is seen as an attacker to the plant and in this study the herbivore utilizes the meristems of the plant to reproduce. The herbivore in question is the gall-forming midge, *Rhopalomyia californica*, which lays its eggs on the meristems of *Baccharis pilularis*, a coastal shrub. The goal of this project was to account for variation in current herbivory rates based upon previous levels of attack on *B. pilularis*. Field tests were preformed in Berkeley, California, in a plot at the University of California Botanical gardens. In these tests, *R. californica* females were allowed to lay their eggs on *B. pilularis* plants with four different previous attack rates (Four groups: 0 galls, 1 gall, 2 galls, 3 galls). Egg counts and numbers of galls formed were compared between the different groups. It was expected that a similar amount of eggs will be laid on each plant, but fewer galls will form on plants which have previously experienced a higher rate of attack. No significant difference was found in the number of eggs released between the groups. There was also no significant difference found in the number of galls formed.

Introduction

Several studies have attempted to explain why there is variation in herbivory rates between plants in a local population (Inbar et al. 2001, Latto and Briggs 1995, Price 1991, Waring and Price 1990). One system in which studies have been done is that of the host plant *Baccharis pilularis* and the host specific gall-forming midge *Rhopalomyia californica*.

In the context of this study, herbivory will be considered as a form of attack on a plant. This attack could be as simple as feeding on certain parts of a plant, but in this system *Rhopalomyia californica* lays its eggs on the meristems of *Baccharis pilularis*. The larvae eventually hatch and enter the meristem, inducing the plant to forming a spongy tissue around the larvae. This spongy tissue is a called a gall, and it protects and nutures the larvae until they hatch as adults. The number of galls found on a plant at a time will be called the galling rate or gall attack rate.

A study attempting to explain variation in galling rate involved plants of two separate *B. pilularis* populations originating from two areas with different abiotic conditions. A lab sample reared from seed and a field sample of both populations compared galling rates. While field comparisons identified variation in galling rates between the populations, the lab setting which made abiotic conditions a constant found no variation in galling rates (Miller and Weis 1999). This indicates galling rates are somewhat sensitive to differences in abiotic factors of the local environment.

Gall attack rate has also been compared with levels of stress the attacked plant is experiencing. Physical stresses will likely lead to deterioration in plant health, and a comparison of variably stressed plants may serve as predictor of gall formation compared with plant health. Water availability was a factor used to test the effect of environmental stress on galling rate. The results found that plants with low water availability experienced not only slower plant growth rates but also fewer galls found on plants (Waring and Price 1990). This introduces another hypothesis, the plant vigor hypothesis, which suggests plants that are growing more vigorously are more likely to be a target of herbivore attack (Price 1991).

Multiple factors affecting the distribution of midge populations were considered by Latto and Briggs (1995). These include plant suitability, oviposition success, growth rate of plants, as well as egg predation and parasitism which were both found to have a distinguishable impact on gall size and density. Dispersal of midges and associated parasitoids was also found to have an important role in regulating gall density and population size (Briggs and Latto 2000).

Some generally less studied possibilities of variation in gall attack rates are midge choice or induced plant responses. It seems that midge choice can be controlled if environmental factors are held constant (Miller and Weis 1999) and suitable growing tips are made available to each midge. With this in mind, it should be possible to detect an induced response of *B. pilularis* without being concerned that midge choice will cloud the results.

Although it appears that not much research has been done in this system looking at induced responses of the infested *B. pilularis*, a few different forms of induced defenses have been considered for this project. Two responses proposed use a similar mechanism involving the production of phenolic (secondary) compounds. Phenolic compounds are known for their use in plant defense (Hartley 1999). The first would be a response that results in negative feedback in which a secondary compound produced by *B. pilularis* is released which deters the midge. This explanation seems unlikely, however, due to the obvious affinity the midge has for *B. pilularis*. Most populations of *B. pilularis* also support populations of *R. californica*. However, this may be a clue to the lack of success of the defensive method, rather than the likelihood of it existing. The second response suggested has yet another negative response for the midge, where the induced plant attracting parasitoids which would control the midge population (Turlings et al. 1990). Turlings and Tumlinson (1991) have reasoned that this situation is a possibility due to the profitability for both parties involved (plant and parasitoid). A third situation involves the plant itself producing some response that instead of preventing the midge from laying it eggs (1st response), it decreases either the survivorship of egg/larvae after ovipostion or the likelihood for galls to be formed.

I have tested for the latter of the three proposed responses. Field observations have made well aware the attraction of *R. californica* to *B. pilularis*, which nearly disqualifies the likelihood of the first scenario mentioned. The introduction of the third trophic level to the question, due to coercion of parasitoids towards galled plants, seems like a genuine response (Turlings and Tumlinson 1991). However, the success of a plant-level defense mechanism appears most likely to be effective and interesting in the context of the system.

Observations in the field have also pointed to this question of susceptibility to gall formation of certain plants within a population. Lower gall densities are regularly found on very leafy bushes or also bushes with large amounts of resin found on branches and leaves (personal observation). However, the formation of this resin seems to be a natural occurrence and there is no evidence that it would be a response to previous gall formation. This example attempts only to establish that the local properties of individual plants can affect the formation of galls on the particular plant.

This study proposes that a plant that has previously been used as a vessel of midge reproduction (has had galls formed upon) will experience a change that results in fewer galls formed in subsequent attacks. It is also expected that a similar number of eggs will be released on plants regardless of previous infestation.

These questions relate to differentiating between midge choice and defense of the plant. A form of midge choice has been documented in the lab, when *R. californica* females were observed exhibiting ovipositional restraint (Ehler 1992). If the female prefers plants that are already galled, this could contribute to difficulty detecting a difference. For example, if this is true, the uninfested plant may receive few or no eggs, so the galled plant will have possibly have more galls resulting due to a higher attack rate. Another problem again occurs from the initial wave of infestation. If the first group of females chose the best sites for oviposition, the question arises of whether an adequate amount of equally good sites were available for the second group of females.

Methods

The project took place in an experimental plot in the Botanical Gardens at the University of California, Berkeley. This area has a low background density of *Rhopalomyia californica*. The experimental plot contained *Baccharis pilularis* from a previous experiment, but the plants have since been mostly left alone. New, young plants grow freely in the plots, and have been used in this project. These plants were assumed to be of common age also because they are all the same size and grow in an area that was completely free of *Baccharis pilularis* two years ago (pers. communication, John Latto). It was also assumed that the each plant's current level of galling is the only amount of galling the plant has experienced. This is reasonable because plants generally begin being galled upon at one year old, and galls can survive on plants for up to a year (resulting in the total two years since the plot was completely empty).

First, plants of equal size were found throughout the site. Then, these plants were inspected for galls and categorized based on the number found. To determine if previous infestation affects the number of galls formed in subsequent infestations, midges were released on the plants. These midges were captured from galls collected in the Bolinas Lagoon Preserve in Bolinas, California. Using a mesh bag (10 by 20 cm) to ensure the midges will remain at the intended plant, female midges will be added to growing tips of individual plants. Three midges were added to each plant over a three-day period, for a total of one hundred and twenty midges.

The day after the release of the first wave, each of the bags containing the growing tips (one from each plant) from each galling class will be collected. The eggs were then counted and the number recorded. The numbers of eggs laid were compared between each galling class. After two months, galls found on tips in the mesh bags that were release points for midges were counted and collected. The numbers of successful galls produced were correlated with the previous level of galling experienced by the plant, and these results were complemented by the numbers from the egg count.

Results

The numbers of eggs released by midges for each gall attack rates (0, 1, 2, and 3 galls on plant) did not vary significantly in an ANOVA test (F=0.7125, df = 3,36, P=0.5509).

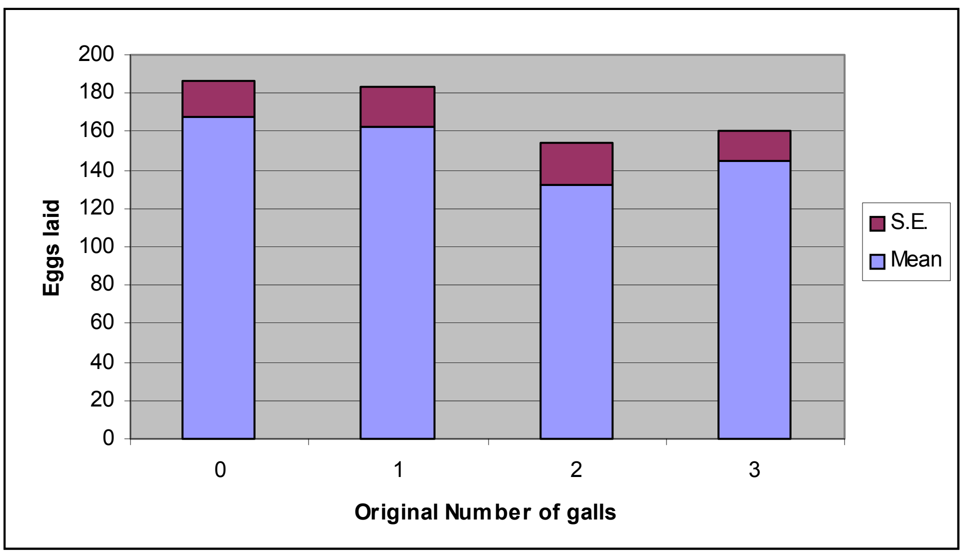

Figure 1. Mean of eggs laid for each galling level +/- s.e.

The numbers of galls formed also did not vary significantly in a Kruskal-Wallis test (P=0.6890).

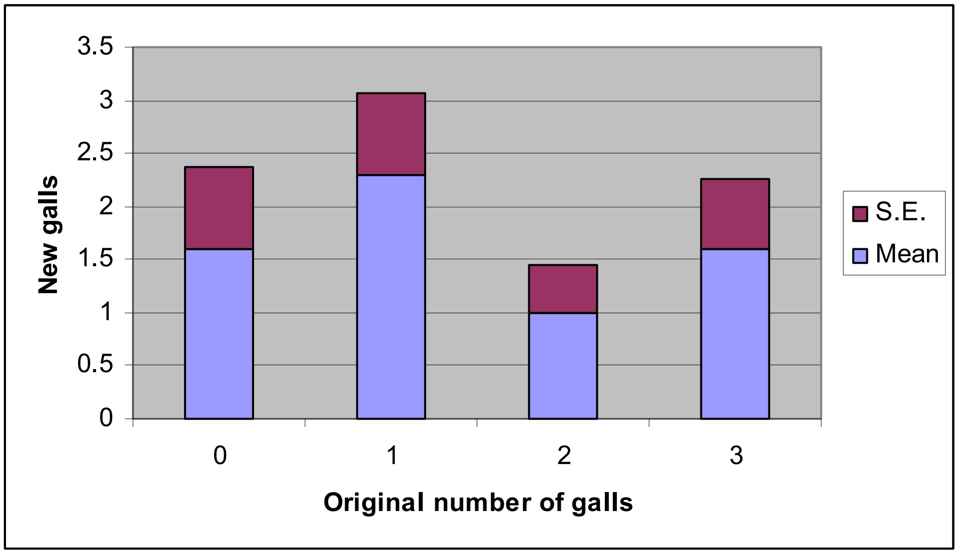

Figure 2 – mean of new galls formed for each galling level +/- s.e

Discussion

In the introduction of this paper, three scenarios of possible *R. californica* control by *B. pilularis* were discussed involving different mechanisms of induced defense. One of these ideas proposes that *B. pilularis* would deter the midge from laying its eggs on the plant. If this were true, the deterrent would take the form of a compound not necessary for the plants regular functions. This substance would be what is known as a secondary compound, such as phenolic compounds, more commonly found being used for plant defense (Hartley 1999). By placing the midges on a specific branch midge choice has been removed from the experiment. The hypothesis that the number of oviposited eggs per midge in each level of galling would be similar was supported by the data.

While this attempts to remove midge choice from the list of variables, it does also force the midges to lay eggs on shoots they may not have chosen for reasons that are not entirely clear. Karban (1990) acknowledged in a similar experiment that criteria for host selection that are not accounted for in an experiment may lead to results that do not reflect a naturally occurring pattern. Perhaps there are certain characteristics of a successful plant that are determined by a midge female prior to oviposition.

The second situation discussed in the introduction also involves the use of a secondary compound by *B. pilularis*. However, instead of repelling the midge from the plant, this compound would attract parasites that feed on *R. californica*. The end response would reduce the number of the midge female's surviving offspring. Although the data from this project can in no way affirm this response, it also cannot be discounted as a possibility in a natural setting. This project has been constrained to the point were parasites of the midge larva and eggs could not be included. However, the presence of parasitoids in the natural *B. pilularis* setting suggests that more research opportunities are available for this aspect of the system.

The hypothesis that fewer galls would form on plants that have already been galled upon was not supported, as the results show no significant difference between the four galling levels. As a result, this project does not prove any defensive reaction by the plant which would be manifested sometime after the eggs are laid.

An applicable explanation suggested by Karban (1990) concerns the distinctions between galling level of the plants. Only four classes were used (0,1,2,3). It is possible that it would very difficult to detect a difference at this level. For example, if there is an induced defense, the response may be equal for plants at this low level of attack. The induced defense might be more evident if a plant with a large number of galls formed previously was compared a plant that has absolutely no galls formed.

However, is scale of attack an issue? Kearsley and Whitham found that herbivory resistance for a cottonwood tree species increased greatly in a two-year period (1989). This resistance was shown to develop independent of herbivore attack. This idea lead to an alternate explanation found in Karban's (1987, 1990) articles suggesting another hypothesis for induced resistance. This hypothesis introduces the possibility that the development of resistance is related to the maturation of the plant. Tests on plants of different age classes would be needed to for this information. Again, a greater distinction between galling levels and age classes might be needed to detect a significant difference.

In his 1991 article, Price identifies some questions on herbivory that could relate to this project. One of these questions involving duration of defense adds an element of time that was not looked at here. Specifically, the defensive response could be short term, lasting not very after the initial attack, or long term, altering the activities of the plant for the rest of its lifetime. It is not always clear how long defensive mechanisms would remain active. However, it seems that this would be partially explained by the costs and benefits of the plant. As the plant utilizes energy and nutrients to create defenses, there is a tradeoff as those resources are diverted from normal use, such as plant growth. It appears that efficient defensive mechanisms would depend on the time length and magnitude of attack.

In the same article, Price acknowledges that attack rates of herbivores may not be entirely clear, and gradients may exist which reflect individual plant characteristics (1991). Some identified variables contributing to this gradient are age of the individual plant as well as current rate of growth. Roininen et al. 1993 also cited plant age and vigor as factors that would affect gall distribution. It was also acknowledged that it would be difficult to remove these two variables from each other, as they are often closely related (Roininen et al. 1993). Although Price is discussing these gradients in terms of herbivore nutrition and plant response, it seems that a connection could be made to the context of this project. As mentioned earlier, visits to local areas of wild *B. pilularis* populations have resulted in the observation that very small, young plants often have many galls where as it has been noticed that larger, mature plants are not found as often with a great number of galls (Rob Wellbrock, pers. obs.). It would be interesting to attempt to quantify the effects of plant age and vigor on a larger scale. A good project testing these variables would have accurately established age classes and a method for modifying vigor of the plant.

One proposed explanation for this perceived representation of more galls on smaller plants would be the orientation of available (and preferred) galling locations on the individual plants. Larger plants have many shoots, but not all of these shoots are currently experiencing high growth because not all the branches are new. The newly growing shoots are more interspersed, leading to questions regarding the midges' decision on where the eggs will be laid. However, on smaller plants, most of the shoots are new and suitable for oviposition. These shoots are also in a smaller, more concentrated area. There is little effort needed to seek out a desirable spot to lay eggs. A survey of large and small *B. pilularis* could tell if this pattern has been observed because the small size of younger plants make galls more apparent, or if the smaller plants actually have a significantly higher amount of galls in comparison to older, larger plants.

This project cannot conclude that induced defense is likely to contribute to gall distribution patterns on *B. pilularis*. Limitations of this project can obviously be found in the fact that only one trial was performed, and in that one trial only two midges were added per plant. This resulted from poor timing as well as difficulties arising from midge collection and handling. Furthermore, the life history of the individuals used in the project is not truly known; rather it has been estimated from size and the contemporary level of galling when the project began (amount of galls actually on the plant at the time of midge release). It was assumed that the galls found on the plants were not only all of the galls currently found on the plant, but also these were all the galls ever to exist on the plant. This assumption particularly reintroduces the question of timing and duration of defense reactions. Growing plants from seed in a more controlled setting might relieve future projects of these issues regarding life history of the plants. A more rigorous experiment controlling for these variables would lead to a better understanding of the system, and a truthful interpretation of variation in gall levels in *B. pilularis* populations.

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