

Full Length Research Paper

Exploration of climate influences on the abundance of galls on red willow (*Salix laevigata*) across two riparian communities in Southern California

Tauras Vilgalys¹, Rachael Sears², Emily Hand³, Sara Morledge-Hampton¹ and Víctor D. Carmona-Galindo^{1*}

¹Biology Department, Loyola Marymount University, 1 LMU Dr. MS 8220, Los Angeles, CA 90045, United States.

²Environmental Science Program, Loyola Marymount University, 1 LMU Dr. MS 8220, Los Angeles, CA 90045, United States.

³Natural Science Department, Loyola Marymount University, 1 LMU Dr. MS 8220, Los Angeles, CA 90045, United States.

Received 14 January, 2014; Accepted 7 March, 2014

In Southern California, the red willow (*Salix laevigata* Bebb) hosts a variety of gall-inducing parasitic insects. However, little is known about the ecology of these parasites, particularly the characterization of their microclimate preferences. This study explores the relationship between microclimate and gall frequencies in *S. laevigata* in the Ballona Wetlands and Temescal Canyon, and gall count correlated with biotic and abiotic factors such as soil pH, soil moisture and willow density. Significantly more galls per leaf were found at Temescal Canyon than Ballona Wetlands. Although the number of galls per leaf correlated negatively with soil pH, soil moisture content and canopy openness, only site and gall location were found to significantly predict the number of galls. These results suggest that additional or interacting microclimate factors may influence gall frequencies between Temescal Canyon and the Ballona Wetlands.

Key words: Insect galls, microclimate, plant vigor hypothesis, self-thinning rule.

INTRODUCTION

Willows (*Salix* spp.) are the host of parasitic interactions with a variety of other organisms. Some of these interactions produce abnormal plant growths, more commonly known as galls, which are formed as parasites manipulate the hormone levels of the host plant. Typically, gall formation relies on a mix of environmental and chemical signals that assist the parasite in identifying

and infecting the host plant. More than 15,000 organisms can produce stem and leaf galls (Hartley and Lawton, 1992) and the identification of willow galls poses a monumental taxonomic challenge (Russo, 2006). Among willows, it is known that sawflies, midges, mites and fungi can cause galls although the complete number of species is unknown and the current list is far from exhaustive

*Corresponding author. E-mail: vcarmona@lmu.edu. Tel: +13103381968.

(Russo, 2006). Additionally, little is understood regarding the environmental requirements of gall-producing organisms (Russo, 2006). Gall formation is an important form of plant-herbivore interaction in that gall inducing insects affect the developmental pattern of plant organs (Larson and Whitham, 1991) and the physiology of the plant tissue surrounding the developing insect (Kopelke et al., 2003; Nymen and Roininen, 2000).

According to the plant vigor hypothesis, plants with optimal resource availability are most suited for gall formation (Price, 1991). Having a large amount of available energy, they create a nutrient-rich environment for the formation of galls that makes them ideal targets for parasitism (Price, 1991). Many factors can contribute to the distribution of gall-forming insect larvae, such as the distribution of secondary chemistry within or between host plants, environmental conditions, and weather-related factors (Kopelke et al., 2003). As such, characterizing the surrounding microclimate is an important tool in understanding the variability of gall formation between a singular plant species growing in various localities.

The main objective of this study was to evaluate the relationship between gall frequencies and microclimate in red willows (*Salix laevigata* Bebb) growing in two riparian communities in Southern California, Temescal Canyon and Ballona Wetlands. We hypothesized gall count would correlate with biotic and abiotic factors such as soil pH, soil moisture and willow density. Based on previous studies, we predicted that higher soil pH would correlate with higher stem gall count and a lower leaf gall count in *S. laevigata*. We also expected gall count would increase with soil moisture and canopy openness, but decrease as willow density decreases.

METHODS

Site description

Temescal Canyon is a protected chaparral area that experiences regular drought conditions. Alternately, Ballona Wetlands is a complex habitat comprising of estuarine and brackish marshes, freshwater marsh, and riparian corridor with a managed fresh water and tidal inputs (Carmona-Galindo et al., 2013). Additionally, the two communities are in different successional stages with Temescal Canyon being an unmanaged ecosystem and Ballona Wetlands having been restored and planted in 2003 (Friends of Ballona, personal communication). Temescal Canyon annually receives 18 to 28 cm of rain (Santa Monica Mountain Conservancy, personal communication) and Ballona Wetlands 18 to 38 cm of rain (Friends of Ballona, personal communication).

Gall counts

Thirteen (13) *S. laevigata* samples were collected at Ballona Wetlands and 12 at Temescal Canyon in Los Angeles, CA during November. Focal plants throughout each environment were randomly selected from both solitary plants and groups of trees. No

more than a single tree from any cluster was selected. For each focal plant, a stem approximately 1.3 m from the ground was randomly selected and the galls on the last 50 cm of the stem were counted. Twenty leaves were selected from each stem and the number of galls per leaf were counted. The area of each leaf was measured via digital image analysis using Sigma Scan Pro (v5).

Environmental characterization

While collecting data, soil samples were taken from the base of each plant and stored in a cold room at 5°C until the time of analysis. Soil pH was measured by suspending 15 g of soil in 30mL of deionized water. Soil suspensions were agitated for 30 s and allowed to settle for 15 min. The pH of the supernatant was then measured using a Multi-Parameter Tester 35 pH meter. Soil moisture was measured by taking an initial wet soil weight for 10-20 g of soil, desiccating the soil in the Labconco FreeZone 4.5 L freeze dryer for 48 h, and weighing the soil again to obtain a dry weight.

Willow density was measured by counting the number of *S. laevigata* within a 2 m radius of each focal plant. Distance to the nearest woody-plant neighbor was also measured, as well as the base diameter of each focal tree. Canopy openness was measured by averaging four densiometer readings taken at the base of each focal plant.

Statistical analysis

Soil pH, soil moisture content, canopy openness, galls per leaf, galls per leaf area, stem galls, base diameter, distance from the nearest willow, distance from the nearest woody plant and willow density were tested for normality using a Shapiro-Wilks test. Correlations between these variables were also determined using Spearman Rank tests. Additionally, character traits were compared between the Temescal Canyon and Ballona Wetlands sites using Mann-Whitney U tests. The influence of variables on gall abundance was calculated using a general linear model. All analysis were conducted in Statistica (v9.1).

RESULTS

Data distribution

Soil pH ($W=0.909$), soil moisture content ($W=0.891$), canopy openness ($W=0.890$), galls per leaf ($W=0.714$), leaf gall density, stem galls ($W=0.712$), base diameter ($W=0.812$), nearest willow ($W=0.581$), nearest woody plant ($W=0.764$), and willows within two meters ($W=0.641$) followed a non-normal distribution ($p<0.05$).

Correlations

The number of galls per leaf correlated positively with leaf gall density and negatively with soil pH, soil moisture content and canopy openness (Table 1). Leaf gall density also correlated negatively with pH and canopy openness (Table 1).

The number of willows within a 2 m radius correlated negatively within the distance to the nearest woody plant, distance to the nearest willow, and willow base diameter (Table 1). The distances to the nearest woody plant and the nearest willow correlated positively and soil moisture correlated positively with soil pH (Table 1). The number

Table 1. Correlational relationships between biotic and abiotic microclimate variables and gall abundance. N.S. is used to designate insignificant relationships and r-values are given for significant correlations ($p < 0.05$).

	Gall density per leaf	Willow density	Nearest woody plant	Nearest willow	Soil pH	Percent moisture	Stem galls	Canopy openness	Base diameter
Galls per leaf	0.98	N.S.	N.S.	N.S.	-0.61	-0.43	N.S.	-0.80	N.S.
Gall density per leaf		N.S.	N.S.	N.S.	-0.63	N.S.	N.S.	-0.78	N.S.
Willow density			-0.67	-0.69	N.S.	N.S.	N.S.	N.S.	-0.58
Nearest woody plant				0.75	N.S.	N.S.	N.S.	0.52	N.S.
Nearest willow					N.S.	N.S.	N.S.	N.S.	N.S.
Soil pH						0.43	N.S.	0.65	N.S.
Percent moisture							N.S.	0.51	N.S.
Stem galls								N.S.	N.S.
Canopy openness									N.S.

Table 2. Analysis of covariance for the number of gall with respect to main effects and interaction terms. Asterisk (*) denotes covariate variables. Double asterisks (**) denotes significant effect on gall abundance ($p < 0.05$).

Source of variation	DF	MS	F	p
*Soil pH	1	30.1932	2.4244	0.1267
*Moisture	1	2.3976	0.1925	0.6630
*Canopy openness	1	11.0762	0.8893	0.3509
Site	1	107.8604	8.6609	0.0052**
Gall location	1	106.6338	8.5624	0.0055**
Site x gall location	1	207.4338	16.6564	0.0002**
Error	43	12.4537		

of stem galls did not correlate with any other measured variable (Table 1).

Analysis of covariance

A general linear model identified the site (Ballona or Temescal), gall location (leaf or stem), and the interaction between site and gall location as the only factors that predicted a significant amount of the variance in gall count (Figure 1; Table 2; $p < 0.01$). At Ballona Wetlands, there was no difference in the number of stem and leaf galls with a mean of 0.65 ± 1.72 galls per plant on both the stem and shoot. However at Temescal Canyon, there was a difference between leaf tissues with the leaves of a plant containing 9.06 ± 6.64 galls and the stems containing 1.75 ± 2.77 galls (Figure 1).

Site differences in gall densities and microclimate factors

Significant differences were detected in galls per leaf

($U = 0.5$, $p < 0.0001$; Figure 2a) and galls per leaf area ($U = 2.0$, $p < 0.0001$; Figure 2b) between the two sites. At Temescal Canyon, there were significantly more galls per leaf than at the Ballona Wetlands. Canopy openness was significantly greater at Ballona Wetlands than at Temescal Canyon ($U = 0.0$, $p < 0.0001$; Figure 2c). Soil moisture content ($U = 30.0$, $p < 0.01$; Figure 2d) and soil pH ($U = 7.0$, $p < 0.001$; Figure 2e) were both significantly greater at Ballona Wetlands.

DISCUSSION

The number of galls per leaf had a significant negative correlation with soil pH, soil moisture and canopy cover. Since the leaf gall density is strictly correlated to leaf area, the number of gall per leaf area behaved similarly to the number of galls per leaf. Additionally, among the environmental factors, it was positively correlated with soil pH and soil moisture while negatively correlated with base diameter and willow density. The analysis of covariance shows that site, location of the gall, and location by site interaction were found to explain

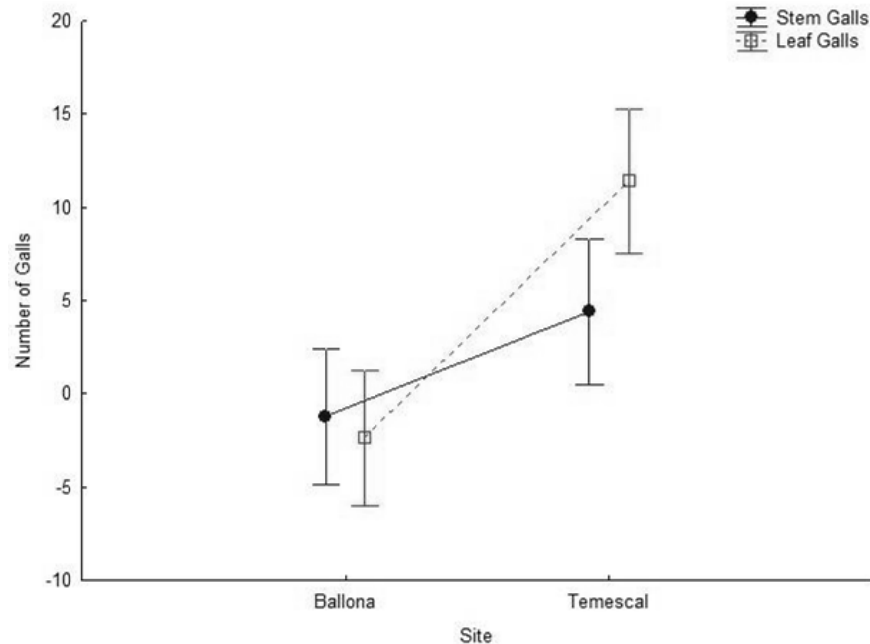


Figure 1. Average number of stem and leaf galls at the Ballona Wetlands and Temescal Canyon. The site, gall location and site x gall location each differed significantly ($p < 0.01$). Bars denote 95% confidence intervals.

variations in the quantity of galls. As covariates, soil pH, soil moisture and canopy cover did not explain a significant proportion of the variation in the numbers of galls. Temescal Canyon had significantly more galls per leaf, greater canopy cover, less soil moisture and a lower pH than Ballona Wetlands.

Based on the analysis of covariance, none of the site differences had an effect independent of site. It is unclear what microclimate characteristics contributed the change in gall abundance between sites. However, it is possible that the interactions between biotic and abiotic factors that were measured may be more important than individual variables (Bollinger et al., 1991). For example, studies of soil moisture and gall formation have shown no relationship (Bauer, 2010) and a negative relationship (Sumerford et al., 2000), suggesting that the interactions of multiple factors may be important in explaining changes in gall abundance. Additionally, there may be alternate factors in the plant chemistry (e.g. semiochemicals) that influence gall formation between the two sites. Examining differences in semiochemicals and biochemistry of plants at the two sites may help further in explaining the variation in gall abundance and formation. Furthermore, additional variation may be explained by differences in the species of gall present.

An important difference between Ballona Wetlands and Temescal Canyon is their relative secondary successional stage. The Ballona Wetlands are a heavily managed environment and was recently revegetated while Temescal Canyon is a preserved area of older riparian

growth (Carmona-Galindo et al., 2013). Au (2013) found that successional changes influenced *Syzygium malaccense* gall formation as different biological communities dominated the landscape. Successional stages may influence gall dispersion and more established areas may be more likely to have resident gall forming insect populations that invaded in earlier successional stages.

There may be a possible tradeoff between plant vigor and insect distribution in that the number of galls did not correlate with either trunk diameter or willow density. According to the plant vigor hypothesis, galls should grow on the trees with the greatest amount of available energy. This hypothesis suggests that galls would be the most prevalent on older, healthier individuals. However, these more mature trees are often the result of a self-thinning process and decreased tree density may reduce opportunities for insect dispersion.

Consistent with self-thinning rule, we observed a significant inverse relationship between trunk diameter and willow density. This indicates there may be a tradeoff between willow size and tree density and an optimal intermediate condition that would promote the greatest amount of gall formation.

Conflict of Interests

The author(s) have not declared any conflict of interests.

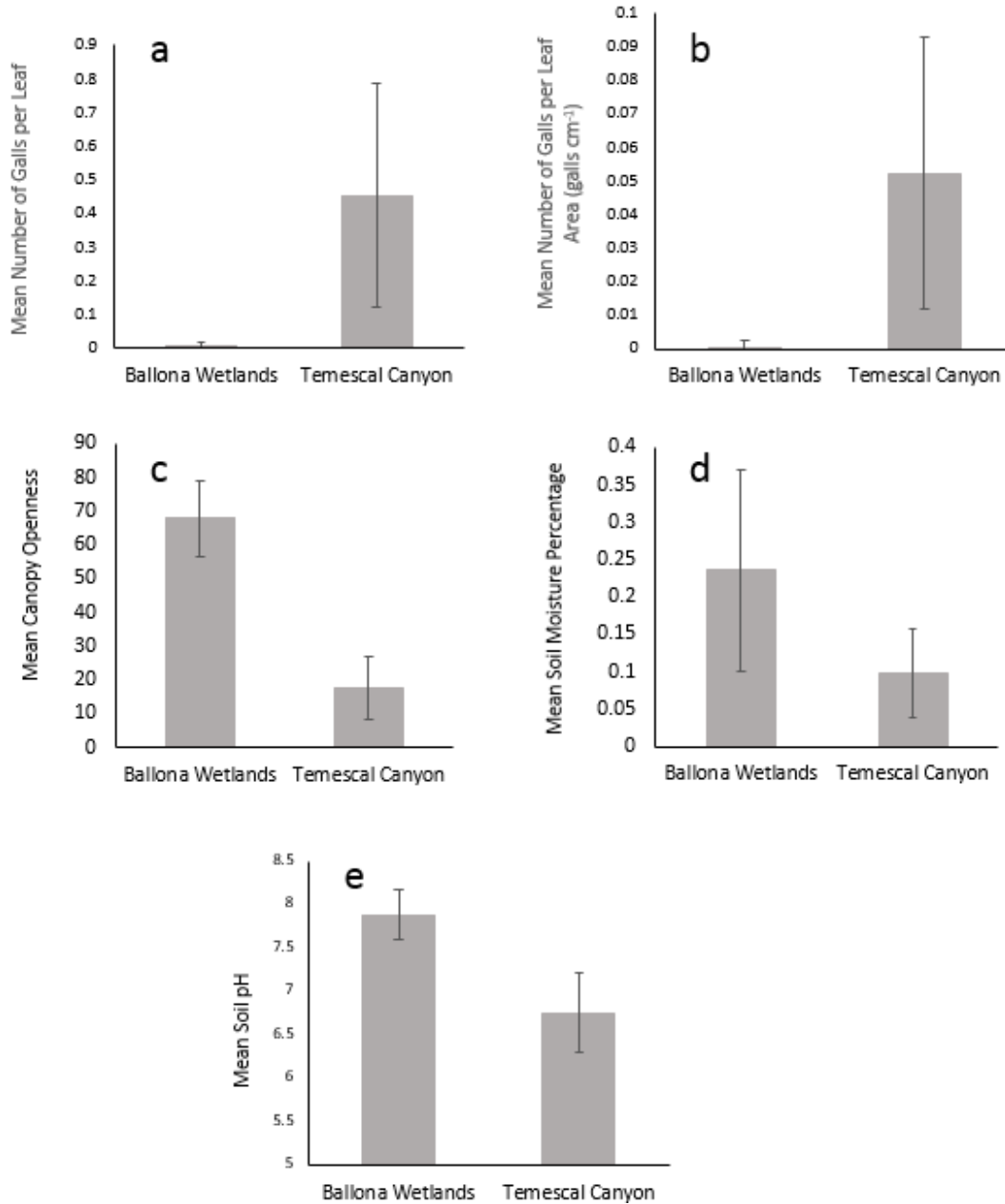


Figure 2. Site differences in microclimate and gall frequencies between the Ballona Wetlands and Temescal Canyon for a) the number of galls per leaf, b) the number of galls per leaf area, c) canopy openness, d) soil moisture and e) soil pH. Bars denote ± 1 S.D.

REFERENCES

Au S (2013). Succession in galls on *Syzygiummalaccense* and their impact on leaf aging. UC Berkeley Library. Retrieved November 21, 2013 from <http://escholarship.org/uc/item/847704sb>.
 Bauer T (2010). The densities of goldenrod galls (*Eurostasolidaginis*) and their goldenrod host plants (*Solidago canadensis*) while directly related to each other, are not impacted by soil nitrogen or soil moisture. Deep Blue. Retrieved November 21, 2013 from http://deepblue.lib.umich.edu/bitstream/handle/2027.42/78445/Bauer_Tatia_2010.pdf?sequence=1.
 Bollinger EK, HarperSJ, Barrett GW (1991) Effects of seasonal drought on old-field plant-communities. *American Midland Naturalist* 125:

114-125. <http://dx.doi.org/10.2307/2426374>.
 Carmona-Galindo VD, Hinton-Hardin D, Kagihara J, Pascua MRT (2013). Assessing the Impact of Invasive Species Management Strategies on the Population Dynamics of Castor bean (*Ricinus communis* L., Euphorbiaceae) at Two Southern California Coastal Habitats. *Nat. Areas J.* 33(2):222-226. <http://dx.doi.org/10.3375/043.033.0212>
 Hartley SE, Lawton JH (1992) Host-plant manipulation by gall insects: a test of the nutrition hypothesis. *J. Anim. Ecol.* 61: 113-119. <http://dx.doi.org/10.2307/5514>.
 Kopelke JP, Amendt J, Schonrogge K (2003). Patterns of interspecific associations of stem gallers on willows. *Divers. Distributions* 9: 443-453. <http://dx.doi.org/10.1046/j.1472-4642.2003.00037.x>.

- Larson KC, Whitham TG (1991) Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. *Oecologia* 88:15-21. <http://dx.doi.org/10.1007/BF00328398>
- Nymen T, Widmer A, Roininen H (2000) Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). *Evolution* 52(2): 526-533. [http://dx.doi.org/10.1554/00143820\(2000\)054\[0526:EOGMAH\]2.0.CO;2](http://dx.doi.org/10.1554/00143820(2000)054[0526:EOGMAH]2.0.CO;2); <http://dx.doi.org/10.1111/j.0014-3820.2000.tb00055.x>
- Price PW (1991). The plant vigor hypothesis and herbivore attack. *Oikos* 62:244-251. <http://dx.doi.org/10.2307/3545270>.
- Russo R (2006). Field guide to plant galls of California and other Western states. University of California Press, Berkeley, pp. 20-22, 205-216.
- Sumerford DV, Abrahamson WG, Weis AE (2000) The effects of drought on the *Solidagoaltissima*-*Eurostasolidaginis*-natural enemy complex: population dynamics, local extirpations, and the measures of selection intensity on gall size. *Oecologia* 122: 240-248. <http://dx.doi.org/10.1007/PL00008852>