

**IMPLICATIONS OF THE WITHIN-CANOPY OVIPOSITION PREFERENCE,  
ABUNDANCE, AND LARVAL PERFORMANCE PATTERNS OF A HOST  
SPECIFIC CYNIPID GALLFORMER**

THESIS

Presented to the Graduate Council of  
Southwest Texas State University  
in Partial Fulfillment of  
the Requirements

For the Degree

Master of SCIENCE

By

Preston S. Galusky

San Marcos, Texas  
December 2000

## ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Dr. Jim Ott, Dr. Paul Barnes, and Dr. Kevin Jones, for their guidance in this endeavor. Their unique perspectives were simultaneously stimulating and frustrating in testament to their integrity and respective talents as both educators and scientists. Thanks to friends and colleagues Richard Reynolds, Tracy Boussetot, Jay McGhee, Valentin Cantu, and Jim Ott for mercifully enduring numerous bouts of my neuroses as well as combating the relentless tedium of data collection. The SWTSU Biology Department and Graduate College, Texas Parks and Wildlife, Texas Opportunity Graduate Fellowship program, Sigma Xi, and National Science Foundation were extremely generous in their support of this work.

Submitted on September 1, 2000

## TABLE OF CONTENTS

	Page
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
ABSTRACT.....	viii
INTRODUCTION.....	1
Mediation of herbivory patterns within plants.....	1
Gallformer constraints.....	2
Effects of intraspecific competition.....	4
Objectives.....	4
MATERIALS AND METHODS.....	6
Study system.....	6
Sampling methods.....	7
Canopy-level statistical analysis.....	8
Leaf-level and host vigor analysis.....	10
RESULTS.....	12
Canopy-level patterns of abundance, ovipuncture density, and performance.....	12
Canopy-level preference-performance linkage.....	21
Host vigor and relationships between leaf size, abundance, and performance.....	22
DISCUSSION.....	24
Canopy-level abundance, ovipuncture density, and performance patterns.....	24
Influence of canopy-level gall density on herbivory patterns.....	26
Canopy-level preference-performance linkage.....	26
Leaf-level herbivory patterns and host vigor.....	28
Conclusions.....	29
LITERATURE CITED.....	30

## LIST OF TABLES

	Page
Table 1. Spearman correlation coefficients by year between indicated gallformer parameters estimated at the level of stratum by direction cells (n = 12 per tree) within tree canopies.....	21
Table 2. Pearson correlation coefficients by tree in 1998. Column headings represent correlated parameters.....	23
Table 3. Leaf size and gallformer performance relationships as determined by Spearman correlation analysis according to gallformer density at the leaf level. Each cell represents the number of trees exhibiting a particular relationship. All correlation coefficients were calculated with samples of at least 5 leaves. Significant correlation coefficients ranged in magnitude from 0.14 (n = 254) to 0.43 (n = 17).....	23

## LIST OF FIGURES

	Page
Figure 1. Untransformed mean ( $\pm$ SE) gallformer abundance indices among plateau live oaks according to height within canopies. Low strata abundance was greater than higher strata in both years (REFGWQ test, $p < 0.05$ ).....	13
Figure 2. Untransformed mean ( $\pm$ SE) ovipuncture density indices among host plants according to height within canopies. Low strata ovipuncture density was significantly greater than that of higher strata in both years (REFGWQ test, $p < 0.05$ ).....	14
Figure 3. Untransformed mean ( $\pm$ SE) larval performance indices according to height within host canopies. Upper strata performance was significantly greater than lower strata in both years (REFGWQ test, $p < 0.05$ ).....	16
Figure 4. Mean ( $\pm$ SE) gallformer abundance indices according to compass direction within canopies of host plants. In 1997, differences in means were not significant (REFGWQ test, $p > 0.05$ ). In 1998, mean abundance in the northeast was greater than western canopy regions and southeastern abundance was greater than that of the southwest (REFGWQ test, $p < 0.05$ ). Upon inspection of tree x direction interactions however, patterns were not consistent among trees.....	17

Figure 5.	Mean ( $\pm$ SE) ovipuncture density indices by compass direction within host-plant canopies. In 1997, differences in means were not significant (REFGWQ test, $p > 0.05$ ). In 1998, ovipuncture density in the northeast was greater than in the southwest (REFGWQ test, $p < 0.05$ ). Upon inspection of tree x direction interactions however, patterns were inconsistent among tree.....	18
Figure 6.	Mean ( $\pm$ SE) larval performance indices according to compass direction within host plant canopies. No differences were significant in either year (REFGWQ test, $p > 0.05$ ).....	19

## ABSTRACT

IMPLICATIONS OF THE WITHIN-CANOPY OVIPOSITION PREFERENCE,  
ABUNDANCE, AND LARVAL PERFORMANCE PATTERNS OF A HOST  
SPECIFIC CYNIPID GALLFORMER

by

Preston Galusky, B.S.  
Southwest Texas State University  
August 2000

Supervising Professor: James R. Ott

Gallformers oviposit at the site of larval development and thus promote selection for a linkage between the oviposition behavior of fertile females and larval performance. The host vigor hypothesis (HVH) predicts this linkage will occur on host tissues with relatively high growth rates that can be identified by large tissue size. However, both predictions may be subject to system-specific developmental and/or intra-specific competition constraints that may occur at different scales within host plants. We examined patterns and relationships of oviposition preference, gall abundance, and larval performance of the leaf-galling generation of *Belonocnema treatae* Mayr (Cynipidae:

Hymenoptera) on its host plant, *Quercus fusiformis* Small at both within-canopy and within-leaf levels. Over two consecutive gallformer generations, leaves were collected from 16 trees according to previously documented within canopy patterns of carbon uptake (upper > mid > lower and SE > SW = NE > NW) which, along with abiotic correlates, are known to influence phytophage habitat suitability. At each of the 12 strata x direction sites/tree, ovipuncture density, initiated gall density, gall size, and gall-former performance (# mature galls/ovipuncture) were estimated from an arbitrary sample of 100 leaves (total 1200 leaves per tree) to document within-canopy herbivory patterns. Three-way ANOVA was used to test for main effects and interactions of direction, strata, and host plant on ovipuncture density, initiated gall density, and performance followed by post-hoc REFGWQ tests to detect consistent herbivory patterns among trees. To examine within-leaf herbivory patterns and test the HVH, leaf area was estimated for all second year leaves attacked by *B. treatae* and correlated with gallformer performance and abundance. At the within-canopy level, gall and ovipuncture densities were highest in the low stratum, whereas performance was highest in the upper stratum. Leaf-level performance and abundance were not consistently correlated with each other or leaf size. These results suggest that larval performance is enhanced on more physiologically active regions within host canopies, but preferred oviposition sites are not linked to larval performance and neither oviposition preference nor larval performance is influenced by tissue size.

## INTRODUCTION

Descriptions of within-host phytophagous insect distribution and performance patterns are fundamental to understanding the ecology of plant-insect interaction systems. As such, herbivory patterns within host plants may be used to identify the conditions that mediate herbivore success (Faeth 1990, Rowe and Potter 1996, Whitham and Kearsley 1998, Blatt and Borden 1999) as well as predict large-scale herbivore population dynamics (Price et al. 1990, Price 1992). Here we examine the distribution and performance of a host and tissue specific gallformer for evidence of non-random within-host patterns and discuss their implications.

### Mediation of herbivory patterns within plants

Plant physiological processes and abiotic conditions known to influence herbivore habitat quality commonly vary along canopy level gradients (Kull and Kruijt 1999) and between adjacent tissues within host plants (Taper and Case 1987, Suomela 1996). Within tree canopies, for example, photosynthesis rates typically vary at both scales and consistently correlate with temperature and humidity extremes, incident radiation, leaf nitrogen and sugar content as well as leaf toughness and secondary metabolites (Lawton

1983, Dudt and Shure 1994, Suomela 1996, Kull and Kruijt 1999). As a result of consistent interactions between such plant-mediated factors and herbivore dispersal, enemy avoidance, and mating strategies, herbivory patterns may be non-random among and within hosts (Askew 1962, Lawton 1983, Damman 1987, Taper and Case 1987, Fernandez and Price 1991, Rowe and Potter 1996, Whitham and Kearsley 1998).

### Gallformer constraints

Though system-specific adaptations and developmental constraints make *a priori* predictions of within-plant herbivory patterns difficult (Fay and Whitham 1990, Schultz 1992), gallformer life history suggests strong selection for a positive relationship between the oviposition behavior of fertile females and larval performance (Price 1991). Specifically, gallformer larvae develop at the site of oviposition and thus experience micro-environmental conditions largely pre-determined by adult oviposition behavior (Weis et al. 1988, Price et al. 1992). This constraint thus promotes preference-performance linkage whether larvae adapt to or are pre-adapted to the conditions inherent to preferred oviposition sites (Price 1991, Faeth 1990, Pretzler and Price 1995, Carr et al. 1998, Pires and Price 2000, but also see Fay and Whitham 1990, Cronin and Abrahamson 1999). Positive relationships between indices of oviposition preference, herbivore abundance, and larval performance suggest preference-performance coupling at scales ranging from suspected host-plant species to tissues within known hosts. For example, abundance and performance of *Euura lasiolepis*, a shoot-galling sawfly, are greater within plants and among host populations containing relatively high concentrations of

preferred oviposition sites (Price 1992) indicating preference-performance linkage at both host population and among plant levels. However, as potential host cues identifying differences in site suitability vary with scale, the degree to which herbivores are capable of discriminating between oviposition sites of various quality may be limited (Whitham and Kearsley 1997, Cronin and Abrahamson 1999). Whitham and Kearsley (1997) suggest, for example, that the leaf-galling aphid, *Pemphigus betae*, prefers upper zones of birch canopies due to higher concentrations of more suitable leaves in the upper canopy; but within this region, fundatrices may be unable to detect finer scale variation in leaf quality. Despite such potential limitations, discrimination between various oviposition sites by herbivores at the host-tissue level is not uncommon (Whitham 1980, Bultman and Faeth 1986, Fritz et al. 1987, Price et al. 1987). And, the host vigor hypothesis predicts that herbivores which depend on plant growth processes for larval development will prefer and perform better on relatively fast growing tissues which consequently attain a large size (see for review Price 1991, Kimberling et al. 1990, Pires and Price 2000). Using shoot length as an index of vigor, Craig et al (1989) showed both oviposition preference for, and increased performance on, relatively vigorous host tissues by a stem-galling sawfly. Thus an understanding of the relationships and implications of system-specific gallformer distribution and performance patterns, including linkage between oviposition behavior and larval performance, may require description of herbivory patterns at a variety of scales.

## Effects of intraspecific competition

Intraspecific herbivore competition may directly influence the behavior and performance patterns of insects within host plants (Weis et al. 1988, Whitham 1986, Auerbach and Simberloff 1989, Faeth 1990). In sawfly populations, for example, females avoid ovipositing on previously attacked shoots suggesting a behavioral mechanism to reduce larval competition (Craig et al. 1990, Price 1992). In endophagous systems where multiple larvae may feed on a single host organ, enhanced individual performance at sites corresponding to optimal and preferred micro-environments may be offset by relatively high densities of conspecifics (Whitham 1986, Faeth 1990, but see also Sitch et al. 1988). As a result, within-host herbivore performance patterns as well as linkage between larval performance and oviposition preference may exist under herbivore density constraints.

## Objectives

In this study, we examine within canopy preference, abundance, and performance patterns of the host and tissue specific gallformer, *Belonocnema treatae* (Cynipidae), on its host plant *Quercus fusiformis*. Our goals are to: 1) determine whether *B. treatae* is distributed non-randomly within and among tree canopies according to previously documented gradients of photosynthesis rates, 2) examine linkage between oviposition preference and larval performance at both canopy and tissue levels by correlating herbivore distributions with performance, 3) test the host vigor hypothesis and examine

tissue-level herbivory patterns by correlating leaf size with *B. treatae* abundance and performance indices, and 4) document patterns of gallformer density among trees and determine if gallformer densities among plants influence the patterns described above.

## MATERIALS AND METHODS

### Study system

The host and tissue specific gallformer, *Belonocnema treatae* Mayr (Cynipidae: Hymenoptera), exhibits heterogony (Lund et al. 1998), a life cycle common to cynipids which consists of alternating generations between sexual and parthenogenic adults. In early spring, coincident with bud-break of its host plant, the sexual generation of *B. treatae* emerges from root galls and females oviposit on the underside of immature *Q. fusiformis* leaves. Gall development is arrested until leaves have matured and galls form along prominent lateral leaf veins before lignifying in late August to early September (Lund et al. 1998). Each attacked leaf, identified by the presence of characteristic ovipuncture scars, may hold 0 to over 30 galls that reach a maximum size of 9mm in diameter (pers. obs.). Based on haphazard samples of at least 1000 leaves per tree, estimated within-tree leaf gall densities range from 0 to over 3 galls per leaf (unpub. data). Parthenogenic females emerge from leaf galls in late fall and oviposit on root tissue to complete the cycle. Emergence from leaf galls varies from less than 0.1% to approximately 5% among infected trees and is a positive exponential function of gall size (Lund et al. 1998, Reynolds and Ott in prep).

The host plant, *Q. fusiformis*, is a late seral stage tree reaching heights of over 25m but more typically forming clonal clusters less than 10m in height. *Quercus fusiformis* is generally restricted to the Edwards Plateau region of central Texas and is considered a wintergreen species, retaining annual leaves until late February to early April. At a location approximately 150km to the southwest of our study sites, a survey of photosynthetic patterns within the canopy of *Q. fusiformis* demonstrated that carbon uptake rates decrease by as much as 40% from upper to lower strata and by as much as 20% along a gradient from the southeast to northwest side of the canopy (Owens 1996). According to that study, greatest differences in photosynthetic rates occur during the summer months and thus correspond with the development phenology of *B. treatae* leaf galls.

### Sampling methods

In fall 1997, ten trees were selected at random from each of 4 sites in the Edwards Plateau region of central Texas. Sites were at least 50km apart and individual trees selected within sites were no closer than approximately 50m. According to photosynthesis patterns within tree canopies detected by Owens (1996) at the more arid site described above, leaves were collected per tree from 12 areas corresponding to 3 heights (0-2m, 2-4m, and 4-6m) and 4 compass directions at each height (NE, SE, SW, and NW). Leaves (n = 100 per height x direction cell; n = 1200 per tree) were examined individually under a dissecting microscope for the number of *B. treatae* ovipuncture scars and distinguishable leaf galls of any size (initiated galls). After preliminary estimations

of gallformer densities (mean # galls/leaf) trees with greater than 0.05 initiated galls per leaf were selected for further study. In each of these 16 trees, initiated gall diameters were measured to the nearest 0.1mm. In Fall 1998, the same 16 hosts were sampled and scored as in 1997 with additional measurements of leaf length and width (nearest 0.1mm) recorded for each attacked leaf.

#### Canopy-level abundance, oviposition preference, and performance indices

Gallformer abundance was indexed on a galls per leaf basis by dividing the total number of initiated galls scored within each direction x stratum cell (n = 12 per tree per year) by the number of leaves (n = 100) within each cell. Similarly, the mean number of ovipunctures (initiated galls + ovipuncture scars) per leaf per cell indexed within-canopy oviposition preference. Larval performance within canopy regions was indexed as the number of galls 4.5mm in diameter or greater to the total number of ovipunctures within each canopy cell. Mature *B. treatae* emergence from field collections of galls has not previously been recorded for galls less than 4mm in diameter (Lund 1998 et al.). Hence, our measure of canopy-level gallformer performance is the ratio of initiated galls from which *B. treatae* is capable of emerging to the total number of oviposition attempts.

#### Canopy level statistical analysis

To determine general within-host patterns of *B. treatae* distribution and performance at the canopy level, indices were compared by stratum and direction among

trees. Height x direction cells lacking sufficient vegetation for sampling due to unequal crown development were assigned parameter estimates based on within-tree stratified grand means. Mean replacement of missing data more evenly accounted for both the large variation expected among trees and anecdotal evidence of stratified differences. The general linear model (GLM) approach to ANOVA was employed for detection of omnibus effects of tree, stratum, direction, and all two-way interactions on each index by year. Prior to analysis, preference and abundance indices were natural log transformed and performance estimates were arcsine square root transformed to meet the distribution assumptions of ANOVA (Sokal and Rohlf 1969). All tree effects were considered random with fixed effects assigned to stratum, direction, and stratum x direction. Main effects were explored further with post-hoc (REFGWQ) tests for individual mean differences. The REFGWQ test controls experiment wise error rates ( $\alpha=0.05$ ) and uses modified  $q$  statistics based on full model MSE to calculate difference ranges among groups from 1 to  $p$  (SAS Institute 1989). If significant, interaction terms were examined via graphical inspection.

The influence of overall gallformer density on canopy level distribution and performance patterns was examined by assigning trees to high ( $> 0.4$  galls/leaf) and low ( $< 0.4$  galls/leaf) density categories according to tree-wise differences detected by REFGWQ post-hoc tests in the original analysis. Density groups were analyzed separately and by year as above to see if density mediated patterns.

To examine linkage between oviposition preference and larval performance at the within-canopy level, Spearman correlation coefficients between preference, abundance and performance estimates per canopy cell were calculated across all trees for each year.

To examine the influence of tree density on the above patterns, trees were again grouped according to high ( $> 0.4$  galls/leaf) and low ( $< 0.4$  galls/leaf) densities and analyzed separately.

#### Leaf-level abundance, performance, and relative leaf area indices

Leaf-level gallformer abundance was indexed as the number of initiated galls on each leaf. Larval performance at the leaf level was indexed as the number of galls 4.5mm or larger divided by the total number of initiated galls per leaf. Leaf-level performance is thus the ratio of galls capable of rearing a mature gallformer to the total number of initiated galls present on any particular leaf. Length and width measurements of individual leaves were multiplied to approximate leaf area. In *Q. fusiformis*, leaves are generally oval in shape and approximated areas were thus over-estimated as indexed. However, we have confidently assumed that relative leaf size was maintained due to the consistent shape of leaves within trees.

#### Leaf-level and host vigor analysis

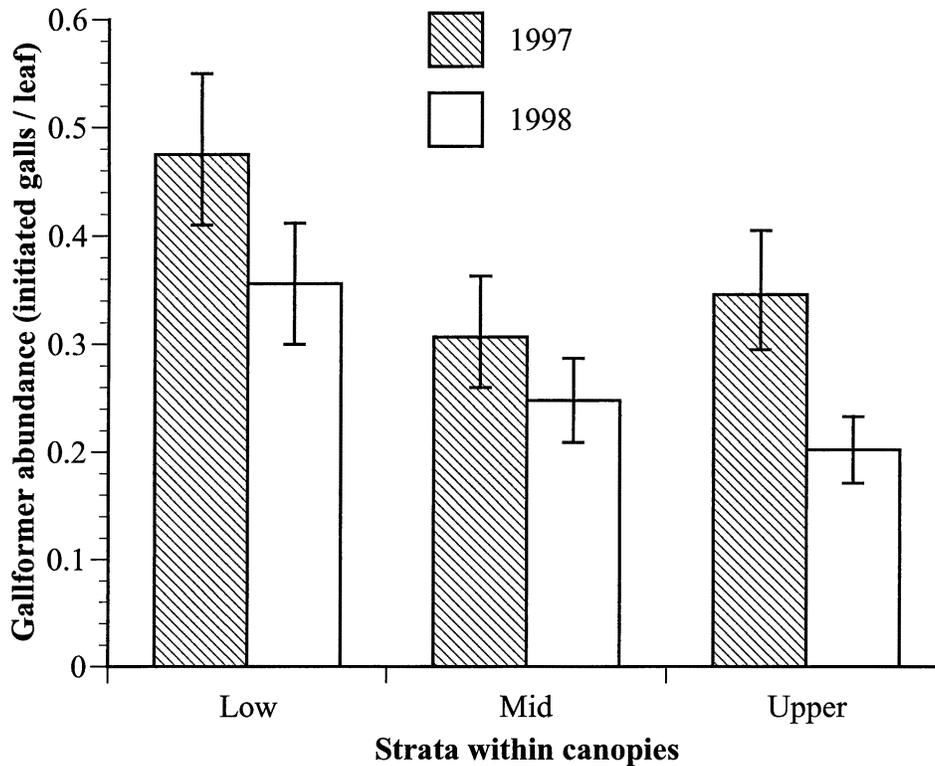
To test the host vigor hypothesis and examine leaf-level gallformer abundance and performance patterns, Spearman correlation coefficients were calculated between relative leaf area, gallformer abundance per leaf, and performance per leaf for each tree sampled in 1998. Leaf-level linkage of oviposition behavior to larval performance regardless of leaf size within individual trees was examined via correlation coefficients

between gallformer abundance and performance. To determine if the strength of relationships between leaf size and gallformer performance were influenced by gall density per leaf, Spearman correlation coefficients between leaf size and performance were calculated according to 4 gall density categories per leaf (1, 2-3, 4-6, >6) within each tree. Density dependent leaf area and gallformer performance relationships would suggest the degree to which intra-specific competition interacts with tissue size to mediate tissue-level habitat suitability.

## RESULTS

### Canopy level patterns of abundance, ovipuncture density, and performance

Patterns of gallformer abundance were well described in both 1997 ( $R^2 = 0.83$ ,  $p < 0.001$ ) and 1998 ( $R^2 = 0.93$ ,  $p < 0.001$ ) by a model including tree, strata, direction, and all two-way interactions as predictors of abundance. The majority of variation in both years was due to among tree differences (1997 = 65%,  $p < 0.001$ ; 1998 = 76%,  $p < 0.001$ ) indicating that leaf gall densities vary dramatically between trees. Canopy stratum was also a significant predictor of abundance in both years ( $F_{2,84, 1997} = 4.97$ ,  $p < 0.01$ ;  $F_{2,90, 1998} = 19.81$ ,  $p < 0.001$ ); and counter to gradients of photosynthesis rates, gallformer abundance was generally highest in the lower canopy stratum compared to higher strata by an average of 37.2% and 45.5% in 1997 and 1998 respectively (Fig. 1). There were significant interactions between tree and stratum in 1998 ( $F_{30,90} = 1.97$ ,  $p = 0.0012$ ) but not in 1997 ( $F_{28,84} = 0.59$ ,  $p = 0.94$ ). Upon inspection of 1998 tree x strata interaction plots (not shown), 11 of 16 trees exhibited highest gallformer abundance in the low stratum and were thus consistent with overall stratified abundance trends. Of the 5 remaining trees, patterns of abundance included: low = mid = upper (3 trees) and low = mid < upper (2 trees). The relative consistency of stratified abundance patterns within and between years indicates that lower canopy strata generally harbor more gallformer larvae per leaf than higher canopy regions.

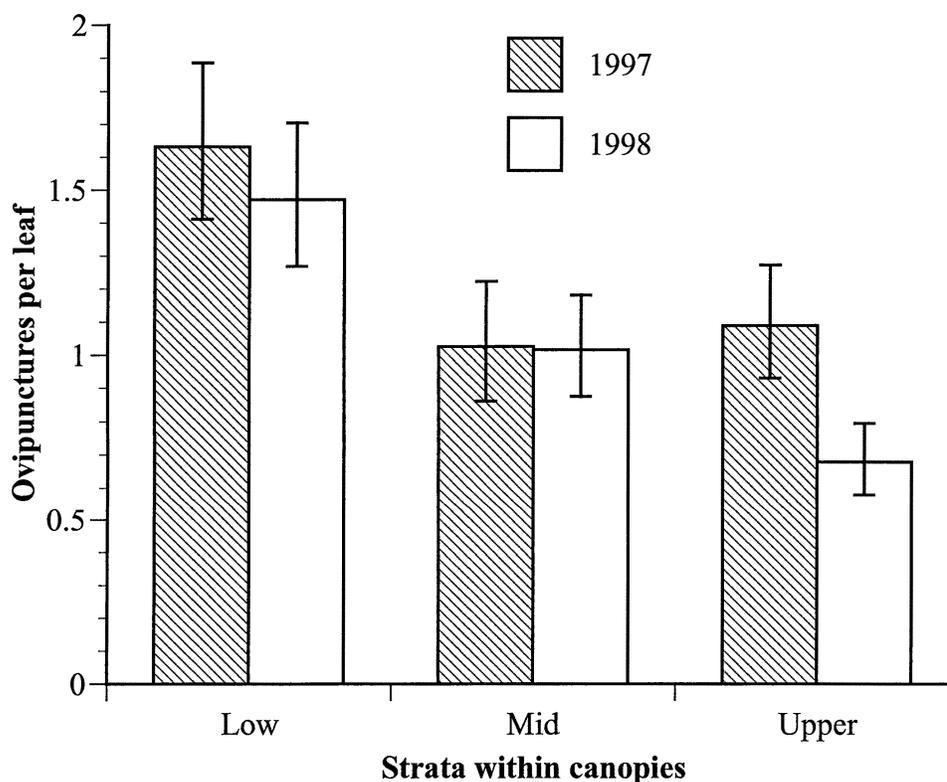


**Fig. 1** Untransformed mean ( $\pm$  SE) gallformer abundance indices among plateau live oaks according to height within canopies. Low strata abundance was greater than higher strata in both years (REFGWQ test,  $p < 0.05$ ).

Ovipuncture density (initiated galls + ovipuncture scars) per leaf was also well explained by a model with tree, stratum, direction, and two-way interactions as predictors ( $R^2_{1997} = 0.86$ ,  $R^2_{1998} = 0.95$ ). Mirroring gallformer abundance analyses and patterns, between tree differences accounted for the majority of variation (1997 = 69%,  $p < 0.001$ ; 1998 = 78%,  $p < 0.001$ ) with stratum as the only other significant predictor of ovipuncture density in both years ( $F_{2,84,1997} = 7.18$ ,  $p < 0.01$ ;  $F_{2,90,1998} = 59.32$ ,  $p < 0.001$ ). General stratified ovipuncture density patterns were counter to photosynthesis gradients and consistent with gallformer abundance patterns in that ovipuncture density was 42.8% (1997) to 55.1% (1998) higher on average in the lower canopy stratum than higher strata

(Fig. 2). Tree x strata interactions not present in 1997 ( $F_{28,84} = 0.60$ ,  $p = 0.94$ ) were highly significant in 1998 ( $F_{30,90} = 2.60$ ,  $p < 0.001$ ). Examination of 1998 tree x strata interaction plots yielded patterns consistent with the main effect pattern in 12 of 16 trees. Of the remaining 4 trees, stratified ovipuncture density patterns included: low = mid > upper (1 tree); low = mid < upper (1 tree); and low < mid = upper (2 trees). Oviposition attempts by *B. treatae* were thus generally more frequent in the lower regions of host canopies than in either middle or upper regions.

**Fig. 2** Untransformed mean ( $\pm$  SE) ovipuncture density indices among host plants

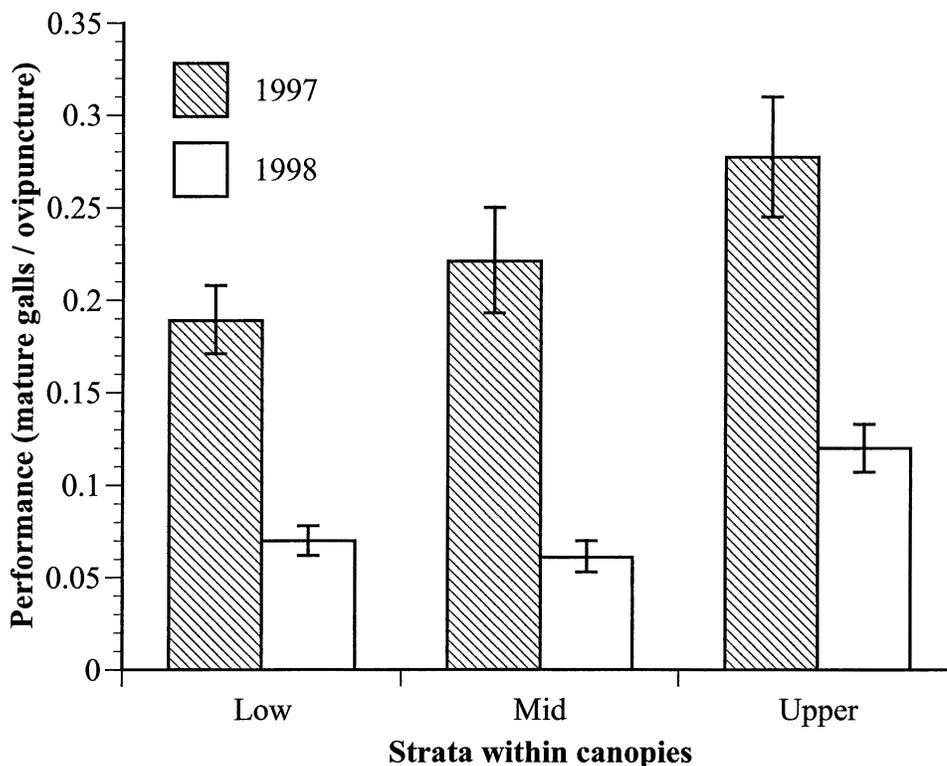


according to height within canopies. Low strata ovipuncture density was significantly greater than that of higher strata in both years (REFGWQ test,  $p < 0.05$ ).

Models with tree, strata, direction, and two-way interactions predicting gallformer performance (# mature galls per initiated gall) explained 79% and 77% of the total

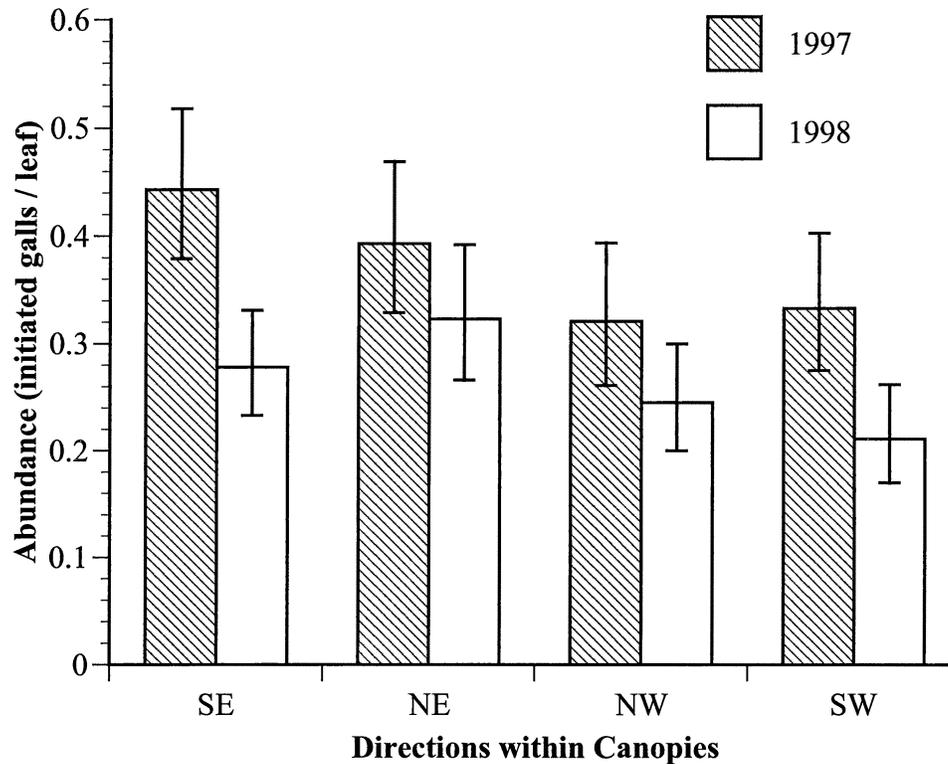
variation in 1997 and 1998 respectively. Among tree differences accounted for the majority of variation (1997 = 66%,  $p < 0.001$ ; 1998 = 46%,  $p < 0.001$ ) and strata was the only other consistent predictor between years ( $F_{2,84,1997} = 5.91$ ,  $p < 0.01$ ;  $F_{2,90,1998} = 5.50$ ,  $p < 0.01$ ). In accord with photosynthesis gradients yet counter to trends in abundance and ovipuncture density, performance indices were 30.2% (1997) to 34.9% (1998) higher in the upper versus middle and lower strata of host plant canopies (Fig. 3). Galls thus tended to reach sizes capable of producing *B. treatae* more frequently per oviposition attempt in upper, more photosynthetically active regions of tree canopies.

By direction, patterns of gallformer abundance (Fig. 4) and ovipuncture density (Fig. 5) were inconsistent among and within tree canopies between years. Direction was not a significant predictor of gallformer abundance ( $F_{3,84} = 2.00$ ,  $p = 0.12$ ) or ovipuncture density ( $F_{3,84} = 1.63$ ,  $p = 0.19$ ) in 1997. And, despite significant direction main effects in 1998 for both abundance ( $F_{3,90} = 5.78$ ,  $p < 0.01$ ) and ovipuncture density ( $F_{3,90} = 3.45$ ,  $p < 0.05$ ), general patterns were not consistently exhibited among trees. For example, upon inspection of tree x direction interactions associated with predicting gallformer abundance in 1998 ( $F_{45,90} = 2.07$ ,  $p < 0.01$ ), only 5 of 16 trees exhibited abundance patterns consistent with that indicated by Fig. 4. Of the remaining 11 trees, 9 unique patterns of abundance by direction were recorded with no pattern common to more than 2 trees. Similar results were obtained from inspection of significant tree x direction interactions present in the 1998 ovipuncture density analysis ( $F_{45,90} = 2.28$ ,  $p < 0.001$ ).

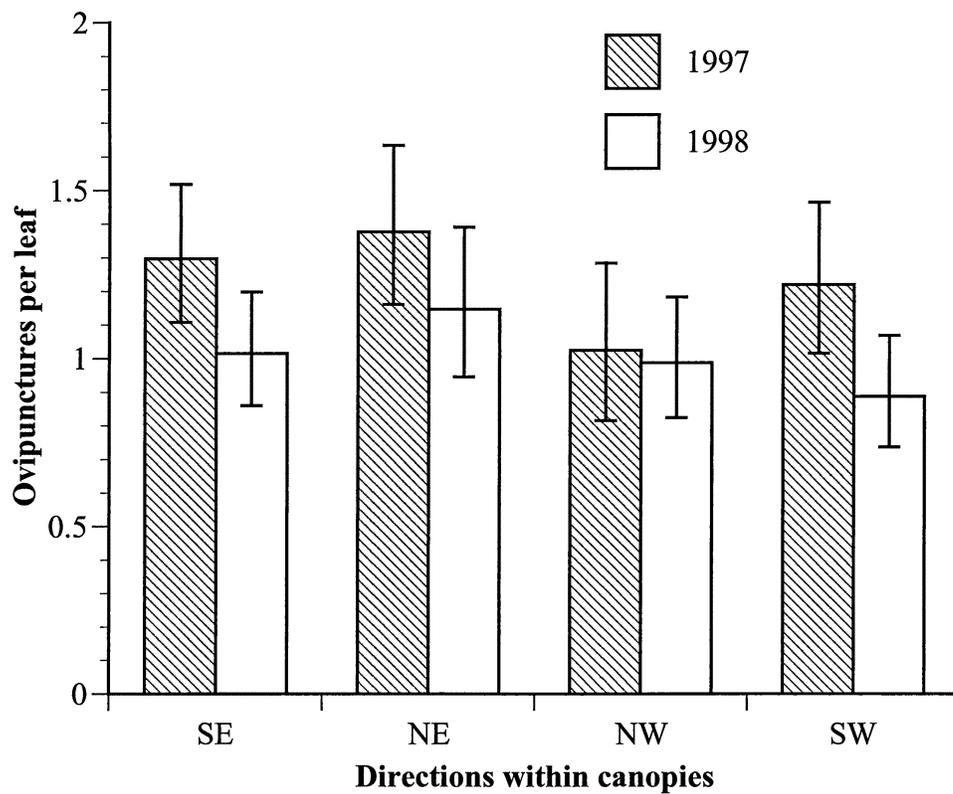


**Fig. 3** Untransformed mean ( $\pm$  SE) larval performance indices according to height within host canopies. Upper strata performance was significantly greater than lower strata in both years (REFGWQ test,  $p < 0.05$ ).

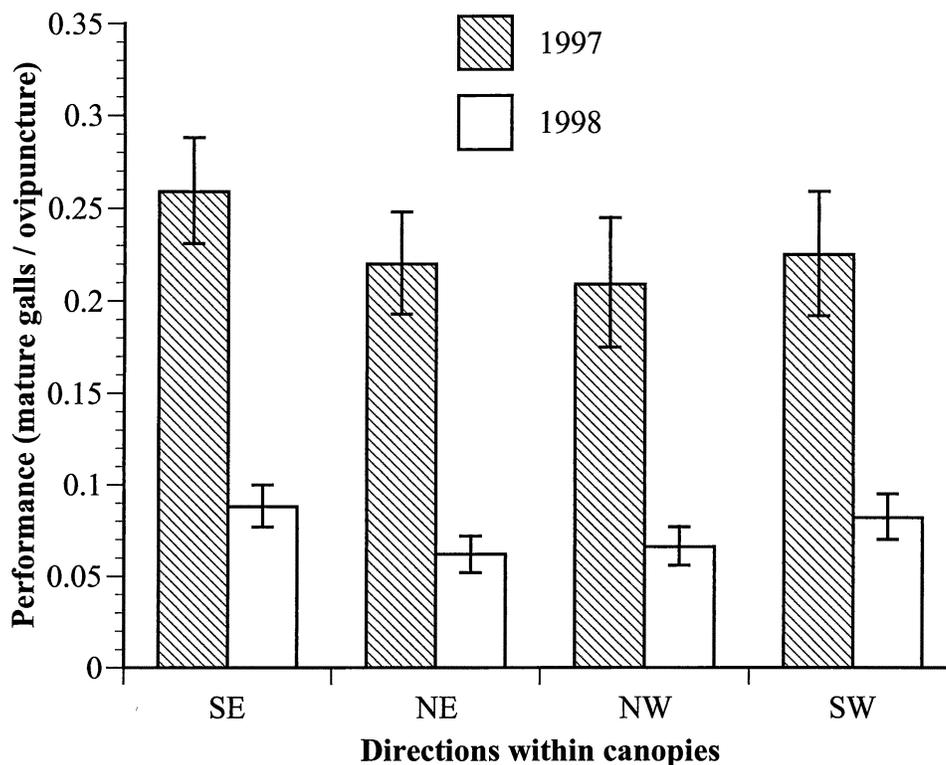
Direction was not a significant predictor of larval performance in 1997 ( $F_{3,72} = 1.01$ ,  $p = 0.40$ ) and was marginally significant in 1998 ( $F_{3,90} = 2.33$ ,  $p = 0.08$ ). Though effect sizes were insignificant at the  $\alpha = 0.05$  level, the magnitudes of average performance values were highest on the south side of tree canopies in both years (Fig. 6). Since photosynthesis rates are likely higher on the south sides of canopies, this result does not preclude a relationship between performance and relatively active regions of carbon uptake within tree canopies as suggested by stratified patterns.



**Fig. 4** Mean ( $\pm$  SE) gallformer abundance indices according to compass direction within canopies of host plants. In 1997, differences in means were not significant (REFGWQ test,  $p > 0.05$ ). In 1998, mean abundance in the northeast was greater than western canopy regions and southeastern abundance was greater than that of the southwest (REFGWQ test,  $p < 0.05$ ). Upon inspection of tree  $\times$  direction interactions however, patterns were not consistent among trees.



**Fig. 5** Mean ( $\pm$  SE) ovipuncture density indices by compass direction within host-plant canopies. In 1997, differences in means were not significant (REFGWQ test,  $p > 0.05$ ). In 1998, ovipuncture density in the northeast was greater than in the southwest (REFGWQ test,  $p < 0.05$ ). Upon inspection of tree  $\times$  direction interactions however, patterns were inconsistent among trees.



**Fig. 6** Mean ( $\pm$  SE) larval performance indices according to compass direction within host plant canopies. No differences were significant in either year (REFGWQ test,  $p > 0.05$ ).

The overall density of galls per tree did not alter gallformer abundance and ovipuncture density trends. In both high ( $> 0.4$  galls/leaf/tree) and low ( $< 0.4$  galls/leaf/tree) density categories analyzed by year, abundance and ovipuncture density was highest in low versus mid and upper strata. However, despite fewer trees within high ( $n_{1997} = 6$ ;  $n_{1998} = 7$ ) versus low ( $n_{1997} = 9$ ;  $n_{1998} = 9$ ) density categories, stratified effects were generally stronger in high density trees ( $p_{abund\ 1997} = 0.0013$ ;  $p_{abund\ 1998} = 0.0001$ ;  $p_{ovip\ 1997} = 0.0033$ ;  $p_{ovip\ 1998} = 0.0001$ ) than in low density trees ( $p_{abund\ 1997} = 0.076$ ;  $p_{abund\ 1998} = 0.0025$ ;  $p_{ovip\ 1997} = 0.026$ ;  $p_{ovip\ 1998} = 0.0001$ ). The magnitude of difference in gallformer

abundance and ovipuncture density between strata thus tends to increase as overall densities increase within tree canopies. However, the percentage of within-canopy leaf galls and ovipunctures found in the lower stratum within low density trees (42 to 47%) was virtually identical to that of high density trees (40 to 46%) indicating similar proportional differences between strata regardless of density. Thus the stronger high density effects were an artifact of effect sizes associated with wide ranges of average abundance (0.54 to 1.21 galls / leaf) and ovipuncture density (1.78 to 3.76 ovipunctures / leaf) relative to those of low density trees (0.09 to 0.24 galls / leaf and 0.32 to 0.90 ovipunctures / leaf). Though mean performance indices were consistently highest in the upper stratum across all density by year groups, signals were lost in high density 1997 trees ( $p = 0.31$ ) and low density 1998 trees ( $p = 0.15$ ). The lack of tree x strata interactions in the full analysis of performance described above suggests that signals were lost due to a reduction in sample size. Directional patterns of gallformer abundance, ovipuncture density, and performance according to density categories were either random or confounded by tree dependent interactions as described in the previous analysis.

### Canopy-level preference–performance linkage

As suggested by the above trends, preference–performance linkage did not exist at the within canopy level according to formal correlation analysis (Table 1). High density trees, in fact, exhibited highly significant negative correlation coefficients between ovipuncture density and performance suggesting an oviposition preference for relatively low performance regions within canopies. Ovipuncture density and abundance indices were highly correlated across density groups suggesting similar rates of gall initiation regardless of location within tree canopies.

**Table 1** Spearman correlation coefficients by year between indicated gallformer parameters estimated at the level of stratum by direction cells (n = 12 per tree) within tree canopies.

Density		1997		1998	
		Preference	Performance	Preference	Performance
Low n = 84; 1997 n = 120; 1998	Abundance	0.85***	-0.02	0.92***	0.26**
	Preference	-	-0.18	-	0.13
High n = 72; 1997 n = 72; 1998	Abundance	0.92***	-0.41***	0.85***	-0.20
	Preference	-	-0.67***	-	-0.54***

\*\* p < 0.01, \*\*\* p < 0.001

## Host vigor and relationships between leaf size, abundance, and performance

Within half of the study trees, one or more weak yet significant correlation coefficients were detected between indices of leaf size, gallformer abundance, and performance; however, few relationships were consistent among trees. The most common correlation occurred in 7 of 16 trees and described a positive relationship between abundance and leaf area. Though such a relationship is predicted by the host vigor hypothesis, the concomitant predictions of positive performance–leaf size and performance–abundance correlation coefficients were not realized together within any single tree (Table 2). This also suggests that leaf size alone is a poor predictor of both gallformer abundance and performance within tree canopies. When examined within gall density per leaf categories (1, 2-3, 4-6, and > 6 galls/leaf) within each tree, significant performance and leaf size relationships remained inconsistent and rare (Table 3). Therefore, the strength of relationships between relative performance and leaf size within host canopies is neither influenced by nor exists non-randomly among trees under potential gall density per leaf constraints.

**Table 2** Pearson correlation coefficients by tree in 1998. Column headings represent correlated parameters.

Tree Designation	Abundance / Leaf Area	Performance / Leaf Area	Performance Abundance
F0 (n=532)	0.262***	0.022	0.015
F1 (n=281)	0.029	-0.012	-0.069
F5 (n=376)	0.157**	0.018	0.081
H0 (n=148)	0.276***	-0.357***	-0.056
H1 (n=320)	0.071	-0.054	0.044
H2 (n=741)	0.238***	0.080*	-0.017
H3 (n=126)	-0.202*	-0.180*	-0.051
H4 (n=127)	0.032	0.070	-0.080
H5 (n=86)	-0.006	-0.045	-0.103
H6 (n=205)	0.075	0.003	0.022
H7 (n=220)	0.165*	-0.047	0.143*
H8 (n=446)	0.093*	-0.082	-0.014
H9 (n=167)	-0.098	0.020	-0.090
K3 (n=325)	0.228***	-0.143**	0.021
P2 (n=27)	-0.108	-0.110	-0.224
P9 (n=135)	0.086	-0.045	0.025

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

**Table 3** Leaf size and gallformer performance relationships as determined by Spearman correlation analysis according to gallformer density at the leaf level. Each cell represents the number of trees exhibiting a particular relationship. All correlation coefficients were calculated with samples of at least 5 leaves. Significant correlation coefficients ranged in magnitude from 0.14 (n = 254) to 0.43 (n = 17).

Correlation trends	Initiated leaf gall density per leaf			
	1 gall	2-3 galls	4-6 galls	>6 galls
None	11	11	11	3
+	0	2	0	1
-	5	3	1	0

## DISCUSSION

### Canopy-level abundance, ovipuncture density, and performance patterns

Consistently stratified patterns of *B. treatae* ovipuncture density, gallformer abundance, and larval performance suggest that sexual females under field conditions prefer to oviposit in the lower regions of their host canopies but that oviposition in higher regions is more likely to produce galls capable of rearing mature progeny. Given these contradictory preference / performance patterns and the ground to canopy dispersal path of sexual females emerging from root galls (Lund pers. comm.), oviposition on or near leaves initially encountered by searching females may explain the stratified oviposition preference patterns. Risks such as the exhaustion of parent energy reserves and increased predation rates typically associated with extended dispersal flights, however, have yet to be evaluated in this system at the canopy level. Anecdotally, captive adults remain active under environmentally controlled conditions for over two weeks, are surprisingly strong fliers, and rarely (once by a crab spider) has predation been observed (pers. obs.). Thus, the relative contributions of selection pressures and developmental constraints underlying stratified oviposition preference patterns remain unclear at present. Stratified performance patterns, contrary to those of oviposition preference, suggest the possibility of a positive relationship between *B. treatae* performance and the micro-environmental correlates of relative photosynthetic rates. Though generally applied to the relative

growth rates of host tissues, many gallformer systems exhibit positive relationships between larval performance and the physiological activity of their host tissues (Price 1991). Since *B. treatae* galls develop after leaves mature and are therefore unexposed to growth rate variation, the increased physiological activity associated with greater photosynthesis rates in upper canopy leaves may influence the stratified pattern of performance. Alternatively however, the relationship of leaf gall size to gallformer survivorship used as a basis for our performance index is heavily dependent on rates of parasitism by over a dozen known parasitoids and inquilines of *B. treatae* (Lund et al. 1998, Reynolds and Ott in prep). As yet, within-canopy attack patterns of natural enemies are unknown and may therefore interact with or subsume bottom-up influences to mediate detected performance patterns.

Directional patterns of gallformer abundance and ovipuncture density tended to be tree dependent and inconsistent between years. The inconsistent nature of these patterns suggests that differences in oviposition behavior by direction cannot be generalized among or within trees at the canopy level. However, such patterns are consistent with the constraints imposed by sexual female dispersal in that directional patterns within individual trees would depend on local conditions such as wind currents, root gall distributions near host plants, and their random interactions with the particular timing of emergence by individual females.

Relative larval performance by direction, unlike observed patterns by strata, did not coincide statistically with the assumed gradient of photosynthesis rates. The magnitudes of mean performance values were higher however on the more photosynthetically active south side of canopies in both years suggesting perhaps an

undetectable directional effect with the given sample size (Fig. 6). If physiological activity associated with photosynthesis of the host leaves does influence larval performance, the relatively small directional gradient of photosynthetic activity would be less dramatic than stratified signals. It is likely, in fact, that given the more mesic conditions of our study sites relative to that of Owens (1996), the realized directional photosynthesis gradients in our study trees could be significantly less than those reported by Owens (Barnes pers com).

#### Influence of canopy-level gall density on herbivory patterns

General trends of herbivore abundance, oviposition preference, and larval performance remain consistent across gall density levels among trees examined in this study suggesting no modification to current canopy-level herbivory patterns as a result of intra-specific competition. Furthermore, middle stratum gallformer abundance and ovipuncture density estimates were generally consistent with those of the upper stratum while middle stratum performance estimates more closely approximated those of the lower stratum (Figs. 1, 2, 3). This suggests that the higher performance within trees in the upper versus lower stratum is not solely a function of lower herbivore density.

#### Canopy level preference-performance linkage

The significance of preference-performance and abundance-performance relationships described by formal correlation analysis was dependent on density groups

(Table 1). With the exception of low density 1998 trees however, the negative (high density) and insignificant (low density 1997) correlation coefficients suggest, as do stratified patterns, that canopy level oviposition preference is not linked to larval performance. Perhaps as a consequence of drought, overall performance indices were much lower in 1998 and 16% of the performance values for low density trees were zero. This result may have confounded more general trends considering that, without the zero values, the remaining data exhibit relationships (abundance-performance  $r = -0.011$ ,  $p > 0.10$ ; preference-performance  $r = -0.158$ ,  $p > 0.10$ ) similar to those of low density 1997 trees. Given the stratified trends of low density trees, a lack of significant negative correlation coefficients between herbivore distribution indices and performance is likely an artifact of both low sample size and a limited range of distribution indices. It appears therefore that at least some degree of selection potential promoting the linkage between oviposition preference behavior and larval performance exists counter to the maintenance of current stratified herbivory patterns. However, assuming our performance index translates to higher gallformer emergence rates as expected, the strength of the implied selection for preference-performance linkage would depend on the absolute difference in emergence rates as well as the relative survivorship and fecundity of post-emergent adults from upper versus lower canopy strata. Actual modification of current canopy level herbivory patterns in response to this selection potential is subject to the capacity for either sexual females to discriminate between strata or larvae to specialize on lower strata leaves. Both potential mechanisms associated with the maintenance of larval performance patterns, i.e. relative physiological activity within host plants and natural enemy attack strategies, suggest relatively severe developmental constraints to larval

specialization on lower strata leaves. Conversely, oviposition preference for upper canopy leaves by sexual females would require seemingly simple modifications to current dispersal strategy and thus appears to be the more likely avenue of potential changes in current herbivory patterns.

#### Leaf-level herbivory patterns and host vigor

Strict interpretation of the host vigor hypothesis states that host tissue size indicates relative vigor and will predict relative larval performance and oviposition preference in gallformer systems (Price 1991). In *B. treatae*, leaf size was not a consistently significant predictor of either gallformer abundance or larval performance among canopies and leaves of various gall densities. Though gallformer abundance was positively correlated with leaf size in nearly half of the trees examined, the lack of corresponding leaf size–performance relationships suggests that little selection potential exists to promote host vigor hypothesis predictions as stated above. The positive abundance–leaf size correlation coefficients may instead be an artifact of sexual female preference for ovipositing on lower stratum leaves which are generally larger due to shading effects than higher strata leaves. However, in the sawfly system used to model the host vigor hypothesis (Craig et al. 1989), galls and host shoots develop simultaneously suggesting a relationship between larval performance and the relative physiological activity of its host tissue. The utility of tissue size as a predictor of vigor and, more specifically, physiological activity may be lost in the *B. treatae*–*Q. fusiformis* complex due to the fact that gall development is delayed until after leaves have attained their mature sizes. Thus,

while growth rates may indeed be higher for larger leaves during development, unisexual *B. treatae* larvae are not exposed to the physiological differences of host leaves until growth has ceased.

## Conclusions

Interpreting host vigor more broadly as a reference to relative physiological activity, stratified performance patterns do imply a positive relationship between relative host vigor and larval performance thus supporting one component of the host vigor hypothesis. The combination of patterns and relationships described above, however, suggest that preference-performance linkage on vigorous tissues or otherwise is not manifest at either the leaf or canopy levels in *B. treatae*. Selection pressures seemingly inherent to the life history of gallformers may thus be unrealized under system specific antagonistic pressures and/or developmental constraints (Fay and Whitham 1990, Cronin and Abrahamson 1999). The canopy and tissue level patterns of *B. treatae* herbivory described here do not necessarily preclude preference-performance linkage and/or potential gall density constraints on distribution and performance patterns from occurring at intermediate scales. Other leaf galling herbivores have been shown, for example, to rely on, and compete for resources from integrated networks of nearby leaves and branches (Fay et al. 1996, Larson 1998). Further exploration of the mediating factors underlying the various patterns described here will, however, help resolve the relative significance of antagonistic selection pressures imposed by the top-down and bottom-up dynamics of *B. treatae* and similar herbivores.

## LITERATURE CITED

- Askew RR (1962) The distribution of galls of *Neuroterus* (Hym: Cynipidae) on oak. *J Anim Ecol* 31: 439-455
- Auerbach M, Simberloff D (1989) Oviposition site preference and larval mortality in a leaf-mining moth. *Ecol Entomol* 14: 131-140
- Blatt SE, Borden JH (1999) Physical characteristics as potential host cues for *Leptoglossus occidentalis* (Heteroptera: Coreidae). *Environ Entomol* 28: 246-254
- Bultman TL, Faeth SH (1986) Leaf size selection by leaf-mining insects on *Quercus emoryi* (Fagaceae). *Oikos* 46: 311-316
- Carr TC, Roininen H, Price PW (1998) Oviposition preference and larval performance of *Nematus oligospilus* (Hymenoptera: Tenthredinidae) in relation to host plant vigor. *Environ Entomol* 27: 615-625
- Craig TP, Itami JK, Price PW (1989) A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology* 70: 1691-1699
- Craig TP, Itami JK, Price PW (1990) Intraspecific competition and facilitation by a shoot-galling sawfly. *J Anim Ecol* 59: 147-159

- Caouette MR, Price PW (1989) Growth of arizona rose and attack and establishment of gall wasps *Diplolepis fusiformans* and *D. spinosa* (Hymenoptera: Cynipidae). *Environ Entomol* 18: 822-828
- Cronin JT, Abrahamson WG (1999) Host-plant genotype and other herbivores influence goldenrod stem galler preference and performance. *Oecologia* 121: 392-404
- Dudt JF, Shure DJ (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology* 75: 86-98
- Faeth SH (1990) Aggregation of a leafminer, *Cameraria sp. nvo.* (Davis): consequences and causes. *J Anim Ecol* 59: 569-586
- Fay PA, Whitham TG (1990) Within-plant distribution of a galling adelgid (Homoptera: Adelgidae): the consequences of conflicting survivorship, growth, and reproduction. *Ecol Entomol* 15: 245-254
- Fay PA, Preszler RW, Whitham TG (1996) The functional resource of a gall-forming adelgid. *Oecologia* 105: 199-204
- Fritz RS, Gaud WS, Sacchi CF, Price PW (1987) Patterns of intra- and interspecific association of gall-forming sawflies in relation to shoot size on their willow host plant. *Oecologia* 73: 159-169
- Kearsley MJC, Whitham TG (1997) The developmental stream of cottonwoods affects ramet growth and resistance to galling aphids. *Ecology* 79: 178-191
- Kimberling DN, Scott ER, Price PW (1990) Testing a new hypothesis: plant vigor and phylloxera distribution on wild grape. *Oecologia* 84: 1-8
- Larson KC (1998) The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. *Oecologia* 115: 161-166

- Lawton JH (1983) Plant architecture and the diversity of phytophagous insects. *Ann Rev Entomol* 28: 23-39
- Lund JN, Ott JR, Lyon RL (1998) Heterogony in *Belonocnema treatae* Mayr (Hymenoptera: Cynipidae). *Proc Wash Entom Soc*
- Owens MK (1996) The role of leaf and canopy-level gas exchange in the replacement of *Quercus virginiana* (Fagaceae) by *Juniperus ashei* (Cupressaceae) in semiarid savannas. *Am J Bot* 83: 617-623
- Pires CSS, Price PW (2000) Patterns of host plant growth and attack and establishment of gall-inducing wasp (Hymenoptera: Cynipidae). *Pop Ecol* 121: 392-404
- Prezler RW, Price PW (1995) A test of plant-vigor, plant-stress, and plant-genotype effects on leaf-miner oviposition and performance. *Oikos* 74: 485-492
- Price PW, Roininen H, Tahvanainen J (1987) Why does the bud-galling sawfly, *Euura mucronata*, attack long shoots?. *Oecologia* 74: 1-6
- Price PW, Cobb N, Craig TP, Fernandes GW, Itami JK, Mopper S, Prezler RW (1990) Insect herbivore population dynamics on trees and shrubs: new approaches relevant to latent and eruptive species and life table development. In: Bernays EA (ed) *Insect-plant interactions*, vol 2. CRC Press, Boca Raton, FA, pp 1-38
- Price PW (1991) The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244-251
- Price PW (1992) Plant resources as the basis of insect herbivore population dynamics. In: Hunter MD, Ohgushi T, Price PW (eds) *Effects of resource distribution on animal-plant interactions*. Academic Press Inc, San Diego, pp 139-173
- Rowe WJ II, Potter DA (1996) Vertical stratification of feeding by Japanese beetles within linden tree canopies: selective foraging or height per se?. *Oecologia* 108: 459-466

- SAS Institute (1989) SAS/STAT user's guide, version 6, 4<sup>th</sup> ed, vol 2. SAS Institute Inc, Cary, NC
- Shultz JC (1992) Factoring natural enemies into plant tissue availability to herbivores. In: Hunter MD, Ohgushi T, Price PW (eds) Effects of resource distribution on animal-plant interactions. Academic Press Inc, San Diego, pp 175-197
- Sitch TA, Grewcock DA, Gilbert FS (1988) Factors affecting components of fitness in a gall-making wasp (*Cynips divisa* Hartig). *Oecologia* 76: 371-375
- Sokal RR, Rohlf FJ (1969) Biometry: the principles and practice of statistics in biological research. WH Freeman and Co, San Francisco
- Suomela J (1996) Within-tree variability of mountain birch leaves causes variation in performance for *Epirrita autumnata* larvae. *Vegetatio* 127: 77-83
- Taper ML, Case TJ (1987) Interactions between oak tannins and parasite community structure: unexpected benefits of tannins to cynipid gall-wasps. *Oecologia* 71: 254-261
- Weis AE, Walton R, Crego CL (1988) Reactive plant tissue sites and the population biology of gall makers. *Ann Rev Entomol* 33: 467-486
- Whitham TG (1980) The theory of habitat selection: examined and extended using *Pemphigus* aphids. *Am Nat* 115: 449-466
- Whitham TG (1986) Costs and benefits of territoriality: behavioral and reproductive release by competing aphids. *Ecology* 67: 139-147

## VITA

Preston Galusky was born in Kalamazoo, Michigan on June 4, 1970, son of Ed and Lynn Galusky. After graduating from Garland High School in 1988, he entered Texas A&M University and received the degree of Bachelor of Science in May 1993. He entered the Graduate School of SWTSU in August of 1997 to pursue a Master of Science degree in General Ecology. During his tenure at SWTSU, Preston was employed as a teaching assistant.

Permanent address: 1810 Meridian Way  
Garland, TX 75040

This thesis was typed by Preston Galusky.