

THE ROLE OF NATURAL ENEMIES IN DETERMINING THE RELATIONSHIP
BETWEEN GALL SIZE AND EMERGENCE SUCCESS OF A HOST-SPECIFIC
CYNIPID

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ABSTRACT

THE ROLE OF NATURAL ENEMIES IN DETERMINING THE RELATIONSHIP BETWEEN GALL SIZE AND EMERGENCE SUCCESS OF A HOST-SPECIFIC CYNIPID

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Large gall size is considered an adaptation to avoid parasitism for a number of insect gall-formers when gall size is positively correlated with emergence success and negatively correlated with the probability of parasitism. This interpretation is warranted only if gall size at maturity is not an artifact of parasitism before the potential benefit of this structural defense is fully realized. Here I investigate the significance of gall size variation for the parthenogenetic generation of the host-specific gall-former, *Belonocnema treatae* (Hymenoptera: Cynipidae), which induces galls on the leaves of

Quercus fusiformis, and is subject to high levels of mortality from a diverse community of parasitoids. Employing a field study and a parasitoid exclusion experiment I tested two primary hypotheses: (1) gall-former emergence success increases with gall size and (2) the distribution of gall sizes at maturity is independent of parasitism. To test hypothesis one, I collected mature galls in 1998, prior to gall-former emergence, from 5 trees in each of 3 natural populations (n = 21,000 galls total). Galls were measured, housed individually, and scored for gall-former emergence. Additional galls sampled from the same trees in 1998 and 1999 were used to investigate the relationship between mean gall size per tree and emergence success per tree. In all populations the probability of gall-former emergence increased exponentially with gall size. Moreover, mean gall size varied significantly among trees ($p = 0.0001$) and significantly non-uniform emergence success per tree ($p < 0.05$) was positively correlated with mean gall size per tree ($r = 0.72$, $DF = 13$, $p < 0.01$) in 1998 but not in 1999 ($r = 0.39$, $DF = 13$, $p > 0.10$). To test hypothesis two, I manipulated levels of parasitism in the field in 1999. On each of 6 *Q. fusiformis* trees, 15 branches were bagged prior to oviposition and coincident with the advent of the oviposition period bags were stocked with equal numbers of newly emerged and freshly mated female *B. treatae*. Following oviposition, parasitoid exclusion, bag control, and open treatments were created. When parasitoids were removed from the system emergence success was independent of gall size. Parasitoid attack significantly reduced mean gall size by 18% relative to unparasitized galls and generated bimodal gall size distributions which are significantly different ($p = 0.0001$) from the positively skewed gall size distributions of unparasitized treatments. Thus, a substantial portion of galls are parasitized before they reach full size

and the interpretation of increasing gall size as an adaptation to thwart parasitoids is not fully supported in this study system. In contrast to other studies purporting the adaptive significance of large gall size in avoiding insect parasitism, this investigation highlights the difficulty in establishing clear links between gall size dependent selection by parasitoids and evolutionary response to this selection in gall former systems associated with complex parasitoid communities which attack galls during all stages of gall development.

Introduction

Structural attributes of insect induced galls including gall size function as a last line of defense against predation by natural enemies, thus the interaction of gall morphology, natural enemy attack and the consequences thereof are a central feature of the ecology of gall-forming insects (Price et al., 1980; Cornell, 1983; Abrahamson and Weis, 1997). In these natural systems gall size (diameter) commonly varies both within and among host plants, and a major source of gall-former mortality is natural enemies (parasitoids) (Washburn and Cornell, 1981; Weis and Abrahamson, 1985; Weibs-Rijks and Shorthouse, 1992). It has been shown explicitly for a number of gall-formers that gall size and the probability of gall-former death due to parasitism interact through the limiting condition gall size places on parasitoid ovipositors in reaching gall-formers (Weis et al., 1985; Price and Clancy, 1986). The outcome of this interaction produces the commonly observed positive relationship between gall size and gall-former emergence success and a corresponding negative relationship between gall size and levels of parasitism (Jones, 1983; Weis et al., 1985; Weis and Abrahamson, 1985; Price and Clancy, 1986; Price, 1988; Zwolfer and Arnold-Rinehart, 1994).

Gall size plays a major role in determining the survival and hence reproductive contribution of gall-formers in study systems that exhibit tight correspondence between emergence success and gall size. The consistent, size dependent mortality due to parasitism and the apparent immunity large galls afford their inhabitants from parasitism

strongly suggests that large gall size is an adaptation to thwart parasitoid attack. The existence of additive genetic variance for the trait “gall size” has been shown for *Eurosta solidaginis* (Weis and Abrahamson, 1986; Weis and Gormon, 1990) and therefore is plausible in other gall forming insects. The consequence of extreme gall size dependent mortality during successive generations has profound implications for gall-former populations. Given the heritable nature of gall size, under a positive directional selection regime, the trait gall size could evolve in response to predation pressure from parasitoids.

Support of the notion that gall size evolution is a response to natural enemies would be especially satisfying in cynipid gall-formers. These study systems are characterized by species rich and abundant parasitoid guilds that inflict a correspondingly high level of mortality (Washburn and Cornell, 1981; Cornell, 1983; Askew, 1984; Weibs-Rijks and Shorthouse, 1992; Schonrogge et al., 1996). Cynipid gall-formers also exhibit a wide variety of gall morphological features which appear to protect gall-formers from natural enemies (e.g., hairy galls, hard galls, large galls, ephemeral galls, galls with air spaces) (Cornell, 1983; Price et al., 1987). These features of cynipid systems are logical requirements for proposing that gall morphological evolution is in response to parasitoids. Therefore, clearly demonstrating that increasing gall size is an adaptation to thwart parasitoids would support the argument that gall size may evolve in response to gall size dependent attack by parasitoids.

Attributing the adaptive significance of large gall size to parasitoid avoidance requires testing the assumption that parasitoids only attack mature galls; i.e., parasitoids respond to the natural range of gall size. If parasitoids attack galls during early gall development and subsequently halt further gall growth, the gall size distribution at

maturity is an artifact of levels of parasitism early in gall growth phenology. In this instance large galls, instead of representing extreme gall-former genotypes prone to large gall growth, may simply represent the subset of galls that by fortune alone escaped early parasitism. Consequently, gall-former genotypes capable of inducing large galls would be removed randomly from populations by parasitoid attack early in gall development, and the response to directional selection for gall size would be substantially diluted. If it were shown that parasitoids determine gall size, the adaptive significance of large gall size would be more complicated and the frequent explanation of gall size evolution as a response to parasitoids should be questioned.

In prior research with *Belonocnema treatae* (Hymenoptera: Cynipidae) host-specific on *Quercus fusiformis*, Plateau Live Oak, emergence success was shown to increase exponentially with gall size within a single study site (Lund, 1998). This finding in combination with the high level of mortality inflicted by the guild of natural enemies (>95% for 1995, 1996, and 1997-*unpublished data*) have led to an current interest in this system of how to interpret the exponential relationship between emergence success and gall size. The objectives of the research presented herein are (1) to corroborate the prior result at multiple sites (populations) that gall size and emergence success are positively related and (2) to test the null hypothesis that parasitoids do not determine gall size by manipulating levels of parasitism in the field. If gall size and successful emergence are linked at three separate sites I can conclude that this relationship is a general feature of the ecology of *B. treatae*. In addition, it would support the interpretation of large gall size as an adaptation to thwart parasitoids. However, if parasitoids determine gall size this would complicate the interpretation of large gall size as solely an adaptation to thwart

parasitoids and possibly question this interpretation in other study systems where the assumption that parasitoids do not determine gall size has not been tested rigorously.

Natural History of Study System

Belonocnema treatae (Hymenoptera: Cynipidae) is a host-specific phytophagous insect which induces gall formation on both leaf and root tissue of its host plant Plateau Live Oak, *Quercus fusiformis*. *B. treatae*, like many cynipids, exhibits heterogony with an all female parthenogenetic generation, in which larvae develop in unilocular leaf galls located on the lateral veins of the undersides of leaves, and a sexual generation in which males and females develop in multilocular galls located on underground root tissue (Lund et al., 1998). In early spring coincident with *Q. fusiformis* bud-break (March to April), the sexual generation emerges from root galls, mates, and females oviposit on immature leaves. Gall growth commences when leaves have matured (May to June) and proceeds for approximately four weeks. Galls lignify in late August through September and female parthenogenetic *B. treatae* subsequently emerge from leaf galls starting in mid-October and lasting to mid-December. These females descend to *Q. fusiformis* roots and oviposit thereby completing the life cycle.

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 of central Texas and is considered a wintergreen species, retaining annual leaves until late February to early April. Gall density (# galls per leaf) may vary considerably within and among host plants. Individual leaves may hold 0 to over 30 galls that reach a maximum size of 9 mm 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 (Galusky, 2000).

Previous study has shown that extremely low emergence success from leaf galls is a general feature of this study system (1996: 0.45%; 1997: 0.86%). The bulk of this mortality is attributable to 13 known species of parasitic hymenoptera comprising 7 families (n = 3 species parasitoids: Ormyridae, Torymidae, Pteromalidae; n = 4 species inquilines: Cynipidae, Eurytomidae; n = 1 species hyperparasitoid: Eurytomidae; n = 5 species life habit unknown: Braconidae, Eulophidae) and a number of unidentified non-hymenopteran species, all reared from randomly collected galls at a single site in each of two years (1996 and 1997) (Lund, 1998). Also shown was that gall-former emergence success increased exponentially with gall size ($R^2 = 0.92$), the minimum gall size in which *B. treatae* emerged was 5.25 mm, and 95% of *B. treatae* emerge from galls in the upper 50% of all gall sizes. Regardless of the life habit of the attacking species (i.e., inquiline or parasitoid), *B. treatae* has never been reported to emerge from the same gall following the emergence of a natural enemy. Therefore, all natural enemies that emerge from leaf galls are hereafter referred to as parasitoids. The emergence phenology of these parasitoids is roughly bimodal (Sep-Oct and May-Jul) with a third less pronounced emergence window (Feb-Apr) all occurring within a single year. Thus, parasitoids are capable of attacking *B. treatae* galls during all periods of gall development (initiation: May to June; growth: June to July; maturation: August to September) constantly threatening the successful development and emergence of individual gall-formers.

Methods

The two primary objectives were addressed in separate research phases. The first phase consisted of a one-year (1998 field season) gall sampling study at three geographically distinct sites (5 trees/site), Freeman Ranch (FR), Devil's Backbone (DB), Honey Creek (HC), which allowed me to examine the relationship between gall-former emergence success and gall size at the individual gall level. All sites are located in the Eastern portion of the Edwards Plateau region of Central Texas (FR, Hays Co, 98° 00' WLong, 29° 55' NLat; DB, Comal Co, 98° 11' Wlong, 29° 56' NLat; HC, Comal Co, 98° 29' Wlong, 29° 52' NLat). Additional galls were sampled from each study tree for two consecutive field seasons (1998 and 1999) to investigate overall gall-former emergence success per tree, to measure among tree and between year variation in gall size, and to determine the relationship between mean gall size per tree and emergence success per tree. The second phase consisted of a parasitoid exclusion experiment, conducted on 6 trees at FR during the 1999 field season, to compare the distribution of gall sizes at maturity and the relationship between emergence success and gall size in the presence and absence of parasitoids.

Field Study

Individual gall level variation in gall size. To confirm the generality of *Belonocnema treatae* emergence success as an exponential function of gall size I first collected haphazard samples of leaf galls from each of 5 trees at 3 geographically distinct

sites (populations) during the 1998 field season just prior to the onset of *B. treatae* emergence in October 1998. By timing the point of gall collection immediately before *B. treatae* emergence, galls were exposed to maximum levels of natural enemy attack possible, thus ensuring that emergence success estimates corresponded to natural levels of emergence in the field. To even out possible canopy level variation on emergence success of the gall former, galls were collected from three tree strata: low (2 meters), middle (4 m), and upper (7 m). Galls were then removed from leaves, sorted by size class, and those galls > 3.35 mm (prior study showed no *B. treatae* emerge from galls < 5.25 mm) were placed individually into gelatin capsules and incubated where light and temperature regimes were set to reflect current field conditions. Capsules were monitored and scored for emergent *B. treatae*, starting immediately after they were incubated and lasting until mid-December. At the conclusion of the emergence period all incubated galls were measured to 0.01 mm with digital calipers. By collecting galls just prior to emergence and individually incubating them I can link individual gall-formers' fate (success/failure) to gall size.

The relationship between gall size and emergence success was assessed for each site by pooling information from individual galls over all five trees within the study sites. Student's *t*-tests were used on a per study site basis to compare mean gall size between failed and successful galls. A total of 21,000 galls were collected from the three sites but because no *B. treatae* have been observed to emerge from galls less than 5.25 mm (see above study system section) nor have they emerged from damaged galls (wrinkled or partially broken), only intact galls, > 3.35 mm diameter were used for these analyses. Thus, the actual sample size was 12,969 galls ($\bar{X}_{\text{tree}} = 865$ galls; $\bar{X}_{\text{site}} = 4323$ galls).

Removing small or damaged galls insured that mean comparisons and regression analyses performed were conservative. The relationship between *B. treatae* emergence success and gall size at each study site (data pooled across 5 trees/site) was determined by plotting percent success in 0.50 mm gall size bins [(#emergent *B. treatae*/ # galls in bin)*100] with each bin size value. Using the incubated galls, emergence success estimates were also calculated on a per tree basis [(#emergent *B. treatae* per tree/ # galls sampled per tree)*100]. These data allowed me to address the possibility that the incubator may bias emergence success estimates by subsequently comparing emergence success per tree in incubated samples between emergence success estimates of additional samples of galls not reared inside of the incubator (see below).

Host plant level variation in gall size. For the two year survey of *B. treatae* emergence success per tree and gall size per tree the additional samples of galls taken from each study tree were returned from the field to the laboratory where all galls were removed from leaves and placed into collection devices. These collection devices were modified 1qt Mason jars containing an inverted plastic vial attached to an inverted funnel glued to the lid of the jar, and these jars were stored outside in ambient conditions. Emergent *B. treatae* and parasitoids readily flew into the plastic vials. Vials were periodically removed, and their contents were scored for adult *B. treatae*. Emergence success per tree (# emergent insects/# of galls in sample*100) was determined and compared to emergence success per tree from galls in the incubator (G-test). Emergence success estimates per tree were independent of method of insect rearing ($G = 2.84$, $DF = 13$, $0.999 < p < 0.995$). This comparison thus ensured that the rearing conditions of the incubator (capsules vs. jars) did not bias success estimates.

Sources of gall size variation were examined by comparing mean gall size per study tree of subsampled galls ($n = 85$) among sites, trees, and between years. Gall size data from subsampled galls were analyzed using ANOVA specifying study tree as a random effect, year as a fixed effect, a site by year interaction term, and tree(site) as a nested effect. Finally, each study year's gall size and emergence success data were used to correlate average gall size per tree with emergence success per tree thereby testing the hypothesis that a relationship exists between emergence success and gall size at the host plant level.

Field Bagging Experiment

To test the null hypotheses that parasitoids do not affect gall size and that the relationship between emergence success and gall size is equivalent in the presence and absence of parasitoids I conducted a manipulative field experiment. The experiment was performed using six Plateau Live Oaks during the 1999 field season at Southwest Texas State University's Freeman Ranch (FR). I began by enclosing 90 oak branches (15 bags/tree on each of 6 trees) with Nytex screening bags prior to spring leaf flush and *B. treatae* bisexual generation emergence. Bags were constructed of nytex screening (0.67 m X 0.45 m), and were constructed with Velcro closures at each end. At the onset of new leaf growth on the experimental trees, 10 newly emerged *B. treatae* females which had been allowed to mate in the lab were added to each bag. Oviposition was verified for all bagged branches by examining leaves for oviposition scars which appear shortly after the time of oviposition. Following this inspection five bags were removed from each tree to create the (+) parasitism treatment (control). Velcro ends were unfastened from five

additional bags per tree to create a (+) parasitism treatment in which leaves with galls were still covered by the nytex screening (bag control). Bags were left secured to the remaining five branches to create the (-) parasitism treatment. Treatments were present from March to October during the entire period of gall development: initiation, growth and maturation. Efficacy of the (-) parasitism treatment was confirmed after galls were harvested and examined closely for parasitoid emergence holes; 9 of 2234 galls = 0.4% in the (-) parasitism treatment contained parasitoid emergence holes compared to 1158 of 1888 = 61.3% in the (+) parasitism treatment.

Just prior to unisexual generation emergence (early October 1999) experimental branches were harvested and returned to the laboratory where galls were removed from leaves, sieved, and the larger galls (>3.35mm in diameter) were placed individually into gelatin capsules and incubated. Capsules were scored for emergent insects (every 2-3 days). At the conclusion of *B. treatae* emergence the diameters of all galls were measured to 0.01mm.

Analysis of variance of the effects of treatment and tree on gall size was performed using the Statistical Analysis System. I used a mixed model two way factorial design specifying treatment as a fixed effect and tree and tree X treatment as random effects. Tree was specified as a random effect since the experiment trees were a random sample of the population of *Q. fusiformis* attacked by *B. treatae* in the Central Texas, Hill Country savanna. Additionally, I was more concerned with among treatment effects on gall size than among tree effects on gall size. I used the interaction effect MSE as the denominator of the F statistic in testing the significance of the fixed treatment effect. Means comparisons were made among treatment levels pooled over all trees and among

treatment levels within each individual tree using the procedure REGWQ. The difference in mean gall size between the (-) parasitism and bag control is the effect due to parasitoids. The difference in mean gall size between the (+) parasitism treatment and bag control is the plant effect on gall size via leaf shading of the nytex screening. The possible confounding effect of gall density on gall size was addressed by correlating mean gall size per leaf with gall density per leaf within each treatment.

B. treatae emergence success was determined within 0.50 mm gall size classes (# of emergent *B. treatae* / # galls per size bin*100) and on a per treatment basis (# of emergent *B. treatae* per treatment / # galls per treatment*100). Expected emergence success values, assuming equal emergence rates in all size classes, were generated by multiplying the overall emergence rate for each treatment by the number of galls in each 0.50 mm size bins. Observed emergence rates were compared to expected emergence rates on a per tree basis using Chi Square goodness of fit tests. If observed emergence rates were not significantly different from expected in each treatment then *B. treatae* emergence success was deemed independent of gall size.

Results

Field Study

Individual gall level variation in gall size. The positive relationship between emergence success and gall size is a general pattern in the ecology of *Belonocnema treatae*, repeatable in space and time. At all study sites, *B. treatae* emerged more frequently from larger galls than smaller galls suggesting that individuals inhabiting large galls are at a selective advantage to those in small galls. At each of the three sites (populations) successful galls were significantly larger than failed galls (Figure 1) (FR: $\bar{X}_{\text{succ}} = 6.66$ mm, $\bar{X}_{\text{fal}} = 5.56$ mm ($t = 8.55$, DF = 5370, $p = 0.0001$); HC: $\bar{X}_{\text{succ}} = 7.19$ mm, $\bar{X}_{\text{fal}} = 5.62$ mm ($t = 9.13$, DF = 2564, $p = 0.0001$); DB: $\bar{X}_{\text{succ}} = 6.91$ mm, $\bar{X}_{\text{fal}} = 5.58$ mm ($t = 16.43$, DF = 4809, $p = 0.0001$). At each site 50% of emergent *B. treatae* came from galls in the upper 10% of the size frequency distributions. These simple gall size contrasts emphasize the great disparity between failed and successful gall diameters indicating that gall size is a major factor influencing the successful emergence of gall formers.

Emergence success increased exponentially with increasing gall size at all study sites corroborating the results obtained at the Freeman Ranch site in 1997 (Figure 2). Consequently, the probability of gall-former emergence was highest for inhabitants of the largest galls (up to 50% emergence success). In contrast, no adults emerged from the smallest galls, and moreover, emergence rates were miniscule for all galls in the lower 50th percentile of gall sizes. Overall emergence success at each site was 0.95% at FR,

0.97% at HC, and 3.10% at DB, or equivalently percent mortality of 99.5%, 99.3%, and 96.9%, respectively again corroborating high mortality estimates for this study system in previous years. Parasitoids are the root cause of the bulk of this mortality (Lund, 1998) which apparently is focused on relatively small galls. The relatively high probability of emergence success from large galls in comparison to extreme gall former mortality in smaller galls supports the contention that for *B. treatae* large gall size is an adaptation to thwart parasitoids.

Host plant level variation in gall size. Given the exponential relationship between emergence success and gall size at the individual gall level I can predict that at the host plant level emergence success per tree and gall size per tree will be positively related. Emergence success per tree was positively correlated with mean gall size in 1998 ($r = 0.72$, $DF = 13$, $p < 0.01$) but not in 1999 ($r = 0.39$, $DF = 13$, $p > 0.10$) (Figure 3) indicating that the strong link between emergence success and gall size evident at the individual gall level (Figure 2) can translate to the host plant level. In 1998 percent emergence success was significantly non-uniform among trees ($G = 145$, $DF = 14$, $p < 0.05$) varying by over an order of magnitude (0.11% to 7.36%). Mean gall size varied significantly among trees within sites (nested effect tree(site): $F = 48.05$, $DF = 12$, $p = 0.0001$) and emergence success was higher on host plants which had large average gall size. This result is consistent with data from individual galls that larger galls realize an exponentially higher probability of emergence success (i.e., lower probability of parasitism) than smaller galls. However, the association of gall size and emergence success at the host plant scale was not observed in 1999 because levels of gall former emergence were extremely low. In fact, only 8 galls produced emergent *B. treatae* in

1999 compared to 208 galls in 1998. No *B. treatae* emerged from galls sampled from 11 of 15 trees in the 1999 field season resulting in zero emergence success estimates thereby precluding the ability to detect positive correlation between emergence success per tree and mean gall size per tree.

Since field levels of gall-former mortality are extremely high (> 99%), and the probability of natural enemy attack is higher for smaller galls, low average gall size and/or low gall productivity could result in zero emergence success estimates. While the year effect did not significantly account for variation in mean gall size ($F = 4.08$, $DF = 1$, $p = 0.1806$) the year X site interaction effect was highly significant ($F = 89.04$, $DF = 2$, $p = 0.0001$). Inspection of the interaction plot shows mean gall size does vary between years but the magnitude of this variation is site dependent (Figure 4). At all sites mean gall size was significantly lower in 1999 than 1998, raising the possibility that lower gall size facilitated extreme mortality from natural enemies (1 *B. treatae* emerged from galls collected at these two sites combined). Moreover, gall abundance was extremely low in some study trees (sample sizes ranged from 9 to 1,180 galls per tree), thus despite equally intensive sampling effort, measurable success estimates were precluded by small sample size.

That I detected a positive relationship between *B. treatae* emergence and mean gall size per tree suggests that the host plant may mediate the susceptibility of gall-formers to natural enemy attack through plant-induced variation in mean gall size, but this relationship may only be observable in years when conditions for *B. treatae* emergence are favorable (e.g., 1998). This result directs attention to a central issue in the interpretation of these positive relationships between gall size and emergence success and

the corresponding interpretation of the apparent adaptive nature of large gall size.

Does parasitism occur after galls are full grown and/or does parasitism occur when galls are immature halting further gall growth. If parasitoids respond to the natural range of gall sizes at maturity then gall size determines susceptibility to parasitoid attack, but if parasitoids attack prior to gall maturity and halt gall growth then the probability of parasitism could be independent of gall size and the positive relationship between gall size and emergence success could be an artifact of levels of parasitism early in gall development.

Field bagging experiment

Parametric and non-parametric comparisons of gall size distributions under manipulated levels of parasitism showed that parasitism influences gall size. This result does not support, in contrast to results from the gall sampling study, the gall diameter hypothesis and casts doubt on parasitoid avoidance as an adaptive explanation of large gall size for *B. treatae*. The shape and location parameter of gall size distributions at maturity, were significantly different among gall groups exposed to different levels of parasitism (Kruskal Wallace test, $\chi^2 = 1523.5$, DF = 2, $p < 0.0001$). Gall size was distributed bimodally in the bag control and (+) parasitism treatments (Figure 5). In contrast, gall size in the (-) parasitism treatment was skewed towards the larger gall size classes. The striking deviations in the shape and locations between parasitized and unparasitized gall size distributions provide evidence that parasitism has an effect on gall size and that the source of this effect arises from the preponderance of galls smaller than 3.00 mm only present in the parasitized treatments, (+) parasitism and bag control. This

result suggests that parasitism may occur when galls are immature and those galls that are attacked are prevented from fully developing.

Parametric analysis of the mean gall size data showed that the effect of parasitism significantly reduced mean gall size. The overall model was highly significant ($F = 7.27$, model $DF = 17$, $P = 0.0001$) and accounted for 65% of the total variation in mean gall size. The fixed effect, level of parasitism (bag control, (-) parasitism, (+) parasitism) accounted for the most mean gall size variation ($P = 0.0001$), followed by the random effect tree ($P=0.0020$), but the interaction effect, tree X treatment, was not significant ($P = 0.25$). Mean gall size was significantly smaller in the bag control ($\bar{X}_s = 4.41$) than in the (-) parasitism treatment ($\bar{X}_s = 5.37$), but significantly larger than in the (+) parasitism treatment ($\bar{X}_s = 3.59$) (Figure 6).

Since the average level of parasitism (percent of galls with parasitoid emergence holes) was unequal between the bag control treatment and the (+) parasitism treatment (bag control: 35%; (+) parasitism: 68%) it was difficult to interpret the plant effect on gall size via shading from the bag). It is possible that the bag compromised local plant defenses against herbivory resulting in more carbon allocated for gall growth. However the observed increase in bag control mean gall size relative to the (+) parasitism treatment may have been the outcome of lower levels parasitism; i.e., fewer absolute numbers of galls were parasitized prior to maturity. Nevertheless, controlling for the positive influence of the bag on gall size, the effect of parasitism reduced mean gall size by 18.1%.

The relatively high frequency of small galls (<3.00mm) was primarily responsible for reducing gall size in the parasitized treatments (bag control and (+) parasitism) and

correspond to the lower end peak in the bimodally distributed frequency of gall sizes in those treatments. Analysis of the mean gall size data without this subset of galls using the same model as above resulted in non-significant main effects of (treatment, $P = 0.1135$; tree, $P = 0.2252$) and a significant tree X treatment interaction term ($P = 0.0015$). Therefore, when the small subset of galls was not present in the analysis the effect of parasitism went away which suggests the source of parasitism's negative effect on gall size originates from these galls. This analysis also supports the argument that the source of the bag effect on gall size was the disparate level of parasitism between the bag control and (+) parasitism treatments since no treatment effect on gall size was detected when the small subset of galls was removed from the analysis.

Assuming the adaptive significance of large gall size was parasitoid avoidance then in the (+) parasitism treatment I would expect (1) observed emergence success to exhibit positive deviations from expected in large galls and negative deviations from expected in small galls; i.e. a positive relationship between emergence success and gall size, and (2) mean gall size of successful emergents should be significantly larger than failures. In the (-) parasitism treatment (1) observed and expected emergent success should not be significantly different; i.e. emergence success is uniform with respect to gall size class, and (2) mean gall size of successful emergents should not be significantly larger than failures.

Emergence success from the (+) parasitism treatment, when pooled across all trees, was extremely low (12 emergents/ 2142 galls = 0.56%). This low emergence mirrored emergence from galls sampled from unmanipulated branches on the experimental trees and all other field estimates of emergence in previous years

(*unpublished data*). In the (+) parasitism treatment observed emergence differed significantly from expected ($\chi^2 = 34.1$, $DF = 10$, $p < 0.005$) with higher than expected observed emergences from large galls and lower than expected from small galls. The nature of these deviations resembled predictions given the well-defined exponential relationship between emergence success and gall size and implies that gall larva mortality is influenced by gall size when parasitoids are present. Moreover, successful galls were significantly larger than failed galls ($t = 3.61$, $DF = 2140$, $p = 0.0003$), a result consistent with emergence success data from the fall 1998 gall sampling study.

Emergence success was an order of magnitude higher from galls in the (-) parasitism treatment (139 emergents/ 2163 failures = 6.04%). No parasitoids emerged from these galls (*personal observation*) so the reason 94% of the gall formers perished in the (-) parasitism treatment was not due to the bags being ineffective barriers against parasitoids. While observed emergence success deviated significantly from expected in the (-) parasitism treatment ($\chi^2 = 22.5$, $DF = 10$, $0.05 < p < 0.025$) observed emergence success coarsely tracked changes in expected emergence success, and the nature of the observed vs. expected deviations were dissimilar from the (+) parasitism treatment (Figure 7). More wasps than expected emerged from mid-sized galls and fewer than expected emerged from large galls. Moreover, mean gall size of successful galls was significantly smaller than failed galls ($t = -2.74$, $DF = 2300$, $p = 0.0062$). These data together suggest the well defined exponential relationship between gall size and emergence success disappeared when parasitoids were prevented from attacking galls. Therefore, the inconsistent deviation in observed and expected emergent rates between (+) parasitism and (-) parasitism treatments, and the size differences between failed and

successful galls within each treatment supports the assertion that large gall size could be an adaptation to thwart parasitoids, but only for the subset of galls that completes their development prior to being parasitized.

Discussion

For *Belonocnema treatae* gall size at maturity is a reliable predictor of adult emergence. My results indicate that the mean gall size of emergents is significantly larger than galls in which no *B. treatae* emerge, the probability of emergence is an exponential function of gall size, and that this relationship disappears in the absence of parasitoids. Together these results support the argument that increasing gall size provides increasing protection from parasitism. In this respect my results are consistent with other gall insect studies, and provide additional evidence that gall size influences emergence success and that parasitoids cause this relationship (Jones, 1983; Weis et al, 1985; Price, 1988; Zwolfer and Arnold-Rinehart, 1994; Stiling and Rossi, 1996). My results provide further evidence that this relationship between the second and third trophic levels is a general phenomenon in the ecology of a variety of gall formers and their associated complex of parasitoid taxa. Considering that large galls are at a selective advantage to smaller galls which are more apt to be parasitized suggests the adaptive significance of large gall size is to thwart parasitoids (Weis and Abrahamson, 1985; Price and Clancy, 1986). However the feasibility of evolutionary response to the apparent size selective attack by parasitoids depends on the nature of the interaction between the attack phenology of the suite of parasitoids and the phenology of gall development.

Manipulating field levels of parasitism allowed me to show that parasitism has a negative effect on gall size at maturity complicating our interpretation of the adaptive significance of large size in this natural system. This interpretation is unusual

considering the profusion of studies attesting that parasitoids are major selective agents responsible for the evolution of the wide array of cynipid gall morphologies (Askew, 1975; Cornell, 1983; Stone and Cook, 1998), and the documented direct role of large gall size in thwarting natural enemies in gall forming diptera and hymenoptera (Weis et al., 1983; Price and Clancy, 1986). Moreover this interpretation is contrary to other evidence from this work of the defensive role of gall size in thwarting insect parasitism.

Applying the parasitoid avoidance hypothesis to cynipid systems may be problematic because in these systems a number of species in the parasitoid community attack galls when they are immature, kill the gall larvae, and halt their further development (Askew, 1961; Askew, 1975; Wiebesrijks, 1982; Schonrogge et al., 1996). Thus the benefits of the structural defense of gall size is never realized for a large percentage of initiated galls. The process of parasitoid attack prior to gall maturity may give rise to the pattern of bimodal gall size distributions frequently observed in these cynipid systems (Neth J Zoo 32: 112-116; Washburn and Cornell, 1981; Plantard and Hochberg, 1998). Similarly, I discovered that *B. treatae* gall size distributions are bimodally distributed and those galls comprising the left handed peak were the source of the negative effect of parasitism on gall size. Therefore for a nontrivial proportion of *B. treatae* galls attacked when they are immature, gall diameter at maturity does not play a role in structural defense against parasitism.

This complication is absent from natural systems characterized by low parasitoid community diversity in which the few parasitoids that do exist attack galls when they are mature (Abrahamson and Weis, 1997). In these instances the adaptive significance of large gall size in thwarting insect parasitoids is tenable as is the clear feasibility of

evolutionary response to size selective parasitism. Therefore the widely held interpretation of the adaptive nature of large gall size in solely thwarting insect parasitism likely represents a simple view of nature while in reality gall-former host plant systems may be much more complex harboring parasitoid communities in which constituent taxa partition the gall resource at all times and sizes throughout gall development.

I have learned that the associated parasitoid community of *B. treatae* induces bimodally distributed gall size frequency distributions under field conditions, however it remains to be determined which parasitoid taxa or taxon prevents the full growth of in many cases a considerable subset of initiated galls. Parasitoid emergence data from 1996 and 1997 asexual generation galls showed that *Synergus* is present during the initial period of gall growth and comprises 50% of parasitoid individual abundance, making this species a likely candidate taxon responsible for stopping gall growth (Lund, 1998). Relating the phenology of *Synergus* attack to phenology of gall development which may vary among host plants will clarify the pattern of these bimodal distributions and could provide more insight into the evolutionary ecology of this tri-trophic level natural system. Currently our lab is investigating this issue through a reciprocal field bagging experiment designed to delineate the time periods of attack and emergence, in relationship to the developmental phases of gall growth, for each member taxon of the complex community of parasitoids associated with *B. treatae*.

The complex interactions of *B. treatae* and its natural enemies occur on the background of the host plant, *Quercus fusiformis* that in addition to parasitoids is a significant source of gall size variation. A positive relationship existed between gall size/tree and emergence success/tree in 1998 indicating the host plant, by mediating gall

size and hence attack by parasitoids, directly influences the successful emergence of gall formers. This interpretation is consistent with the acknowledged role of bottom up factors in indirectly determining the strength of 2nd and 3rd trophic level interactions (Price et al., 1980; Price and Clancy, 1986; Roininen et al., 1996). Considering that in the 1998 field season emergence success of *B. treatae* varied by more than an order of magnitude (7.9% to 0.11%) among host plants (explained by host plant variation in gall size) it would be intriguing to know (1) Does parthenogenetic generation adult abundance predict sexual generation abundance within host plants and (2) given the strong correspondence between emergence success/tree and gall size/tree of the asexual generation is the same relationship a central feature of the sexual generation. Moreover does the gall diameter hypothesis apply to the sexual generation? Further study will determine if the complex interactions of the host plant, gall former, and natural enemies of the parthenogenetic generation is coupled or uncoupled with the sexual generation.

I suggest that there exists two main sets of the original cohort of initiated galls; (1) those galls prevented from attaining maximum size due to early larval death from parasitism and (2) those galls which escape early parasitism but are then subjected to attack at or after maturity. Gall size as a structural defense does not matter at all for set (1) while the benefits of this defense is conferred to members of (2) which escaped early parasitism. While the parasitoid avoidance hypothesis can not be applied to *B. treatae* galls as a whole, my data indicate that gall size is a major determinant of *B. treatae* adult emergence and that escape from parasitism is at the root of this relationship. Therefore factors (possibly interacting) which influence gall size and hence emergence success are critical in understanding the evolutionary ecology of *B. treatae* on its host plant.

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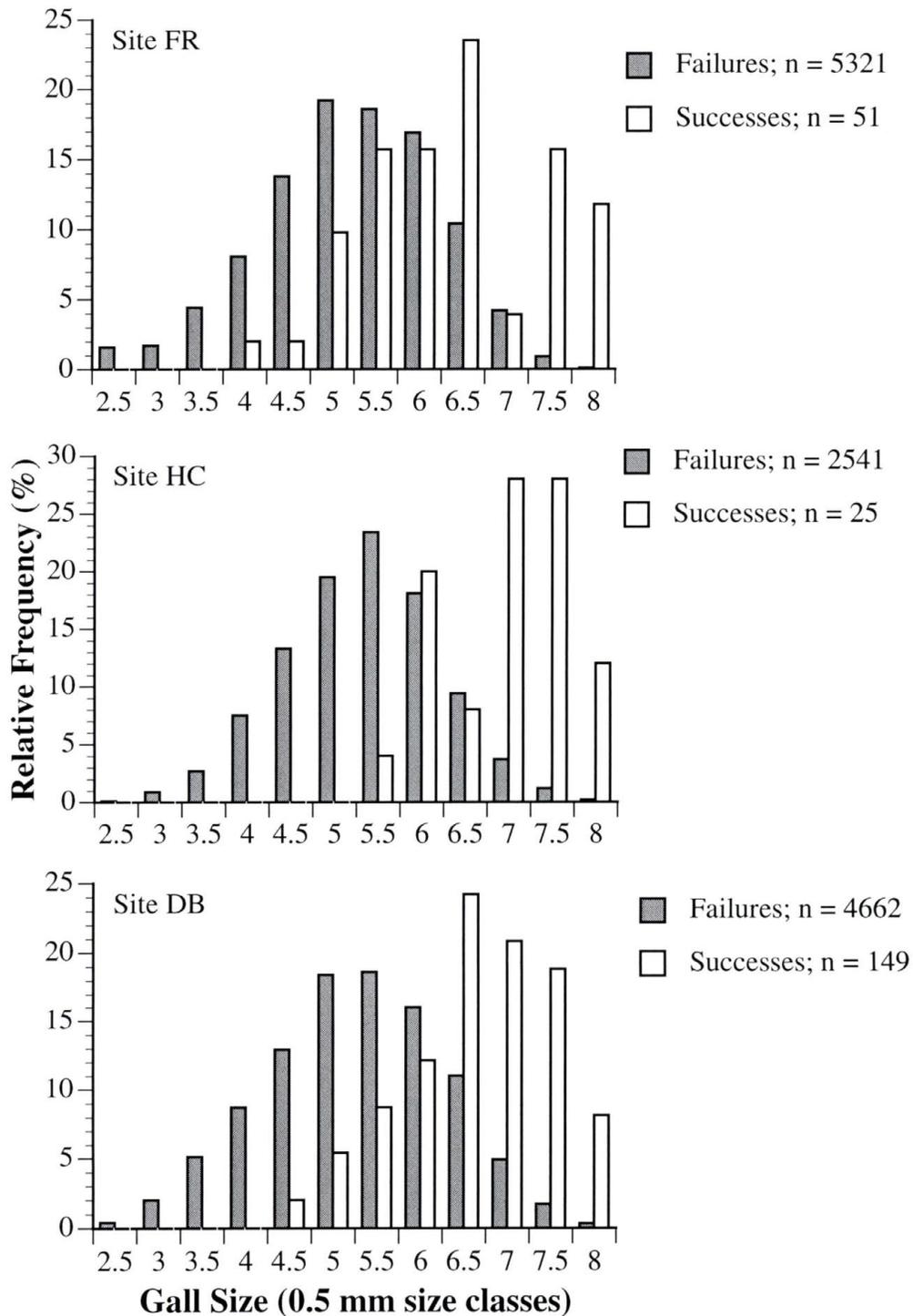


Figure 1. Gall size distributions of successful (open bars) and failed emergents (shaded bars) at each of 3 sites (Freeman Ranch (FR), Honey Creek (HC), Devil's Backbone (DB)) pooled over 5 trees per site. At each site mean gall size of successful galls is significantly larger than failed galls ($p = 0.0001$).

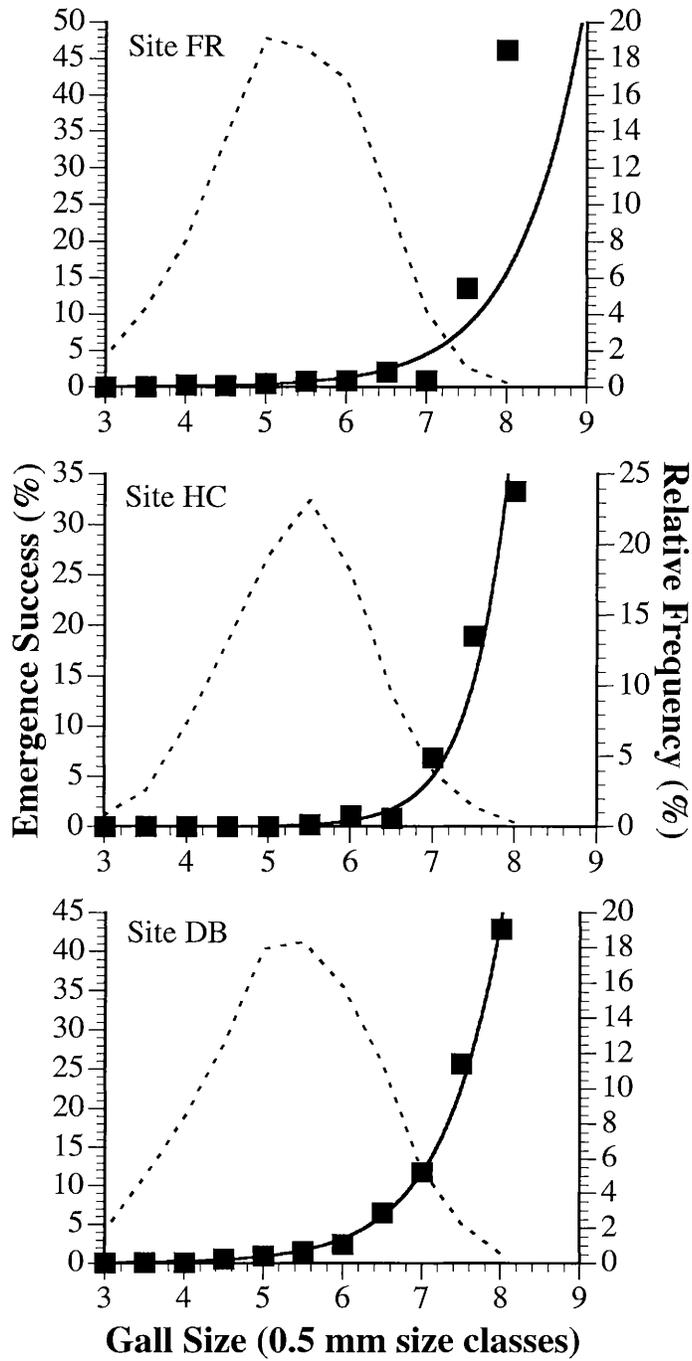


Figure 2. The exponential relationship between gall size and *B. treatae* emergence success (solid line) at each of 3 sites (Freeman Ranch (FR): $R^2 = 0.82$, $y = 7.4E-4 * \exp(1.2x)$; Honey Creek (HC): $R^2 = 0.94$, $y = 1.8E-6 * \exp(2.1x)$; Devil's Backbone (DB): $R^2 = 0.99$, $y = 1.2E-3 * \exp(1.3x)$) pooled over 5 trees per site and corresponding gall size distributions (hatched lines). The left ordinate represents *B. treatae* emergence success and the right ordinate represents percent relative frequency of galls.

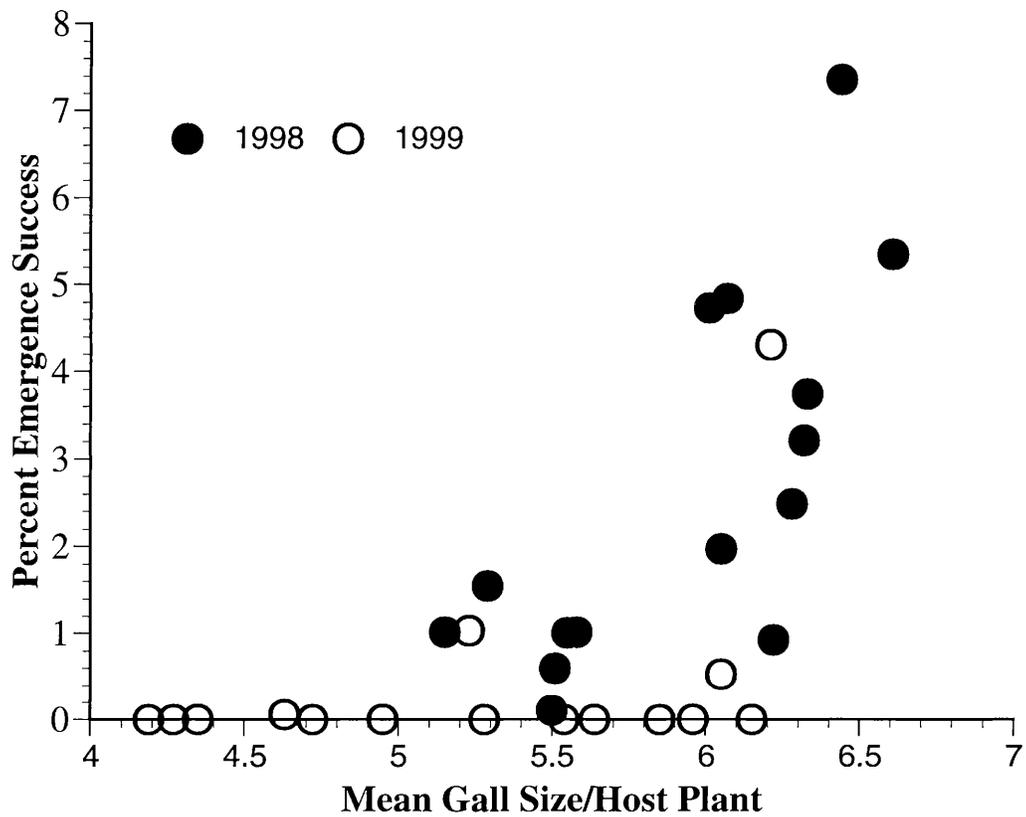


Figure 3. The correlation between mean gall size per tree and *B. treatae* emergence success per tree in 1998 (solid circles; $r = 0.72$, $p < 0.01$) and 1999 (open circles; $r = 0.39$, $p > 0.10$). Each point reflects mean gall size and emergence success data from a single host plant ($n=15$) during the 1998 field season.

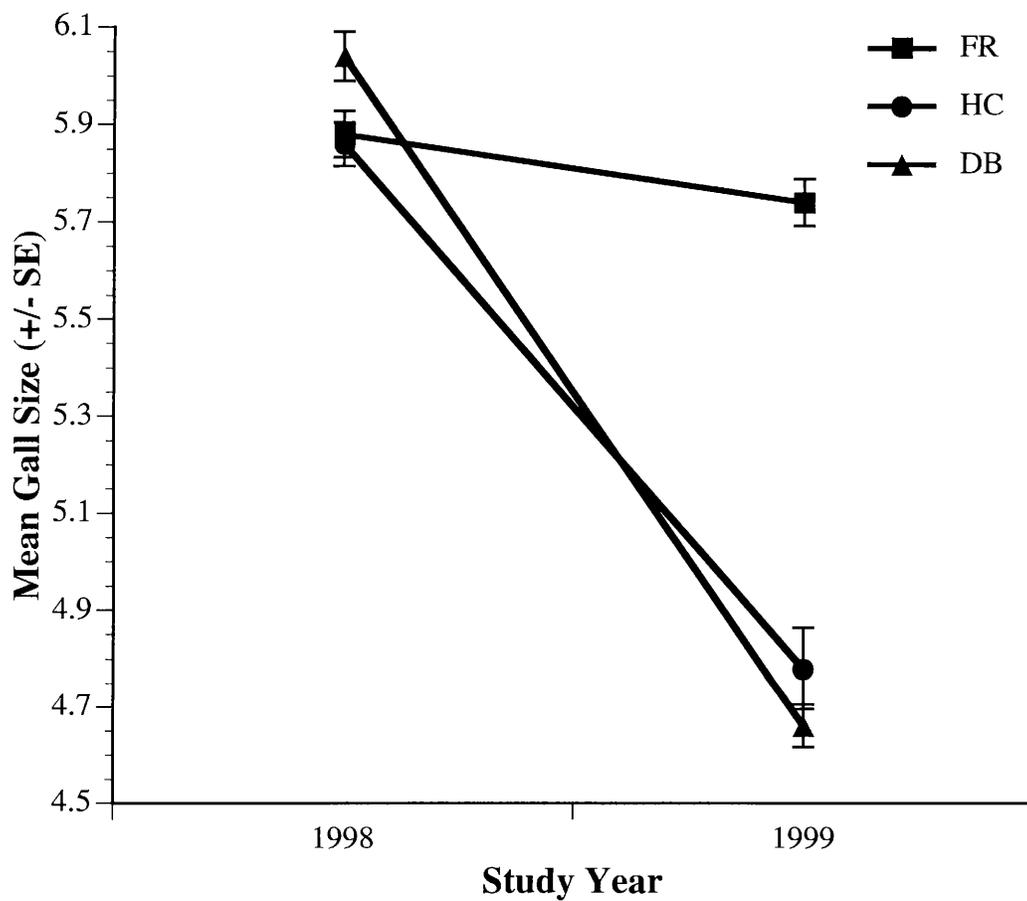


Figure 4. Mean gall size (+/-) 1SE in 1998 and 1999 at each site (Freeman Ranch (FR), squares; Honey Creek (HC), circles; Devil's Backbone (DB), triangles). Mean gall size is significantly lower in 1999 than 1998 at all sites (FR: $p < 0.035$; HC: $p = 0.0001$; DB: $p = 0.0001$).

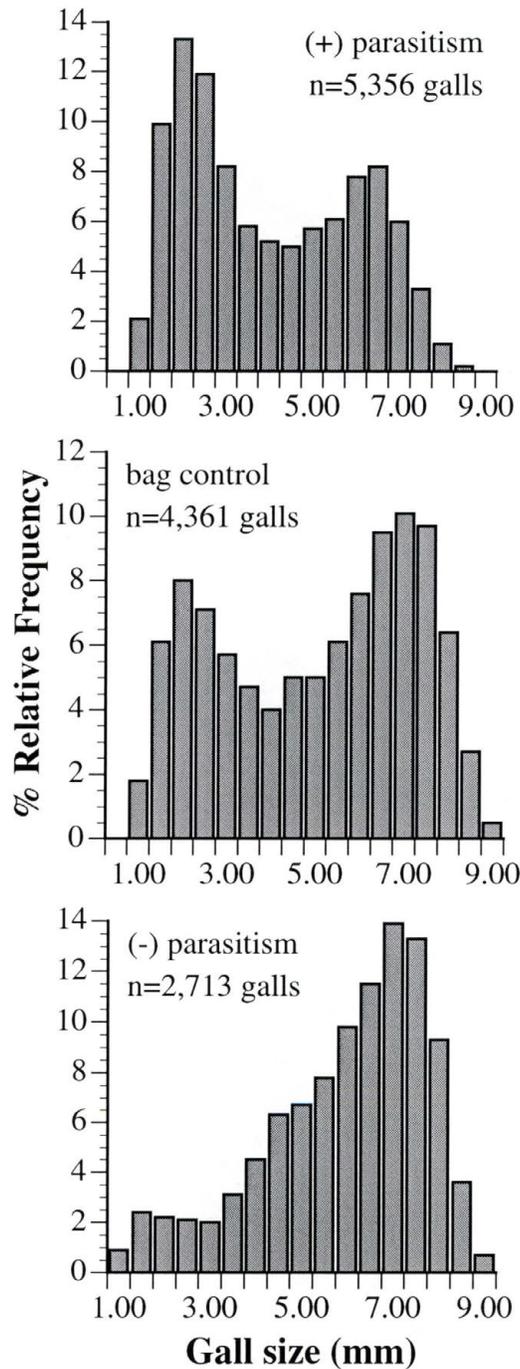


Figure 5. Gall size distributions from experimental treatments ((+) parasitism, bag control, (-) parasitism) pooled across $n = 6$ trees. Galls in parasitized treatments (bag control and (+) parasitism) are distributed bimodally, but gall sizes in (-) parasitism treatment exhibit a positively skewed distribution. These distributions are significantly different in shape and location ($p = 0.0001$).

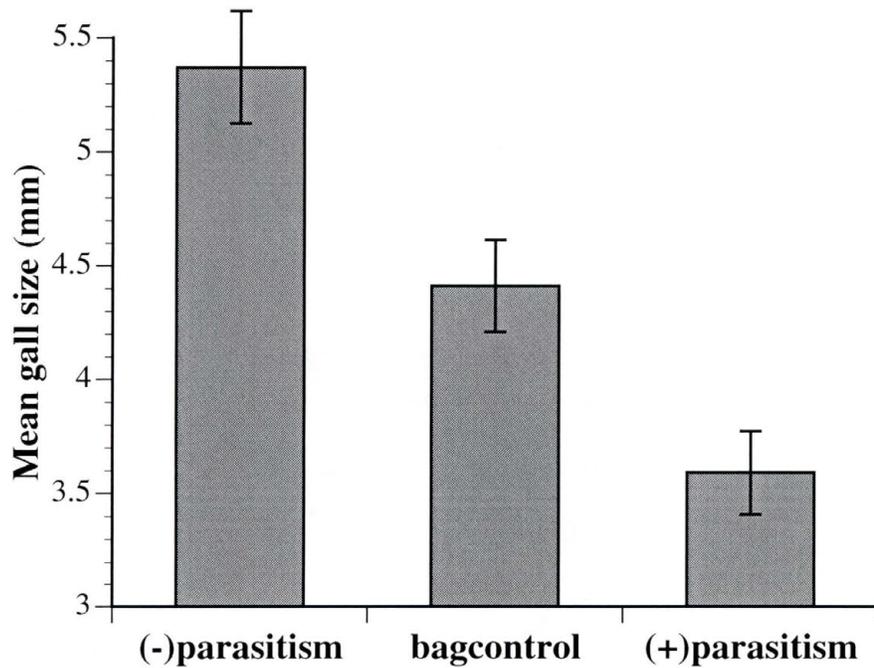


Figure 6. Mean gall size (\pm 1SE) across $n=6$ experimental trees for each treatment. (-) parasitism ($n=25$ observations mean gall size/branch), bag control ($n=30$ observations mean gall size/branch), and (+) parasitism ($n=29$ observations mean gall size/branch). All means are significantly different (REGWQ procedure).

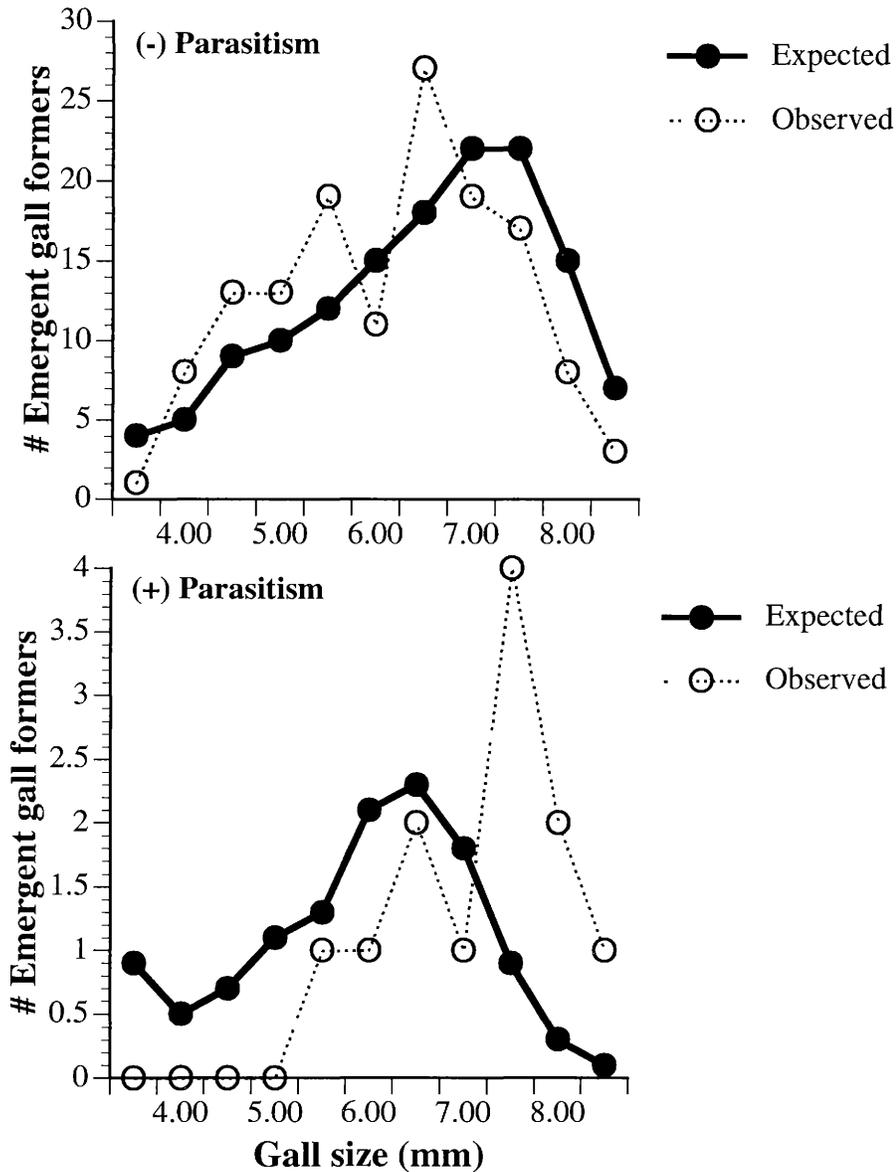


Figure 7. Observed (open circles) and expected (closed circles) numbers of emerging *B. treatae* plotted versus gall size for the (-) parasitism and (+) parasitism treatments. Information from each treatment was pooled across $n = 6$ trees. In both the (-) parasitism ($0.05 < p < 0.25$) and (+) parasitism ($p < 0.005$) treatments observed numbers of emergents deviated significantly from expected (assuming equal emergence rates across all size classes). For the (+) parasitism treatment the observed deviations are much higher than expected in the large gall size classes and much lower than expected in the low gall size classes. In contrast observed and expected emergences roughly track each other across all gall size classes in the (-) parasitism treatment.

VITA

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