ECOLOGY, BEHAVIOR AND BIONOMICS

Cynipid Gall Growth Dynamics and Enemy Attack: Effects of Gall Size, Toughness and Thickness

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Dinâmica de Crescimento de um Cinipídio e Ataque por Inimigos Naturais: Efeitos do Tamanho, Dureza e Espessura da Galha

RESUMO- Insetos galhadores desenvolveram várias estratégias para evitar ou reduzir o ataque por inimigos naturais, tais como maior espessura da parede no início de formação, e maior diâmetro e dureza da parede em estágios posteriores do desenvolvimento. Neste estudo, analisamos a eficiência de cada um desses parâmetros na redução da mortalidade das galhas de *Atrusca caprone* Weld (Hymenoptera: Cynipidae) em *Quercus turbinella* Greene (Fagaceae), durante os três meses de seu desenvolvimento (junho-agosto). O diâmetro e a dureza das galhas aumentaram do primeiro para o terceiro mês de estudo (ANOVA, diâmetro: $F = 88.73$, $p < 0.0001$; ANOVA, dureza: $F = 26.13$, $p < 0.0001$). Entretanto, a espessura das galhas apresentou um aumento de junho para julho, diminuindo de julho para agosto (ANOVA, $F = 35.84$, $p < 0.0001$). A análise de regressão linear múltipla mostrou que apenas a dureza das galhas influenciou a susceptibilidade das larvas ao ataque por parasitóides ($r^2 = 0.52$, $F = 13.84$, $p < 0.01$, $n = 15$). A sobrevivência das galhas foi elevada no primeiro mês, diminuindo com o tempo, provavelmente devido à menor dureza da parede. Estes resultados sugerem que o primeiro mês seja crítico para o estabelecimento das galhas, sendo que galhas não parasitadas neste estágio são menos suscetíveis a um ataque posterior.

PALAVRAS CHAVE: Insecta, galhador, *Atrusca caprone*, parasitóides, *Quercus turbinella*, janela de vulnerabilidade.

ABSTRACT- Galling insects have developed many strategies to preclude or reduce the attack by natural enemies, such as an increased wall thickness early in the season, and switching to larger and tougher walls later in the maturation stage. In this study, we observed the efficiency of each one of these parameters in reducing mortality of the leaf galling wasp *Atrusca caprone* Weld (Hymenoptera: Cynipidae), on *Quercus turbinella* Greene (Fagaceae), during the three months of gall development (June-August). Gall diameter and wall toughness increased from the first to the third month of the study (ANOVA, Diameter: $F = 88.73$, $p < 0.0001$; ANOVA, Toughness: $F = 26.13$, $p < 0.0001$). However, gall wall thickness increased from June to July, decreasing in August (ANOVA, F =

35.84, $p < 0.0001$). Gall survivorship was only 2 % in June, increasing to 10 % in July and to 29.3 % in August. Multiple regression analyses showed that only gall wall toughness influenced gall susceptibility to parasitoid attack ($r^2 = 0.52$, $F = 13.84$, $p < 0.01$). Gall survivorship was very low in the first month due probably to low wall toughness, which led to a higher success of oviposition by parasitoids during this phase. These results suggest that the first month is critical to gall establishment, and the galls which are not parasitized at this stage are less likely to be attacked later.

KEY WORDS: Insecta, gall development, parasitoids, *Atrusca caprone*, window of vulnerability, *Quercus turbinella.*

The induction of galls by insects has been shown to be of adaptive nature. Gall formation may protect its inducers from harsh microenvironmental factors, such as dryness, and temperature (Price *et al.* 1987). In addition, galls can provide enemy-free space to the gall-inducer, by offering a protective refuge against predators and parasites (Askew 1961, Hodkinson 1984, Price *et al.* 1987, Price & Pschorn-Walker, 1988, Fernandes & Price 1992; but see Fernandes *et al*. 1987, Hawkins 1988).

Several factors may affect the rates of parasitism on gall-forming insects. Gall size may interfere with parasitoid oviposition success in an inverse relationship, since ovipositors of limited length are unable to reach the larvae inside the gall chamber (e.g., Weis & Abrahamson 1985, Rossi *et al.* 1992, Rossi & Stiling 1995). The toughness of gall tissue may also preclude parasitoid attack, if the gall wall is not easily penetrable (Cornell 1983, Craig *et al.* 1989). Finally, the thickness of the gall walls is also important to reduce parasitism rates, since the success of oviposition by a parasitoid is related to the length of the ovipositor and to the depth of the larvae in the gall (Askew 1961, Price 1972, Weis *et al.* 1985, see also Brandl & Vidal 1987).

Parasitoids may synchronize their activity pattern with gall development, by attacking early in the gall growing season (Askew 1975, Wiebes-Rijks 1982, Jones 1983, Schonrögge *et al.* 1996). This should be related to their ability to overcome gall size and toughness in time. With increasing age the gall tissue becomes harder and the gall larger, therefore precluding parasitism (Weis *et al.* 1985). In this way, a "window of vulnerability" (Cornell 1983) appears to exist early in the season, when the developing gall is smaller and softer. However, these early galls may preclude or decrease mortality by natural enemies if they have thicker walls which act as a barrier to the parasitoids' ovipositors. We postulate that galls may have different 'ontogenetic' barriers to avoid parasitoid attack, if their walls are thicker in the early season and harder and larger in the late season.

We tested this hypothesis observing the phenological development and the survivorship rates of a leaf galler, *Atrusca caprone* Weld (Hymenoptera: Cynipidae), on *Quercus turbinella* Greene (Fagaceae). This study aimed to answer the following questions: a) How does gall development affects gall toughness, gall size, and gall thickness of *A. caprone* ? b) Which of these variables are more effective against the natural enemies of *A. caprone* ?

Material and Methods

Quercus turbinella is a shrub widely distributed in the chaparral vegetation of Arizona- USA (McDougall 1973). Galls were found throughout the distribution of the host plant, and were more abundant between 1,200 and 1,500 meters altitude (adjacent to highways I-17, and 89 A South) in northern Arizona.

The cynipid galls occured on the abaxial leaf surface, were spherical, glabrous, and one-chambered with only one gall-making larva per chamber (Fernandes *et al.* 1990). Galls varied from pale yellow to red. Young galls are solid, but the centrally located larval chamber becomes separated from the gall walls during development. However, the larval chamber remains attached to the gall walls by fibers of conductive tissue during gall development (see Fernandes *et al.* 1990).

Galls were first observed in early June 1987**,** at the beginning of their development, and by the end of September all of the galls were mature. Galls belonged to the same cohort. These galls were induced early in the season, when plant leaves are flushing. We randomly removed 100 galls in June, 50 galls in July and 75 galls in August, from several plants in the same patch. Galls were dissected and analyzed for survivorship, diameter, wall thickness, and wall toughness. Gall mortality was caused by several different species of hymenopteran parasitoids. Gall diameter was measured with a caliper (mm), while gall wall thickness was measured under a dissecting scope (mm). Gall wall toughness was obtained by using a Volander Texture Analyzer, which measured the force needed to push a needle probe 2 mm into the gall at a speed of 1 mm/s. The device was calibrated in "grams" of force; measurements were converted to Newtons of force, using the conversion factor of 1 "gram-force" = 9.80665 mn (Craig *et al.* 1990). All these parameters were compared between the three months using a one-way ANOVA followed by the Tukey test (Zar 1984).

We used linear regression analyses to verify the relationship between gall diameter and survivorship rates. Since there was no relationship between these variables (r^2 = 0.005, F = 1.22, p > 0.05, n = 225), we divided gall survivorship, thickness, and toughness into eight classes of diameter at each month. This procedure was conducted in an attempt to transform gall survivorship from a binomial to a continuous variable. Data on gall survivorship were then arcsine-square-root transformed, and data on gall thickness and toughness were log transformed to meet normality (Zar 1984). We used stepwise multiple regression analysis to observe the influence of gall age, gall wall thickness, and toughness on the survivorship of *A. caprone* galls (Zar 1984).

Results

Galls of *A. caprone* varied in diameter, wall thickness, and toughness during development (Fig. 1). On average, gall diameter and gall wall toughness were significantly higher at the end of maturation period (August) (ANOVA and Tukey test, Table 1; Fig. 1). Thus, gall development influenced positively these variables. However, gall wall thickness increased from June to July, decreased from July to August, and attained a significantly lower average at the end of the gall developmental period (ANOVA and Tukey test, Table 1, Fig. 1).

Gall survivorship, on the other hand, increased with gall development (Table 1; Fig. 2). In June, survivorship rates were very low, with only 2% of the galls remaining alive, increasing to 10 % in July and 29.3 % in August. The stepwise multiple regression analyses showed that only gall wall toughness presented a significant positive relationship with gall survivorship (r^2 = 0.52, F = 13.84, p < 0.01, n = 15, Fig. 3). Therefore, all the other variables were excluded from the model, as they did not show significant relationships with gall survivorship (p > 0.05, all). Thus, the higher gall survivorship in the later stage of development may be related to an increase in the toughness of gall walls.

Discussion

The growth dynamics of *A.caprone* experienced a change from thicker and softer walls at the initial stage of development to thinner but harder walls later in the development. However, the higher gall wall thickness did not seem to be efficient to preclude parasitoid

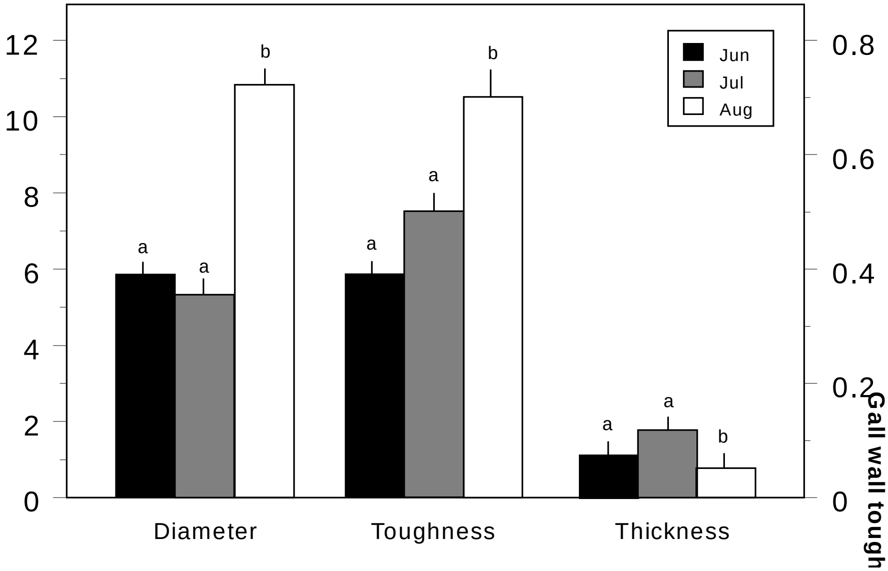

Figure 1. Average diameter, wall toughness, and wall thickness of the galls of *A. caprone* on *Q. turbinella* during three months of study. Statistically significant differences are represented by different letters above the bars (ANOVA and Tukey test, $p < 0.05$).

attack, since mortality of the earlier galls was almost 98 %. Our results suggest that gall wall toughness is more effective as a defense against natural enemies in *A. caprone* galls, assuming that rates of parasitoid attack are similar over gall developmental period. In fact, even longer ovipositors may generally be found broken off in many galls, if they are not strong enough to overcome gall wall toughness (see also Askew 1965). In addition, Craig *et al.* (1990) stated that larval inaccessibility (i.e., larval depth inside the gall) is less important than gall wall toughness in determining parasitism rates.

The existence of this window of vulnerability may decrease the range of resource

Table 1. One-way ANOVA comparison of variations on gall diameter, gall wall toughness, thickness and survivorship rates of galls of *A. caprone* on *Q. turbinella* between the three months of study.

Variable	DF	F	P
Gall Diameter	2	88.73	< 0.0001
Gall wall toughness	2	26.13	< 0.0001
Gall wall thickness	2	35.84	< 0.0001
Gall survivorship	2	16.42	< 0.0001

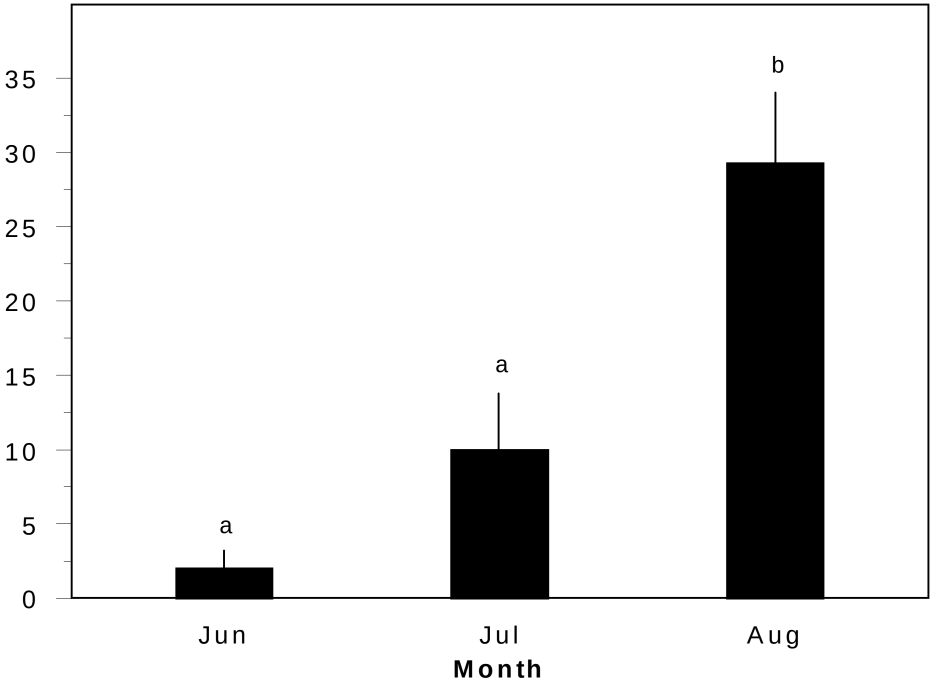

Figure 2. Average mortality rates of the galls of *A. caprone* on *Q. turbinella* during three months of study. Statistically significant differences are represented by different letters above the bars (ANOVA and Tukey test, $p < 0.05$).

availability, decreasing the efficiency of natural enemies (Craig *et al.* 1990). However, Schonrögge *et al.* (1996) suggested that parasitoids are morphologically, phenologically and/or physiologically well adapted to their host galls, being able to circumvent any protective function offered by the galls. Thus, gall wall toughness may diminish the parasitoid attack, but is far from precluding it completely (gall mortality, even in the third month, remained higher than 70 %).

The higher gall mortality at the initial stage of development is probably due to a higher success of oviposition by natural enemies during this phase. This pattern was already observed in other systems (Wiebes-Rijks 1982, Craig *et al.* 1990, Shorthouse *et al.* 1990). Since gall wall thickness is not efficient in precluding parasitoid attack, and gall toughness is still low on the first month after gall induction, this should be the most critical phase of gall establishment. The galls remaining alive in the second and third months of development have tougher walls, possibly excluding certain species of parasitoids with ovipositors of limited strength, and this factor seems to determine the vulnerability of the galls. In this way, if the larvae of galling insects escape detection when galls are soft and within reach of parasitoids, they are protected once imbedded inside hard maturing galls.

We concluded that gall wall thickness of *A. caprone* can not avoid the "window of vulnerability" in the initial phase of development. Thus, the mortality rates of the cynipid larvae are very high in this stage, probably due to a

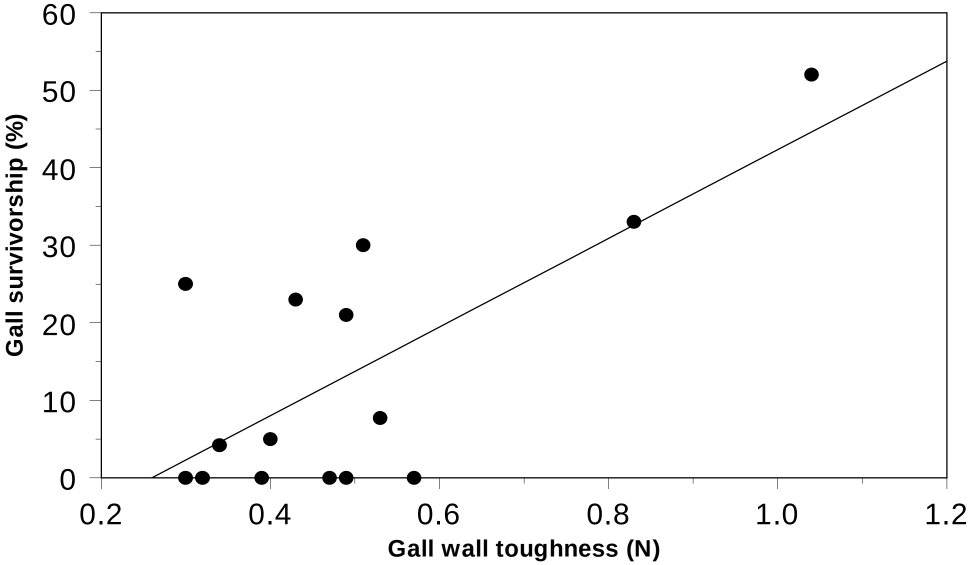

Figure 3. Linear regression analyses showing the relationship between survivorship rates and wall toughness of *A. caprone* galls ($r^2 = 0.52$, $y = 0.006x + 0.163$).

higher oviposition success by parasitoids. Gall wall toughness seems to limit larva susceptibility to parasitism and, as this parameter increased with gall age, galls in the late season are less likely to be affected by the pressures of natural enemies.

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