Oecologia
DOI 10.1007/s00442-009-1492-9

PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Developmental plasticity and reduced susceptibility to natural enemies following host plant defoliation in a specialized herbivore

Glen R. Hood · James R. Ott

Received: 1 September 2009 / Accepted: 20 October 2009

Abstract Host-specific phytophagous insects that are short lived and reliant on ephemeral plant tissues provide an excellent system in which to investigate the consequences of disruption in the timing of resource availability on consumer populations and their subsequent interactions with higher tropic levels. The specialist herbivore, *Belonocnema treatae* (Hymenoptera: Cynipidae) induces galls on only newly flushed leaves of live oak, *Quercus fusiformis*. In central Texas (USA) episodic defoliation of the host creates variation in the timing of resource availability and results in heterogeneous populations of *B. treatae* that initiate development at different times. We manipulated the timing of leaf flush in live oak via artificial defoliation to test the hypothesis that a 6- to 8-week delay in the availability of resources alters the timing of this gall former's life cycle events, performance and survivorship on its host, and susceptibility to natural enemies. *B. treatae* exhibits plasticity in development time, as the interval from egg to emergence was significantly reduced when gallers oviposited into the delayed leaf flush. As a consequence, the phenologies of gall maturation and adult emergence remain synchronized in spite of variation in the timing of resource availability. Per capita gall production and gall-former performance are not significantly affected by the timing of resource availability. The timing of resource availability and natural enemies interact, however, to produce strong effects on survivorship: when exposed to natural enemies, *B. treatae* developing in galls initiated by delayed oviposition exhibited an order-of-magnitude increase in survivorship. Developmental plasticity allows this gall former to circumvent disruptions in resource availability, maintain synchrony of life cycle events, and results in reduced vulnerability to natural enemies following defoliation of the host plant.

Keywords Cynipidae · Gall former · Tri-trophic interactions · Phenology

Communicated by Thomas Hoffmeister.

Present Address:
G. R. Hood
Department of Biological Sciences, University of Notre Dame, Galvin Life Sciences Building, Notre Dame, IN 46556, USA

G. R. Hood · J. R. Ott (✉)
Population and Conservation Biology Program, Department of Biology, Texas State University-San Marcos, San Marcos, TX 78666, USA
e-mail: JimOtt@txstate.edu

Introduction

Synchronization of consumers with the resources upon which they depend is of fundamental importance to consumer fitness. Short-lived phytophagous insect species that exploit specific plant tissues are excellent study systems in which to explore the ecology and evolution of synchrony as in these specialized consumers, phenological synchronization with plant resources is linked to the timing of life cycle events, life history patterns, and ecological dynamics (Mopper 2005; Van Asch and Visser 2007). Such synchonization is likely under selection (Clancy and Price 1986; Eliason and Potter 2000a; Yukawa and Akimoto 2006; Forkner et al. 2008). Of general interest then to understanding consumer–resource synchrony are both the causes and consequences of asynchrony between plant resource availability and insect life cycles and the responses of phytophagous insect populations to disruptions in synchrony.

Published online: 15 November 2009

One of many causes of asynchrony of plant resources and herbivorous insect populations is prior herbivory. Prior herbivory from other insects, particularly during outbreaks that produce partial to complete defoliation (Crawley and Akhteruzzaman 1988; Krause and Raffa 1995; Parry et al. 2003) can have cascading effects for insect species that use defoliated hosts before and/or after defoliation. Consumption of leaf tissue, particularly at outbreak densities, can eliminate previously deposited eggs or larvae of other insects dependent on the plant (Mopper and Simberloff 1995), as well as potential sites for feeding, mating and oviposition, and refuge from natural enemies (Barbosa and Schultz 1987). Moreover, partial to complete defoliation can also alter host plant quality (Karban and Baldwin 1997) with either positive (Rieske and Raffa 1998; Wallin and Raffa 2001) or negative (Tikkanen and Julkunen-Tiitto 2003) effects on associated host-specific phytophagous insect populations.

For those plant species that respond to outbreaks of herbivores by refoliation (Potter and Redmond 1989), the effects of defoliation extend to inducing time lags in the availability of resources. The subsequent use of these resources can result in variation in the realized timing of insect life cycle events. As a consequence, variation in tissue availability can create the template for selection favoring synchronization of insect and plant life cycle events within populations or selection favoring divergence in the timing of life cycle events among populations experiencing differences in the timing of resource availability (Horner et al. 1999; Blair et al. 2005; Joy and Crespi 2007). Life cycle events closely tied to host plant phenology and thus likely altered by variation in the timing of tissue availability include the timing of mating (Cushman et al. 1994; Mopper 1996), oviposition (Ohgushi 1991), the rate of larval development (Kaitaniemi et al. 1997; Hicks et al. 2007), and the timing of eclosion (Komatsu and Akimoto 1995; Van Dongen et al. 1997; Rehill and Schultz 2002). Variation in the timing of plant tissue formation can also directly affect mortality due to natural enemies through changes in enemy abundance (Yukawa and Akimoto 2006), attack timing (Briggs and Latto 1996; Van Nouhuys and Lei 2004), and community structure (Kaitaniemi and Ruohomaki 1999; Yukawa and Akimoto 2006). The response of insect herbivores to resource uncertainty can extend to include plasticity in development and/or bet-hedging strategies (Nylin and Gotthard 1998; Danks 2006).

Gall-forming insects are a specialized and diverse group of herbivores that redirect plant resources to induce nutritive rich tissue within which the insects feed and develop (Price et al. 1987). For these consumers, the timing of host plant tissue availability is particularly important because gall formers are host and tissue specific, restricted to ovipositing into newly formed tissue, sessile during development, and short lived during the oviposition phase of the life cycle (Weis et al. 1988). As a consequence, gall formers exhibit tightly synchronized phenological schedules with their host plants (Yukawa 2000). Thus, temporal variation in tissue formation can alter the timing of, or even prevent, gall-former oviposition and/or subsequent development (Kaitaniemi and Ruohomaki 1999; Stone et al. 2002). Because gall formers are associated with diverse natural enemy communities (Askew 1971; Hayward and Stone 2005) and exhibit windows of vulnerability to portions of their natural enemy communities (Clancy and Price 1986; Craig et al. 1990), a change in the timing of resource availability leading to variation in oviposition timing and gall-former development potentially alters the effects of natural enemies synchronized to attack gall formers at specific developmental stages (Parry et al. 2003). While available evidence suggests the importance of changes in the timing of tissue availability for gall formers, it is not clear at present, for any gall-former system, what the effects of prior defoliation of the host plant are on gall-former life cycle events and subsequent susceptibility to natural enemies.

Herein we report the results of an experiment in which we manipulated the timing of leaf flush via artificial defoliation to study the effects of variation in the timing of tissue availability on the timing of life cycle events, measures of performance, and susceptibility to natural enemies for a host-specific gall former. By examining the effects of delayed oviposition across this suite of response variables we were able to assess the potential impact that episodic defoliation of this gall-former's host plant (Stewart and Bailey 1993; Drees 2004) has on the timing of life cycle events and the population ecology of this host-specific phytophagous insect.

Materials and methods

Study system

Belonocnema treatae Mayr (Hymenoptera: Cynipidae) is a host-specific gall former of plateau live oak, *Quercus fusiformis* Small (Muller 1961) in the Edwards Plateau region of central Texas, USA (Lund et al. 1998). *B. treatae* exhibits heterogony wherein an asexual generation developing within unilocular leaf galls alternates with a sexual generation that develops within root galls (Lund et al. 1998). Coinciding with spring leaf flush, the short-lived sexual females oviposit into newly flushed leaves (Lund et al. 1998). Each ovipositor insertion produces a permanent scar documenting potential oviposition (Egan and Ott 2007). The sexual generation typically emerges and

oviposits from March up to and including May (Cryer 2003). However, emergence phenology and oviposition can be extended following defoliation (Hood 2009). Asexuals develop through the summer, emerge from November up to and including February (Lund 1998; Hood 2009) and then oviposit into oak rootlets. Leaf galls are characterized by changes in consistency and color during development with mature galls being lignified and brown. Only mature galls were used in the experiment described herein because harvesting galls earlier in development halts growth and can result in gall-former death (Lund 1998; Egan and Ott 2007). Asexual generation *B. treatae* larvae are attacked by a diversity of parasitoids, and the leaf galls are attacked by inquilines (Lund et al. 1998; Hall 2001). As *B. treatae* does not emerge from galls following inquiline attack, both inquilines and parasitoids are natural enemies (Hall 2001).

Quercus fusiformis flushes new leaves each spring as the previous year's leaves are dropped. In central Texas, live oaks can experience defoliation during outbreaks of two species of oak leaf roller, *Archips semiferana* and *Sparganothis pettitana* (Lepidoptera: Tortricidae; Stewart and Bailey 1993). Defoliation events are episodic and severity varies within and among live oak populations (Hood 2009). Because caterpillar feeding coincides with leaf flush and *B. treatae* oviposition, these defoliators also consume *B. treatae* eggs and eliminate oviposition sites. Partial to complete defoliation induces a second leaf flush 6–8 weeks later. This second flush is oviposited into by later emerging *B. treatae* (Hood 2009). Thus, *B. treatae* leaf galls developing on partially defoliated trees likely represent both pre- and post-defoliation oviposition events, whereas galls developing on defoliated trees likely represent post-defoliation oviposition events. Our observations of periodic widespread defoliation of live oak in conjunction with variation in both the phenology of oviposition by sexual generation *B. treatae*, and the emergence of asexuals (Lund 1998; Hood 2009) motivated the experiment described below.

Treatment design

Six live oak trees each possessing suitably high root and leaf gall densities were chosen at Texas State University's Freeman Ranch in Hays County, Texas, USA (29°55′N, 98°00′W). In spring of 2008, root galls housing sexual generation *B. treatae* were collected from the six trees and pooled. One half of the galls were incubated at seasonally adjusted light and temperature regimes and the other half stored at 9°C to delay emergence. On each tree, 48 Nytex screen (BioDesign, Carmel, N.Y.) enclosures (replicates) measuring 30 cm × 46 cm were secured around branches prior to leaf flush to exclude natural enemies and *B. treatae* not involved in the experiment. Following leaf flush, 24 enclosures/tree were opened and branches stripped of leaves to mimic localized defoliation. Manual defoliation standardized the timing and extent of defoliation and, as observed with natural defoliation, induced a second leaf flush 6–8 weeks later. This manipulation created a natural early spring leaf flush [henceforth "early" (E)] and a second, artificially stimulated, late-spring flush [henceforth "delayed" (D)], each with 24 replicates/tree. Immediately following natural leaf flush (≈week of 31 March 2008), *B. treatae* emerging from husbanded root galls were collected daily and allowed to mate for 1 day. Three to five mated females were then placed inside each of the 24 early flush enclosures/tree and allowed to oviposit. The procedure was repeated 6–8 weeks later using the cohort of *B. treatae* whose emergence was delayed by cold storage. Oviposition scars were verified in all enclosures 5 days after stocking. For each leaf flush, 12 enclosures/tree were then removed after a 5-day oviposition period to allow natural enemy attack to occur throughout leaf galler development. The other 12 enclosures/tree remained closed throughout development. Thus each tree consisted of two temporally distinct leaf flushes, and hence oviposition events (E and D) with 12 replicates per leaf flush either exposed to (+NE) or protected from (−NE) natural enemies. Galls within all replicates of the four treatments (E−NE, E+NE, D−NE, D+NE) were then allowed to develop throughout the fall–winter of 2008. On each experimental tree unbagged (control galls) resulting from naturally occurring oviposition and exposed to natural enemies were monitored on three to six randomly selected branches at each census date for development, gall-former emergence phenology, and survivorship (see below). One tree that failed to produce sufficient leaves following defoliation was dropped from the study. Throughout the spring the Lepidoptera-specific pesticide, *Bacillus thuringiensis kurstaki* was applied to each experimental tree to guard against defoliation by caterpillars. Defoliation from caterpillars was not detected for the six trees, and defoliation above 25% was not observed for any tree surveyed at the study site in 2008 (Hood 2009).

Variable definitions and sample processing

Three categories of response variables (life cycle timing events, performance measures, and survivorship) were monitored to test the general prediction that asynchrony (delayed oviposition) altered the outcome of the interaction of *B. treatae* with its host plant and natural enemies. To test the prediction that galls initiated from early oviposition reach maturity sooner than do the galls induced by delayed oviposition, the phenology of gall maturation was monitored in situ within three E-NE and three D-NE randomly selected replicates per tree. The percent of mature galls in

each replicate was scored at 2-week intervals beginning with the onset of maturation (early October) up to and including December, by which time all galls were mature. For comparison the maturity of naturally occurring galls on each experimental tree was also scored at each interval. Tests of the effects of delayed oviposition for all other variables required that galls be collected. To guard against the possibility that gall-former emergence and survivorship were dependent on the timing of collection, one-third of replicates/treatment, along with galls from control branches, were collected successively during the first week of December 2008, January 2009, and February 2009. Subsequent tests showed that collection date did not significantly affect any variable, thus data were pooled across collection dates. At each collection branches housing replicates were cut from trees and galls were processed and monitored as described below.

To test the hypothesis that the timing of sexual generation oviposition controls the timing of emergence of asexuals from leaf galls, the phenology of emergence of asexuals produced in the early and delayed oviposition treatments was compared. Because natural enemies greatly reduce *B. treatae* survivorship (Reynolds 2000) we compared emergence phenologies using only the E−NE and D−NE treatments. All galls were scored at the time of collection for the presence of an emergence hole to determine the number of *B. treatae* that had emerged prior to collection. Those galls from which *B. treatae* had not yet emerged (those without holes) were then placed outdoors in collection traps at the Texas State University greenhouse. Further emergence was then monitored by scoring the number of emergents/replicate per week for 16 weeks. The percent of *B. treatae* that emerged by each survey date was determined by dividing the number of emergents from each survey by the total number of *B. treatae* that emerged over the course of study in each replicate. Development time was estimated as the number of days from the midpoint of the 5-day spring oviposition window for each replicate to the midpoint of the survey week in which each gall former emerged. Development time was then averaged per replicate. The oviposition date midpoints for early and delayed oviposition were 5 April and 26 May, respectively. The mean number of galls produced per female per treatment was assessed by counting the galls produced in each enclosure and dividing by the number of females added per enclosure.

Because parasitism influences the distribution of gall sizes (Reynolds 2000) only galls from the E−NE and D−NE treatments were used to test whether early and delayed oviposition differentially affected gall size. Gall size was measured as greatest gall diameter using digital callipers to 0.01 mm. The above comparison, while free from natural enemy effects on gall size, still includes galls in which the gall former died (possibly before completing gall growth). Thus, each gall was also scored for the presence of a *B. treatae* emergence hole at the end of the emergence season to identify those galls that, by virtue of having produced a gall former, had reached their maximum attainable size based on the gall former × tree genotype interaction (Weis and Abrahamson 1986). We then again tested the size hypothesis using maximal attainable gall size**.** To test the hypothesis that delayed oviposition alters susceptibility of *B. treatae* to natural enemies via an influence on gall size, we tabulated the percent of mature galls equaling or exceeding the natural enemy threshold size of 5.82 mm for each E−NE and D−NE replicate and then compared treatment means. This threshold size represents the minimum size galls must attain to have a ≥5% chance of producing a gall former in the presence of natural enemies (Egan and Ott 2007).

Percent survivorship was scored as the number of emergent *B. treatae* divided by the number of galls per replicate at the end of the emergence season and was evaluated as a function of oviposition timing, exposure to natural enemies, and interaction. All replicates per treatment were processed and surveyed for surviving gall formers following the methods used to monitor phenology. For the E−NE and D−NE treatments the number of survivors equaled the number of galls/enclosure with wasp exit holes at the time of collection plus the number of *B. treatae* subsequently collected in emergence traps. For these treatments each gall with an exit hole equaled an emergent gall former because only one *B. treatae* emerges per leaf gall (Hall 2001) and parasitism within the −NE enclosures was 0.01%. For the E+NE, D+NE treatments and controls, however, an exit hole at the time of collection could indicate either an emergent *B. treatae* or a parasitoid. Thus, we estimated survivorship by monitoring the subset of galls per replicate without emergence holes at the time of collection. We also estimated survival for these two treatments by adjusting estimates by the proportion of *B. treatae* that had emerged within the treatments protected from natural enemies by the time of collection. However, both procedures yielded estimates that were in close accord and the choice of estimator did not alter ANOVA results for tests of hypotheses, thus we present the simplest method.

Statistical analysis

The variables, percent of galls mature per survey date, and the percent of *B. treatae* that had emerged by each survey date, were both arcsine-square-root transformed and analyzed using repeated-measures ANOVA. Both designs were balanced and for each analysis tree was the experimental unit (subject), survey date was the random repeated

factor (time), and the timing of oviposition was the main effect. A preliminary ANOVA showed no difference in the cumulative emergence phenologies of *B. treatae* among the December, January, and February collection dates for early ($F_{2,25} = 1.11$; $P = 0.35$) or delayed ($F_{2,25} = 0.33$; $P = 0.72$) oviposition. Thus data were pooled across three collection dates within each tree to calculate percent emergence per survey date. This single emergence phenology for each of the early and delayed oviposition events per tree provided the data for the repeated-measures ANOVA.

Percent survivorship and percent of galls exceeding the natural enemy threshold were transformed using the empirical logistic transform (Cox 1970). This transform weights the observed value/replicate by the inverse of its variance (i.e., a function of sample size and binomial outcome). Transformed means and SEs are not readily back transformed; thus means and SEs of untransformed percentages are presented.

Two-way ANOVAs were used to assess the effects of a delay in oviposition and tree on the following performance measures: (1) development time, (2) number of galls produced per female, (3) gall size, (4) maximum attainable gall size, and (5) percent of galls exceeding the natural enemy threshold. Each variable (with the exception of variable 2 was tested using only the data from the −NE treatments to avoid confounding effects of oviposition timing and parasitism. For variable 2, a preliminary ANOVA showed that gall production per female did not differ between +NE and −NE treatments ($F_{1,158} = 1.21$ $P = 0.27$), thus, the hypothesis that delayed oviposition influences per capita gall production was tested using data from both the +NE and −NE treatments. A three-way ANOVA was used to assess survivorship as a function of oviposition timing, exposure to natural enemies, and tree. Preliminary ANOVAs conducted within the treatments exposed to (E+NE, D+NE), and protected from natural enemies (E−NE, D−NE) showed that survivorship was not affected by collection date ($F_{2,76} = 0.61$, $P > 0.548$ and $F_{2,80} = 1.77$, $P > 0.176$) or a collection date by oviposition timing interaction ($F_{2,76} = 0.20$, $P > 0.820$ and $= F_{2,80} = 0.35$, $P > 0.704$). Hence replicates from all three collection dates were used to estimate survivorship in each treatment.

All ANOVAs were performed using mean values per replicate weighted by sample size and predictor variables (timing of oviposition, exposure to natural enemies, and tree) were considered fixed. All analyses were performed using JMP Version 7 (SAS Institute 2007). We considered gall density as a covariate within replicates but found no significant effect for any variable. Results are presented throughout as means ± SE and the accompanying *n* gives the number of replicates used to estimate parameters.

Results

Experimental outcomes

Oviposition occurred in all 240 replicates across the five trees and galls were established and monitored in 71% (85/120) and 69% (83/120) of the early and delayed replicates, respectively. The average number of galls produced per replicate was 74 ± 4, range 4–298. Enclosures effectively excluded natural enemies, as a single parasitoid emerged from the 6,751 galls that developed in the −NE enclosures. Experiment-wide 12,208 galls were produced and scored and 3,022 *B. treatae* reared. An additional 2,819 naturally occurring galls were monitored as controls (mean = 564; range 300–716 per tree) from which 39 *B. treatae* emerged.

Oviposition timing and life cycle event timing

If development time was of fixed length, both the timing of gall maturation and emergence of *B. treatae* from galls induced by early and delayed oviposition would be predicted to differ by 6–8 weeks. However, development time differed by 7.4 weeks between the treatments mirroring the time lag between early and delayed oviposition cohorts ($F_{1,85} = 487.6$, $P = 0.0001$). The mean number of days from oviposition to emergence for E−NE and D−NE treatments was 287.6 ± 0.4, $n = 44$, and 235.6 ± 0.2, $n = 42$, respectively. As a consequence, the phenologies of gall maturation for the E−NE and D−NE treatments did not differ as evidenced by the nonsignificant treatment ($F_{1,131} = 0.044$, $P = 0.96$) and treatment by time interaction ($F_{1,131} = 0.003$, $P = 0.96$; Fig. 1a). Within both treatments gall development closely paralleled development of the natural control galls. Galls in the early and delayed treatments and controls all reached the 50th and the 95th percentiles for maturation within 5 and 8 days, respectively (Fig. 1a). Ninety percent of galls produced by females within enclosures were mature at the time of the first of three collections.

Similarly, repeated-measures ANOVA of the emergence phenologies of cohorts of *B. treatae* produced by early and delayed oviposition showed that the timing of emergence of the leaf gall generation in the fall was not affected by changes in the timing of oviposition in the spring ($F_{1,128} = 0.205$, $P = 0.65$; Fig. 1b). There was no evidence of a time-by-treatment interaction ($F_{15,128} = 0.26$, $P = 0.99$). *B. treatae* emergence from leaf galls occurred from early December to late February and was bimodal with peaks occurring in early January and February for both oviposition events. The 50th and the 95th percentiles for emergence were 13 January and 6 February for the E−NE treatment and 10 January and 4 February for the D−NE treatment, respectively. The phenology of

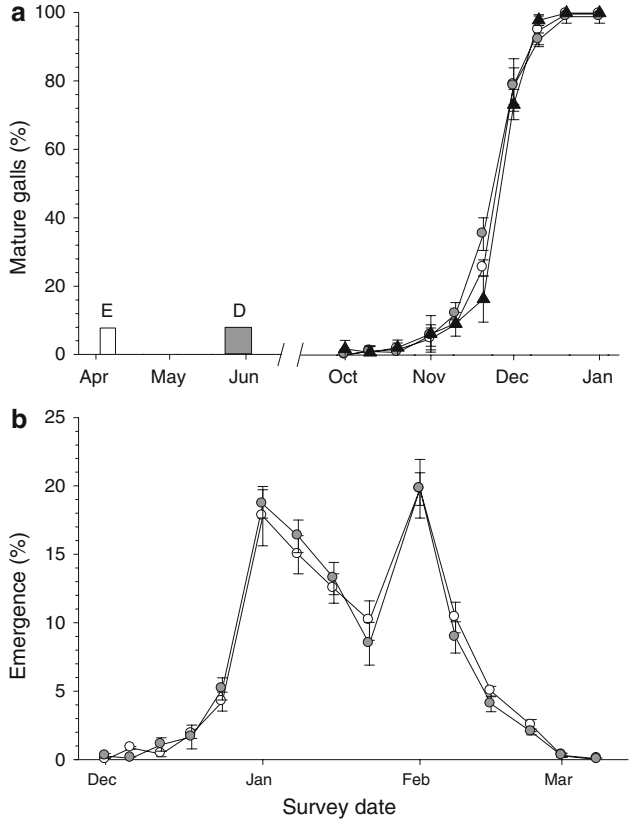

Fig. 1 **a** Gall maturation schedules (back-transformed means ± 95% confidence interval) for early (E; *open circles*), delayed (D; *filled circles*), and naturally occurring (*filled triangles*) oviposition events. **b** Phenology of emergence of *Belonocnema treatae* from leaf galls initiated by E (*open circles*) or D oviposition (*filled circles*) when protected from natural enemies (−NE). Phenologies were based on 1,158 and 1,371 *B. treatae* that emerged from the E−NE and D−NE treatments, respectively. *Errors bars* denote back-transformed means ± SEs. Eight percent of gall formers emerged prior to collection. The phenology of emergence from control galls, based on 39 surviving *B. treatae,* is not shown but followed the above pattern. The *bars* labeled E and D denote the time interval of early and delayed oviposition

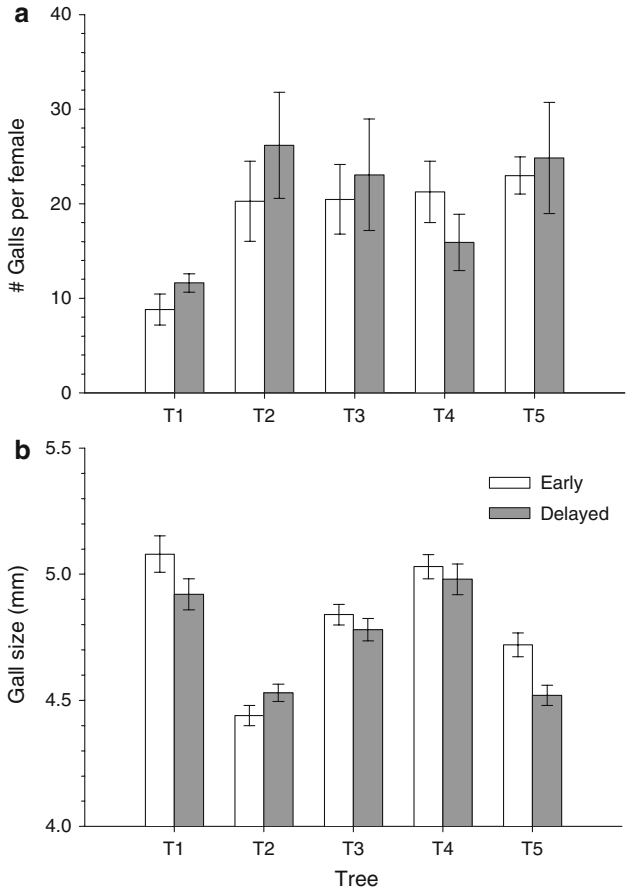

Fig. 2 **a** Mean number of galls produced per female (±SE) in the E (*open bars*) and D oviposition (*filled bars*) cohorts on each of five trees. **b** Mean size of galls produced by female *B. treatae* (±SE) for both E and D oviposition when protected from natural enemies on each of five trees. Means in both **a** and **b** represent the average of 7–10 replicates/treatment per tree with 79 ± 64 (SE) galls measured/replicate. For abbreviations, see Fig. 1

emergence for the 1.3% of *B. treatae* that survived in the controls paralleled the patterns shown in Fig. 1b.

Oviposition timing and performance

While per capita gall production differed significantly among trees ($F_{4,158} = 6.19$, $P < 0.0001$), the number of galls produced by females was not influenced by the delay in oviposition timing: $E = 18.3 \pm 1.2$ ($n = 85$) and $D = 19.5 \pm 1.3$ ($n = 83$; $F_{1,158} = 0.42$, $P = 0.52$; Fig. 2a). These main effects are directly interpretable as there was no evidence of an interaction between timing of oviposition and tree on per capita gall production ($F_{4,158} = 0.44$, $P = 0.78$). Similarly, the size attained by galls that completed development while protected from natural enemies did not differ between the E−NE (4.82 ± 0.06 mm, $n = 44$) and D−NE treatments (4.77 ± 0.04, $n = 42$; $F_{1,76} = 2.26$, $P > 0.14$). Gall size did differ among trees ($F_{4,76} = 17.00$, $P < 0.0001$; Fig. 2b), again with no interaction between tree and oviposition timing ($F_{4,76} = 1.30$, $P = 0.28$). ANOVA based on just those galls that produced a *B. treatae* showed that maximal attainable gall size decreased by 2.1% when oviposition was delayed (E−NE = 4.93 ± 0.04 mm, $n = 44$ and D−NE = 4.83 ± 0.04 mm, $n = 42$; $F_{1,76} = 3.97$, $P = 0.05$). However, a delay in the timing of oviposition does not significantly alter predicted susceptibility of the gall former to natural enemies through a direct effect on gall size as the percent of galls exceeding the natural enemy threshold did not differ between early (19.9%) and delayed (18.7%) oviposition ($F_{1,76} = 0.826$, $P = 0.37$).

Oviposition timing and susceptibility to natural enemies

Natural enemies and the timing of oviposition interacted strongly to affect gall-former survivorship. When protected from natural enemies, two-way ANOVA showed the gall-former survivorship was not influenced by oviposition timing ($F_{1,76} = 0.021$, $P = 0.88$; Fig. 3a). Average survivorship for the E−NE and D−NE treatments was 58% ± 3.60, $n = 44$, and 57.3% ± 3.0, $n = 42$, respectively. Survivorship varied significantly among trees ($F_{1,76} = 6.84$, $P = 0.0001$; range 48.7–64.3%). Three-way ANOVA showed that exposure to natural enemies further reduced survivorship in both the early and delayed oviposition cohorts ($F_{1,148} = 490.51$, $P = 0.0001$; Fig. 3b). However, as illustrated in Fig. 3b and supported by the test of the oviposition timing by natural enemy interaction term, the impact of natural enemies was highly asymmetrical. Gall formers produced by early oviposition and exposed to natural enemies throughout development experienced lower survivorship by greater than an order of magnitude (1.44% ± 0.59, $n = 41$) compared with gall formers produced by delayed oviposition (23.67% ± 1.20, $n = 41$; $F_{1,148} = 100.78$, $P < 0.0001$). The difference in survivorship of *B. treatae* between early and delayed oviposition when exposed to natural enemies did not differ among trees as shown by the test of the tree × NE interaction term ($F_{4,148} = 0.865$, $P = 0.49$). Average survivorship of *B. treatae* reared from control galls collected from the five experimental trees in 2008 was 1.3% and ranged from 0.52 to 2.27% per tree. Survivorship of *B. treatae* in these naturally produced galls exposed to natural enemies paralleled survivorship of *B. treatae* within experimental galls produced by early oviposition that were exposed to natural enemies (Fig. 3b).

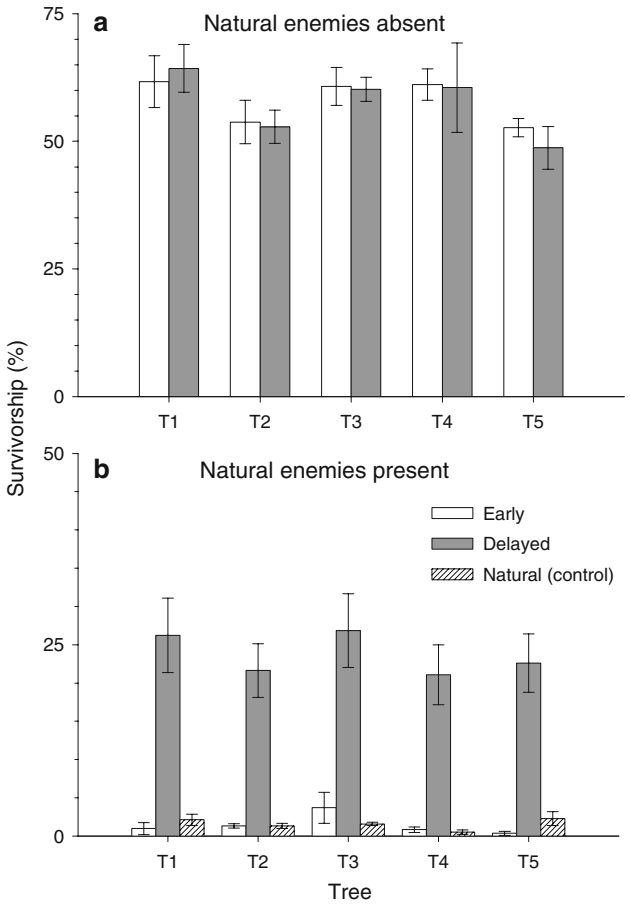

Fig. 3 *B. treatae* survivorship (mean ± SE) for E and D oviposition in the absence of natural enemies (**a**) and in the presence of natural enemies (**b**) on each of five trees. Note the change in scale of the *y*-axis in **a** and **b**. Means and SEs per treatment/tree were based on 7–10 replicates/treatment per tree. The total number of galls surveyed to estimate survivorship for each treatment was: E−NE = 3,224; D−NE = 3,557; E+NE = 2,735; and D+NE = 2,692. Also shown in **b** is the survivorship of *B. treatae* within naturally occurring galls that developed on each tree in the presence of natural enemies (mean sample size/tree = 564, range 300–716). For abbreviations, see Fig. 1

Discussion

Partial to complete defoliation of host plants is encountered by herbivorous insect species that share hosts with herbivores capable of reaching outbreak densities. Given the reliance of gall formers on specific and ephemeral tissues for gall induction and tightly synchronously life cycles, the broad hypothesis that defoliation disrupts life cycle events, reduces performance and survivorship on the host plant, and alters the impact of natural enemies is a logical one for this diverse group of herbivores. To our knowledge ours is the first study to examine simultaneously the direct effects of a delay in tissue formation produced by local defoliation on development time, phenology of emergence, measures of performance, and survivorship on the host plant and the indirect effects of defoliation on susceptibility to natural enemies for a gall-forming insect.

Variable development and conserved emergence times

Belonocnema treatae exhibits developmental plasticity in the time required for the asexual generation to complete development from egg to emergence. Gall formers produced by females forced to oviposit into the delayed second leaf flush exhibit development times that averaged 7.4 weeks shorter than the development times of asexuals produced by females that oviposited into the early (naturally occurring) leaf flush. As a consequence of plasticity in development time (Danks 2006) both the phenology of leaf gall maturation and the emergence of asexual adults are unaffected by variation in the timing of sexual generation oviposition.

How was plasticity in development time expressed? In *Callirhytis cornigera* (Eliason and Potter 2000b) and other gall-formers emergence timing is dependent on the accumulation of degree-days. However for *B. treatae* this mechanism does not apply as the effect of delayed oviposition is opposite to the direction predicted by the degree-day model. One hypothesis is that leaf quality following defoliation was increased allowing gall formers to compensate for the delay in the timing of gall initiation by accelerated growth on the second flush of leaves (Yukawa 2000). In this and other gall-former systems gall size and development rate can be functions of plant resource quality (Price et al. 1987; Egan and Ott 2007). However, our cursory observations of early gall development showed no difference between E and D treatments in the time at which galls first attained three-dimensional form, and our subsequent monitoring showed no difference in the phenology of gall maturation. Moreover, if leaf quality following defoliation were increased, we would have expected to see an increase in gall size as a corollary. We observed no evidence of increased gall size following delayed oviposition. In fact the size of galls from which *B. treatae* emerged was marginally (2%) smaller in the delayed oviposition cohort. A second hypothesis is that variation in oviposition timing does not influence gall development so long as oviposition occurs prior to the onset of rapid gall growth. This hypothesis is bolstered by our observations in previous years that while oviposition can occur as early as mid-March, galls do not develop to three-dimensional form until May. Finally, if gall formers develop to the penultimate stage within mature galls and then await for signals to emerge, so long as the interval between early and delayed oviposition is not longer than the interval between attaining the penultimate stage and emergence, any effects of variation in oviposition timing on emergence timing are likely to be swamped. The bimodal peaks in the emergence phenologies of both early and delayed cohorts shown in Fig. 1b coincided with mid-winter warming trends in central Texas (National Climatic Data Center) and suggest a major role for weather-related events in controlling the immediate timing of emergence of adult *B. treatae* from leaf galls. These hypotheses could be tested by further delaying oviposition by the sexual generation to shorten the time available for development. To better understand how *B. treatae* compensates for variation in the timing of oviposition, both detailed observations of early development and the maturation of gall formers within galls leading up to emergence are needed.

Host plant-mediated effects on gall-former survival

Gall formers face challenges to survivorship that are mediated by the host plant from the time of oviposition up to and including emergence (Denno et al. 1995; Horner et al. 1999; Egan and Ott 2007). Both the early and delayed cohorts of *B. treatae* faced such challenges as evidenced by: (1) mortality rates of ~42% when protected from natural enemies, and (2) significant variation in mortality rates among trees (range 36–51%). These estimates of plant-mediated mortality (1—the percent of galls that produced a *B. treatae*) measure the stage-specific mortality that takes place between the appearance of galls and the emergence of adults. Thus these estimates represent minimum estimates of plant-mediated mortality as they exclude unwitnessed mortality that takes place from the time of oviposition to the development of galls to measurable size. For example, when *B. treatae* develops while protected from natural enemies only a fraction of oviposition scars (each potentially a site of egg insertion) translate into galls and the percentage varies significantly among trees (Egan and Ott 2007). Our estimates of plant-mediated mortality show that the 6- to 8-week delay in oviposition had no effect on the percent of asexual *B. treatae* that survived to emerge from galls. Importantly, this result suggests that there are no intrinsic penalties associated with oviposition into leaves produced following defoliation (such as insufficient time or resources to complete development) that affect survivorship once galls are established. In a related analysis using the distributions of gall sizes from which *B. treatae* emerged and failed to emerge for each of the five experimental trees, we show that plant-mediated mortality (in the absence of natural enemies) is associated with stabilizing selection for intermediate gall size at the time of emergence (S. Egan et al., unpublished data).

Delayed oviposition and natural enemy attack

Typical of cynipids (Stone et al. 2002), the asexual generation of *B. treatae* experiences high mortality from natural enemies. As shown by Lund (1998) and Hall (2001), survivorship within naturally occurring leaf galls is typically in the single-digit range with estimates of zero percent survivorship on individual trees not uncommon even with extraordinary sampling effort (Reynolds 2000; Hood 2009). Our estimates of survivorship for naturally occurring galls did not exceed 2.3% for any tree and reinforce this finding. Survivorship of gall formers produced by early ovipositing females dropped to the level observed in the control galls when exposed to natural enemies. This result confirms that our bagging experiment provided realistic estimates of survivorship for early cohort gall formers and highlights the relative importance of host plants versus natural enemies as mortality agents for this gall-former species. While natural enemies significantly decreased survivorship in both oviposition treatments, *B. treatae* developing in galls induced by delayed oviposition exhibited an order of magnitude greater survival.

Our experiment allowed us to evaluate two competing hypotheses to explain the order of magnitude increase in survival of the delayed cohort when exposed to natural enemies: increased survivorship is due to an increase in the size of galls produced by the delayed cohort, increased survivorship is due to a decrease in the time that the delayed cohort was exposed to natural enemies. For *B. treatae* (Reynolds 2000) as for other gallers (Stone et al. 2002; Craig et al. 2007), gall size is related to survivorship when gall formers are exposed to natural enemy attack. Anticipating the possibility of an effect of delayed oviposition on vulnerability to natural enemies via an effect on gall size, we tracked and then compared the final sizes of galls produced by the early and delayed cohorts. Because parasitism influences gall size (Reynolds 2000), we avoided confounding cause and effect by testing hypothesis one using just those galls that developed while protected from natural enemies. We rejected the gall size hypothesis as a likely explanation for the difference in survivorship between early and delayed cohorts as we detected no biologically significant effect of oviposition timing on the three measures of gall size known to be related to variation in gall-former survival when exposed to natural enemies: average gall size, maximal size, and the percent of galls that exceeded the natural enemy threshold. Consideration of the biology of *B. treatae* and this species' parasitoid community provides support for the second hypothesis: increased survivorship of delayed cohort progeny is due to a decrease in the time that galls produced by delayed oviposition were exposed to natural enemies. Asexual generation *B. treatae* are attacked by a diverse community of natural enemies of which six species attack *B. treatae* coinciding with oviposition and extending through early development (i.e., prior to *B. treatae* larvae producing three-dimensional, measurable-size galls; Hall 2001). Parasitism in this phase does not prevent galls from developing to measurable size (Hall 2001). The early and delayed cohorts differed only in that the early cohort was exposed to an additional 6–8 weeks of natural enemy attack during early development. Because the progeny produced by both early and delayed oviposition were fully exposed to natural enemies from the time that galls attained three-dimensional form to emergence, we interpret the asymmetry in survivorship as the consequence of increased natural enemy attack during the initial phase of gall development for progeny of gall formers that oviposited early in the season.

We found no difference in per capita gall production for females as a function of the timing of oviposition (or natural enemy exposure). The above considerations of the interaction of the gall former and its natural enemies also explain why the increased mortality of progeny of early ovipositing gall formers did not decrease per capita gall production: *B. treatae* eggs and/or larvae parasitized early in the gall-induction process survive to maintain gall development to a size at which galls are measurable.

Population biology of variation in oviposition timing and natural enemy attack

The order of magnitude difference in survival between gall formers developing on the initial and delayed leaf flush has intriguing implications for gall-former populations that experience episodic shifts in the timing of availability of young leaves due to defoliation in central Texas. At the population level, the increased survival of asexuals that develop in galls on leaves produced following defoliation could maintain *B. treatae* populations in years in which defoliators reach outbreak densities and essentially consume the initial pulse of developing gall formers. Rapid rebound of asexual generation populations decimated by defoliators in high defoliation years may be possible. The contribution of sexual generation females whose emergence coincided with the second flush of leaves to the overall size of the asexual generation that survives to emerge from leaf galls would be expected to be a function of the degree of defoliation and the percent of females emerging on the delayed schedule.

Cryer (2003) working in a year in which defoliators were not at outbreak densities, showed that the emergence phenology of sexual generation *B. treatae* centered on the initial spring leaf flush which spanned a 45-day interval from late March to early May. At the time the study was terminated dissection of root galls revealed that 18% of living *B. treatae* still had not emerged, thus the realized emergence window even in non-outbreak years is perhaps wider still. We do not know if there is a cause and effect relationship between live oak defoliation and delayed emergence of sexual generation *B. treatae*. Thus, in years where defoliation occurs, we do not know whether those *B. treatae* that oviposit into the post-defoliation flush of leaves represent the right-hand tail of an emergence phenology timed to coincide with the initial flush of oak leaves or whether later ovipositing females represent a subgroup whose emergence was either intrinsically timed or conditionally triggered to coincide with a possible second flush. Our experimental delay of 6–8 weeks produced emergent wasps that overlap with the right-hand tail of the emergence window described by Cryer (2003). We do know that completely defoliated trees can support significant densities of leaf galls following the second flush of leaves (Hood 2009). It is tempting to speculate that defoliation acts as a cue mediated by the tree to modulate root galler development and resynchronize galler emergence and leaf flush.

The consequences for leaf gallers developing under variable conditions are important for addressing how selection may operate to influence the timing of life cycle

events in *B. treatae*. We found no evidence for an effect of oviposition timing on any component of gall-former fitness other than the strong effect on survival. However, the observed differences in the survivorship of asexuals related to oviposition timing will contribute significantly to fitness variation of sexual generation females that emerge throughout the spring. Our observations of variation in asexual survivorship and variation in defoliation intensity within and among live oak populations suggest that the timing of emergence and oviposition may be subject to natural selection that fluctuates in direction and intensity both temporally and spatially. Because sexuals are short lived, individual females experience either the initial (pre-defoliation) leaf flush or the second leaf flush in defoliation years. Hence each sexual female's offspring likely experience the survivorship associated with initial or delayed oviposition. When live oak populations exhibit variation in intensity of defoliation the population of leaf gallers developing on undefoliated, partially defoliated, and completely defoliated trees will differ in the mix of progeny produced by sexual generation females that emerged and oviposited prior to or following defoliation. In years of little or no defoliation, selection would be expected to favor sexual generation females who emerge in synchrony with the initial leaf flush. *B. treatae* emerging as late as the 6- to 8-week delay we imposed (a delay we have observed in nature in defoliation years; Hood 2009) would be severely disadvantaged for two reasons. First, leaves capable of supporting gall induction constitute an ephemeral resource as galls can be initiated only on young leaves. While leaf flush varies among trees, virtually all live oaks at the study site complete leaf flush within a 2- to 3-week span (G. H., personal observation). Thus suitable leaves are a limiting resource. Second, oviposition sites on those leaves that are available likely become limiting as the emergence–oviposition season progresses and the ratio of gall formers to suitable leaves increases. In years when gall formers are abundant up to 100% of leaves on live oak can exhibit oviposition scars, and both oviposition scar and gall density per leaf can reach very high levels (Lund 1998). In contrast, in years in which defoliators have a significant impact on live oak foliage, selection would be expected to favor sexual generation females who emerge in synchrony with the delayed leaf flush. The survivorship of progeny of sexual generation *B. treatae* emerging in synchrony with the initial leaf flush would be reduced relative to *B. treatae* emerging in synchrony with the delayed leaf flush due to both defoliators and the heightened impact of natural enemies. Under all conditions whether *B. treatae* populations could respond to continued selection would depend on the genetic basis of variation in emergence timing of the sexual generation.

In summary we have shown that asexual generation *B. treatae* express plasticity in development time. This plasticity allows populations of gall formers that initiate development asynchronously on individual live oaks as a consequence of prior defoliation to later emerge in synchrony. Developmental plasticity re-establishes synchrony in defoliation years between the gall-former generations by linking the emergence of the asexuals to the initiation of the sexual generation. While the host plant exerts strong influences on survivorship there are no intrinsic penalties to delayed oviposition that are mediated through the host plant. However, gall formers that develop from delayed oviposition experience greatly increased survival in the face of natural enemies, via a reduction in exposure to enemy attack. Differential mortality linked to delayed resource availability suggests the possibility of alternating directional selection for oviposition timing as a consequence of episodic defoliation of this specialized herbivore's host plant.

Acknowledgments We thank S. Egan, M. Eubanks, J. Fordyce, C. Nice, and E. Silverfine for reviews of this manuscript, B. Weckerly for statistical advice, and R. Porter and N. Medina for help with fieldwork. The Department of Biology and the College of Science of Texas State University–San Marcos, the Freeman Ranch Advisory Board, and a Theodore Roosevelt Memorial Grant from the American Museum of Natural History provided support to G. Hood. This study complied with the laws and regulations of the United States of America.

References

Askew RR (1971) Parasitic insects, 1st edn. Elsevier, New York

Barbosa P, Schultz JC (1987) Insect outbreaks, 1st edn. Academic Press, San Deigo

Blair CP, Abrahamson WG, Jackman JA, Tyrrell L (2005) Cryptic speciation and host-race formation in a purportedly generalist tumbling flower beetle. Evolution 59:304–316

Briggs CJ, Latto J (1996) The window of vulnerability and its effect on relative parasitoid abundance. Ecol Entomol 21:128–140

Clancy KM, Price PW (1986) Temporal variation in three-trophic-level interactions among willows, sawflies and parasites. Ecology 67:1601–1607

Cox DR (1970) The analysis of binary data. Methuen, London

Craig TP, Itami JK, Price PW (1990) The window of vulnerability of a shoot-galling sawfly to attack by a parasitoid. Ecology 71:1471–1482

Craig TP, Itami JK, Horner JD (2007) Geographic variation in the evolution and coevolution of a tritrophic interaction. Evolution 61:1137–1152

Crawley MJ, Akhteruzzaman M (1988) Individual variation in the phenology of oak trees and its consequence for herbivorous insects. Funct Ecol 2:409–415

Cryer G (2003) Temporal and spatial patterns of parasitoid attack on a root-galling cynipid. Masters thesis, Department of Biology, Texas State University, San Marcos

Cushman JH, Boggs CL, Weiss SB, Murphy DD, Harvey AW, Ehrlich PR (1994) Estimating female reproductive success of a

threatened butterfly: influence of emergence time and host plant phenology. Oecologia 99:194–200
Danks HV (2006) Key themes in the study of seasonal adaptations in insects. II. Life cycle patterns. Appl Entomol Zool 41:1–13
Denno RF, McClure MS, Ott JR (1995) Competition revisited and resurrected. Annu Rev Entomol 40:297–331
Drees BM (2004) Oak leaf roller and springtime defoliation of live oak trees. Texas Cooperative Extension E-206
Egan S, Ott JR (2007) Host plant quality and local adaptation determine the distribution of a gall-forming herbivore. Ecology 88:2869–2879
Eliason EA, Potter DA (2000a) Budburst phenology, plant vigor, and host genotype effects on the leaf-galling generation of *Callirhytis cornigera* (*Hymenoptera*: *Cynipidae*) on pin oak. Environ Entomol 29:1199–1207
Eliason EA, Potter DA (2000b) Biology of *Callirhytis cornigera* (*Hymenoptera*: *Cynipidae*) and the arthropod community inhabiting its galls. Environ Entomol 29:551–559
Forkner RE, Marquis RJ, Lill JT, Corff JL (2008) Timing is everything? Phenological synchrony and population variability in leaf-chewing herbivores of *Quercus*. Ecol Entomol 33:276–285
Hall MC (2001) Community structure of parasitoids attacking leaf galls of *Belonocnema treatae* on *Quercus fusiformis*. Masters thesis, Department of Biology, Texas State University, San Marcos
Hayward A, Stone GH (2005) Oak gall wasp communities: evolution and ecology. Basic Appl Ecol 6:435–443
Hicks BJ, Aegerter JN, Leather SR, Watt AD (2007) Asynchrony in larval development of the pine beauty moth, *Panolis flammea*, on an introduced host plant may affect parasitoid efficiency. Arthropod Plant Int 1:213–220
Hood GR (2009) Effects of prior defoliation on the timing of life cycle events and susceptibility to natural enemies of a host specific gall-former. Masters thesis, Department of Biology, Texas State University, San Marcos
Horner JD, Craig TP, Itami JK (1999) The influence of oviposition phenology on survival in the host races of *Eurosta solidaginis*. Entomol Exp Appl 93:121–129
Joy JB, Crespi BJ (2007) Adaptive radiation of gall-inducing insects within a single host-plant species. Evolution 61:784–795
Kaitaniemi P, Ruohomaki K (1999) Effects of autumn temperature and oviposition date on timing of larval development and risk of parasitism in a spring folivore. Oikos 84:435–442
Kaitaniemi P, Ruohomaki K, Haukioja E (1997) Consequences of defoliation on phenological interaction between *Epirrita autumnata* and its host plant, mountain birch. Funct Ecol 11:199–208
Karban R, Baldwin IT (1997) Induced responses to herbivory, 1st edn. University of Chicago, Chicago
Komatsu T, Akimoto S (1995) Genetic differentiation as a result of adaptation to the phenologies of individual host trees in the galling aphid *Kaltenbachiella japonica*. Ecol Entomol 20:33–42
Krause SC, Raffa KF (1995) Defoliation intensity and larval age interact to affect sawfly performance of previously injured *Pinus resinosa*. Oecologia 102:24–30
Lund JN (1998) The biology and ecology of *Belonocnema treatae* (*Hymenoptera*: *Cynipidae*) on its host plant, *Quercus fusiformis.* Masters thesis, Department of Biology, Texas State University, San Marcos
Lund JN, Ott JR, Lyons R (1998) Heterogony in *Belonocnema treatae* Mayr (*Hymenoptera*: *Cynipidae*). Proc Entomol Soc Wash 100:755–763
Mopper S (1996) Adaptive genetic structure in phytophagous insect populations. Trends Ecol Evol 11:235–238
Mopper S (2005) Phenology—how time creates spatial structure in endophagous insect populations. Ann Zool Fenn 42:327–333
Mopper S, Simberloff D (1995) Differential herbivory in an oak population: the role of plant phenology and insect performance. Ecology 76:1233–1241
Muller CH (1961) The live oaks of the series Virentes. Am Midl Nat 65:17–39
Nylin S, Gotthard K (1998) Plasticity in life-history traits. Annu Rev Entomol 43:63–83
Ohgushi T (1991) Lifetime fitness and evolution of reproductive pattern in the herbivorous lady beetle. Ecology 72:2110–2122
Parry D, Herms DA, Mattson WJ (2003) Responses of an insect folivore and its parasitoids to multiyear experimental defoliation of aspen. Ecology 84:1768–1783
Potter DA, Redmond CT (1989) Early spring defoliation, secondary leaf flush, and leafminer outbreaks on American holly. Oecologia 81:192–197
Price PW, Roininen H, Tahvanainen J (1987) Why does the bud-galling sawfly, *Euura mucronata*, attack long shoots? Oecologia 74:1–6
Rehill B, Schultz J (2002) Opposing survivorship and fecundity effects of host phenology on the gall-forming aphid *Hormaphis hamamelidis*. Ecol Entomol 27:475–483
Reynolds R (2000) The role of natural enemies in determining the relationship between gall size and emergence success of a host-specific cynipid. Masters thesis, Department of Biology, Texas State University, San Marcos
Rieske LK, Raffa KF (1998) Interactions among insect herbivore guilds: influence of thrips bud injury on foliar chemistry and suitability to gypsy moths. J Chem Ecol 24:501–523
SAS Institute (2007) JMP version 7.0. SAS Institute, Cary, NC
Stewart JW, Bailey B (1993) Defoliation of live oak trees by the oak leaf roller and a closely related moth. Texas Agricultural Extension UC-021
Stone GN, Schronrogge K, Atkinson RJ, Bellido D, Pujade-Villar J (2002) The population biology of oak gall wasps (*Hymenoptera*: *Cynipidae*). Annu Rev Entomol 47:633–668
Tikkanen OP, Julkunen-Tiitto R (2003) Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operophtera brumata*. Oecologia 136:224–251
Van Asch M, Visser ME (2007) Phenology of forest caterpillars and their host trees: the importance of synchrony. Annu Rev Entomol 52:37–55
Van Dongen S, Backeljau T, Matthysen E, Dhondt AA (1997) Synchronization of hatching date with budburst of individual host (*Quercus robur*) in the winter moth (*Operophtera brumata*) and its fitness consequences. J Anim Ecol 66:113–121
Van Nouhuys S, Lei G (2004) Parasitoid-host metapopulation dynamics: the cause and consequences of phenological asynchrony. J Anim Ecol 73:526–535
Wallin KF, Raffa KF (2001) Effects of folivory on subcortical plant defenses: can defense theories predict interguild processes? Ecology 82:1387–1400
Weis AE, Abrahamson WG (1986) Evolution of host-plant manipulation by gall makers: ecological and genetic factors in the *Solidago-Eurosta* system. Am Nat 127:681–695
Weis AE, Walton R, Crego CL (1988) Reactive plant tissue sites and population biology of gall makers. Annu Rev Entomol 33:467–486
Yukawa J (2000) Synchronization of gallers with host plant phenology. Popul Ecol 42:105–113
Yukawa J, Akimoto K (2006) Influence of synchronization between adult emergence and host plant phenology on the population density of *Pseudasphondylia neolitseae* (Diptera: Cecidomyiidae) inducing leaf galls on *Neolitsea sericea* (Lauraceae). Popul Ecol 48:13–21