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Life History, Natural Enemies, and Management of *Disholcaspis quercusvirens* (Hymenoptera: Cynipidae) on Live Oak Trees

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ABSTRACT Live oak (*Quercus virginiana* Mill.) trees are hosts to a complex of gall making arthropods. However, the bullet galls produced by the asexual generation of the cynipid *Disholcaspis quercusvirens* (Ashmead) can esthetically and physically damage nursery and street trees, and thus reduce tree value. We sought to describe the unknown sexual generation of *D. quercusvirens*, describe the development of galls from both generations, record adult cynipid and parasitoid activity periods, and evaluate the efficacy of several insecticides to suppress the gall makers and prevent additional gall formation. The oviposition period for asexual females occurred from late November to January in both years of the caging study. Eggs laid into dormant buds resulted in small bud galls in which the sexual generation developed for 4–5 mo. Sexual adults emerged and laid eggs in young elongating shoots in April. Bullet galls began protruding from branches in June, and asexual wasps emerged 5–7 mo later. Cynipids that emerged from the bullet (asexual generation) and bud (sexual generation) galls were genetically identical. Both generations were heavily parasitized. Targeting asexual females with an early December treatment of bifenthrin or acephate significantly reduced the number of bud galls, but control did not extend to the next generation of bullet galls, possibly because of reinvasion from neighboring infested trees.

KEY WORDS gall wasp, bullet gall, alternating generation, *Quercus virginiana*

Species of *Disholcaspis*, or bullet gall wasps, attack several oak species (Fagaceae: *Quercus*) in North and Central America (Eckberg and Cranshaw 1994, Melika and Abrahamson 2002). These cynipids have alternating generations (Melika and Abrahamson 2002), in which the asexual wasps develop singly or in clusters of detachable, spherical branch, or root galls. The sexual-generation eggs are laid in leaf or flower buds, which become small thin-walled galls (Morgan et al. 1983, Melika and Abrahamson 2002). Clusters of bullet galls are referred to as “compound galls”. An occasional cluster of bullet galls is considered harmless (Gilman and Watson 2011), but severe infestations may partially or completely girdle branches, thus reducing tree quality and value. Succulent bullet galls also produce a sticky exudate that attracts stinging insects, which creates a hazard for sensitive and al-

lergic individuals (Felt 1940, Seibert 1993; J.P. Bird and E.A. Buss, personal observations).

Disholcaspis quercusvirens (Ashmead) infests southern live oak (*Quercus virginiana* Mill.), sand live oak (*Quercus geminata* Small), and running oak [*Quercus minima* (Sarg.) Small] (Price et al. 2004) in South Carolina, Georgia, Florida, Mississippi, Louisiana, and Texas (Krombein et al. 1979). Only wasps from its asexual, stem, or “bullet” gall generation have been taxonomically described (Ashmead 1881), and little of the wasp’s biology is known. As part of a gall maker complex in Florida, *D. quercusvirens* has contributed to the decreased production and sale of live oak clones, the return to seedling tree production, and has increased the number of treatments needed to produce marketable trees (E.A. Buss, personal observations). Knowledge of the life cycle and natural enemy complex of *D. quercusvirens* may help minimize labor-intensive corrective pruning (Eliason and Potter 2000c) and help synchronize insecticide applications with adult or larval susceptibility to prevent full gall formation.

In this study, we identified the sexual generation of *D. quercusvirens*, described gall development of both asexual and sexual generations, determined adult cynipid and parasitoid activity periods, and evaluated the efficacy of three contact insecticides against active asexual adults to disrupt the life cycle.

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Materials and Methods

Study Sites. The biology, development, and management of *D. quercusvirens* were studied primarily at a tree nursery in Penney Farms (Putnam County), FL, from 2007 to 2008. Blocks of similarly sized (≈ 5 m in height, ≈ 6 cm diameter at breast height), field grown, clonal Cathedral live oak trees that were heavily infested with *D. quercusvirens* stem galls were selected for use. A secondary site with Cathedral live oak trees (University of Florida Horticultural Tree Unit, Gainesville, FL) was used to augment bud gall collections in 2008 (trees were ≈ 5 m in height, ≈ 11 cm diameter at breast height).

Asexual Bullet Gall Development and Inhabitants. Four to 10 compound bullet galls of the asexual generation assumed to be actively growing were periodically cut from randomly selected live oak trees at the tree nursery from August to December 2007 and from July to November 2008 to monitor mean gall growth. Bullet galls were then frozen and later examined under a binocular dissecting scope. To rear and identify arthropods inhabiting the bullet galls, another 5–10 compound galls were collected every 2–3 wk from early July through mid-December 2007. The number of bullet galls per compound gall was counted, and the mean maximum compound gall length, individual bullet gall diameter (two measurements taken perpendicular to each other and averaged) and height (from base of stem gall after removal from branch to the highest point), and mean diameter of the branch apical to the compound gall were measured with a digital caliper. Dimensions of individual and compound galls were associated with the insects reared from them to determine if the inhabitant altered bullet gall morphology.

To rear specimens, bullet galls were placed individually into scintillation vials, held in the laboratory (a photoperiod of 13:11 [L:D] h; 23.7°C; and $\approx 48\%$ relative humidity [RH]), and checked weekly. All specimens collected were preserved in 95% ethyl alcohol (EtOH) and identified by G.M. The effects of bullet gall inhabitant (i.e., gall maker, parasitoid, or inquiline) on mean maximum gall diameter and height, and number of galls per compound gall, were compared by using multivariate analysis of variance (ANOVA) (SAS Institute, version 9.2, 2008). In addition, the abdomens of 20 asexual female *D. quercusvirens* stored in 95% EtOH were dissected under a binocular dissecting scope (20 \times magnification) and the number of mature eggs were counted (potential fecundity). Previtellogenic eggs were not present.

Asexual Female Longevity. Ten branches containing one to four compound bullet galls were collected weekly from the tree nursery on 2–3-yr-old unpruned trees starting ≈ 20 October 2008. Compound galls were held in 0.12-liter plastic Solo cups with vented lids (≈ 2.5 -cm, in diameter, hole covered with white chifon mesh) to allow air flow. Galls in cups were held under partial shade in a shelf (0.3 by 0.3 by 0.6 m) with a solid roof to better approximate outdoor conditions.

Cups were checked daily for *D. quercusvirens* emergence.

To determine the longevity of individual *D. quercusvirens* females, a no-choice test was conducted during early (21 November to 2 December 2008) and late (4–9 January 2009) wasp emergence from bullet galls ($n = 30$ wasps per treatment at each time interval). Within 24 h of emergence from a bullet gall, a female was placed into a clear plastic deli container (0.47 liter; 7 cm in height, 8.5 cm bottom diameter) with a plastic Solo cup (29.6 ml) secured to the bottom. Each Solo cup contained water, a 10% honey solution, or nothing (control). The water and honey solutions were soaked into a 5.1-cm-long dental wick projecting through each lid, and solutions were replaced every 2 d. Because a variable number of *D. quercusvirens* emerged daily, single replicates were prepared over time. Containers were held in a rearing chamber (a photoperiod of 13:11 [L:D] h; 26.4°C; $\approx 40\%$ RH). The effects of diet on gall maker longevity were compared by using PROC LIFETEST (SAS Institute, version 9.2, 2008).

Confirmation of the Alternate Generation. To minimize parasitism, maximize gall wasp survival, and prevent invasion by other gall makers, we caged young compound galls of the asexual generation on their branches in the lower crown (≤ 2.2 m) ($n = 30$) and upper crown (≈ 2.2 –4.3 m) ($n = 20$) of infested live oaks in June and July 2007. Cages consisted of a fine white organza mesh. Asexual *D. quercusvirens* females eventually emerged and oviposited within the cages. Because dormant buds were anticipated to be the targets for oviposition, the initial sizes of 30 buds present during the asexual wasp emergence period were recorded. Caged branches were removed in late March 2008. Any new galls found on those branches were presumed to contain the alternating sexual generation.

In April 2008, three male and two female cynipids recovered from bud galls within the cages were preserved in 95% EtOH and, based on their morphology, were confirmed as a species of *Disholcaspis*. That the cynipids from the bullet and bud galls comprised a single species lifecycle was then tested by using a DNA barcoding approach using mitochondrial and nuclear sequence data for the three male sexual *Disholcaspis* specimens and three female asexual *D. quercusvirens* specimens (for an example of this approach see Stone et al. 2008). Molecular work was carried out at the NERC Genepool facility at the University of Edinburgh, United Kingdom.

Bud Gall and Gall Maker Development. On 9, 11, and 16 March 2009, 10 branches were selected randomly from trees at the nursery near Penney Farms. Twenty bud galls of the sexual generation per date were dissected to determine gall maker survival and development, and to document gall morphology. The length and width of galled and ungalled buds while bud scales completely enclosed the galls were compared by using a *t*-test (SAS Institute, version 9.2, 2008). To minimize variability, bud galls that were visible because leaves already had started to expand and bud scales were falling off were excluded. The bud

stage (i.e., dormant, swelling, green tip, or expanded leaves [Eliason and Potter 2000b]), bud length and width, gall length and width (after bud scales were removed), and gall maker life stage were recorded.

Bud Gall Inhabitants. To determine the survival of the sexual generation of *D. quercusvirens* in bud galls and describe its natural enemy complex, bud galls were collected weekly from 4 April to 14 May 2008 from the lower crowns of live oak trees at the nursery in Penney Farms and the UF Horticultural Tree Teaching Unit. Bud galls ($n = 480$) were carefully removed from branches and placed individually into clear gelatin capsules (size 0) (Shorthouse 1972) in the laboratory (a photoperiod of 13:11 [L:D] h; 23.7°C; and $\approx 48\%$ RH). Cynipid and natural enemy emergence was monitored daily. All specimens were preserved in 95% EtOH and identified by G.M.

The activity period of the sexual generation emerging from bud galls in 2009 was also monitored. Ten branches (≈ 10 cm long) were collected on 9 March 2009, before bud galls were visible to the collector, from live oaks at the nursery in Penney Farms, and each bud was placed individually into a gelatin capsule (size 0), held at room temperature, and insect emergence dates and species were recorded. In addition, at least 20 bud galls were collected on 16, 19, and 24 March; 17 and 21 April; and 4 and 11 May 2009, and reared in a similar manner. Twenty sexual-generation females reared from bud galls collected in mid-May were later dissected under a binocular dissecting scope to count the number of eggs per female abdomen.

Insecticide Trial. Three contact insecticides were evaluated for their ability to either kill asexual *D. quercusvirens* during emergence or before ovipositing, thus preventing bud gall formation. Treatments included acephate (1.17 liters/ha, Orthene TTO 97, Valent USA Corporation, Walnut Creek, CA), bifenthrin (1 ml/liter, Bifen XTS, Control Solutions, Inc., Pasadena, TX), carbaryl (292.3 ml/ha, Sevin SL, Bayer Environmental Science, Research Triangle Park, NC), and an untreated control.

Heavily galled trees within a block of field-grown live oaks at the nursery in Penny Farms were selected for this test. Three untreated tree rows separated each treatment row to minimize insecticide drift and contamination. Within a treatment row ($n = 6$ replicates/rows), four sets of five to seven heavily galled trees were selected and each was randomly assigned one treatment (e.g., acephate, bifenthrin, carbaryl, or control). Insecticides were applied to the foliage of two trees in a set either once (24 November 2008, after most field-collected bullet galls contained adult *D. quercusvirens*, based on monitoring) or twice (24 November and 15 December 2008, before peak asexual adult emergence was expected) to the other two trees. Within each set, one to three buffer trees separated each pair of treatment trees. Treatments were sprayed to runoff with a two-nozzle (XR Tee Jet 110/2 VS) 1-m boom by using a CO₂ backpack sprayer at 0.22 MPa (Weed Systems Inc., Hawthorne, FL).

To evaluate efficacy, two branches (≈ 23 – 25 cm in length) from each cardinal direction in the upper and lower canopy of each tree were cut, bagged, brought to the laboratory on 24 March 2009, and all bud galls on each branch were counted. Intact bud galls of the sexual generation with no emergence holes were placed individually into clear gelatin capsules and held in the laboratory (a photoperiod of 13:11 [L:D] h; 23.7°C; and 48% RH) to record the percentage of cynipid and parasitoid emergence. Proportion of insect emergence data were arcsine-transformed, treatment differences were analyzed by ANOVA, and, when significant, means were separated by Tukey's honestly significant difference test (PROC GLM, SAS Institute, 2008). Data were presented as a percentage of the insects that emerged.

On 12 August 2009, subsequent bullet gall development was estimated on trees that had been treated only once in November 2008, during early asexual *D. quercusvirens* emergence (trees treated twice were excluded because results from the bud gall generation did not statistically vary with single-treatment trees). The approximate total number of young red-colored bullet galls per tree was counted for 60 s by J.B. and E.B. (one on the east half and the other on the west half of each tree). Results were analyzed with an ANOVA (PROC GLM, SAS Institute, 2008), and means were separated by Tukey's honestly significant difference test.

Results and Discussion

Stem Gall Development and Inhabitants. Bullet galls were initiated by the sexual generation of *D. quercusvirens* during early shoot expansion in the spring. Slight stem swellings were visible by June, and small red-colored bullet galls with soft and spongy tissue (Fig. 1A) emerged through the bark by August. A sweet and sticky exudate occurred on the bullet galls from August to mid-October, when only larvae were present inside the galls. Similarly, stem gall secretions on branches of *Quercus gambellii* Nutt. coincided with *Diaporthe pernicioso* Bassett larval development (Seibert 1993). This exudate attracted red imported fire ants (*Solenopsis invicta* Buren), velvet ants (Hymenoptera: Mutillidae) (Fig. 1B), and paper wasps (Hymenoptera: Vespidae), and supported sooty mold growth (Fig. 1C). Despite oaks not having floral or extrafloral nectaries, certain gall makers (e.g., *Disholcaspis eldoradensis* Beutenmüller) can cause their host trees to secrete a honeydew-like substance. This can result in a mutualism with ants that protect the gall makers from parasitism (Inouye and Agrawal 2004). *D. quercusvirens* bullet galls were mature and woody (Fig. 1D) in the fall, reaching their maximum diameter by November (Fig. 2).

Mean maximum bullet gall diameter was 7.7 ± 0.1 mm in 2007 and 4.8 ± 0.1 mm in 2008 on the trees at the nursery (Fig. 2). Compound galls were 30.4 ± 1.6 mm in length with 9.3 ± 0.6 bullet galls per compound gall (range: 1–44 bullet galls) in 2007 and 25.5 ± 1.4



Fig. 1. Galls of the asexual (A–D) and sexual (E and F) generations of *D. quercusvirens*. (A) Young red bullet galls in August 2007. (B) Older bullet galls tended by ants. (C) Bullet galls covered with sooty mold and mealybug wax. (D) Fully mature bullet galls. (E) Young bud gall in early March 2009, starting to emerge through bud scales. (F) Bud gall with *D. quercusvirens* exit hole. (Photo credit: L. Buss.) (Online figure in color.)

mm in length with 11.0 ± 0.9 bullet galls per compound gall (range: 1–52 bullet galls) in 2008.

Asexual females (Fig. 3A) emerged during cold weather periods from late-November through January and oviposited into dormant buds (1.3 ± 0.07 mm in length, 1.2 ± 0.09 mm in width at base). Females ($n = 20$) carried 64.0 ± 3.0 eggs (range: 43–88 eggs). All eggs within female abdomens appeared to be of similar size, but dimensions were not measured.

In total, 1,650 bullet galls were collected from 1 August to 18 December 2007, and 91.3% were parasitized (Table 1). The natural enemy complex included species of *Synergus* (Cynipidae), *Eupelmus* (Eupelmidae), *Eurytoma* and *Sycophila* (Eurytomidae), *Ormyrus* (Ormyridae), and *Acaenacis* (Pteromalidae) (Fig. 4). Of the insects reared from bullet galls, 8.7% were *D. quercusvirens*, 27.3% were inquiline (species of *Synergus*, Fig. 4A and B), and 63.9%

were parasitoids (Hymenoptera: Eupelmidae, Eurytomidae, Ormyridae, and Pteromalidae) (Table 1). The activity period of the different parasitoid species was not determined.

Of the 1,470 bullet galls collected from the tree nursery in 2008, many did not develop properly, had smaller diameters than bullet galls measured in 2007, and gall inhabitants were unable to complete development on the trees. Only five inquiline specimens were reared (three of an undescribed *Synergus* species and two *Synergus ficigeriae* Ashmead). Seibert (1993) similarly reported that a tree had aborted all 24 compound galls of *D. pernicioso* after it had supported many viable compound galls the previous year. Why this occurred is unknown, but we speculate that tree susceptibility or ability to support galls may vary from year to year (Morgan et al. 1983, Frankie et al. 1992). Other mortality factors could include the introduction

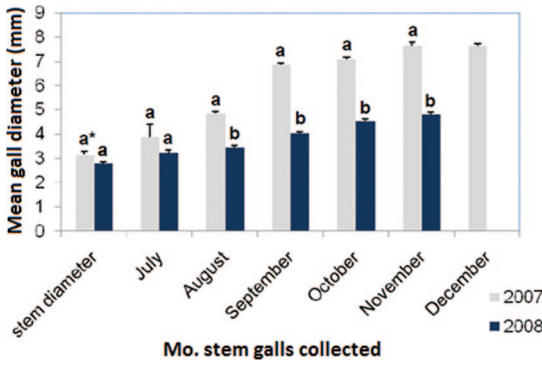


Fig. 2. Mean diameter (millimeters) of bullet galls collected from live oak trees (Clay Co., FL) from July to December 2007 and July to November 2008. *Means \pm SEM within columns followed by a different letter are significantly different at $\alpha = 0.05$ (ANOVA; $F = 260.25$; $df = 12, 3264$; $P < 0.0001$). Letters only represent differences comparing diameters within a month for each year (2007 and 2008), not diameters across months within the same year. (Online figure in color.)

of pathogens that could contaminate the gall tissue or infect the gall maker (Seibert 1993), or parasitoids could have inserted their ovipositors and injured the gall maker, but not deposited an egg, which would have stopped gall growth at that time.

Bullet gall height, diameter, and number of bullet galls per compound stem gall were influenced by the

gall inhabitant (Table 2; multivariate ANOVA, Wilks' $\lambda = 0.8032$; $df = 6, 316$; $P < 0.0001$). Bullet galls containing *D. quercusvirens* and parasitoids in 2007 were significantly wider than those containing inquilines. Bullet galls with live *D. quercusvirens* were significantly taller than those containing parasitoids or inquilines. Furthermore, compound galls with more than five bullet galls in a cluster produced more *D. quercusvirens* than compound galls having five or fewer bullet galls (data not shown).

Bullet galls from which gall makers successfully emerged did not produce any parasitoids or inquilines. However, two or more parasitoids and/or inquilines emerged from many *D. quercusvirens* bullet galls and presumably killed or out-competed the gall maker. Although inquilines are phytophagous, their development may cause gall maker death because of overcrowding or competition (Askew 1984, Csóka et al. 2005). These results are similar to those found in other studies on cynipids, in which all gall makers died because of the occupation of the gall by a *Synergus* inquiline (Washburn and Cornell 1981, Frankie et al. 1992, Seibert 1993, Plantard et al. 1996). Frankie et al. (1992) documented that species of *Synergus* actually changed the morphology of the galls they inhabited by creating denser galls with fewer empty air chambers compared with those galls occupied by parasitoids or gall makers. Similar responses have been observed in rose cynipid galls colonized by *Periclistus* inquilines (Brooks and Shorthouse 1998). This phenomenon of

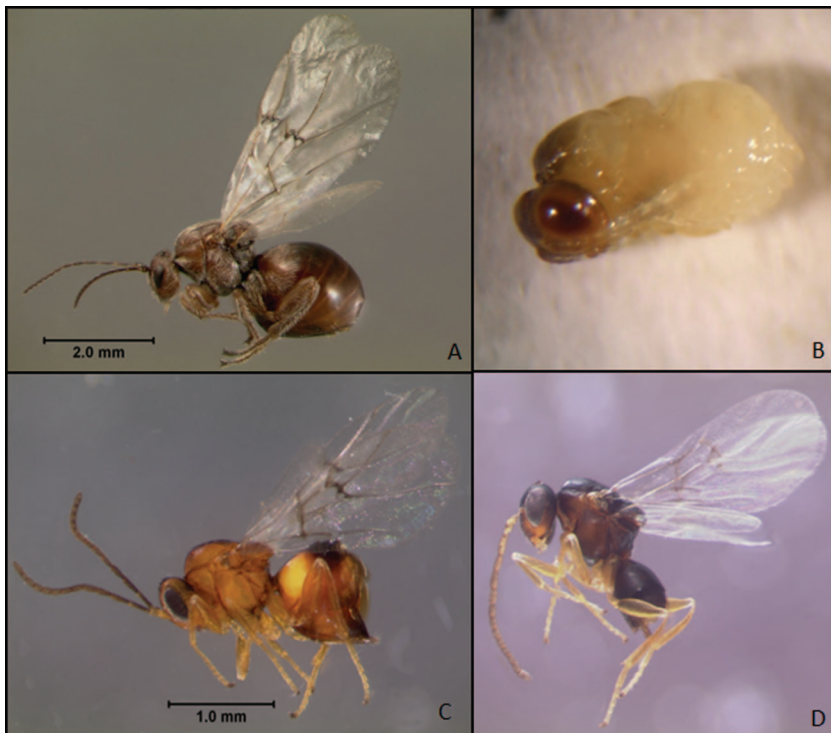


Fig. 3. Life stages of *D. quercusvirens*. (A) Adult asexual female. (B) Pupa of sexual female. (C) Adult sexual female (photo credit: H. Ferrand). (D) Adult sexual male (photo credit: H. Ferrand). (Online figure in color.)

Table 1. Total number of gall makers, inquiline, and parasitoids reared from bullet and bud galls collected from live oak trees, 2007–2009

Family	Taxon	Number from bullet galls (Aug.-Dec. 2007)	Number from bud galls	
			Apr.-May 2008	Mar.-May 2009
Cynipidae	<i>Disholcaspis quercusvirens</i>	16	2	152
	<i>Synergus ficigeriae</i> Ashmead	11	0	0
	<i>Synergus succinipedis</i> (Ashmead)	30	0	0
	<i>Synergus</i> sp. 1	9	0	0
Eulophidae	<i>Aprostocetus</i> species	0	3	2
	<i>Baryscapus</i> species	0	24	585
	<i>Pediobius</i> species	0	1	
Eupelmidae	<i>Brasema auratus</i> (Ashmead)	0	181	460
	<i>Brasema gemmarii</i> (Ashmead)	0	0	101
	<i>Eupelmus</i> sp. 1	31	0	
Eurytomidae	<i>Eurytoma hecale</i> Walker	37	0	0
	<i>Eurytoma</i> species	5	0	0
	<i>Sycophila</i> species	2	0	16
	Unknown species	0	0	53
Ormyridae	<i>Ormyrus hegeli</i> (Girault)	2	0	13
Pteromalidae	<i>Acaenacis lausus</i> (Walker)	40	1	1,002
Total		183	212	2,384

altered gall morphology because of *Synergus* inhabitants was not observed in this study.

Asexual Female Longevity. Female survival in the laboratory ranged from an average 2.5 to 8.1 d (Table

3). For both trials, *D. quercusvirens* provided with water or honey-water lived significantly longer than unfed (control) wasps (Table 3; Trial 1: $\chi^2 = 16.843$, $df = 2$, $P < 0.0002$; Trial 2: $\chi^2 = 17.443$, $df = 2$, $P <$

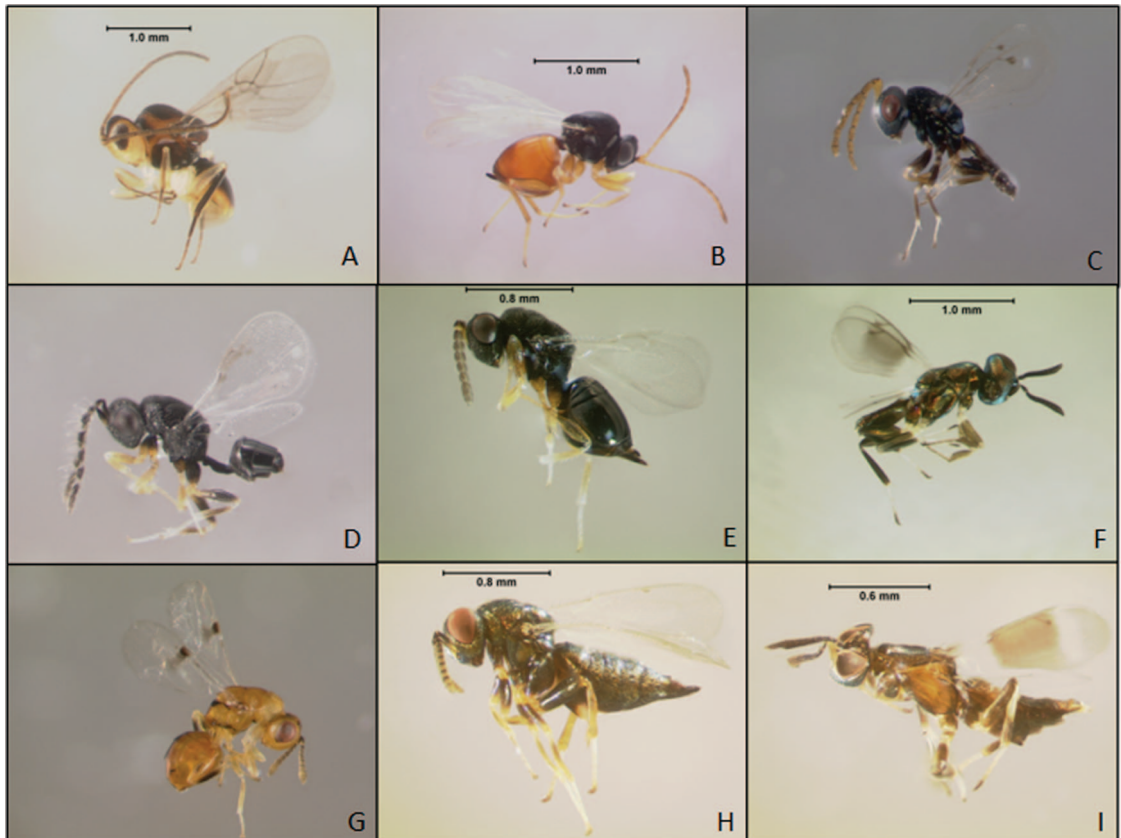


Fig. 4. Parasitoids and inquiline reared from *D. quercusvirens* galls. (A) *Synergus ficigeriae* male, (B) *Synergus succinipedis* female, (C) *Acaenacis lausus* male, (D) *Eurytoma* spp. male, (E) *Eurytoma* spp. female, (F) *Brasema auratus* male, (G) *Sycophila* spp. female, (H) *Ormyrus hegeli* female, and (I) *Brasema gemmarii* female. (Photo credits: H. Ferrand.) (Online figure in color.)

Table 2. Individual bullet and compound gall dimensions when inhabited by the asexual gall maker female, or at least one parasitoid or inquiline

Stem gall inhabitant	No. bullet galls in compound gall	Mean bullet gall diam. \pm SEM (mm)	Mean bullet gall ht \pm SEM (mm)
<i>D. quercusvirens</i>	24.3 \pm 2.7a	8.2 \pm 0.3a	7.2 \pm 0.4a
Parasitoid	15.4 \pm 1.1b	7.5 \pm 0.2a	5.6 \pm 0.1b
Inquiline	18.5 \pm 1.6ab	6.4 \pm 0.3b	5.3 \pm 0.2b

^a Multivariate ANOVA for comparisons of gall inhabitant on gall size and size of compound gall. Means marked with different letters represent significant differences at $\alpha = 0.05$ (Wilks' $\lambda = 0.8032$; $df = 6, 316$; $P < 0.0001$).

0.0002). Wasps in Trial 1 survived significantly longer than the wasps in Trial 2 ($t = 9.56$; $df = 177$; $P < 0.001$), possibly because cynipids in Trial 2 may have spent more time developing inside the bullet galls. In contrast, *Disholcaspis cinerosa* Basset adults may live 3–6 wk (Morgan et al. 1983). To extend their survival in the field, *D. quercusvirens* may find accessible water after a rainstorm, or honeydew from various honeydew-producing hemipterans in the oak trees (Eliason and Potter 2001), although we did not directly observe this behavior. Other environmental conditions (e.g., ambient temperature, relative humidity, or photoperiod) also may affect adult wasp longevity. Because cynipid eggs are generally ready to be laid on asexual adult emergence (Seibert 1993, Eliason and Potter 2000a), feeding by adults may not be necessary for egg development or maturation.

Confirmation of the Alternate Generation. DNA sequencing of the mitochondrial cytochrome *b* gene and the nuclear ITS2 gene revealed identical sequences for the sexual and asexual wasps. This DNA barcode testing, along with production of sexual offspring by caged asexual *D. quercusvirens* and morphological examination, confirmed that the asexual generation developing in the bullet galls and the sexual generation from the bud galls were of the same species. Details of the morphological and DNA analyses demonstrating the alternate generations of *D. quercusvirens* have been described by Melika et al. (2013).

Bud Gall and Sexual Generation Development. Small bud galls developed beneath bud scales (Fig. 1E) and were located on the branches at the base of

a petiole, hidden between young leaves (Fig. 1F). Bud galls were 2.5 ± 0.02 mm in length and 1.1 ± 0.01 mm in width. They likely were initiated by asexual wasp oviposition (i.e., November–January), and remained on trees until May. Bud gall tissue was soft and spongy and continued to grow and harden until the bud scales fell off the galls. The tips of young bud galls, sometimes red in color (Fig. 1E), were visible in early- to mid-March. Emergence of *D. quercusvirens* ended by mid-April, and parasitoid emergence continued until all bud galls fell off the trees in May. Bud galls were fragile after hardening, and, similar to the *D. cinerosa* system (Frankie et al. 1992), could be easily dislodged after adult emergence or inclement weather.

Galled (2.8 ± 0.1 mm length, 1.7 ± 0.08 mm width) and nongalled (2.5 ± 0.1 mm length, 1.6 ± 0.05 mm width) buds collected on 9 March 2009 did not significantly differ in length ($F = 1.70$; $df = 1$; $P = 0.1983$) or width ($F = 2.44$; $df = 1$; $P = 0.1251$). Live oak buds collected on 9 March 2009 ranged in development from dormant to green-tipped.

Sexual generation pupae (Fig. 3B) were present in bud galls by 11 March 2009, and 65% of the bud galls collected on 16 March contained white pupae. First adult emergence (Fig. 3C and D) occurred on 21–23 March 2009 from laboratory rearings, and emergence continued through ≈ 21 April 2009. Although longevity of the sexual generation of *D. quercusvirens* was not evaluated, mated females of *D. cinerosa* were reported to live for ≈ 1 wk (Frankie et al. 1992). Sexual-generation *D. quercusvirens* females had 87.2 ± 3.0 eggs within their abdomens. All eggs appeared to be of the same size and maturity, although measurements were not taken. The ratio of males to females was $\approx 2:3$.

Bud Gall Arthropod Inhabitants. In total, 480 bud galls were collected from 4 April to 14 May 2008, at the end of the sexual *D. quercusvirens* emergence period. Parasitoids comprised 99.1% and *D. quercusvirens* comprised 0.9% of the specimens reared (Table 1). Parasitoids reared from these bud galls included a species of *Aprostocetus*, *Baryscapus*, and *Pediobius* (Eulophidae); *Brasema auratus* (Ashmead) (Eupelmidae); and *Acaenacis lausus* (Walker) (Pteromalidae). The most abundant species was *B. auratus*. No inquilines were reared from the bud galls; a feature shared with some other small and structurally simple oak cynipid galls (Stone et al. 1995, Schönrogge et al. 2000).

From 19 March to 14 May 2009, 3,884 bud galls were collected. These bud galls had a more diverse parasitoid complex (Table 1), which included a species of *Aprostocetus*, *Baryscapus*, and *Pediobius* (Eulophidae); *B. auratus* and *Brasema gemmarii* (Ashmead) (Eupelmidae); *Sycophila* and an unknown species (Eurytomidae); *Ormyrus hegeli* (Girault) (Ormyridae); and *A. lausus* (Pteromalidae). Only one specimen was reared from each bud gall (e.g., bud galls were monothalamous), as observed in other small sexual-generation oak cynipid galls (e.g., Stone et al. 1995, Schönrogge et al. 2000). The most abundant parasitoid in 2009 was *A. lausus*, and it was reared also from bullet galls in 2007. Pteromalid wasps in cynipid galls are

Table 3. Number of days that asexual *D. quercusvirens* females survived when provided different diets in the laboratory

Treatment	Trial 1 ^a	Trial 2
Control	5.1 \pm 0.4a ^b	2.5 \pm 0.2a ^c
Water	8.1 \pm 0.6b	3.3 \pm 2.6b
Honey	7.3 \pm 0.7b	3.6 \pm 0.2b

^a Trials 1 and 2 were significantly different at $\alpha = 0.05$ (TTEST; $F = 9.56$; $df = 177$; $P < 0.0001$).

^b Means \pm SEM within columns with different letters represent significant differences at $\alpha = 0.05$ (Trial 1: LIFETEST; $\chi^2 = 16.843$; $df = 2$; $P < 0.0002$).

^c Means \pm SEM within columns with different letters represent significant differences at $\alpha = 0.05$ (Trial 2: LIFETEST; $\chi^2 = 17.443$; $df = 2$; $P < 0.0002$).

Table 4. Effects of four insecticides on *D. quercusvirens* bud and bullet gall formation when entire field-grown trees were treated in a nursery

Treatment	Trade name	Rate	Total no. bud galls per branch per tree ^a	Total no. bullet galls per tree ^b
Acephate	Orthene TTO 97	1.17 liters/ha	11.5 ± 7.4b	98.3 ± 21.9a
Bifenthrin	Bifen XTS	1 ml/L	1.8 ± 2.7a	90.5 ± 19.5a
Carbaryl	Sevin SL	292.3 ml/ha	15.4 ± 9.7c	96.0 ± 35.6a
Control	—	—	16.7 ± 12.3c	87.8 ± 12.1a

^a ANOVA for means ± SEM within columns followed by a different letter were significantly different at $\alpha = 0.05$ ($F = 8.87$; $df = 27, 347$; $P < 0.0001$).

^b ANOVA for comparison of mean ± SEM bullet galls counted ($F = 0.05$; $df = 3, 20$; $P = 0.987$).

typically parasitoids, and their larvae feed on the eggs or larvae of their hosts (Krombein et al. 1979, Askew 1984). *O. hegeli* (Girault) (Ormyridae) was also reared from both bud galls in 2009 and bullet galls in 2007.

The parasitoid species *Brasema ficigeriae* (Ashmead), *Eupelmus quercus* Ashmead, *Eurytoma querciglobuli rubra* Bugbee, *Eurytoma studiosa* Say, *Torymus elegantissimus* (Ashmead), *Torymus fagopirum* (Provancher), and *Torymus lissus* (Walker) were previously reared from the bullet galls of *D. quercusvirens* (Krombein et al. 1979), but were not found in the current study. *Eurytoma* and *Tetrastichus* (Eulophidae) can feed on developing larvae of *D. perniciosus* in bullet galls (Seibert 1993). It is assumed that the parasitoids reared from the *D. quercusvirens* bud galls also fed on the gall maker larvae or pupae.

Insecticide Trial. We demonstrated that one well-timed application of a contact insecticide before asexual adult emergence can sufficiently reduce the next generation by preventing eggs from being laid. Applications of bifenthrin and acephate at the beginning of asexual *D. quercusvirens* emergence significantly reduced the number of bud galls on sampled branches when compared with carbaryl and the control, with bifenthrin having the greatest reduction of bud galls (Table 4; $F = 8.87$; $df = 27, 347$; $P < 0.0001$). Number of applications (one or two) was initially included in the model, but did not significantly affect the number of bud galls formed. Treatments in November did not reduce the number of new bullet galls per tree in the following year when compared with the control (Table 4; ANOVA, $F = 0.05$; $df = 3, 20$; $P = 0.987$).

These results suggest that one application of bifenthrin or acephate applied in late November or December can significantly reduce bud gall formation

compared with the control, without reducing parasitism in the following bullet gall generation. This knowledge could minimize the number of applications made by live oak tree growers during the winter months. However, one winter application did not reduce bullet gall formation the following summer, which could justify the addition of a spring application aimed against the sexual-generation adults or their offspring. In this test, gall makers from surrounding buffer (untreated) trees may have immigrated to treated trees in the spring, after residues broke down, increasing the numbers of bullet galls that developed in the subsequent generation. Under commercial conditions, entire blocks of trees would be treated, which could minimize tree reinfestation. Targeted applications at or after bud break or repeated applications over two or more years may be necessary to significantly increase tree quality by minimizing gall makers and their galls. Future trials should assess gall maker control in blocks or fields to minimize the potential of reinfestation from neighboring trees.

Effect on Natural Enemy Complex. Treatments had no significant effect on the percentage of parasitoids ($F = 0.58$; $df = 3, 43$; $P = 0.632$) or *D. quercusvirens* ($F = 2.33$; $df = 3, 43$; $P = 0.0875$) that emerged from bud galls. Overall, more parasitoids emerged from bud galls than did *D. quercusvirens* ($F = 98.07$; $df = 1, 22$; $P < 0.0001$). Unfortunately, gall maker emergence is underestimated in this test because branches were harvested during the period in which gall makers emerged from galls. All *D. quercusvirens* adults that emerged in the bags before bud galls were placed into rearing containers were excluded from the data set.

Of all parasitoids reared from intact galls, *Acaenacis lausus* (Hymenoptera: Pteromalidae) was the most abundant, followed by *B. auratus* (Hymenop-

Table 5. Number of *D. quercusvirens* and parasitoids reared from intact bud galls sampled from the insecticide trial

Family	Genus	No. insects reared from bud galls			
		Acephate	Bifenthrin	Carbaryl	Control
Cynipidae	<i>D. quercusvirens</i>	13	11	21	21
Eulophidae	<i>Baruscapus</i> species	98	17	108	125
Eupelmidae	<i>Brasema auratus</i>	114	20	90	97
	<i>Brasema gemmarii</i>	14	0	25	35
Eurytomidae	<i>Sycophila</i> species	1	1	6	2
	<i>Eurytoma</i> species	13	3	18	14
Ormyridae	<i>Ormyrus hegeli</i>	1	0	4	3
Pteromalidae	<i>Acaenacis lausus</i>	227	51	217	350

tera: Eupelmidae), *Baryscapus* (Hymenoptera: Eulophidae), *Sycophila* sp. (Hymenoptera: Eurytomidae), *O. hegeli* (Ormyridae), and *Eurytoma* sp. (Eurytomidae) (Table 5).

The authors, outside the study site at the tree nursery in Penney Farms (Putnam Co.), reared another two species from the bud galls of *D. quercusvirens*: *Brasema flavovariagatus* (Ashmead) and a *Eurytoma* sp. different from a *Eurytoma* sp. reared from bullet galls.

When insecticide applications coincided with asexual adult emergence and were applied to whole trees, results were similar to those of Eliason and Potter (2000c), who showed that treating the stem gall (asexual) generation of *Callirhytis cornigera* (Osten Sacken) reduced the leaf gall (sexual) generation. The numbers of sexual-generation galls for both *C. cornigera* and *D. quercusvirens* were significantly reduced on sampled shoots and branches for treated tree canopies when compared with the control (untreated) trees, whereas the percentages of parasitized galls and the number of bullet galls formed the following year did not differ between treatments.

This is the first study to associate the asexual- and sexual-generation galls and adults of *D. quercusvirens*, identify their natural enemy complex, and describe gall development and the emergence periods of the alternating generations. This information will help live oak nurserymen and landscape managers better formulate a management program to minimize tree infestations and improve tree quality.

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