

LIFE HISTORY AND MANAGEMENT OF A BULLET GALL WASP, *DISHOLCASPIS QUERCUSVIRENS* (HYMENOPTERA: CYNIPIDAE) ON CATHEDRAL[®] LIVE OAK (*QUERCUS VIRGINIANA*) IN NORTHERN FLORIDA

By

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Abstract of Thesis Presented to the Graduate School
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The stem galls of a bullet gall wasp, *Disholcaspis quercusvirens* (Ashmead), are disfiguring to live oak (*Quercus virginiana* Mill.) trees in Florida nurseries and landscapes. The presence of galls reduces tree aesthetics and value, alters tree architecture, and concerns clients enough to return tree shipments to the growers. Little was known about stem gall and asexual wasp development, and the sexual generation wasps and their gall type and location were undescribed. Thus, the life history, seasonality, gall development and management practices of both *D. quercusvirens* generations were determined. Stem galls on Cathedral® live oak trees in Clay Co., Florida, were periodically cut and inhabitants were reared from June 2007 to November 2009. The asexual generation developed in 7-8 months. Asexual wasps laid eggs into dormant buds in November through January, which initiated the sexual generation. Sexual wasps developed for 4-5 months and emerged in April, after bud break and during shoot elongation. Species identification was confirmed by DNA analysis. Both generations were heavily parasitized.

Insecticide trials targeting the asexual generation were conducted as a branch trial (2007) and whole tree trial (2008) to determine the best management practices. The efficacy of three insecticides (acephate, carbaryl and bifenthrin) was tested against emerging gall makers (2007) so the adults would not lay eggs, thus reducing the formation of bud galls and possibly future stem galls (2008). No treatment had a significant effect on asexual wasp emergence, death or parasitism in the 2007 branch trial. Bifenthrin and carbaryl successfully reduced the formation of bud galls in the whole tree trial, however, there were no differences between treatments and the control for number of stem galls counted per tree. These results show that some reduction of galls may occur with correct timing, but long term studies need to be conducted to determine the efficacy of insecticide applications over multiple years.

CHAPTER 1 REVIEW OF LITERATURE

Gall Overview

Galls are created when a plant reacts to the presence and activity of a foreign substance, causing abnormal plant growth (Dreger-Jauffret and Shorthouse 1992). These abnormal structures have fascinated humans for thousands of years. In the Middle Ages, the mysterious growths were thought to predict the future based on what was found inside (Fagan 1918). Later, galls were used for medical remedies, food, and, most importantly, in inks and dyes (Fagan 1918). Recently, galls have been studied for their links to pathological problems, to determine their developmental biology, and the specific interactions between the gall maker and the plant (Mani 1992). Other areas of study concerning galls are based in community ecology and focus on the evolution of the inducer, its host and other gall inhabitants, the importance of competition within gall communities, and the predictability of gall communities (Wiebes-Rijks and Shorthouse 1992).

A gall can be induced by various organisms such as bacteria, viruses, fungi, nematodes, mites, or insects (Mani 1992). These structures can be found on every plant organ (Mani 1992) including roots, stems and leaves, with leaves being the most highly galled organ (Dreger-Jauffret and Shorthouse 1992). The complexity of galls can vary greatly from being a simple indentation in plant tissue to a highly structured growth with cells and tissues that have high levels of specialization and differentiation (Mani 1992). Since the plant receives no benefit from the presence of the organism and its gall, gall inducers are considered to be parasitic (Abrahamson and Weis 1997).

The interactions of the gall maker and the host plant actually change the growth and differentiation of plant cells so that the polarity is no longer in relation to the plant, but towards the insect (Ananthakrishnan 1984, Dreger-Jauffret and Shorthouse 1992, Abrahamson and Weis 1997, Stone and Schönrogge 2003). Although galls may have originally been induced as a protective measure by the plant, the gall inducer has evolved to receive nourishment, protection and even microclimate regulation from the gall (Ananthakrishnan 1984, Askew 1984, Price et al. 1987, Stone and Cook 1998). Usually, galls are induced on plant areas that have a high capacity for growth. The structure of the gall is actually determined by a combination of both the gall inducer and the host plant genotypes, such that the gall-inducer's genes code for the gall inducing stimulus and the host plant's genes code for the growth that occurs (Abrahamson and McCrae 1986, Abrahamson and Weis 1997). However, gall wasps (Hymenoptera: Cynipidae) that form galls on multiple species of closely related oak trees usually have the same gall structure. This could lead one to believe that gall structure is directed almost solely by the insect itself or is an extension of the wasp's genotype (Stone and Cook 1998).

Gall inducers occur in several key families within Coleoptera (family Curculionidae), Diptera (families Cecidomyiidae and Tephritidae), Hemiptera (families Aphidae, Psyllidae, and Tingidae), Hymenoptera (families Agonidae, Cynipidae, Pteromalidae, and Tenthredinidae), Lepidoptera (families Gelechiidae and Tortricidae), and Thysanoptera (families Phlaeothripidae and some Thripidae) (Gronemann 1930, Abrahamson and Weis 1987, Ananthakrishna 1992, Dreger-Jauffret and Shorthouse 1992). Over 70% of the galls in North America are made by gall midges (Diptera:

Cecidomyiidae) and gall wasps (Abrahamson and Weis 1987). These two families also have the greatest gall complexity (Abrahamson and Weis 1987). All cynipid galls and most cecidomyiid galls contain tissue that has differentiated into nutritive and sclerenchyma cells, thus forming a new organ (Dreger-Jauffret and Shorthouse 1992).

Gall Classification

Gall inducers can often be identified by their host plant and gall, but this is not always reliable. Many different terms have been created to classify galls based on structure alone (Dreger-Jauffret and Shorthouse 1992). Küster (1911) used these terms: *organoid gall* (the plant organ where the gall develops remains recognizable), *histoid gall* (the plant organ where the gall develops does not remain recognizable), *kataplastic gall* (the size and shape of the galls are irregular and their tissues are barely differentiated), and *prosoplasmic gall* (very complex, gall tissue goes through differentiation) (Dreger-Jauffret and Shorthouse 1992). *Monothalamous* describes a gall that has only one larva developing or only one chamber with larvae living inside and *polythalamous* describes galls that contain multiple larvae developing in multiple chambers within the same gall (Felt 1940). Another classification is based on how the gall is formed and the insect's initial position (Dreger-Jauffret and Shorthouse 1992). Most of the following galls are histoid and prosoplasmic (Dreger-Jauffret and Shorthouse 1992):

- *Bud and rosette galls* can vary in their different degrees of gall complexity. Some larvae may simply cause a bud to swell where others may cause a growth that resembles a bud, pine cone or pineapple.
- In *covering galls*, the inducers act externally and become enclosed in leaf or stem tissue with an exterior opening.
- *Filz galls* are hairs or hair-like outgrowths usually on the undersides of leaves.

- *Mark galls* are more complex than the previously mentioned galls and occur on both leaves and stems. The egg is deposited directly into the plant tissue and the insect larva is completely enclosed throughout its development.
- *Pit and blister galls* are characterized by a slight arching or blistering in the leaf.
- *Pouch galls* are usually hollow bulges or intensely arched areas of leaves.
- *Roll and fold galls* occur when a leaf is rolled in an upward direction or appears pinched or folded over at the edge.
- *Stem and twig galls* either partially or completely encircle a twig or stem and are not detachable (Felt 1940).
- *Bullet galls* are solid, single-celled, detachable galls, usually on oak twigs, and their shape roughly resembles a bullet (Felt 1940).

One of the most complex families of gall inducing insects is Cynipidae. Cynipid galls can range in form from a single gall ≤ 1 in mm diameter to compound galls ≥ 10 cm long (Cornell 1983). Usually, galls are symmetrical with only one larva per chamber, but some may be polythalamous (Rohfritsch 1992).

Gall Development

There are four phases in the development of cynipid galls: initiation, growth and differentiation, maturation, and dehiscence (Rohfritsch 1992). Cynipid gall initiation begins when the larva hatches from the egg, begins feeding and is surrounded by plant tissue (Askew 1984, Wiebes-Rijks and Shorthouse 1992). For growth and differentiation to occur, the stimulus of the gall inducer must be present and functioning (Mani 1992). It is thought that unidentified morphogens in the larval saliva are responsible for the stimulation of plant tissue (Askew 1984, Stone and Cook 1998). These morphogens stimulate cell division and enlargement, and may mimic the plant's naturally occurring auxins (Rohfritsch 1992).

Through the growth and differentiation stage, specialized tissue forms along the inner surface of the larval chamber (Rohfritsch 1992). The maturation stage is where the gall divides into two distinct regions: the inner gall and outer gall. The inner gall consists of nutritive tissue and is controlled solely by the gall maker. The outer gall is controlled mainly by the plant. It is in the maturation stage when the larva reaches its last instar and consumes most of the nutritive tissue (Rohfritsch 1992). These cells are usually high in starch, sugar, protein and lipids (Dreger-Jauffret and Shorthouse 1992). Even though the larvae are continually feeding and growing, they will not defecate until immediately before pupation to avoid contaminating the larval chamber (Askew 1984, Bronner 1992). Cynipids pupate directly within the larval chamber, so no pupal case is produced.

Once the larval feeding stimulus has ceased (due to death or pupation), gall development ceases (Askew 1984), and gall dehiscence begins. During this final stage, the gall maker is essentially cut off from the host plant; the gall is no longer a nutrient sink (Rohfritsch 1992). Finally, the adult will chew an exit hole and emerge. In contrast to some gall midges, cynipid larvae do not initiate the exit hole before pupation (Askew 1984).

Gall Defense Mechanisms

Parasitoids, inquilines and insectivorous vertebrates are often successful in consuming either gall tissue or the gall maker itself, but galls and their inducers have developed many different defense mechanisms. Galls may be covered with hairs, creating a surface difficult for parasitoids and inquilines to survey through antennation, which, in return, can complicate oviposition (Askew 1984). Some galls exude a sugary secretion that deters parasitoids directly by creating a sticky surface or indirectly

through recruiting ants or other stinging insects that may tend and ultimately protect the galls (Askew 1984, Washburn 1984, Abe 1992, Seibert 1993, Inouye and Agrawal 2004). Inouye and Agrawal (2004) found when ants were excluded from collecting the gall secretion, parasitism increased by 36% and gall maker emergence decreased by 54%. Broken off ovipositors of chalcid parasitoids (Hymenoptera: Chalcidoidea) have been found in extremely woody or hard galls, and air spaces within galls may serve as false chambers, confusing parasitoids and inquilines (Askew 1984). However, parasitoids may target galls during early gall formation when galls are usually smaller with more succulent tissue (Askew 1975, Washburn and Cornell 1981, Cornell 1983). Parasitoids attacking young galls may have shorter ovipositors than those parasitoids attacking more mature galls (Wiebes-Rijks and Shorthouse 1992). Larger galls or galls with a thick wall may provide greater protection for gall makers (Plantard and Hochberg 1998, Eliason and Potter 2000a). Tannins in galls may also deter vertebrate predators, other herbivores, and even act as antifungal agents (Taper et al. 1986). In addition to physical attributes, gall makers may be protected by avoiding parasitoids through time and space. Galls that develop early in the season may complete development before natural enemies are active, thus increasing their own survival (Askew 1984).

Cynipid Galls

Many cynipid species have a complex life cycle with an alternation of generations, also known as heterogeneity, where the asexual (all female) and sexual (male and female) generations are not only morphologically different, but also have dissimilar galls on different plant organs (Askew 1984). These differences in wasp and gall morphology have resulted in the different generations being described as separate species and sometimes only one generation (usually the asexual generation since their

galls tend to be more noticeable) is known (Pujade-Villar et al. 2001). DNA sequencing procedures have been developed to associate both generations of gall wasps. 28S ribosomal DNA, cytochrome *b* (Cytb) from the mitochondrial genome and/or cytochrome oxidase I (COI) can be sequenced and then paired to confirm that the asexual and sexual generations are both the same species without (or in addition to) conducting rearing and caging studies (G. Melika, personal communication). These methods are currently being used to construct phylogenetic relationships between cynipids (Stone and Cook 1998, Rokas et al. 2002).

Gall Inhabitants

Proper identification of the gall maker may be complicated by the simultaneous development of other arthropods in the gall tissue (Wiebes-Rijks and Shorthouse 1992). Inquilines (e.g., *Synergus* spp. or *Ceroptres* spp.) are cynipids that cannot create galls, but feed within existing gall tissue. Many times, when multiple inquiline eggs are laid into a gall, chambers with nutritive tissue can form around the inquilines, separating the larvae from each other and possibly changing the gall's shape (Askew 1984, Wiebes-Rijks and Shorthouse 1992). The inquilines feed on the nutritive tissue, but do not usually feed on the gall inducer themselves (Askew 1984). Gall inducer death may occur when the inquiline egg is oviposited into the larval chamber or if the inquiline outcompetes the inducer. In rare occasions, consumption of the gall maker may occur after it dies, but is not directly killed through consumption (Askew 1984, Wiebes-Rijks and Shorthouse 1992).

Parasitoids (e.g., Eulophidae, Ormyridae, Pteromalidae, etc.) may also occupy galls, but kill the gall inducer (and even other inquilines) through direct consumption (Wiebes-Rijks and Shorthouse 1992). Two kinds of parasitoids may exist: those that

are monophagous (e.g., the parasitoid consumes only the gall maker or inquiline, and arrives early in gall development) or polyphagous (e.g., all larvae inside the gall are fed on indiscriminately, usually occurring later in gall development) (Askew 1984, Wiebes-Rijks and Shorthouse 1992). Washburn and Cornell (1979) found that *Eurytoma* spp. were the most prevalent parasitoids that emerged from leaf galls of *Acraspis hirta* Bassett on *Quercus prinus* L. because they consumed the gall maker and other parasitoids. As a result of this complex system, parasitoids that emerge from a gall could have attacked the gall maker, an inquiline, or another parasitoid. These hyperparasitoids are the greatest cause of mortality for gall makers in galls that were successfully initiated.

Common Cynipid Host Plants

Over 90% of galls are formed on dicots, especially those plants belonging to the families of Rosacea, Asteracea and Fagacea (Abrahamson and Weis 1987). Fagacea, the plant family containing oak trees (*Quercus* spp.), hosts a variety of gall makers, primarily cynipids (Abrahamson and Weis 1987). In North America, ~87% of all cynipid galls use an oak species (or a few related species) as their host (Abrahamson and Weis 1987).

One important oak species in Florida is southern live oak (*Q. virginiana* Mill.). Live oak is distributed throughout the lower coastal plain of the southeastern United States (Carey 1992). Texas live oak [*Q. virginiana* var. *fusiformis* (Small) Sarg.] and sand live oak [*Q. virginiana* var. *geminata* (Small) Sarg.] are two naturally-occurring varieties (Harms 1990). Cultivars (i.e., *Q. virginiana* Cathedral®, Highrise® and Millennium®) are propagated by vegetative cuttings, and are most commonly grown in nurseries due to their uniform growth patterns.

Southern live oaks are important street and landscape trees because they are tough, resilient, and provide abundant shade (Carey 1992, Gilman and Watson 1994). Live oaks can reach a height of 12-18 m with a canopy diameter of 18-30 m and DBH of 200 cm (Burns and Honkala 1990, Gilman and Watson 1994). These evergreens are usually found in sandy soils and can tolerate salt spray and high soil salinity (Burns and Honkala 1990). Bud break usually occurs in mid-March and three flushes may occur each year (in early March, early July and late summer). Some common genera of gall inducing cynipids on live oak trees include *Atusca* Kinsey, *Bassettia* Ashmead, *Belonocnema* Mayr, *Biorhiza* Westwood, *Callirhytis* Foerster, *Chilaspis* Mayr, *Disholcaspis* Dalla Torre and Kieffer, *Dryocosmus* Giraud, *Eumayria* Ashmead, and *Eumayriella* Melika and Abrahamsom (Melika and Abrahamson 2002).

***Disholcaspis* Spp.**

Disholcaspis spp., or bullet gall makers, occur throughout North and Central America (Melika and Abramson 2002). They have alternating sexual and asexual generations, and usually only the asexual generations are known (Melika and Abramson 2002). The galls of the asexual generation are the most noticeable, and are usually “detachable, woody bullet-like stem” or root galls and the galls of the sexual generation are usually “small thin walled bud galls” (Melika and Abramson 2002). The only study of a *Disholcaspis* sp. in North America was by Morgan and Frankie (1982) in Texas. *Disholcaspis cinerosa* Bassett develops on two oak species in Texas (*Q. fusiformis* Small and *Q. virginiana*), and has two alternating generations (Morgan et al. 1983). In December and January, asexual females emerge from spherical branch galls and lay eggs in swollen leaf buds. The newly emerged females usually live for 3-6 wk. Sexual galls (3-5 mm) develop as the leaves expand and the sexual adults emerge in

early April. Once mated, females lay eggs in new branch growth on which beige stem galls develop (5-25 mm), containing the asexual generation (Morgan and Frankie 1982).

Unlike *D. cinerosa*, the life cycle of *D. quercusvirens* (Ashmead) has not been studied, and only the asexual generation has been described (Ashmead 1881). It has been renamed three times. It was originally named *Cynips q. virens* Ashmead in 1881 and then changed names twice in 1885 to *Andricus virens* (Ashmead) and then to *Cynips quercus ficigera* (Ashmead) (Krombein et al. 1979). Ashmead described the galls and the only adult female wasp that he could rear from one of almost 200 galls. The galls of the asexual generation were described as small (0.4 - 0.6 cm), globular, attached to the underside of the leaf, light brown and monothalamous (Ashmead 1881). We suspect this is inaccurate because the galls are attached to young branches, not leaves. The female wasp emerged from the gall in February, was 0.4 cm in length with a reddish brown head, 13-jointed antennae and the thorax was brown, coarsely punctuate and pubescent (Ashmead 1881). The abdomen was dark reddish brown, and the legs were a little lighter in color (Ashmead 1881). The woody bullet galls of *D. quercusvirens* have been found on *Q. virginiana*, *Q. geminata* and *Q. minima* (Price et al. 2004), and have been collected in South Carolina, Georgia, Florida, Mississippi, Louisiana, and Texas (Krombein et al. 1979).

Gall Management

Gall infestations are usually harmless and chemical control is not recommended (Gilman and Watson 1994) unless severe damage occurs. The aesthetic damage of *D. quercusvirens* stem galls is especially a problem for nurseries. According to the Florida Grades and Standards for Nursery Plants (1998), the crown and trunk and/or branches

should have little or no evidence of insect infestation. Intense galling can reduce the grade of the plant, leading to economic loss for nursery owners. Nursery owners are concerned that dieback may occur apical to a stem gall, and that galled branches may be weaker, more likely to break, and reduce tree form. Also, developing *Disholcaspis* spp. stem galls have a sticky exudate that can attract stinging insects and ants in late summer, which can be hazardous for nursery workers (Felt 1940).

Proper management of either physically or aesthetically damaging galls depends on knowing the gall maker's life cycle (Eliason and Potter 2000c). Since the asexual generation is most often known, targeting adults at their emergence period could prevent the next generation from developing, although it may not be the most damaging life stage (Pujade-Villar et al. 1999, Eliason and Potter 2000c). One management technique is to prune infested branches, however on a large scale, such as a nursery setting, it is too labor intensive (Eliason and Potter 2000c). Other techniques include foliar sprays or trunk or soil injections at different times in the gall maker's life cycle (Eliason and Potter 2000c). Timing will be crucial for these management practices to be successful, therefore the life cycle of *D. quercusvirens* must be determined.

Objectives

Because of the need for more information on the life cycle of *D. quercusvirens* and optimal ways to manage it on live oak trees, the objectives of my research were:

1. To describe the development and seasonality of both *D. quercusvirens* generations, their galls and associated natural enemy complex, and
2. To test the efficacy of three insecticides against emerging asexual *D. quercusvirens* to reduce bud gall, and later stem gall, formation.

CHAPTER 2
BIOLOGY, DEVELOPMENT AND NATURAL ENEMY COMPLEX OF *DISHOLCASPIS*
QUERCUSVIRENS

Gall wasps (Hymenoptera: Cynipidae) are one of the most complex families of gall inducing insects. Identifying the gall-maker can be confusing due to simultaneous development of parasitoids and inquilines inside gall tissue and because many cynipids have two alternating generations where the asexual and sexual adults not only look different, but also have dissimilar galls on different plant parts (Askew 1984, Melika and Abramson 2002). This has often resulted in the different generations being described as different species, or only one of the generations is known.

Disholcaspis spp. are prevalent throughout North and Central America (Melika and Abramson 2002). *Disholcaspis quercusvirens* forms woody bullet galls of the asexual generation on live oak, *Quercus virginiana* Mill. The sexual generation has been undescribed. Light gall infestations are usually harmless to the trees, but heavily infested trees are physically and aesthetically damaging, especially in nurseries where gall infestations can lead to decreased tree value (Eliason and Potter 2000c).

Questions and concerns have arisen from nursery owners and home owners on the best management practices to reduce this gall maker population. Since no documented studies of *D. quercusvirens* exist, it was imperative to first determine its complete life cycle and natural enemy complex before any management decisions could be made. Thus, the objective of this study was to describe the development and seasonality of both *D. quercusvirens* generations, their galls and associated natural enemy complex through collection, dissection and rearing of galls.

Materials and Methods

Study sites. A block of 94 Cathedral® live oak trees at Shadowlawn Nursery in Penny Farms, Florida (Clay County), was established from cuttings in 2005 and grown according to the “Roots Plus Growers” standards for ball and burlap trees (rootsplusgrowers.org). Tree height, basal diameter, and diameter at breast height (DBH) were measured on 20 randomly selected trees from the block on 11 July 2007 with a telescoping measuring rod and diameter tape, respectively. The trees were 4.7 ± 0.1 m tall, the basal trunk diameter was 8.7 ± 0.3 cm, and DBH was 6.4 ± 0.3 cm.

A row of nine Cathedral® live oak trees at the Horticultural Tree Teaching Unit in Gainesville, FL (Alachua County), were established in 2002-2003. Trees were 49.0 ± 1.1 cm tall with a caliper (taken at 15.24 cm from the ground) of 11.2 ± 0.2 cm (C. Harchick. pers. comm.)

Asexual Generation (Stem Galls)

Stem gall development and inhabitants. From August to December 2007 and from July to November 2008 four to ten compound stem galls were periodically cut from randomly selected live oak trees at Shadowlawn Nursery, placed in bags, frozen, and later dissected in the laboratory under a binocular dissecting scope. Maximum compound gall length, number of individual stem galls per compound gall, individual bullet gall diameter (two measurements taken perpendicular to each other and averaged) and height (from base of bullet gall after removal from stem to the highest point), diameter of the stem apical to the compound gall, presence of exit holes, and presence of arthropod inhabitants were recorded.

To rear and identify the arthropod complex inhabiting stem galls, five to ten compound stem galls were collected from Shadowlawn Nursery every 2 – 3 wks from

early June through mid-December 2007. Maximum compound stem gall length, number of individual stem galls per compound gall, individual gall diameter and height, and diameter of the stem apical to the compound gall were recorded. Single stem galls were placed into individual scintillation vials and held in the laboratory (13L:11D hrs, 23.7°C, and 48% relative humidity). All insect specimens were preserved in 95% ethyl alcohol (EtOH). Representatives of each morphotype were sent to Dr. George Melika (Pest Diagnostic Laboratory, Plant Protection & Soil Conservation Directorate of County Vas, Tanakajd, Hungary) for identification. Twenty asexual females preserved in 95% EtOH were dissected under a binocular dissecting scope (20X magnification) to determine potential fecundity, and ovipositor length was measured using an ocular micrometer. The effects of stem gall inhabitant [i.e., gall maker (n = 15), parasitoid (n = 100) or inquiline (n = 50)] on gall size (diameter and height) and number of galls in the compound gall were compared using MANOVA (SAS Institute, 9.2, 2008).

Asexual Female Longevity. Ten branches containing one to four compound stem galls were collected weekly from Shadowlawn Nursery on 2 – 3 yr old unpruned trees starting ~ 20 Oct 2008. Galls were reared in 0.12 L plastic Solo cups that had a vented lid (~2.5 cm diameter hole covered with white chiffon mesh) to allow air flow. All Solo cups were held outside in a wire mesh cage (dimensions: 0.3 m x 0.3 m x 0.6 m) that had a solid plastic top to prevent water from entering the Solo cups. Several cages were placed in partial shade beside a building. Galls were checked daily for asexual wasp emergence.

One newly emerged female (<24 hrs old) was placed into an individual 0.47 L plastic deli container (7 cm tall, 8.5 cm bottom diameter). A dental wick (5.1 cm long)

was inserted through the lid of a 29.6 mL Solo cup and secured to the bottom of each deli container. Solo cups were filled with one of the three treatments and replaced every 2 d. Treatments included water, a 10% honey solution, or nothing (control). Replicates (n = 30 wasps per treatment) were set up one at a time, on the day of wasp emergence and held in a rearing chamber (13L:11D hrs 26.4°C, relative humidity of 40%). Wasp survival was monitored daily. Two trials were set up, one at the beginning (21 November to 2 December 2008) and one near the end of the adult emergence period (4–9 January 2009). The effects of diet on cynipid longevity were compared using PROC LIFE (SAS Institute, 9.2, 2008).

Sexual Generation (Bud Galls)

Confirmation of the Alternate Generation. To associate the asexual with the sexual generation of *D. quercusvirens*, 30 developing stem galls were randomly located in the lower crown (≤ 2.2 m) on infested live oak trees at Shadowlawn Nursery from 14 June to 13 July 2007 and caged with a fine white organza mesh. Twenty additional compound stem galls in the upper crown ($\sim 2.2 - 4.3$ m) were caged on 20 July 2007. Caging was done to minimize parasitism and maximize gall maker survival, while also preventing other gall maker access to the branches. All cages were removed on 17 August because mold was forming on the stem gall exudate, but galls were recaged after they had hardened and mold had disappeared (2 November 2007). Cages remained on galled branches, and asexual *D. quercusvirens* females were allowed to emerge and oviposit within the cages. The dimensions of 30 buds that were present during the asexual wasp emergence period were documented. Caged branches were cut from trees in late March 2008 and brought into the laboratory to inspect for gall

formation and any intact cynipid specimens. Any new galls found within the cages were presumed to contain the sexual generation.

In April 2008, three male cynipids recovered from the caged branches and two female cynipids (reared from collected bud galls) were preserved in 95% EtOH and Dr. George Melika determined if they were a *Disholcaspis* spp. The confirmed sexual *Disholcaspis* specimens and 10 asexual *D. quercusvirens* females preserved in 95% EtOH were then given to Dr. James Nicholls (Institute of Evolutionary Biology, Ashworth Labs, Kings Buildings, University of Edinburgh, Edinburgh, United Kingdom) for DNA sequencing of the mitochondrial cytochrome *b* (cyt *b*) gene (J. Nicholls, personal communication) to confirm the field observation that the stem galls and bud galls were made from different generations of the same species.

Bud Gall and Gall Maker Development. On 9, 11 and 16 March 2009, ten branches were randomly selected and 20 bud galls per date were dissected to determine gall maker fate and development, and to document morphology before all bud galls were completely visible. The association between galled and ungalled buds when the bud scales completely enclosed the gall was evaluated using a TTEST (SAS Institute, 9.2, 2008). Bud galls that were visible because leaves had already started to expand were not included. The bud stage [i.e., dormant, swelling, green tip or expanded leaves (Eliason and Potter 2000b)], bud length and width, if the gall was visible before bud dissection, gall length and width (after bud scales were pulled off by hand), and gall maker stage of development were recorded.

Bud Gall Arthropod Inhabitants. To determine gall maker survival and describe the natural enemy complex within the bud galls, bud galls were collected

weekly from 4 April to 14 May 2008 from live oak trees at Shadowlawn Nursery and the Horticultural Tree Teaching Unit. The lower crown of the trees was examined for bud galls for 45 min. or until no bud galls could be found after searching for 15 min. A total of 480 bud galls were collected. Bud galls were carefully removed from branches and placed into clear gelatin capsules (size 0) (Shorthouse 1972) in the lab (13L:11D hrs, 23.7°C, and 48% relative humidity). Emergence of gall makers and parasitoids was monitored daily. All specimens were preserved in 95% EtOH, morphotyped, and a representative sample of each morphotype was sent to Dr. George Melika for identification. Voucher specimens will be deposited at the Florida State Collections of Arthropods (FSCA) at the Division of Plant Industry, Gainesville, FL.

On 9 March 2009, before bud galls were visible, ten branches (~10 cm long) were collected from live oaks at Shadowlawn Nursery, and all buds were placed into individual gelatin capsules (size 0). After bud galls became visible on branches, additional bud galls were collected on 16, 19, and 24 March, 17 and 21 April, 4 and 11 May 2009 from Shadowlawn Nursery for 45 min. If no bud galls were present, searching ended after 15 min. At least 20 bud galls were collected on each sampling date, and placed into individual gelatin capsules. Each gall inhabitant was identified at least to genus, and its emergence date was recorded. In addition, 20 sexual generation females reared from bud galls collected in mid-May were dissected under a binocular dissecting scope to determine the number of eggs per female abdomen and to measure ovipositor length with an ocular micrometer.

Results and Discussion

Asexual Generation (Stem Galls)

Stem Gall Development and Inhabitants. Galls of the asexual generation were initiated after oviposition occurred during early shoot expansion. Slight stem swellings were visible as early as June. Small stem galls emerged from beneath the bark by August. Young stem galls were red in color (Figure 2-1A) with soft, spongy tissue. From August to mid-October, stem galls had a sweet, sticky exudate that attracted imported fire ants (*Solenopsis* spp.), velvet ants (Hymenoptera: Mutillidae), and paper wasps (Hymenoptera: Vespidae) (Figure 2-1B), and supported sooty mold growth (Figure 2-1C). Stem gall secretions by *D. perniciosus* Bassett on *Q. gambellii* Nutt. coincided with the development of its gall maker larva (Siebert 1993), which may also be relevant for *D. quercusvirens*. The *D. quercusvirens* stem galls grew for ~4 mo, with maximum diameter being reached in November (Figure 2-3). At this point, galls were fully mature and woody (Figure 2-1D).

Maximum individual stem gall diameter was 7.7 ± 0.1 mm in 2007 and 4.8 ± 0.1 mm in 2008. The reduced stem gall diameter in 2008 may have been because trees had “outgrown” their susceptibility (Morgan et al. 1983, Frankie et al. 1992), or trees aborted the galls after initiation occurred (Seibert 1993). Compound stem galls were an average of 30.4 ± 1.6 mm long (range, 1 – 44 stem galls; mean = 9.3 ± 0.6 stem galls per compound gall) in 2007 and 25.5 ± 1.4 mm long (range, 1 – 52 stem galls; mean, 11.0 ± 0.9 stem galls per compound gall) in 2008.

Asexual females (Figure 2-3 A) emerged during cold weather periods from late-November through January and oviposited into dormant buds (1.3 ± 0.07 mm long, 1.2 ± 0.09 mm wide at base). Adult female (n = 20) abdomens had 64.0 ± 3.0 eggs (range,

43 – 88 eggs), and mean ovipositor length was 0.08 ± 0.002 mm. All eggs appeared to be one size (e.g., mature), but dimensions were not measured.

Stem galls collected in 2007 were heavily parasitized. A total of 1,650 stem galls were collected from 1 August 2007 to 18 December 2007. The natural enemy complex included *Synergus* spp. (Cynipidae), *Eupelmus* spp. (Eupelmidae), *Eurytoma* spp. and *Sycophila* spp. (Eurytomidae), *Ormyrus* spp. (Ormyridae), and *Acaenacis* spp. (Pteromalidae). *Disholcaspis quercusvirens* comprised 8.7% of the specimens reared from stem galls, inquilines (*Synergus* spp., Figure 2-4 A and B) made up 27.3%, and parasitoids (Hymenoptera: Eupelmidae, Eurytomidae, Ormyridae and Pteromalidae) comprised 63.9% of the specimens reared (Table 2-1).

Stem gall height, diameter and number of stem galls in the compound gall were affected by the gall inhabitant (Table 2-2; Wilks lambda = 0.8032; df = 6, 316; $P < 0.0001$). Galls containing gall makers and parasitoids were significantly wider than galls containing inquilines. The height of stem galls containing gall makers was significantly greater than galls containing parasitoids or inquilines. Compound stem galls having more individual stem galls produced more gall makers than compound stem galls having few individual stem galls.

Stem galls from which gall makers successfully emerged did not support any parasitoids or inquilines. However, two or more parasitoids, inquilines, or both parasitoids and inquilines did emerge from many stem galls, and presumably killed the gall maker. Although inquilines are phytophagous insects, their development may ultimately lead to gall maker death due to overcrowding or by use of available resources (Askew 1984). These results are similar to those found in comparable cynipid studies

where all gall makers died due to the occupation of the gall by a *Synergus* spp. inquiline (Frankie et al. 1992, Plantard et al. 1996, Seibert 1996). Frankie et al. (1992) documented that *Synergus* spp. actually changed the morphology of the gall it inhabited by creating a denser gall, with fewer empty air chambers when compared to those galls occupied by parasitoids or gall makers. This phenomenon of changed gall morphology due to *Synergus* spp. inhabitants was not observed in the *D. quercusvirens* complex.

Stem galls collected in 2008 yielded few natural enemies. Of the 1,470 galls collected, only five inquiline specimens were reared [*Synergus* sp. 1 (n = 3) and *S. ficigerae* Ashmead (n = 2)]. Many galls did not develop properly (i.e., trees aborted galls after initiation had occurred), and gall inhabitants were unable to complete development. Seibert (1993) also reported that a tree had aborted all 24 compound galls of *D. pernicioso* after the same tree supported many viable compound galls the previous year. Openings in gall tissue from the ovipositors of inquilines and parasitoids may cause gall abortion because the gall maker becomes infected with a pathogen (Seibert 1996).

Asexual Female Longevity. For both trials, wasps provided with water or honey-water lived significantly longer than unfed (control) wasps (Table 2-3; Trial 1, $\chi^2 = 16.843$; df = 2; $P < 0.0002$; Trial 2, $\chi^2 = 17.443$; df = 2; $P < 0.0002$). Wasps in trial one survived significantly longer than the wasps in trial two (t = 9.56, df = 177, $P < 0.001$). Wasps may have had a shorter adult life span in trial two than in trial one because of the longer time spent inside the stem galls. For *D. cinerosa* Basset, wasps were said to live 3 – 6 wk (Morgan et al. 1983) although it was not documented if this was under laboratory or natural conditions. Under natural conditions, *D. quercusvirens* may find

accessible water after a rainstorm, or a sugar solution from soft scales or other Hemipterans on the live oak trees (Eliason and Potter 2001) although this was not observed in the *D. quercusvirens* system. Other environmental conditions (e.g., ambient temperature, relative humidity, or photoperiod) may also play a role in adult wasp longevity. Because cynipid eggs are generally ready to be laid upon adult asexual wasp emergence (Seibert 1993, Eliason and Potter 2000a), adult feeding may not be necessary for egg development or maturation.

Sexual Generation (Bud Galls)

Confirmation of the Alternate Generation. DNA sequencing of *cyt b* revealed identical haplotypes for the sexual and asexual wasps (J. Nicholls, pers. comm.). This DNA testing, along with caging asexual *D. quercusvirens* and morphological examination, confirmed that the stem galls of the asexual generation and the bud galls of the sexual generation were of the same species (personal communications, G. Melika and J. Nicholls).

Bud Gall Development. Small bud galls developed beneath bud scales, and were located on the branches at the base of a petiole, hidden between leaves (Figure 2-1 F). Bud galls were 2.5 ± 0.02 mm long and 1.1 ± 0.01 mm wide. They were presumably initiated at asexual wasp oviposition (between November and January), and present 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 (Figure 2-1 E), were visible in early- to mid-March. Gall maker emergence ended by mid-April and parasitoid emergence continued until all bud galls fell off the trees (~May). Bud galls were fragile after galls were completely hardened,

and similar to the *D. cinerosa* system, would fall or break in extreme weather conditions (i.e., heavy rain or strong winds) (Frankie et al. 1992).

Galled (2.8 ± 0.1 mm long, 1.7 ± 0.08 mm wide) and non-galled (2.5 ± 0.1 mm long, 1.6 ± 0.05 mm wide) 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 developmental stages from dormant to green tipped.

Sexual generation pupae (Figure 2-3 B) were present in bud galls by 11 March 2009, and 65% of the bud galls collected on 16 March had white pupae. First adult emergence (Figure 2-3 C and D) occurred 21 – 23 March 2009 in 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 said to live for ~1 wk (Frankie et al. 1992). Sexual generation females had a potential fecundity of 87.2 ± 3.0 eggs, and their ovipositor length was 0.04 ± 0.001 mm long. All eggs appeared to be the same size and maturity although measurements were not taken. More females than males were reared. The ratio of males to females was ~2:3.

Bud Gall Arthropod Inhabitants. A total of 480 bud galls were collected from 4 April to 14 May 2008, which was at the end of the sexual *D. quercusvirens* emergence period. Parasitoids (Hymenoptera: Eulophidae, Eupelmidae, Eurytomidae, Ormyridae and Pteromalidae) comprised 99.1% of the specimens reared, and *D. quercusvirens* comprised 0.01% of specimens reared (Table 2-1). No inquilines were reared from the bud galls.

A total of 3,884 bud galls were collected from 19 March to 14 May 2009. These bud galls had a more diverse parasitoid complex (Table 2-4), which included *Aprostocetus* spp. and *Baryscapus* spp. (Eulophidae), *Brasema* spp. (Eupelmidae) (Figure 2-4 I), *Sycophila* spp. (Figure 2-4 G) and *Eurytoma* spp. (Figure 2-4 D and E) (Eurytomidae), *Ormyrus* spp. (Ormyridae) and *Acaenacis* spp. (Pteromalidae). Only one specimen was reared from each bud gall. The most abundant parasitoid in 2009 was *Acaenacis lausus* (Walker) and it was also reared from stem galls in 2007 (Figure 2-4 C). Pteromalid wasps are typically parasitoids, and their larvae typically feed on the eggs or larvae of their hosts (Krombein et al. 1979). *Ormyrus* sp. 1 (Figure 2-4 H) was also reared from both bud galls in 2009 and stem galls in 2007. *Eurytoma* spp. and *Tetrastichus* sp. (Eulophidae) have been known to feed on developing larvae of *D. perniciosus* in stem galls (Seibert 1996). It is assumed that the parasitoids or inquiline reared from the bud galls of *D. quercusvirens* also fed on the gall maker larva or pupa.

This is the first study to associate the asexual and sexual generation galls and adults of *D. quercusvirens*, identify their arthropod complex, and describe the development and emergence periods of both generations. Additional studies for the natural enemy complex of the asexual generation could be conducted under more natural outdoor conditions, which may increase gall maker survival and emergence. This information will help nurserymen and landscape managers better understand this species and, if necessary, begin to formulate a management program to minimize tree infestations.

Table 2-1: Total number of gall makers, inquilines, and parasitoids reared from stem and bud galls collected from July 2007 to April 2008.

Family	Taxon	No. specimens reared from stem galls	No. specimens reared from bud galls
Cynipidae			
	<i>Disholcaspis quercusvirens</i>	16	2
	<i>Synergus ficigerae</i> Ashmead	11	0
	<i>Synergus succinipedis</i> (Ashmead)	30	0
	<i>Synergus</i> sp. 1	9	0
Eulophidae			
	<i>Aprostocetus</i> sp.	0	3
	<i>Baryscapus</i> sp.	0	24
	<i>Peduibius</i> sp.	0	1
Eupelmidae			
	<i>Brasema auratus</i> (Ashmead)	0	181
	<i>Eupelmus</i> sp. 1	31	0
Eurytomidae			
	<i>Eurytoma hecale</i> Walker	37	0
	<i>Eurytoma</i> sp. 2	2	0
	<i>Eurytoma</i> sp. 3	3	0
	<i>Sycophila</i> sp. 1	2	0
Ormyridae			
	<i>Ormyrus</i> sp. 1	2	0
Pteromalidae			
	<i>Acaenacis lausus</i> (Walker)	40	1
Total		183	212

Table 2-2: The effects of gall inhabitant on individual and compound gall size.

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

*MANOVA 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; $P < 0.0001$)

Table 2-3: The effect of diet on asexual *D. quercusvirens* longevity.

Treatment	Trial	No. days (\pm SEM) wasps were alive
Control	1 ^{a*}	5.1 \pm 0.4 ^{a**}
Water	1 ^a	8.1 \pm 0.6 ^b
Honey	1 ^a	7.3 \pm 0.7 ^b
Control	2 ^b	2.5 \pm 0.2 ^{c***}
Water	2 ^b	3.3 \pm 2.6 ^d
Honey	2 ^b	3.6 \pm 0.2 ^d

*Trial marked with different letters represent significant differences at $\alpha = 0.05$ (TTEST; $F = 9.56$; $df = 177$; $P < 0.0001$).

Means \pm SEM marked with different letters represent significant differences at $\alpha = 0.05$ Trial 1: LIFETEST; $\chi^2 = 16.8434$; $df = 2$; $P < 0.0002$)

*** Means \pm SEM marked with different letters represent significant differences at $\alpha = 0.05$ (Trial 2: LIFETEST; $\chi^2 = 17.4429$; $df = 2$; $P < 0.0002$)

Table 2-4. Total number of specimens reared from bud galls that were collected from March to May 2009.

Family	Genus	No. specimens reared
Cynipidae		
	<i>Disholcaspis quercusvirens</i> Female	63
	<i>Disholcaspis quercusvirens</i> Male	89
	Total	152
Eulophidae		
	<i>Aprostocetus</i> spp.	2
	<i>Baryscapus</i> spp. Female	278
	<i>Baryscapus</i> sp. 1 Male	249
	<i>Baryscapus</i> sp. 2 Male	58
	Total	587
Eupelmidae		
	<i>Brasema auratus</i> Female	367
	<i>Brasema auratus</i> Male	93
	<i>Brasema gemmarii</i> Female	59
	<i>Brasema gemmarii</i> Male	42
	Total	561
Eurytomidae		
	<i>Sycophila</i> sp. 1	7
	<i>Sycophila</i> sp. 2	5
	<i>Sycophila</i> sp. 3	4
	Unknown Female	41
	Unknown Male	12
	Total	69
Ormyridae		
	<i>Ormyrus</i> sp. 1	6
	<i>Ormyrus</i> sp. 2	7
	Total	13
Pteromalidae		
	<i>Acaenacis lausus</i> (Walker) Female	425
	<i>Acaenacis lausus</i> (Walker) Male	577
	Total	1,002

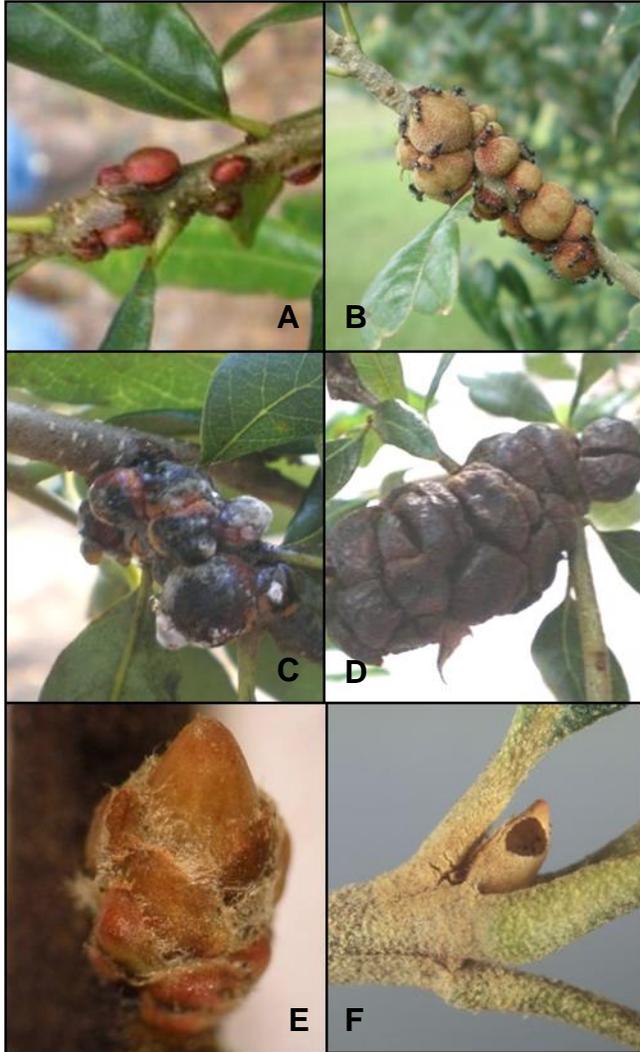


Figure 2-1. Galls of sexual and asexual generations of *D. quercusvirens*. A) Young red stem galls taken Aug 2007. B) Young stem galls being tended by ants, C) Young stem galls with sooty mold due to gall exudates. D) Fully mature stem galls. E) Young bud gall, with reddish tip, starting to emerge through bud scales, taken early March 2009. F) Bud gall with exit hole of the sexual generation. (Photo credit: L. Buss)

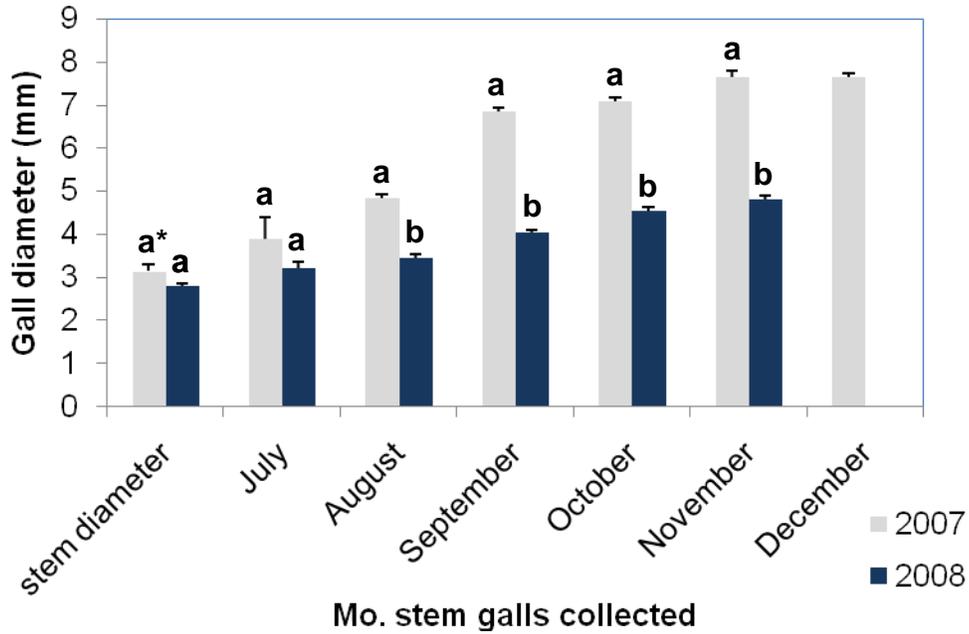


Figure 2-2. Mean individual stem gall diameter (mm) on galls collected from live oak trees (Clay Co., FL) from June to December 2007 and July to November 2008. *Means \pm SEM within columns followed by a different letter are significantly different at $\alpha = 0.05$ (ANOVA; $F = 260.25$; $df = 12, 3264$;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

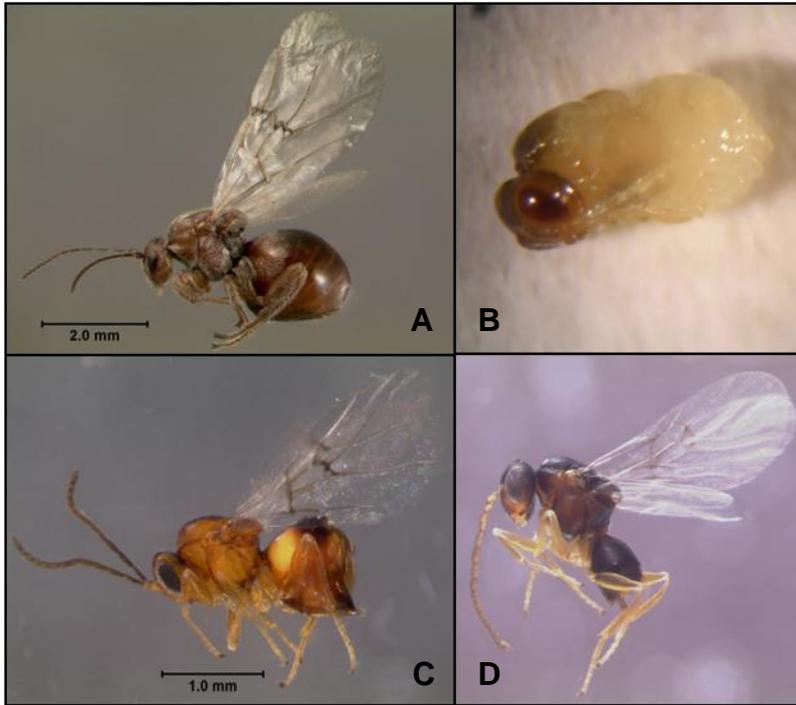


Figure 2-3. *D. quercusvirens* specimens. A) Adult asexual female wasp, B) Pupa of sexual female, C) Adult sexual female wasp (Photo credit: H. Ferrand), D) Adult sexual male wasp. (Photo credit: H. Ferrand)

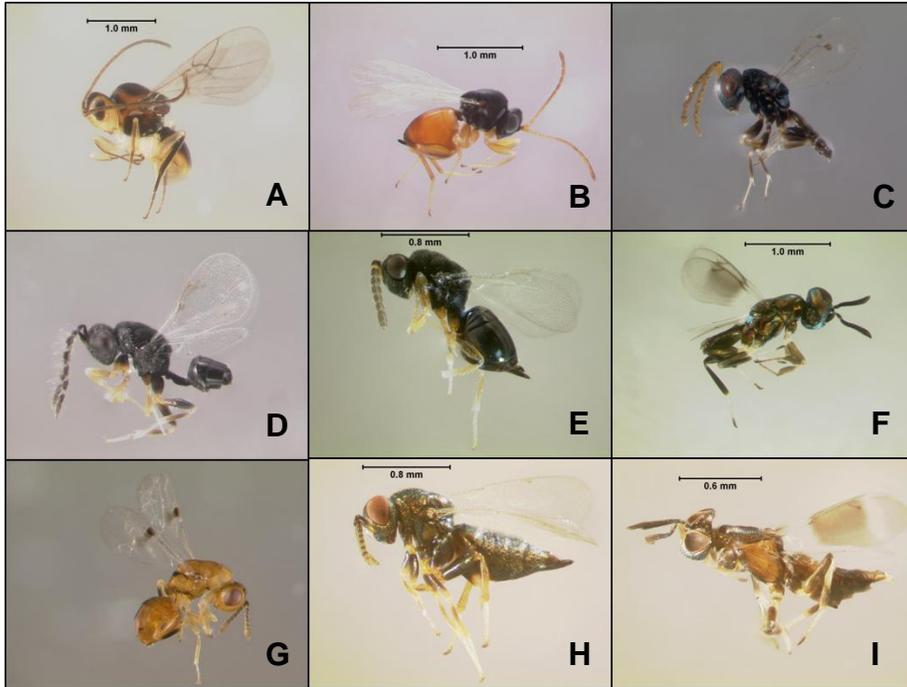


Figure 2-4. Natural enemies of *D. quercusvirens* (Photo credit: H. Ferrand) A) *Synergus ficigerae* female B) *S. succinipedis* female C) *Acaenacis lausus* male D) *Eurytoma* spp. 4 male E) *Eurytoma* spp. 4 female F) *Brasema auratus* male G) *Sycophila* spp. 1 female H) *Ormyrus* spp. 1 I) *B. gemmarii* female

CHAPTER 3
INSECTICIDAL CONTROL OF *DISHOLCASPIIS QUERCUSVIRENS* ON
CATHEDRAL® LIVE OAK TREES IN NORTHERN FLORIDA

Most arthropod galls are only considered minor ornamental plant problems, but some galls can reduce host plant photosynthesis, fruit or seed production, cause premature defoliation, branch dieback or even plant death (Felt 1940, Hodges et al. 2006). Many gall wasps (Hymenoptera: Cynipidae) tend to develop innocuously on oaks (*Quercus* spp.) which are generally robust trees (Felt 1940), but some stem galling cynipids (e.g., *Callirhytis* spp., *Disholcaspis* spp.) can aesthetically and/or physically disfigure their hosts (Ekberg and Cranshaw 1994, Eliason and Potter 2000c). Stem galls of *Disholcaspis* spp. can remain attached to trees for three or more years (Morgan et al. 1983), resulting in an increase in the amount of aesthetic damage that occurs over time.

Chemical control is typically discouraged because many galls are harmless, and gall maker biologies are complex (Gilman and Watson 1994, Hodges et al. 2006). It is difficult for insecticides to penetrate gall tissue to kill the developing insect, and proper timing is critical. Applications at times other than adult emergence periods or early gall development may not kill the gall maker, and could even negatively impact natural enemies that help regulate the pest population (Hodges et al. 2006). Non-chemical controls, such as pruning and destroying infested plant parts, is not feasible in large ornamental plantings or nurseries (Eliason and Potter 2000c, Hodges et al. 2006).

Disholcaspis quercusvirens (Ashmead) is a gall wasp on southern live oak (*Quercus virginiana* Mill.) throughout the southeastern United States (Krombein et al. 1979). The large bullet galls are aesthetically damaging to trees in nurseries, reduce tree value, and a sticky exudate on developing galls attracts stinging insects in late

summer, which can be hazardous for nurserymen. Slight stem swellings become visible in June, increase in diameter through August and September, and reach full size by November (see Chapter 2). Asexual females emerge from mid-November through January and oviposit into dormant buds. The tiny bud galls of the sexual generation develop beneath bud scales and become apparent in March. Adults of the sexual generation emerge from late March to April and females oviposit into the first flush of branch growth.

Several nurserymen requested information on insecticide efficacy and application timing to reduce populations of *D. quercusvirens*. Targeting asexual females during their emergence period could prevent the next generation from developing, although it may not be the most damaging life stage (Eliason and Potter 2000c, Pujade-Villar et al. 2001). Thus, I targeted asexual females emerging from the stem galls with three common insecticides.

Materials and Methods

Insecticides. Insecticides were chosen that were expected to have direct contact or residual activity against wasps, and treatments were timed to coincide with asexual *D. quercusvirens* emergence. Treatments for both tests included acephate (1.17 L/ha Orthene Turf, Tree, and Ornamental Spray 97, Valent USA, Walnut Creek, CA), bifenthrin (1 mL/L Bifen XTS, Control Solutions, Inc., Pasadena, TX), carbaryl (292.3 mL/ha Sevin SL, Bayer Environmental Science, Raleigh, NC), and an untreated control.

Study Site. In-ground Cathedral® live oak trees at Shadowlawn Nursery, Penny Farms, Clay Co., FL, that were heavily infested with *D. quercusvirens* stem galls were selected for use. For the branch trial, twenty randomly selected trees were measured

on 11 July 2007 with a telescoping measuring rod to determine height and a diameter tape to determine basal diameter and diameter at breast height (DBH), but only 12 of the most infested trees were selected for use. The trees averaged 4.7 ± 0.1 m tall with a basal trunk diameter of 8.7 ± 0.3 cm and DBH of 6.4 ± 0.3 cm. For the whole tree trial, 96 heavily galled live oak trees in a different block were selected on 15 October 2008. Trees were on 2.5 m centers within rows, and rows were 3 m apart.

Branch Trial

Each treatment was randomly assigned to one galled branch on the upper and lower canopy of each tree and applied to runoff using a hand-held pump sprayer on 13 – 14 December 2007. Drift was minimized by temporarily surrounding each selected branch with a large plastic bag during application, and then removing the bag. After treatments had dried, each treated branch was caged with a white organza mesh (Bridal Inspirations, China). Air temperature, wind speed, and relative humidity were measured at application.

To determine product efficacy, one caged branch per treatment on each tree was cut on 3 January 2008 and transported to the laboratory. The remaining branches were cut 10 January 2008. All of the insects within the caged branches were dead upon examination, so the number of fully or partially emerged *D. quercusvirens* and parasitoids and inquilines was recorded. Stem galls lacking exit holes were dissected to determine the gall maker's fate (e.g., fungal infection, parasitism). The number of individual stem galls per compound gall on each branch was also determined. At the time of this test, the sexual generation had not been identified, and the number of bud galls per branch was not counted. The successful emergence, death, or parasitism of *D. quercusvirens* was recorded as a proportion of total stem galls per compound gall,

and raw data were arc-sine square root transformed. Treatment differences were analyzed using a MANOVA (PROC GLM, SAS 9.2, 2008).

Site Conditions at Application. Before application, temperature, wind speed, and relative humidity were 29.2°C, <3.2 kph with gusts of 3.2 - 4.8 kph, and 44%, respectively for 13 December 2007, and 33.9°C, <3.2 kph, and 43% respectively for 14 December 2007.

Whole Tree Trial

The goal of this trial was to prevent asexual *D. quercusvirens* from ovipositing, thus reducing the number of sexual wasps developing in bud galls. Treatments were applied either once (on 24 November 2008, after most field collected stem galls contained fully formed adult wasps) or twice (on 15 December 2008, before peak asexual adult emergence was expected). Replications (n = 6) were set up as rows (Figure 3-1). Between each treated row, three untreated rows served as buffers between replicates. Four groups of four heavily galled trees were selected to be treated within each row. At least two buffer trees separated each group of four trees, and treatments were randomly assigned to each group. Within each group of trees, two trees received one application and the other two trees received two applications of the same insecticide. Trees receiving one and two applications were separated by at least one buffer tree. Treatments were applied to run off with a two nozzle (XR Tee Jet 110/2 VS), 1 m-boom to runoff using a CO₂ backpack sprayer at 0.22 MPa (Weed Systems Inc., Hawthorne, FL).

Branches (ca. 23-25 cm long) from each cardinal direction in the upper and lower canopy were cut and taken back to the lab on 24 March 2009. All bud galls on each branch were counted. Bud galls without emergence holes were reared in the laboratory

(13L:11D hrs, 23.7°C, and 48% relative humidity) in size 0 clear gelatin capsules to determine the percentage of gall maker and parasitoid emergence. Treatment differences were analyzed using an analysis of variance and, if significant, means were separated by Tukey's HSD test (PROC GLM, SAS 9.2, 2008).

To evaluate whether the treatments from the single application that reduced bud gall density would also reduce subsequent stem gall density, two observers simultaneously counted stem galls (one on the east and one on the west side of each tree) for 60 seconds from each treatment in each of 6 replicates (total trees, $n = 6$). This was conducted on 12 August 2009, when stem galls were red and easily visible. The total number of galls counted in 60 sec for each tree was recorded and treatment differences were analyzed using an analysis of variance (PROC GLM, SAS 9.2, 2008).

Site Conditions at Application. Before application, temperature, wind speed, and relative humidity were 27.3°C, < 3.2 kph with gusts up to 9.7 kph, and 50% at 10:09 and when applications started and 28.9°C, < 3.2 kph, and 37%, respectively, when applications ended at 12:01 pm on 24 November. Conditions when the applications began on 15 December at 8:15 am included an air temperature of 17.4°C, 88% relative humidity, with wind speed ≤ 3.2 kph, and 25.8°C air temperature, and 58% relative humidity when applications ended at 10:12 am.

Results and Discussion

Branch Trial

No obvious treatment differences occurred among the percentage of successfully emerged, dead within stem galls, and parasitized *D. quercusvirens* galls (Wilks lambda = 0.66; df = 9, 70.729; $P = 0.1835$) (Table 1). Results were impacted by heavy

parasitism and accidental caging of old compound stem galls. All parasitism was thought to have occurred before treatments were applied.

Ideally, compound stem galls would have been caged at initiation to prevent parasitism and the use of galls from previous years' growth. Since all insects that emerged were found dead inside the cages, their cause of death could not be determined (e.g., due to treatment or natural causes). However, it appears that asexual *D. quercusvirens* were able to chew through treated stem gall tissue since emergence was similar between treated and control branches.

Whole Tree Trial

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 to carbaryl and the control, with bifenthrin having the greatest reduction of bud galls (Table 2; $F = 8.87$; $df = 27, 347$; $P < 0.0001$). Number of applications (one or two) was included in the model; however, it did not have a significant effect on number of bud galls formed. Treatments in December did not reduce the number of new stem galls when compared to the control (Table 2; $F = 0.05$ $df = 3, 20$; $P = 0.9868$).

These results suggest that one application of bifenthrin or acephate can significantly reduce the formation of bud galls compared to the control without reducing parasitism. This is important for tree growers, so the number of applications made during the winter can be minimized. However, one winter application did not successfully reduce stem gall formation the following summer on treated trees. Gall makers from surrounding buffer (untreated) trees may have immigrated to treated trees in the spring, impacting the amount of stem galls that developed in the subsequent

generation. Targeted applications around bud break or repeated applications over two or more years may be necessary to significantly reduce stem gall density. Trials where whole fields are treated with one insecticide may also show stronger results and would provide valuable information to growers.

Effect on Natural Enemy Complex. Treatments had no significant effect on the percentage of parasitoids ($F = 0.58$; $df = 3, 43$; $P = 0.6316$) or *D. quercusvirens* ($F = 2.33$; $df = 3, 43$; $P = 0.0875$) that emerged from reared bud galls (Table 3-1). Overall, more parasitoids emerged from bud galls than *D. quercusvirens* ($F = 98.07$; $df = 1, 22$; $P < 0.0001$). Unfortunately, gall maker emergence is underestimated since branches were harvested at time of gall maker emergence. All gall makers that emerged before the evaluation were not included in the data.

Of all intact galls reared, the parasitoid *Acaenacis lauses* (Hymenoptera: Pteromalidae) was the most abundant, followed by *Brasema* spp. (Hymenoptera: Eupelmidae), *Baryscapus* spp. (Hymenoptera: Eulophidae), *Sycophila* spp. (Hymenoptera: Eurytomidae) and unknown Eurytomid and Ormyrid species (Table 4).

These significant results are contrary to those found by Morgan and Frankie (1982) for control of the mealy oak gall (*D. cinerosa* Bass.) in Texas. *Disholcaspis cinerosa* has a life cycle similar to *D. quercusvirens* (see Chapter 1). My findings demonstrated that one well timed application prior to adult emergence is enough to reduce bud gall formation on sampled branches, whereas chemical control had a negative impact on the *D. cinerosa* system. Ideally, this study would be repeated again to see if results were significant over multiple years. Unfortunately, the products and complete methods used in Morgan and Frankie (1982) were not available to compare.

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

Table 3-1. Effects of four treatments on *D. quercusvirens* asexual females emerging from stem galls in the field for the branch insecticide trial.

Treatment	Mean \pm SEM no. of stem galls per compound gall treated per branch	Fate of <i>D. quercusvirens</i> in percent (mean \pm SEM) of individual bullet galls per compound gall *		
		Successfully emerged	Dead	Parasitized
Acephate	18.4 \pm 3.2	10.0 \pm 2.8	7.2 \pm 2.3	53.2 \pm 5.3
Bifenthrin	16.7 \pm 2.4	2.4 \pm 1.4	15.6 \pm 7.6	54.0 \pm 9.3
Carbaryl	26.8 \pm 2.7	14.0 \pm 4.7	7.4 \pm 2.9	53.3 \pm 7.8
Control	22.5 \pm 5.0	5.4 \pm 2.3	1.2 \pm 0.7	66.0 \pm 5.6

***MANOVA** for comparisons of fate of *D. quercusvirens* in percent of individual galls affected per compound gall (Wilks lambda = 0.66; df = 9, 70.729; *P* = 0.1835).

Table 3-2. Effects of four treatments on bud and stem gall formation of sexual *D. quercusvirens* in the field for the whole tree trial.

Treatment	Trade name	Rate	Mean no. \pm SEM bud galls per branch per tree*	Mean no. \pm SEM bullet galls per tree**
Acephate	Orthene TTO 97	1.17 L/ha	11.5 \pm 7.4 b	98.3 \pm 21.9
Bifenthrin	Bifen XTS	1 mL/L	1.8 \pm 2.7 a	90.5 \pm 19.5
Carbaryl	Sevin SL	292.3 mL/ha	15.4 \pm 9.7 c	96.0 \pm 35.6
Control	--	--	16.7 \pm 12.3 c	87.8 \pm 12.1

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

** **ANOVA** for comparison of means \pm SEM stem galls counted ($F = 0.05$; $df = 3, 20$; $P = 0.9868$)

Table 3-3. Effect of treatment on percent emergence of *D. quercusvirens* and parasitoids of reared bud galls.

Treatment	Mean (\pm SEM) no. intact bud galls reared per tree*	Percent (mean \pm SEM) of parasitoids emerged from reared bud galls per tree**	Percent (mean \pm SEM) of <i>D.</i> <i>quercusvirens</i> emerged from reared bud galls per tree***
Acephate	66.1 \pm 7.0 b	58.9 \pm 6.5	1.6 \pm 0.6
Bifenthrin	12.6 \pm 4.0 a	64.5 \pm 10.6	14.5 \pm 6.8
Carbaryl	81.8 \pm 7.1 b	48.7 \pm 7.0	1.9 \pm 0.5
Control	86.2 \pm 7.3 b	64.5 \pm 6.0	1.9 \pm 0.4

* **ANOVA** for Mean \pm SEM within columns followed by a different letter are significantly different at $\alpha = 0.05$ ($F = 25.44$; $df = 3, 43$; $P < 0.0001$).

** **ANOVA** for Mean \pm SEM within columns followed by a different letter are significantly different at $\alpha = 0.05$ ($F = 0.58$; $df = 3, 43$; $P = 0.6316$).

*** **ANOVA** for Mean \pm SEM within columns followed by a different letter are significantly different at $\alpha = 0.05$ ($F = 2.33$; $df = 3, 43$; $P = 0.0875$)

Table 3-4. No. of *D. quercusvirens* and parasitoids reared from intact bud galls sampled for the insecticide trial.

Family	Genus	No. of insects emerged from bud galls			
		Acephate	Bifenthrin	Carbaryl	Control
Cynipidae					
	<i>D. quercusvirens</i>	13	11	21	21
Eulophidae					
	<i>Baruscapus</i> spp.	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> spp.	1	1	6	2
	Unknown spp.	13	3	18	14
Ormyridae					
	Unknown spp.	1	0	4	3
Pteromalidae					
	<i>Acaenacis lauses</i>	227	51	217	350

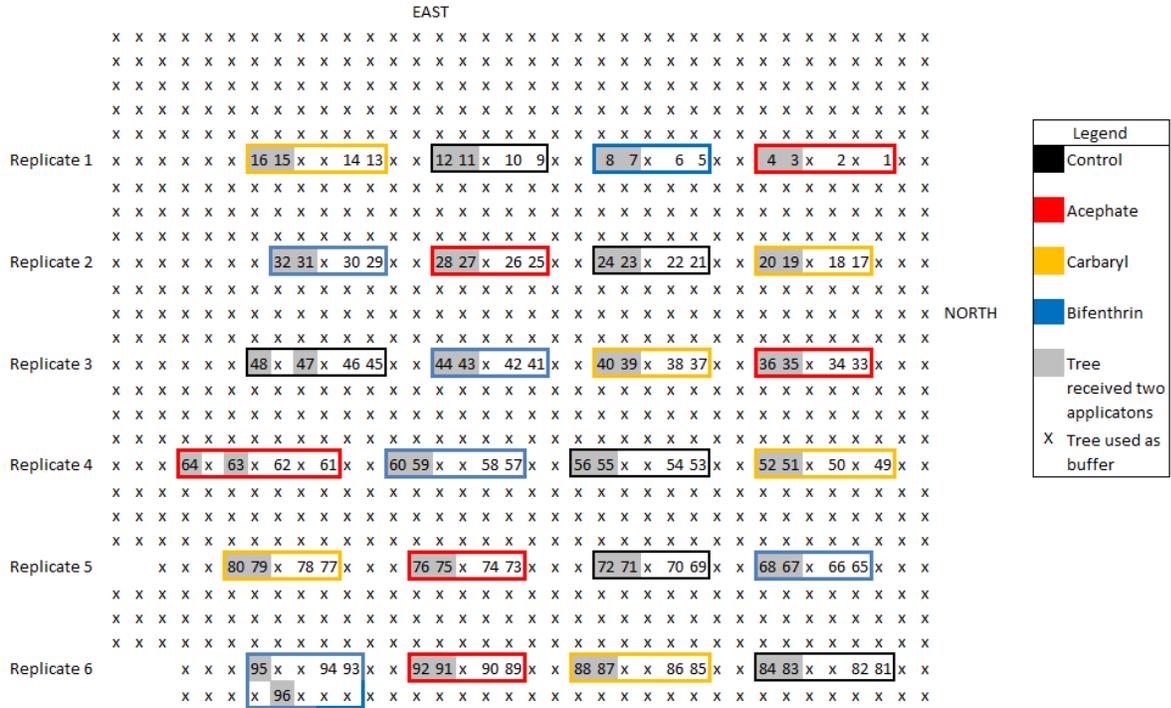


Figure 3-1. Plot map for whole tree insecticide trial.

APPENDIX BUDBREAK PHENOLOGY

Differences in cultivar susceptibility to *Disholcaspis quercusvirens* and other gall makers were suspected by nursery owners and researchers. The following study was done to determine if bud break phenology differed among three cultivars of live oak (Cathedral®, Millennium® and Highrise®) and live oak seedlings.

Materials and Methods

Study Site. Live oak trees at the Environmental Horticultural Teaching Unit in Gainesville, FL (Alachua County) were established in ca. 2002-2003. Four rows of eight to nine trees were used. Each row was comprised entirely of one cultivar or the seedling oaks. Cathedral® oaks were 49.0 ± 1.1 cm tall with a caliper (taken 15.24 cm above ground) of 11.2 ± 0.2 , Highrise® oaks were 67.0 ± 1.3 cm tall with a caliper of 12.3 ± 0.4 , and Millennium® oaks were 65.2 ± 1.0 cm tall with a caliper of 17.9 ± 0.9 when measurements were taken on 11 October 2007 (C. Harchick, pers. comm.). Seedling live oaks were not measured. Gall density and gall maker emergence periods were not assessed on the trees.

Bud Break Phenology. Potential differences in initial bud break phenology among three cultivars (Cathedral®, Highrise®, and Millennium®), and seedling live oaks were evaluated in 2008. Four branches per cardinal direction on each tree per variety were flagged on 10 February 2008 ($n = 40$ buds per tree). The most apical ten buds on each terminal branch were ranked as being dormant (winter stage, with bud scales tightly closed), swelling (bud scales loosely closed), green-tipped (bud scales starting to fall off and green leaf tissue visible), or expanding leaves (small leaves were visible) (Eliason and Potter 2000b) every 2-4 d from 19 February until leaves of Cathedral®

oaks had fully expanded. The percentage of buds per branch in each bud category for each date was summarized.

Results and Discussion

Bud Break Phenology. Bud break phenology appeared to differ among the three cultivars and the seedling Southern live oaks (Figure 4-1). Cathedral® live oaks were the first to flush, and consistently had the most developed buds for each sampling date. Cathedrals® were followed by seedlings, Millennium®, and then Highrise® oaks in order of bud break activity on selected branches. Cathedral® oaks had the first green tipped buds, and they occurred 6, 8 and 11 d before they were present on seedling, Millennium®, and Highrise® trees, respectively. Cathedral® oaks were the first trees to have a majority ($\geq 50\%$) of expanded leaves, which was observed on 12 March 2008. Seedlings had a majority of expanded leaves 12 d later (24 March 2008).

There are many documented instances of herbaceous insects synchronizing their emergence with budbreak or plant phenology (e.g., Watt and McFarlane 1991, Story et al 1992, How et al. 1993, Conner et al. 1994, Eliason and Potter 2000a). These differences in bud break activity could later be used to document if earlier bud break activity correlated with greater gall densities or the gall maker's ability to initiate galls on each type of live oak. Time and length of shoot elongation should also be compared, and correlations between stem gall density and shoot length should also be determined (Fernandes 1998)

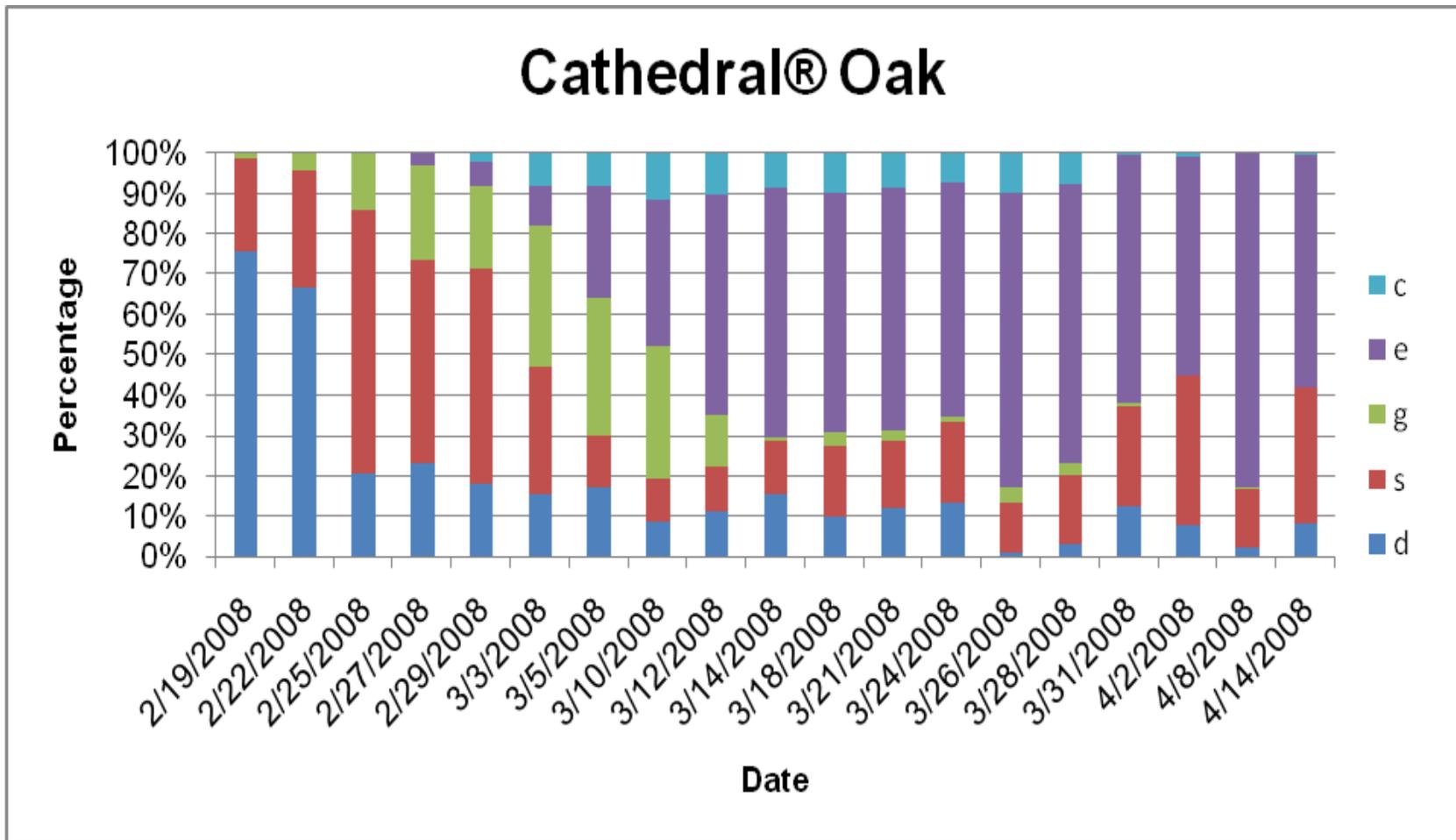


Figure A-1. Stacked column charts showing the percentage of buds in each category for each date measured for Cathedral® live oak.

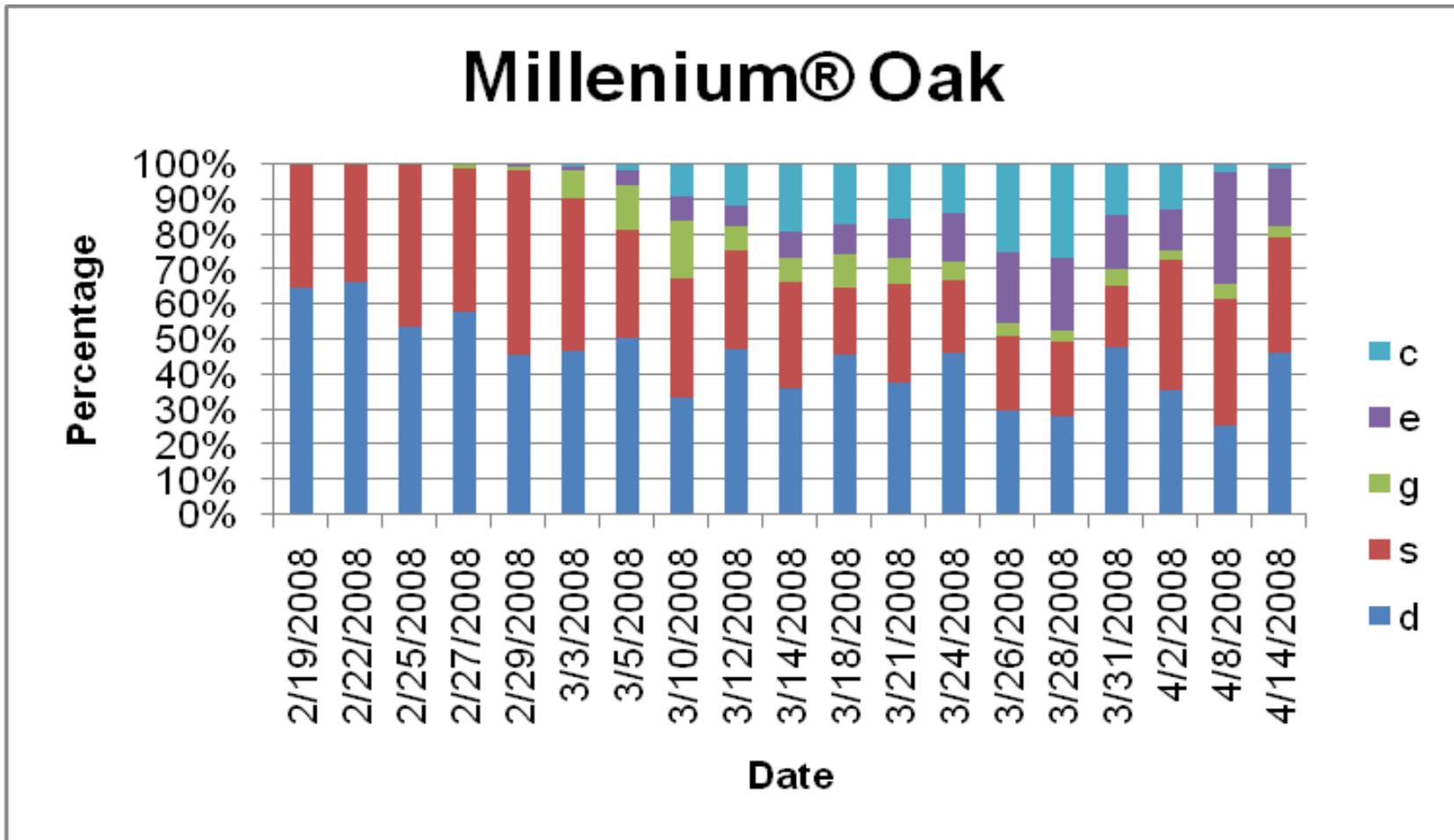


Figure A-2. Stacked column charts showing the percentage of buds in each category for each date measured for Millennium® live oak.

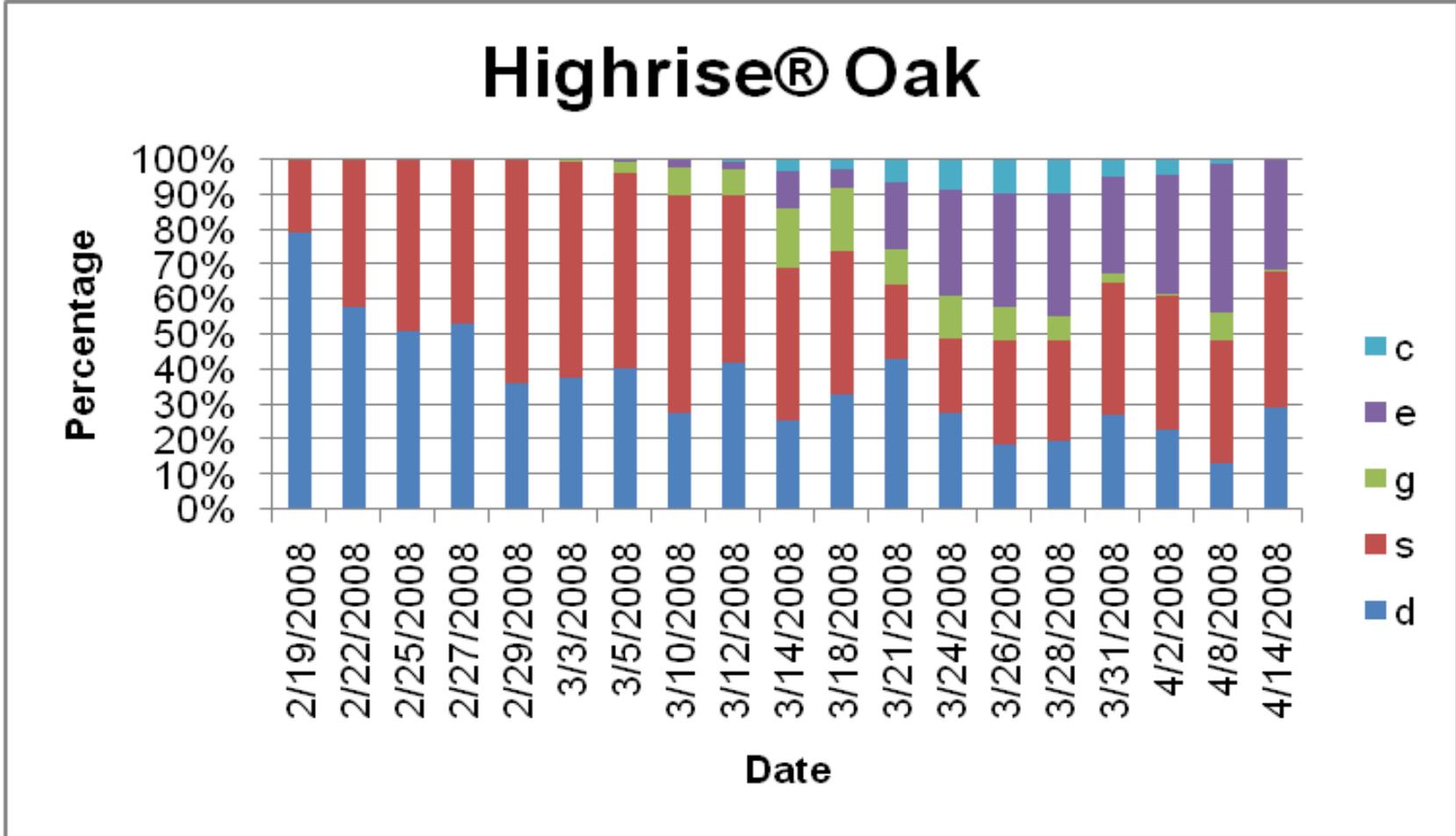


Figure A-3. Stacked column charts showing the percentage of buds in each category for each date measured for Highrise® live oak.

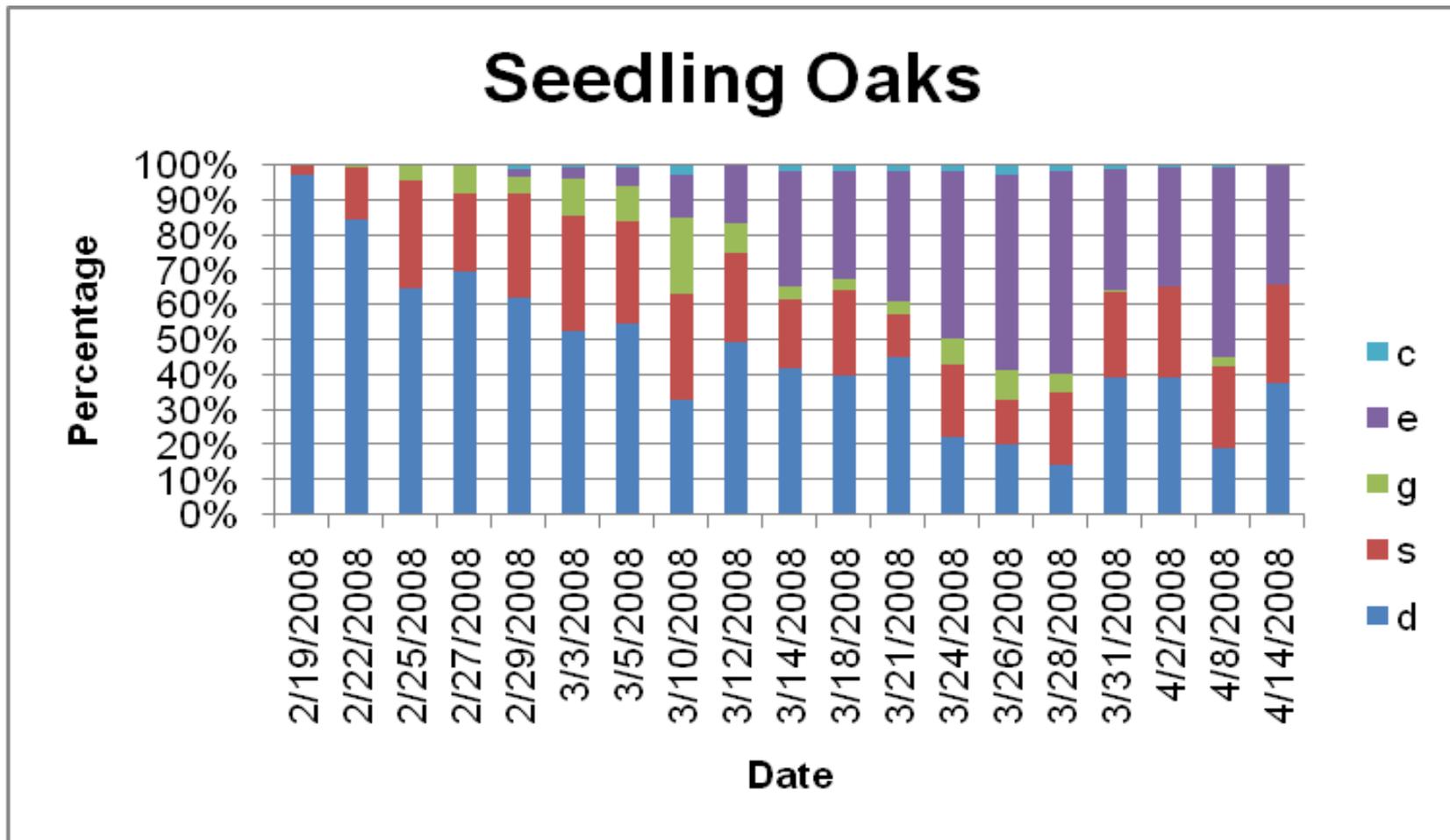


Figure A-4. Stacked column charts showing the percentage of buds in each category for each date measured for seedling live oaks.

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BIOGRAPHICAL SKETCH

Jessica Ellen Platt was born on December 29, 1984 in Jeffersonville, IN. She graduated from Our Lady of Providence High School in Clarksville, IN in 2003. She went on to pursue a Bachelors degree at Purdue University where she discovered that her true calling was not to be a veterinarian. Between her time volunteering within the Entomology department and interning for the Indiana Cooperative Pest Survey program under Dr. Christopher Pierce and later for the National Association of State Departments of Agriculture in Washington D.C., she found her passion for extension entomology and public education. She graduated with a Bachelor of Science in May 2007.

Jessica continued her education at the University of Florida where she became a teaching and research assistant. She graduated with a Master of Science in Entomology in December 2009, and hopes to pursue a career in public service or extension and outreach in agriculture.