BIOLOGY, MOLECULAR SYSTEMATICS, POPULATION DYNAMICS AND CONTROL OF A STEM GALL WASP, ZAPATELLA DAVISAE (HYMENOPTERA: CYNIPIDAE)

Monica Davis
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BIOLOGY, MOLECULAR SYSTEMATICS, POPULATION DYNAMICS AND
CONTROL OF A STEM GALL WASP, ZAPATELLA DAVISAE
(HYMENOPTERA: CYNIPIDAE)

A Dissertation Presented
by
MONICA J. DAVIS

Submitted to the Graduate School of
the University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

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May 2017

Environmental Conservation
BIOLOGY, MOLECULAR SYSTEMATICS, POPULATION DYNAMICS AND
CONTROL OF A STEM GALL WASP, ZAPATELLA DAVISAE
(HYMENOPTERA: CYNIPIDAE)

A Dissertation Presented
By
MONICA J. DAVIS

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DEDICATION
To the two people who have given me more than I will ever be able to give in return.
Thanks Mom and Dad.
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ABSTRACT

BIOLOGY, MOLECULAR SYSTEMATICS, POPULATION DYNAMICS AND CONTROL OF A STEM GALL WASP, ZAPATELLA DAVISAE (HYMENOPTERA: CYNIPIDAE)

MAY 2017

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Gall wasps are phytophagous insects that often go unnoticed, however, when they are released from their natural enemies, they have the capacity to outbreak and cause extensive foliar damage. One such outbreaking pest, *Zapatella davisae*, causes significant damage and mortality to black oak, *Quercus velutina*. In recent years, black oak decline has been documented in Long Island, New York and coastal New England. Little is known about the lifecycle, distribution or population dynamics of *Zapatella davisae* and the taxonomy of the species is still unclear.

My first study described the biology and distribution of *Z. davisae*. *Zapatella davisae* completed one life cycle per year, and emerged in early May. The same proportion of trees were infested in Cape Cod and Long Island, however, the severity of the infestation was significantly greater in Cape Cod, an indication that something may be regulating populations in Long Island.

I evaluated where *Z. davisae* fits within the Cynipidae phylogeny, quantified genetic diversity across geographically isolated populations and identified which loci gave the most taxonomic clarity. Three genes determined that *Z. davisae* is completely invariant across all geographically isolated populations, likely indicative of a founder effect. *Zapatella davisae* may be native, as it was a species-level match to a gall wasp
species from the southeastern, US. LWRh and COI gave the most taxonomic clarity, as they had genera that fell out into distinct clades, whereas 28S increased the incidence of polyphyletic and paraphyletic clades.

After I determined Long Island and Cape Cod populations were both *Z. davisae*, I compared the population dynamics in each location. On Long Island, multiple gall wasp populations exhibited almost 100% parasitism in 2015, which was followed by a near total collapse of the population in 2016. On Cape Cod, parasitism rates were lower and consistent overtime, which may explain greater canopy damage in that region. On Long Island, species-group *Sycophila* species 3 caused the highest level of parasitism, but parasitism from this species was lower on Cape Cod. My results indicate that *Z. davisae* populations are controlled by top-down pressures on Long Island.
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CHAPTER 1
LIFE HISTORY AND POTENTIAL HOSTS OF ZAPATELLA DAVISAE, A RECENT INVADER ON BLACK OAK IN THE NORTHEASTERN UNITED STATES

1.1 Introduction

Oaks are a major component of New England forests, therefore oak pests can have lasting economic and ecological impacts on the region. In particular, a stem gall wasp, *Zapatella davisae* Buffington (Hymenoptera: Cynipidae), has caused extensive mortality and canopy damage to *Quercus velutina* Lam. (Black oak) in Cape Cod, Nantucket and Martha’s Vineyard, Massachusetts (Buffington et al. 2016). Cape Cod is the largest protected coastal area of oak-pine forest or sand-plain vegetation in New England (Eberhardt et al. 2003). It is composed of sandy soil that is often nutrient poor and does not retain water; thus trees that can adapt to dry, low nutrient conditions are the most successful competitors (Neil et al. 2007). Drought-resistant tree species, such as *Pinus rigida* Mill (Pitch pine), *Quercus velutina* Lam. (Black oak), and *Quercus coccinea* Muenchh. (Scarlet oak) make up most of the trees in this forest (Eberhardt et al. 2003). A decline in tree canopy cover due to insect pest outbreaks is of high conservation concern in the Cape Cod region.

*Zapatella davisae* was discovered on Martha’s Vineyard in 2012, but infestations can be tracked back to approximately 2008 based on the inferred ages of damaged twigs and branches. *Zapatella davisae* creates woody stem galls underneath the bark and causes extensive node swelling and twig disfiguration (Buffington et al. 2016) (Fig. 1). Other symptoms include flagging, leaf clumping, canopy dieback, and tree mortality (Pike et al. 2001) (Fig. 2). Although the damage caused by *Z. davisae* has been documented in
individual trees, its life cycle, severity of damage, and host specificity have remained unknown (Buffington et al. 2016).

Part of our research parallels a previous study of an oak gall wasp (previously misidentified as *Bassettia ceropteroides* Bassett) that caused damage and mortality to black oak on Long Island in 1990 (Melika and Abrahamson 2007). Five years after a reported *B. ceropteroides* outbreak developed on Long Island, its population crashed and black oak began to recover. Despite its rapid decline, this gall wasp remains present on Long Island at low densities and causes little environmental harm (M. Davis pers. obs; Pike et al. 2010). Over the past five years, there has been much speculation as to whether *B. ceropteroides* is the same species that is causing extensive tree mortality in Cape Cod, Martha’s Vineyard and Rhode Island. Molecular analyses confirmed that the gall wasp on Long Island is in fact *Z. davisae* (Buffington et al. 2016). Differences between the two populations, specifically host trees and the severity of the infestation, have not been previously evaluated.

*Zapatella davisae* is a member of the Cynipini, a host-specific tribe that contains over 87% of all gall makers on oaks (Abrahamson 1998; Stone et al. 2008). Life cycle descriptions are available for only 85 of 900 species in the Cynipini tribe worldwide (Pujade-Villar et al. 1999). All Cynipini, also known as oak gall wasps, reproduce through either cyclic or obligate parthenogenesis (Stone et al. 2008). Cyclic parthenogenesis in Cynipini consists of the strict alternation between one parthenogenetic generation and one sexual generation (Stone et al. 2008). The alternating generations may occur in the same year or in alternating years and may attack different hosts or different tissues of the same host (Hood and Ott 2001; Stone et al. 2002). Obligate parthenogenesis
is also common in Cynipini, having evolved many times from cyclic parthenogenesis by deletion of the sexual generation (Herbert 1981; Rispe and Pierre 1998). The life cycle of Z. davisae is still unknown; however, the biology of other species of Zapatella may help identify potential life cycle patterns.

Most Zapatella species exhibit obligate parthenogenesis; however, they have various hosts, gall tissue types, and generation times. For example, Zapatella nievesaldreyi Melika and Pujade-Villar induces stem galls on Quercus humboldtii Bonpl. in Colombia, whereas Zapatella oblata Weld creates bud galls on both Quercus coccinea Muenchh and Quercus falcata Michx in Virginia (Pujade-Villar et al. 2012). Preliminary microsatellite data (J. Andersen, University of California Berkley, Berkley, CA, USA. unpubl. data) suggest that sampled populations of Z. davisae are obligately parthenogenetic, but it is unclear whether cyclic parthenogenesis also occurs.

An understanding of the life cycle, severity of the infestation, and potential hosts of Z. davisae will aid in future management efforts and help answer ecological questions regarding Z. davisae community and population dynamics. Our first objective was to describe the life cycle of Z. davisae, specifically its emergence patterns and the phenology of its developmental stages. Our second objective was to compare levels of infestation between Cape Cod and Long Island, and identify any additional host trees in each region. Our final objective was to document the distribution of Z. davisae in Cape Cod. Our research will inform the implementation of different management strategies, as well as identify the geographical distribution and potential hosts of Z. davisae.
1.2 Methods

1.2.1 Life Cycle

To determine emergence patterns of *Z. davisae*, adult gall wasps were captured as they emerged from stem galls in Cape Cod, MA. Individual 11.4 x 17.7 cm organza bags were used to cover new and last year’s growth on 100 branches of 20 infested trees. Bags were checked visually each month from November 2013 to March 2014, and then checked weekly during April and May 2014. At each check, every bag was scored for the presence or absence of *Z. davisae*. The same schedule of bag deployment and visual checks was completed the following year, from November 2014 to May 2015.

Biweekly branch samples were collected from Dennis, Massachusetts and Riverhead, New York to document the stages of development of the stem gall generation. Every other week, five branches were haphazardly selected from the crown of 10 trees at one of the two sites and stored in separate 1-gallon plastic bags. Dissections of the galls were completed on new and last year’s growth under a dissecting microscope (Wild M5A, 6X-50X), and each sample was scored for the presence or absence of each life stage of *Z. davisae*.

1.2.2 Tree Infestation Survey

During the spring and summer of 2016, field surveys were completed on Long Island and Cape Cod. Seven sites per region were randomly chosen as GPS coordinates that contained pitch pine and oak forest vegetation on a GIS topographical map. Each site was at least 10 km from any other site. We checked each site, and if black oak trees were not present, we drove no more than an additional 3 km in search of trees. If no black oak
trees could be found, a new site was randomly chosen in the same manner. At each of the seven sites in both regions, 20 black oak trees were scored for the presence or absence of gall wasp infestation. In addition, at each site, each oak tree that was not a black oak was identified to species and was also scored for the presence or absence of gall wasp infestation. Level of infestation was scored based on field observations. Low-infested trees were defined as trees with small galls that were difficult to find. Moderately-infested trees were defined as those with obvious galls and noticeable canopy damage. Heavily-infested trees were defined as having more than 80% of branches galled, along with severe canopy damage. A Chi-squared test was run in R using RStudio Version 0.99.491 (R Core Team) to compare the number of trees at each infestation level between both regions.

1.2.3 Estimation of Gall Wasp Distribution

Towns in Rhode Island, Cape Cod, Martha’s Vineyard and Nantucket were surveyed by car and by foot to identify places where Z. davisa is present in New England. Visual surveys were completed by the authors and extension personnel in each region. The main focus of this project was Cape Cod, Martha’s Vineyard, and Nantucket; however, infestations reported in Rhode Island were also documented. In Cape Cod, Martha’s Vineyard and Nantucket, Massachusetts all towns were surveyed. In Rhode Island, coastal areas were searched for Z. davisa with local extension personal, but not all towns were evaluated. Z. davisa was scored as being present in a town if Z. davisa damage was sighted on at least five trees. A GIS layer from the Massachusetts Department of Conservation and Recreation was over-layered to compare defoliation
levels with town-level infestation detection. A map was created in QGIS Version 2.180 to identify the current extent of the *Z. davisae* infestation.

### 1.3 Results

#### 1.3.1 Life Cycle

No gall wasp emergence was detected in the fall of 2013 and 2014 of the bag experiment, confirming that *Z. davisae* does not have an autumnal generation. In the spring of 2014, May 7th – 25th was the date range when *Z. davisae* adults emerged on Cape Cod. The same pattern occurred in 2015. It was concluded that *Z. davisae* emerges between the first and third week of May depending on the year.

The life cycle of one generation of *Z. davisae* from August to May is illustrated diagrammatically in Figure 3. The *Z. davisae* life stages we recognized included early and late larval stages, pupae and fully formed adults. Gall cavities were first detected in July, and the early larval stage was present by mid-August, with the late larval stage present in early September. The number of larval instars was not determined. Pupation occurred in mid-September, when both larval stages were still present. In early October, pharate adults were detected. Mature adults were present in early spring of the following year and they emerged in May. *Zapatella davisae* overwintered in several life stages, and individuals became adults by May prior to emergence.
Figure 1.1 *Zapatella davisae* Buffington and Melika 2016, female

Figure 1.2 Damage to black oak, *Quercus velutina* by *Zapatella davisae* (a) Twig gall damage including swollen nodes and stems (b) *Z. davisae* cavities on a branch from black oak (c) Exit holes from *Z. davisae* adults on black oak (d) a heavily damaged black oak tree
1.3.2 Tree Infestation Survey

There was a significant difference in the level of gall wasp infestation on Long Island versus Cape Cod ($\chi^2 = 30.6; \text{df} = 3; P < 0.0001$) (Fig. 4). Long Island had significantly more trees with low-level infestations than trees with medium or heavy infestations. Cape Cod showed the opposite trend, with more heavy infestations, followed by medium and then low infestations. There was a site effect on both Cape Cod and Long Island, confirming that infestation levels varied across both regions (Cape Cod: $\chi^2 = 95.633; \text{df} = 18; P < 0.0001$, Long Island: $\chi^2 = 53.438; \text{df} = 18; P < 0.0001$). No other oak species besides black oak was infested by Z. davisae (Table 1). Most black oaks in both regions harbored some infestation and the proportion of trees infested in Cape Cod versus Long Island was not different (Table 1).
Table 1.1 Number of trees surveyed for each oak species at sites on Cape Cod and Long Island and the proportion of trees in each species that were infested with *Zapatella davisae* (all zero except for black oak)

| Species | White | Red | Scarlet | Chestnut | Pin | English | Black | Count | Infestation
|---------|-------|-----|---------|----------|----|---------|-------|-------|-------------
| Cape Cod | 24 | 9 | 11 | 0 | 0 | 4 | 120 | (0.89 ± 0.027) |
| Long Island | 0 | 4 | 1 | 2 | 2 | 0 | 120 | (0.79 ± 0.037) |

Prop. Infested

0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.84 ± 0.023 |

Figure 1.4 Average proportion of trees at each gall wasp infestation level (low, medium and high) across all sites on Long Island and Cape Cod

1.3.3 Estimation of Gall Wasp Distribution

All towns on Cape Cod contained a *Z. davisae* infestation (Fig. 5). *Zapatella davisae* was present in all towns on Nantucket and Martha’s Vineyard. In Rhode Island, documented infestations were in primarily coastal areas of oak-pine forest, where black oak was the dominant deciduous tree. *Zapatella davisae* defoliation levels reported by the Massachusetts Department of Conservation and Recreation (DCR) in 2015 and 2016
agreed with our survey results; however, *Z. davisae* was found in 12 additional towns where the overhead defoliation data of the DCR survey did not detect *Z. davisae*.

![Map of towns in New England with known Zapatella davisae infestations as a result of ground surveys.](image)

**Figure 1.5** Map of towns in New England with known *Zapatella davisae* infestations as a result of ground surveys. Includes *Z. davisae* defoliation data from DCR mapping flyovers in 2015 and 2016.

### 1.4 Discussion

We found that *Z. davisae* completes one stem gall generation per year, and emerges in May. This information will allow managers to determine the appropriate timing for biological control releases or pesticide applications. *Zapatella davisae* was first noted on Martha’s Vineyard in 2008 and has continued to be spotted in significant portions of the oak-pine forests in coastal New England. Because *Z. davisae* was found to only attack black oak, its range may be limited by the distribution of black oak. In New England, black oak occurs widely, but it is only a dominant deciduous tree in sand plain...
regions of oak-pine forest. Thus far, we have only encountered *Z. davisae* in this forest type.

A comparison of Cape Cod and Long Island *Z. davisae* infestations levels showed differences in black oak tree damage. The proportion of trees infested in both regions was similar, but the level of *Z. davisae* infestation, including gall presence and canopy damage, was much lower on Long Island than on Cape Cod, likely because *Z. davisae* population densities subsided in Long Island in the 1990’s (Pike et al. 2001). These results may provide evidence of possible population regulation by natural enemies of this gall wasp in Long Island, New York. Further research is needed to evaluate the parasitoid communities in both regions.

High density gall wasp populations can have extensive ecological, economical, and social impacts on a region (Stone et al. 2002). Understanding the life cycle and distribution may help improve strategies to manage this pest. *Zapatella davisae* continues to cause high levels of oak mortality on Cape Cod, MA, Martha’s Vineyard, Nantucket and in coastal Rhode Island. The extent of the infestation was significantly larger by 2015 than in 2008 (Buffington et al. 2016). Further survey work should be completed in Rhode Island to determine if *Z. davisae* is present in more inland communities. Mortality of black oaks in coastal New England has caused significant ecological and economic impacts, including the cost of removing and replacing dead trees. Further research needs to be done to evaluate limiting factors of *Z. davisae* population density including overwintering morality and natural enemy regulation.
CHAPTER 2
RECONSTRUCTING CYNIPINI PHYLOGENY AND THE MOLECULAR PLACEMENT OF A STEM GALL MAKER, ZAPATELLA DAVISAE

2.1 Introduction

Gall wasps in the Cynipidae (Hymenoptera: Cynipoidea), a family with over 1,300 species worldwide, create stem, acorn or leaf galls on different plant species (Stone et al. 2002; Rokas et al. 2002). By far the largest of the six tribes in the family is Cynipini, a Holarctic group with over 1000 species worldwide. Cynipini species tend to be highly host-specific and include over 87% of all the gall makers on oaks (Stone et al. 2008; Pujade-Villar et al. 2012). Inconsistencies in the morphological characteristics used to define Cynipini genera have complicated the taxonomic placement of species in the group and their relationships (Melika and Abrahamson 2002).

Genetic markers are useful tools to evaluate diversity, genetic connectivity and phylogenetic relationships of gall wasp species (Ronquist et al. 2015). Previous studies have employed several markers for phylogenetic analysis, with varying success (Rokas et al. 2002; Ronquist et al. 2015). Rokas et al. (2002) determined that the mitochondrial loci tested, such as CO1, had the highest resolution at the species level, whereas nuclear genes (28S and LWRh) were the most useful at the family level. Ronquist et al. (2015) reconstructed three separate phylogenies for the Cynipidae, each based on different combinations of molecular, life history and morphological data. The Cynipini was not well represented in these analyses, as only 7 out of its 34 genera were included. Since Ronquist et al. (2015), CO1 sequences for additional gall wasp species have been published. In addition, new sequences have become available for 28S and LWRh within the NCBI GenBank database. A rise in the number of publically available sequences from
researchers around the world not only increases our ability to accurately match sequences from unidentified gall wasp specimens, it allows us to investigate important questions regarding their population genetics (Collins and Cruickshank 2012).

Gall wasps often go unnoticed; however, when they are separated from their regulating natural enemies, they frequently outbreak to high densities and cause extensive host plant damage and mortality (Schonrogge et al. 1995). A correct taxonomic identification of an outbreaking species is essential for its management, as an identification can provide necessary information about the insect pests’ biology, life history, and range (Yang and Rannala 2012). One species of gall wasp that was absent from the Ronquist et al. (2015) analysis is Zapatella davisae Buffington. This recently described member of the Cynipini has caused extensive black oak Quercus velutina Lam., damage and mortality on Cape Cod, MA; however, the placement and taxonomic status of Z. davisae remains unclear (Buffington et al. 2016).

While the recent outbreak of Z. davisae in the northeastern United States has caused extensive oak mortality, it is not the first time that a gall wasp species has been identified as the source of black oak damage in the Northeast region. In 1900, Basset (1900) reported damage to black oaks in Connecticut and attributed it to a new species Callirhytis ceropteroides (Basset). In the 1990s, black oak damage and mortality by this same species was reported on Long Island, under the name Bassettia ceropteroides (Basset) (Pike et al. 2001). This change in generic assignment was based on differences in the shape of the mesosoma (Melika and Abrahamson 2000). This species was later returned to Callirhytis, along with five other species in 2007 (Melika and Abrahamson, 2007). More recently, several species of Bassettia and Callirhytis were reassigned to a
new genus, Zapatella Pujade-Villar & Melika, based on several characters, including the lack of a malar sulcus and the length of the ventral spine of the hypopygium (Pujade-Villar et al., 2012). Our understanding of the taxonomic placement of species within the Cynipini remains in flux, and we have many questions about the diversity, origin, range and close relatives of Z. davisae. Consequently, it is unknown if Z. davisae, which is currently causing defoliation in Cape Cod, Nantucket, Martha’s Vineyard and Rhode Island, is the same species that previously caused defoliation on Long Island and in Connecticut (Pike et al. 2001).

The purpose of this study is to use molecular systematic tools to better understand the genetic variation, native range and close relatives of Z. davisae. Our specific goals were (1) to determine if morphologically similar populations on Cape Cod, Long Island, Rhode Island, Nantucket and Martha’s Vineyard are the same species, (2) to evaluate where Z. davisae fits in the Cynipini phylogeny, using data from multiple genes, and (3) to identify which genes, if any, best reveal taxonomic relationships within the Cynipini.

2.2 Methods

2.2.1 Specimen Collection

To collect specimens of Z. davisae, branches of Q. velutina were collected at six sites in Massachusetts (four in Cape Cod, one on Martha’s Vineyard, and one on Nantucket), four sites on Long Island, New York, and one site on Cooks Island, Rhode Island, in early spring 2015. Each branch was placed in a 1-gallon zip lock bag and stored in a 4 °C in a growth chamber (Percival Scientific Inc., Perry, IA) for six weeks until adult emergence. After emergence, adult gall wasps or associated species were removed
from the bags, placed in separate 1.5 ul tubes with 99% ethanol and stored at room temperature. All parasitoids and inquilines were removed from the sample before DNA extraction. Voucher specimens are housed at the Smithsonian Institution.

2.2.2 DNA Extraction and Amplification

DNA was extracted from individual gall wasps using the Qiagen DNeasy kit following the manufacturer’s instructions (QIAGEN, Valencia, CA). A fragment of the mitochondrial gene Cytochrome Oxidase I (CO1) was amplified from all individuals using forward primer LepF1 (5'-ATTCAACCAATCATAAAGATATTGG -3’) and reverse primer LepR1 (5'-TAAACTTCTGGATGTCCAAAAATCA -3’) (Hebert et al. 2004) using the following thermocycler protocol. DNA was denatured for 5 min at 95°C, followed by 6 amplification cycles with 60 s denaturing at 94°C, 90 s annealing at 45°C and 75 s extension at 72°C. A second amplification of 36 cycles was completed with 60 s denaturing at 94°C, 90 s annealing at 51°C and 75 s extension at 75°C, followed by a single final extension period of 5 min at 72°C (Hebert et al. 2004).

Fragments of the nuclear loci 28S and long wavelength rhodopsin (LWRh) were amplified for two individuals per location. The 28S gene was amplified using the forward primer s3660 (5'-GAGAGTTMAASAGTACGTGAAAC -3’) and the reverse primer 28b (5'-TCGGAAGGAACCAGCTACTA -3’) (Morse and Normark 2006). A touchdown protocol was used for amplification of the 28S fragment, as described in Morse and Normark (2006). The LWRh gene was amplified using the forward primer LWRhF (5'AAT TGC TAT TAY GAR ACN TGG GT 3’) and the reverse primer LWRhR (5'ATA TGG AGT CCA NGC CAT RAA CCA 3’), according to the thermocycler protocol.
described in Rokas et al. (2002). DNA was denatured for 4 min at 95°C, followed by 35 amplification cycles with 60 s denaturing at 94°C, 60 s annealing at 57°C, and 60 s extension at 72°C, followed by a single final extension period of 5 min at 72°C. PCR products for each locus were visualized on 1.5% agarose gels; prior to sequencing, products were purified using Exonuclease 1 (Thermo Scientific) and Shrimp Alkaline Phosphatase (New England BioLabs) according to the Thermo Scientific PCR and purification protocol. Products were sequenced at the Yale Genomic Lab using an ABI 3730 sequencer (Life Technologies). Forward and reverse sequence reads were then aligned and edited using GENEIOUS 8.1.8 (Kearse et al. 2012), and a consensus sequence was generated for each sample.

2.2.3 Data Concatenation

After editing the consensus sequences for each gene-fragment, sequences were aligned, manually adjusted and truncated to the length of the shortest sequence using GENEIOUS. To identify possible species matches for *Z. davisae* for each locus, we compared our sequences to those published in the NCBI database using the blastn search algorithm. All published cynipid sequences for 28S, LWRh, and COI were downloaded and evaluated for quality, length and duplicate species names. Downloaded sequences were then filtered to include only those sequences belonging to specimens with species-level identification, sequences longer than 500 base pairs, sequences from only a single representative for each nominal species, and sequences of only those species from which at least two of the three target loci could be obtained. A complete list of all species included in the analyses, and the GenBank Accession numbers for sequences from each
included loci is presented in Table 1. Sequences were aligned with *Z. davisae* collectively and truncated to the length of the shortest sequence using GENEIOUS for 28S, LWRh, and COI. After LWRh sequences were aligned, a known intron was removed from the center of the LWRh gene fragment, shortening its total length to 390 base pairs. The three loci were then concatenated using MESQUITE (Maddison and Maddison 2017).

**2.2.4 Phylogenetic Analysis**

Phylogeny was estimated separately for each locus and for the concatenated data set. Before analyses, we identified the optimal model of evolution for each gene fragment to be the Hasegawa-Kishino-Yano (HKY) genetic distance model based on the program JMODELTEST (Darriba et al. 2012), run through the CIPRES Science Gateway (Miller et al. 2010). We then reconstructed phylogenetic relationships using three different search strategies: Neighbor-Joining, Maximum Likelihood, and Bayesian Inference. Neighbor-Joining analyses were conducted in GENEIOUS and support for relationships was estimated using 1000 bootstrap replicates. Maximum likelihood analyses were conducted in PHYML (Guindon et al. 2010), and again node support was estimated using 1000 bootstrap replicates. Bayesian analyses were conducted in MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) using 6 chains, 4 heating parameters, a burn length of 10% and a MCMC chain of 1,000,000 generations. For both the Maximum Likelihood and Bayesian analyses, we used different substitution models for 28S and COI (transversion substitution model [TVM]) compared to LWRh (two-phase substitution model [TPM2]). For an outgroup, we included sequences from *Parnips nigripes* Barbotin (Hymenoptera: Cynipoidea: Figitidae) as this species has been used as an outgroup in previous analyses.
(Ronquist and Nieves-Aldrey 2001). Neighbor-Joining reconstructions were then visualized in FigTree Version 1.4.2 with respective Bayes posterior probabilities and Maximum-Likelihood bootstrap values for each locus and for the results from the concatenated dataset (Rambaut and Drummond 2009).

2.3 Results and Discussion

2.3.1 Molecular Placement of Zapatella davisae

Our analyses were congruent with recent taxonomic revisions based on morphological data (Melika and Abrahamson 2002; Pujade-Villar et al. 2012). As expected Z. davisae fell within the oak gall wasp tribe Cynipini (Figs. 1, 2, 3, 4). Within the Cynipini, Z. davisae was most closely related to Callirhytis species, but did not match any Callirhytis species in the NCBI database (Figs. 2, 4). In the analysis of the concatenated data set (Fig. 4) and the analysis of the LWRh gene fragment (Fig. 2), Z. davisae formed a moderately supported clade with Callirhytis quercussuttoni Dalla Torre and Kieffer and Callirhytis uvellae Weld (0.79 Bayesian Posterior Support from the concatenated analysis and 0.80 Bayesian Posterior Support with 86% Bootstrap Support from the LWRh analysis). Both of these species create gouty stem galls on oaks in California and have not been assigned to Zapatella in recent reclassifications (Dailey 1969; Kinsey and Ayres 1922; Pujade-Villar et al. 2012). Although the biology of Z. davisae and its close matches in Callirhytis appear to be similar, the polyphyly of Callirhytis and the lack of available data from other Zapatella species make it unclear whether the two Callirhytis species or Z. davisae may be misclassified. The 28S analysis placed Z. davisae next to two Heterocous species (with low posterior probability support).
within a clade of *Callirhytis*. All three loci containing sequences for *Callirhytis* species suggest that this genus is polyphyletic (Fig. 4). Recently, it has been claimed that only 15 out of the 115 currently described species of *Callirhytis* have been correctly classified (Melika and Abrahamson 2002), and our molecular results tend to support this conclusion. Each locus contributed to the cumulative understanding of where *Z. davisae* fits in the Cynipini phylogeny and supports the view that *Callirhytis* is polyphyletic and needs to be split up into various genera.

### 2.3.2 Regional Genetic Variation among *Zapatella davisae* Populations

Sequences were obtained from 89 individuals of *Z. davisae*, from 11 populations, and all had identical sequences, likely due to a founder effect (Figs. 1-3). Analyses of the CO1 gene fragment showed that *Z. davisae* is closely related to several unidentified cynipid specimens collected from several locations in North America (Fig. 1). In these analyses, *Z. davisae* was placed in a distinct monophyletic clade (1.0 Bayesian Posterior Support with 100% Bootstrap support for Neighbor-joining and Maximum-likelihood analyses) with specimens from Canada and Florida. Sequences of CO1 from *Z. davisae* were 99.7% similar to those of specimens from Canada and 98.0-98.8% similar to those from Florida. The amount of variation between *Z. davisae* and the Canadian samples is less than the commonly used threshold for separate species (3% genetic diversity) when using the CO1 gene (Hebert et al. 2003; Rubinoff et al. 2006). However, this threshold is simply a suggestion, and should not be used as the sole source of evidence when delimiting species (Hebert et al. 2003; Rubinoff et al. 2006). Given that *Z. davisae* sequences from the northeastern United States are more like those from Florida than
those from eastern Canada, our results imply that *Z. davisae* may be southern in origin. It may have established on Long Island, New York in the 1990s, then spread to New England in the 2000s, or possibly earlier if *Z. davisae* proves to be synonymous with *C. ceropteroides*, which was known from Connecticut in the early 1900’s. No molecular data is available for *Callirhytis ceropteroides* from 1900; therefore, we cannot make any conclusions about its relatedness to *Z. davisae*. The widespread and continuous distribution of black oak across the eastern United States would allow for the natural spread of a native insect that utilizes black oak as a primary host. Further supporting this southern origin, recent morphological work has shown that species within the genus *Zapatella* are native to South America, Central America, and North America (Pujade-Villar et al. 2012). While the definitive location of potential founders for these outbreaking populations is still unknown, we found a complete lack of genetic diversity for all three loci (28S, LWRh, CO1) across 11 populations in Long Island and New England, suggesting that the populations in New England and Long Island are the result of one invasion of a gall wasp species, which may be native to some other part of North America, possibly the southeast.

2.3.3 Inferences into the Oak Gall Wasp Phylogeny

Three tribes of the Cynipidae were incorporated in our phylogenetic analyses, including the Cynipini (oak gall wasps), Aycalini (rose gall wasps), and Synergini (inquilines) (Stone et al. 2002). Across all analyses, the Synergini were monophyletic; however, relationships varied among analyses for the Aycalini and the Cynipini (Table 2). The Cynipini tribe was monophyletic for COI and LWRh, but it was polyphyletic for
28S and the concatenated analysis (Table 2). Aycalini was paraphyletic for CO1, and was polyphyletic for both 28S and the concatenated data set, as seen with previous studies (Table 2) (Ronquist et al. 2015)

Within the Cynipini, our phylogenetic analyses identified several genera besides Callirhytis that may need taxonomic revision, specifically Dryocosmus, Plagiotrochus, Aphelonyx, Andricus and Neuroterus. Neuroterus species were distributed throughout various genera and tribes in the Cynipidae for all three loci. Since sequences used in this study were downloaded from public databases (e.g., NCBI and BOLD), it is possible that some of these sequences may have been misidentified. A thorough investigation of voucher specimens for these sequences should be conducted. Three genera, Dryocosmus, Aphelonyx, and Plagiotrochus, are closely related within the Cynipini. In all analyses, they fell into distinct but related clades that were separate from the rest of the thirteen other Cynipini genera. The relationship between these Cynipini clades varied across loci, reflecting disagreement between loci on the composition and phylogeny of Cynipini. For COI (Fig. 1) and LWRh (Fig. 2), the clades were within the Cynipini making it monophyletic, whereas in 28S (Fig. 3) and the concatenated tree (Fig. 4), they were outside the Cynipini, making it polyphyletic. Andricus species also varied across loci. At CO1 (Fig. 1), Andricus appeared to be monophyletic; however, at LWRh (Fig. 2) and 28S (Fig. 3), Andricus was polyphyletic. These discrepancies may be a function of data availability, as the genera that caused the polyphyletic relationship were not available for COI.
2.3.4 Conclusions

All three loci were useful in identifying where *Z. davisae* fits within the Cynipini and determining genetic diversity across populations. Each locus confirmed that *Z. davisae* populations in Long Island and New England are genetically invariant (Figs. 1-3) suggestive of a founder effect. It is clear that *Z. davisae* is phylogenetically nested within the genus *Callirhytis*; however, *Callirhytis* is polyphyletic and needs taxonomic revision. These results support recent changes to the composition of *Callirhytis*, as per recent work on the genera *Bassettia, Callirhytis, and Zapatella* (Melika and Abrahamson 2000; Melika and Abrahamson 2002; Melika and Abrahamson 2007; Pujade-Villar et al. 2012). CO1 and LWRh analyses were in agreement with each other, and yielded clear results, in which genera fell into distinct clades. The 28S analysis and the concatenated tree yielded more paraphyletic and polyphyletic groups, specifically within the Cynipini and the Aycalini. For the most part, our results demonstrated that many genera in the Cynipini are well supported; however, the placement and identification of a few genera including, *Callirhytis, Plagiotrochus, Dryocosmus, Andricus, Aphelonyx, and Neuroterus* should be reevaluated.

Data accessibility limited our capacity to reconstruct some phylogenies. Future work should be done to streamline the GenBank database, including the removal of incorrectly identified submissions and addition of specimens that increase the diversity of data available (Collins and Cruickshank 2012).
Table 2.1 Species used for all phylogenetic analyses, including GenBank accession numbers for each locus available

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Disholcaspis quercusmamma  Cynipidae  Cynipini  KF554483  KX683823  -
Dryocosmus cerriphilus  Cynipidae  Cynipini  -  DQ217982  DQ286815
Dryocosmus israeli  Cynipidae  Cynipini  -  KX683509  DQ286811
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Isocolus lichtensteini  Cynipidae  Aylacini  DQ012600  -  DQ012644
Isocolus rogenhoferi  Cynipidae  Aylacini  AY368947  -  AY368921
Liposthenes glechomae  Cynipidae  Aulacideini  -  AY371053  AY368915
Liposthenes kernerii  Cynipidae  Aulacideini  -  AY371054  AY368916
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Neaylax versicolor  Cynipidae  Aylacini  DQ012603  -  DQ012647
Neuroterus anthracina  Cynipidae  Cynipini  DQ201493  DQ217947  -
Neuroterus aprilinus  Cynipidae  Cynipini  DQ201488  DQ217949  -
Neuroterus numismalis  Cynipidae  Cynipini  AY368956  -  AY368930
Neuroterus saliens  Cynipidae  Cynipini  DQ201483  DQ217969  -
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Parnips nigipes  Figitidae  -  DQ012605  AY371066  AY368932
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### Table 2.2 Monophyly, paraphyly and polyphyly of Cynipini genera and Cynipidae tribes based on phylogenetic analyses for CO1, LWRh, 28S and a concatenated dataset

<table>
<thead>
<tr>
<th>Tribe</th>
<th>COI</th>
<th>LWRh</th>
<th>28S</th>
<th>Concatenation</th>
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<td>Poly</td>
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<td>Aylacini</td>
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**Cynipini Genera**

<table>
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<th>LWRh</th>
<th>28S</th>
<th>Concatenation</th>
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<td>Poly</td>
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<td>Para</td>
<td>Para</td>
<td>Para</td>
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<td>Para</td>
<td>Mono</td>
<td>Mono</td>
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<td><em>Neuroteras</em></td>
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**Mono = monophyletic; Poly = polyphyletic; Para = paraphyletic**
Figure 2.1 Gene tree reconstruction using Cytochrome Oxidase I (CO1) gene for *Zapatella davisae* and all Cynipidae listed in Table 1. Values above each branch indicate Bayesian Posterior Probability (probability out of 1), and values below each branch indicate Neighbor Joining and Maximum Likelihood Bootstrap Support (support out of 100), respectively. A scale bar (substitutions per site) is shown in the bottom center of the figure. (*Z. davisae* n= 89)
Figure 2.2 Gene tree reconstruction using the Long-Wavelength Rhodopsin (LWRh) gene for *Zapatella davisae* and all Cynipidae listed in Table 1. Support values are reported as in Figure 1. (*Z. davisae* n = 10)
Figure 2.3 Supplementary Tree: Gene tree reconstruction using the 28S gene for Zapatella davisae and all Cynipidae listed in Table 1. Support values are reported as in Figure 1. (Z. davisae n = 10)
Figure 2.4 Gene tree reconstruction using a concatenated data set of 28S, LWRh, and COI. Values above each branch indicate Bayesian Posterior Probability (probability out of 1), and Neighbor Joining Bootstrap Support (support out of 100). Neighbor Joining support is in parentheses. A scale bar (substitutions per site) is shown in the bottom center of the figure. (Z. davisae n = 10)
CHAPTER 3
POPULATION ECOLOGY OF ZAPATELLA DAVISAE
IN CAPE COD, MASSACHUSETTS AND LONG ISLAND, NEW YORK

3.1 Introduction

Most insect herbivores have little impact on their host plant, because their natural enemies keep their densities at levels well below their carrying capacity (Keane and Crawley et al. 2002, Strong et al. 1984). Under certain conditions, however, they can outbreak and cause extensive foliar damage (Stone et al. 2002; Strong et al. 1984). When invasive insects are released from their natural enemies, their population density may increase, leading to host plant damage (Prior and Hellmann 2013). Such natural enemy release explains why plant communities can be greatly affected by the invasion of exotic insects without their native parasitoids or other natural enemies (Didham et al. 2005, Sanders et al. 2003). While many of the organisms that negatively affect community structure are non-native, native species whose ranges are expanding as a result of climate change or other anthropogenic disturbances also have the capacity to alter native plant communities and population dynamics (Prior and Hellmann 2013; Schonrogge et al. 1995).

Gall-making insects that affect trees or other plant hosts can alter native ecosystem communities (Stone et al. 2002). Galls form on various tissues, including stems, leaves and fruits (Raman 2011). This exterior structure provides food and protection for the developing gall maker (Ronquist et al. 2015). Galls support a diverse community of inquilines and parasitoids, which in turn can influence the gall maker’s survival (Ito and Hijii 2004). The Cynipidae family of gall wasps, create galls on trees and plants that are host to a diverse community of insects. Members of the community
include, inquilines or phytophagous insects incapable of producing their own galls, and parasitoids that attack and kill the gall wasp host (Ronquist 1994).

For the most part, native gall formers often go unnoticed and cause little foliar damage (Stone et al. 2002) due to population regulation, which is achieved by the presence of natural enemies, particularly parasitoids (Schonrogge et al. 1996a,b). For example, Otake et al. (1984) found that Torymus sinensis Kamijo caused density-dependent mortality of its host, Dryocosmus kuriphilus Yasumatsu in Japan. Similarly, Xanthoteras politum (Bassett), a gall wasp that attacks sucker shoots on fire-damaged trees in the Pine Barrens of New Jersey, experienced high levels of parasitism (Washburn and Cornell 1979). While parasitoids often regulate gall wasp populations and prevent outbreaks, range expansion as a result of climate change may separate gall makers from their natural enemies (Schonrogge et al. 1995) and lead to gall wasp outbreaks (Prior and Hellmann 2013).

Studies have shown that novel communities containing an introduced pest can form and that they may eventually exhibit the population dynamics present in the pests’ native range (Prior and Hellmann 2013). Schonrogge et al. (1995) studied how parasitism in the gall community of Andricus quercuscalicis Burgsdorf changed along a spatial gradient (from the native range to the introduced range) after it was introduced to Europe in the 1950s. As the distance from the native range increased, percent parasitism decreased. Over time, distant populations along this spatial gradient began to retain the original community structure of A. quercuscalicis (Schonrogge et al. 1995; Stone et al. 2002).

Given the capacity of native gall wasps to expand their distribution and escape
from their native parasitoids, we investigated the population dynamics of a recently described gall wasp, *Zapatella davisae* Buffington (Hymenoptera: Cynipidae) (Buffington et al. 2016). *Zapatella davisae* has been identified as the source of extensive mortality and damage (flagging, leaf clumping, and limb loss) to black oaks (*Quercus velutina* Lamark [Fagales: Fagaceae]), in Long Island, New York, and on Cape Cod, Martha’s Vineyard and Nantucket, Massachusetts (Buffington et al. 2016). *Zapatella davisae* was first documented on Long Island in 1990; however, the population crashed within five years (1995) for reasons that are still unknown (Pike et al. 2001). *Zapatella davisae* was not documented north of Long Island on Cape Cod or Martha’s Vineyard until 2008 (Buffington et al. 2016).

Genetic analyses indicate that populations of *Z. davisae* in New England are likely the result of a founder event and their outbreaks may be attributed to a range shift of a native species (Davis et al. unpublished). Sequences from over 100 individuals of *Z. davisae* from each region were genetically invariant for the mitochondrial gene cytochrome oxidase c subunit I (COI), the nuclear ribosomal gene 28S (28S), and the nuclear protein-coding gene long-wavelength rhodopsin (LWRh) (Davis et al. unpublished). Furthermore, blast results from the NCBI database produced multiple close matches with other cynipids in North America, including individuals collected in Florida, evidence that *Z. davisae* may be native to the southeastern U.S. (Davis et al. unpublished). Field surveys showed that *Z. davisae* infests the same proportion of trees in Long Island and Cape Cod; however, it causes significantly more canopy damage on Cape Cod than Long Island (Davis unpub.). Differences in levels of infestation between the two regions suggest that some factor may be regulating the population on Long
Island.

We have confirmed that gall wasp populations on Long Island and Cape Cod are the same species; however, it remains unclear whether parasitoids were responsible for the collapse of the outbreak population in the 1990s on Long Island and if the same thing is likely to occur on Cape Cod. Therefore, our objectives were to (1) compare percent parasitism of *Z. davisae* in Cape Cod and Long Island; (2) identify the parasitoid community associated with *Z. davisae*, and (3) determine which parasitoid species contribute most to parasitism in each region.

### 3.2 Methods

#### 3.2.1 Rearing of Gall Wasps and Their Parasitoids

Branches were collected at four sites on Long Island and at four sites on Cape Cod in mid-April 2015 and 2016, approximately two weeks before gall wasp emergence (Table 1). At each site, three branches were haphazardly collected from the upper portion of the canopy of each of 10 black oaks, for a total of 120 samples per region. Each branch included the present year’s growth and one previous year’s growth. Upon collection, each branch was placed in an individual Berlese funnel trap (BioQuip, Rancho Dominguez, CA) that was lined with black paper on the bottom half, so insects would be trapped in the top half after emergence. Floral Foam Micro Bricks (Oasis® Floral Products, Kent OH) were placed in the bottom of the 11.4 x 20 cm traps to keep branches alive. Trap containers were held at room temperature until gall wasp or parasitoid emergence was complete in early June (Buffington et al. 2016). Gall wasps and parasitoids were counted for each branch, and emerged specimens were preserved individually in 95% ethanol for
molecular analysis. Twigs were dissected after rearing to detect insects that failed to emerge.

### 3.2.2 DNA Extraction and Amplification

Before DNA extraction, photographs were taken of each parasitoid under a dissecting microscope (Nikon SMZ1000, SPOT graphics), and specimens were sorted into distinct morphological groups (hereafter, “morpho-groups”). DNA was extracted for a total of 250 parasitoids, with 3 to 20 individuals per morpho-group, using the DNeasy tissue extraction kit (QIAGEN, Valencia, CA) for two loci. The mitochondrial Cytochrome Oxidase I (CO1) gene was amplified using forward primer Jerry (5'-CAACATTTATTGGATTATTGG-3') and reverse primer Pat (5'-TCCAATGCACTAATCTGCCATATTA-3') (Ghararieh et al. 2006; Simon et al. 1994). DNA was denatured for 3 min at 94°C, followed by 40 amplification cycles comprising 30 s denaturing at 92°C, 90 s annealing at 52°C, and 120 s extension at 72°C, an extension period for 10 min at 72°C after amplification (Lotfalizadeh et al. 2008). The nuclear 28S gene was amplified using forward primer s3660 (5'-GAGAGTTMAASAGTACGTGAAAC-3') and reverse primer 28b (5'-TCGGAAGGAACCAGCTACTA-3') (Morse and Normark 2006; Whiting et al. 1997). A touchdown protocol was used for amplification of the 28S fragment, as described in Morse and Normark (2006). PCR products for each locus were visualized on 1.5% agarose gels, and before sequencing products were purified using Exonuclease 1 (Thermo Scientific) and Shrimp Alkaline Phosphatase (New England BioLabs) according to the Thermo Scientific PCR and purification protocol. Products were sequenced at the Yale
Genomic Lab using an ABI 3730 sequencer (Life Technologies). Forward and reverse sequence reads were then aligned and edited using GENEIOUS 8.1.8 (Kearse et al. 2012), and a consensus sequence was generated for each sample.

3.2.3 Species Identification and Sequence Comparisons

After editing the consensus sequences for each gene fragment, sequences were aligned, manually adjusted, and truncated to the length of the shortest sequence using GENEIOUS. To differentiate putative species, we used a threshold of 2% variation between individuals for COI and 1% variation for 28S, although many studies suggest that species level differences begin with a divergence greater than 3% (Herbert et al. 2003; Rubinoff et al. 2006). To identify species, we compared our sequences to those published in the NCBI database using the blastn search algorithm. To examine the ancestral relationships of our defined species (defined by DNA, not morphology), we reconstructed a gene tree using the CO1 sequence alignment. The tamura-nei (TrN) genetic distance model was identified as the best substitution model to use for this analysis based on JMODELTEST run through the CIPRES Science Gateway (Miller et al. 2010). Phylogenetic analyses were run in GENEIOUS with a 1000 bootstrap replicates. Bayesian analyses were run using MRBAYES 3.2.6 (Huelsenbeck and Ronquist, 2001) with a transition substitution model (TiM), a burn length of 10%, and a MCMC chain of 1,000,000. A maximum likelihood analysis was completed with a transition substitution model using PHYML (Guindon et al., 2010) and 1000 bootstrap replicates for node support estimation. For an outgroup for all three analyses, we used a Hymenopteran species, Sirex noctilio. A consensus tree, including bootstrap values, posterior probabilities and likelihoods was reconstructed in
FIGTREE Version 1.4.2 (Rambaut and Drummond 2009). We obtained and sequenced DNA from several intact specimens of *Sycophila* (Hymenoptera: Cynipidae) species 3 and 4, which have been submitted to the Smithsonian Institution for identification based on morphological characters. Voucher specimens will be stored here upon identification. We have posted all sequences on Genbank (Add GenBank No. When Approved).

### 3.2.4 Statistical Analyses

Rstudio Version 0.99.491 (R Core Team) was used to complete all statistical analyses based on gall wasp and parasitoid emergence counts. Parasitism rates were calculated as the number of emerged parasitoids divided by the total number of emerged parasitoids and gall wasps. Average percent parasitism was compared between the two regions with a logistic regression. The average number of individuals (gall wasps and parasitoids) that emerged per branch was compared across regions and years using a Poisson model. To determine differences in the amount that each parasitoid species-group contributed to parasitism, we conducted $\chi^2$ tests to separately compare the counts of each parasitoid species-group in each region for the 2015 and 2016 sample.

### 3.3 Results

#### 3.3.1 Population Size and Parasitism

Percent parasitism was significantly higher on Long Island (99.69%) than on Cape Cod (56.53%) ($z = 5.451; \text{df} = 1; P < 0.001$) (Table 2). Parasitism remained steady overtime on Cape Cod, dropping only 3% in 2016. Long Island experienced a decline in parasitism; however, this was due to the fact that the total number of individuals that
emerged from branches collected on Long Island in 2016 was 5 individuals, 3 of which were parasitoids in contrast to the 324 individuals collected in 2015 from the same trees (Table 2).

The total number of parasitoids and gall wasps was used as an estimate of gall wasp establishment, because each parasitoid killed a previously developing gall wasp. Gall wasp establishment density (total number of parasitoids and gall wasps per branch) was significantly larger on Cape Cod than on Long Island for 2015 and 2016 ($z = 7.942$; df = 1; $P < 0.001$) (Table 2). There was a significant difference between years for the Cape Cod and Long Island populations, with each region experiencing a decline in 2016 ($z = -6.873$; df = 1; $P < 0.001$). The Long Island population density declined sharply in 2016, and was significantly lower than the density of the population on Cape Cod, causing a region x year interaction ($z = -7.943$; df = 1; $P < 0.001$) (Table 2).

Table 3.1 GPS coordinates for all sites in Cape Cod (Massachusetts) and Long Island (New York), USA, used to collect emergence rates for Zapatella davisae and its parasitoids.

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<th>Longitude</th>
</tr>
</thead>
<tbody>
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<td>Mellville</td>
<td>40° 46' 31.18'' N</td>
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<td></td>
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<td>70° 1' 51.49'' W</td>
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Table 3.2 Percent Parasitism of *Zapatella davisae* on Cape Cod and Long Island for 2015 and 2016, including gall wasp and parasitoid total counts for each. Also shown is the average number of individuals per branch (parasitoids and gall wasps), which is indicative of initial gall wasp establishment before parasitism.

<table>
<thead>
<tr>
<th>Region</th>
<th>Percent Parasitism</th>
<th>Total No. of Gall Wasps</th>
<th>Total No. of Parasitoids</th>
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<td>183</td>
<td>238</td>
<td>3.56 ± 0.36</td>
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<td>2015 Long Island</td>
<td>99.69</td>
<td>1</td>
<td>323</td>
<td>2.71 ± 0.37</td>
</tr>
<tr>
<td>2016 Cape Cod</td>
<td>53.91</td>
<td>118</td>
<td>138</td>
<td>2.16 ± 0.31</td>
</tr>
<tr>
<td>2016 Long Island</td>
<td>60.01</td>
<td>2</td>
<td>3</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup>—The term individuals includes the number of emerged gall wasps and parasitoids per branch

3.3.2 Species Identification and Relative Contribution to Total Parasitism

Phylogenetic analysis of DNA sequences identified seven parasitoid species from *Z. davisae* on Cape Cod or Long Island in 2015 (Fig. 1). The top blast result for each species-group based on % pairwise sequence identity can be found in Table 3. The 28S gene fragment (length = 780 base pairs) results had the four *Sycophila* (Hymenoptera: Cynipidae) species within the range of 98.9 - 100 percent similar based on nucleotide substitutions, whereas COI (length = 745 base pairs) identified species level differences with 87.8 – 91.4 percent variation between species. For this reason, we chose to use the COI gene in our phylogenetic analysis to best demonstrate species-level differences.

There was a significant difference between the two regions in the proportion parasitized by each species in 2015 ($\chi^2 = 92.782; df = 8; P<0.001$) (Table 3). All parasitoids present on Cape Cod were also present on Long Island, and Long Island contained an additional parasitoid tentatively identified as a *Eurytoma* spp. (Hymenoptera: Eurytomidae). Four distinct *Sycophila* (Hymenoptera: Eurytomidae)
clades were recognized, with high support for intraspecific variation within each species (Fig. 1). *Sycophila* sp. 3 contributed the most to parasitism on Long Island (in all cases of parasitism, 65% were due to *Sycophila* species 3) in 2015 (Table 3). *Sycophila* sp. 3 was not as abundant in Cape Cod, where *Sycophila* sp. 4 contributed the most to total parasitism.

Percent parasitism did not change drastically on Cape Cod from year to year; however, species richness did change overtime with only four of the seven species present again on Cape Cod in 2016. In addition, six individual parasitoids were recovered as singletons on Cape Cod. Each of these individuals was included in the other category in Table 3 and was not present in the 2015 sample. *Sycophila* sp. 3 was still not the most abundant parasitoid on Cape Cod in 2016, as its contribution to parasitism only increased slightly from 34.1% to 40%. Only one parasitoid species was recovered from the Long Island samples in 2016 – *Sycophila* sp. 3.

Table 3.3 *Relative dominance of each parasitoid species as percentage of all parasitoid individuals reared, in each region for 2015 and 2016.* Numbers in parentheses are total parasitoid individuals collected in each region and year.

<table>
<thead>
<tr>
<th>Species</th>
<th>Blast Result ID</th>
<th>2015 Cape Cod (n=279)</th>
<th>2015 Long Island (n=237)</th>
<th>2016 Cape Cod (n=150)</th>
<th>2016 Long Island (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aphelinidae sp. 1</td>
<td>1.0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Aphelinidae sp. 2</td>
<td>1.0</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Eurytoma</em> sp.</td>
<td>0.0</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Sycophila</em> sp. 1</td>
<td>2.9</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Sycophila</em> sp. 2</td>
<td>3.6</td>
<td>9.7</td>
<td>13.3</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td><em>Sycophila</em> sp. 3</td>
<td>34.1</td>
<td>65.0</td>
<td>40.0</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td><em>Sycophila</em> sp. 4</td>
<td>57.3</td>
<td>19.8</td>
<td>42.6</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Other</td>
<td>-</td>
<td>-</td>
<td>4.0</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 3.1 Gene tree reconstruction using Cytochrome Oxidase I (CO1) for all parasitoid species found in the *Zapatella davisae* gall community on Long Island and Cape Cod in 2015 and 2016. Values above each branch indicate Bayesian Posterior Probability (probability out of 1), and values below each branch indicate Neighbor Joining and Maximum Likelihood Bootstrap Support (support out of 100), respectively. A scale bar (substitutions per site) is shown in the bottom center of the figure.

3.4 Discussion

If the climate continues to warm, the potential for insect range expansion or population outbreaks will increase (Stone et al. 2002). The recent geographic expansion of the southern pine beetle, *Dendroctonus frontalis* Zimmermann into New Jersey and New England is one such example (Coulson et al. 1999; Niemiec et al. 2014). The northward expansion of southern pine beetle is similar to what we believe may have
occurred for Z. davisae, which seems to have shifted from Florida and Tennessee to Long Island, and then to Cape Cod and Rhode Island (Davis et al. unpublished).

Gall wasps can be host to a suite of parasitoids that may have the capacity to regulate the population (Stone et al. 2002). Seven species were found to have emerged from Z. davisae galls. The most common parasitoid, Sycophila (Hymenoptera: Eurytomidae) species 3, and the next three most common species were all members of the Eurytomidae (Hymenoptera: Chalcidoidae) (Gómeza et al. 2013). Most Sycophila species are endoparasitic koinobionts that attack gall wasp larvae or pupae (Gómeza et al. 2013). Another identified parasitoid, Eurytoma sp., is also a member of the Eurytomidae. A next step would be to formally identify these species and describe their biology so as to best understand how and when they parasitize Z. davisae.

The successful establishment of insect pests in these newly introduced areas is a function of enemy release, when a population no longer experiences mortality from its natural enemies after it is introduced into an exotic ecosystem (Keane and Crawley et al. 2002; Prior and Hellmann 2013). On Long Island we found multiple gall wasp populations that exhibited almost 100% parasitism in 2015, which was followed by a near total collapse of the population in 2016. On Cape Cod, parasitism rates were lower (about 50%) and consistent overtime, which may explain greater canopy damage in that region (Davis, unpub.). Parasitoid species richness was similar between the two regions in 2015, but the abundance of each species was different. On Long Island, species-group Sycophila species 3 caused the highest level of parasitism, but parasitism from this species was lower on Cape Cod. Its contribution to total parasitism, however, increased slightly on Cape Cod in 2016, suggesting that the population dynamics there may be
converging with those on Long Island. It is very common for introduced insect parasitoid communities to converge with native communities with respect to species richness and abundance (Schonrogge et al. 1995). Since the *Z. davisae* parasitoid communities had similar richness in each region, only increases in abundance are needed for convergence.

We suggest that *Z. davisae* populations on Long Island cause little foliar damage, because they are currently controlled by parasitoids. It is unclear whether populations of *Z. davisae* will return to outbreaking densities in this region. Future estimates of parasitoid richness and abundance should be taken to determine if the outbreak population on Cape Cod will fall to insignificant levels, as it did on Long Island in 2016.
CHAPTER 4
EFFICACY OF SYSTEMIC INSECTICIDES FOR CONTROL OF ZAPATELLA DAVISAE ON BLACK OAK

4.1 Introduction

Oak gall wasps are phytophagous insects that often go unnoticed, but some species have the capacity to reach high densities and cause widespread tree mortality (Stone et al. 2002). Zapatella davisae Buffington and Melika (Hymenoptera: Cynipidae) is a gall wasp associated with black oak, Quercus velutina Lamarck, that has caused extensive canopy dieback and tree mortality on Cape Cod, Nantucket, and Martha’s Vineyard, in Massachusetts. Damaging populations of this species occurred on Long Island, New York in the 1990s (Pike et al. 2001; Buffington et al. 2016). Zapatella davisae induces woody stem galls that cause node and twig distortion (Buffington et al. 2016). Other damage symptoms include flagging, leaf clumping, and canopy dieback (Pike et al. 2001). Z. davisae completes one generation per year, with adult wasps emerging in early May (Buffington et al. 2016). Oak dieback and mortality on Cape Cod and nearby islands is of conservation concern because black oak is the dominant forest tree in the region (Eberhardt et al. 2003). Identifying a successful chemical control agent would contribute to management of Z. davisae on black oak in coastal communities.

Injection of systemic pesticides via the tree bole is increasingly used by arborists to manage pests in urban or suburban areas (Xu et al. 2008, Tattar et al., 1998). When a pesticide is injected, smaller amounts of the active ingredient are needed compared to soil drenches or foliar spray applications, limiting drift and non-target impacts (Tattar et al. 2009). This closed injection system prevents leaching and allows applicators to treat trees adjacent to water (Doccola and Wild 2012). Systemic pesticide application methods can
also reach pests in plant xylem or phloem tissue under the bark, where foliar and soil treatments are not as effective (Doccola and Wild 2012). Systemic insecticides are a logical approach to gall wasp control; however, trials with multiple active ingredients are necessary to determine which formulations will be the most effective.

Imidacloprid and emamectin benzoate have successfully controlled several cynipids (Fischer et al. 2009; Doccola and Wild 2012). In Hawaii, systemic injections of imidacloriprid in coral trees, *Erythrina variegata* L., lowered Erythrina gall wasp, (*Quadrastichus erythrinae* Kim) densities more than emamectin benzoate (Xu et al. 2008). Foliar applications of imidacloprid on *Eucalyptus obliqua* L’Her, made immediately before the emergence of the Eucalyptus gall wasp, *Leptocybe invasa* Fisher and La Sall, lowered adult emergence rates (Kavitha et al. 2009). In Hawaii, emamectin benzoate injection controlled *Josephiella* sp. galls on Chinese banyan (*Ficus microcarpa* L.) for up to 14 months (Bhandari and Zhiqiang 2016). Previous studies have shown that both imidacloprid and emamectin benzoate have the capacity to kill stem gall wasps; however, further research is needed to better understand their effect on oak-specific gall wasps, such as *Z. daviseae*.

We evaluated the efficacy of trunk injections of emamectin benzoate (TREE-äge®) and imidacloriprid (IMA-jet®) against *Z. daviseae* on black oak in Cape Cod, MA, applied either in spring or fall. Since *Z. daviseae* is a new pest, there are no established management plans available. Our findings will help arborists and landowners make future management decisions regarding *Z. daviseae*, and will improve gall wasp chemical control strategies.
4.2 Methods

4.2.1 Tree and Site Selection

4.2.1.1 Fall Injection Trial

Thirty-nine black oak trees distributed over one site in West Harwich (41.7285 N, 70.1869 W) and two sites in Dennis, Massachusetts (41.7370 N, 70.1933 W) were used for a fall injection trial in October 2013. In all three locations, trees were of similar diameter at breast height (DBH), grew in sandy soils and were exposed to substantial sunlight (Table 1). Insecticides were applied to 13 trees per treatment on 4 October 2013; however, after leaf-out, it became apparent that three trees treated with emamectin benzoate were scarlet oak, Quercus coccinea Muenchh., which is not a host of Z. davisae (Davis, unpublished). As a result, three trees were randomly removed from each of the other two treatments for a total of 10 trees per treatment.

4.2.1.2 Spring Injection Trial

A second, spring injection study was conducted on 24 March 2014 with 30 trees at one site in Barnstable, MA (41.9558 N, 70.3098 W) to evaluate whether seasonality of injection affected Z. davisae control. Trees were randomly assigned to one of three treatment options for a total of 10 trees per treatment.

4.2.2 Injection Protocol

The three treatments for both the fall and spring trials were IMA-jet (5% imidacloprid) at 8 ml per 2.54 cm DBH, TREE-äge (4% emamectin benzoate) at 10 ml per 2.54 cm DBH, and the untreated control (Table 1). Rates were those recommended by
Arborjet Inc. and were comparable to those used in previous studies (Doccola et al. 2009, Bhandari and Zhiqiang 2016). Tree sizes and pre-treatment *Z. davisae* infestation levels were distributed evenly across the three treatments in each trial (Table 1). The number of injection sites for a given tree was calculated as the DBH (in centimeters) divided by five. Injection holes, 0.95 cm diameter and 5.1 cm deep, were drilled perpendicular to the surface of the bark and spaced 15 – 20 cm apart on the trunk’s circumference at a height of 20-40 cm above the ground. Into each injection hole, #4 Arborplugs® (Arborjet Inc., Woburn, MA) were inserted at the bark/sapwood interface, through which a syringe deposited a specific amount of each insecticide. Insecticides were administered with either the QUIKjet or Tree I.V. system depending on the tree’s capacity to uptake product (Arborjet Inc., Woburn, MA). Both systems applied the insecticide systematically at the base of the tree.

### 4.2.3 Measurements of Efficacy

#### 4.2.3.1 Estimation of Gall Wasp Establishment

Branches were collected before and after treatment to evaluate changes in new gall numbers for both the fall and spring injection trials. Evidence of new gall formation was determined by counting gall cavity chambers, which develop as a result of larval feeding (Raman 2011). Cavities were counted in March 2014 and March 2015 at all sites in the spring injection trial. Data from the March 2014 branches were used in the analysis as the pre-treatment sample, being the generation that established in July 2013 before treatment. The pre-treatment generation emerged in May 2014 and new galls of the following generation were not evident until July 2014. The number of cavities in the
2014 generation was determined in the post-treatment sample collected in March 2015. At each sample date, three branches (45 cm long) were cut with a pole pruner from the lower crown (<10m) of each tree and placed in a 4-liter Ziploc bag. Branches were dissected in the laboratory and the number of gall wasp cavities (indicators of gall wasp establishment) and the lengths of both the current and previous year’s growth were recorded. The numbers of cavities were then compared among the three treatments for both the fall and spring trials.

**4.2.3.2 Adult Gall Wasp Emergence**

Numbers of new, emerging gall wasps were counted for the fall injection trial only, as it was part of an additional larger study with trees at the fall injection sites. To estimate the number gall wasps that emerged from branches of injected trees, two branches were taken from each of the 10 trees for all three treatments in April 2015. Samples were placed individually in Berlese funnel traps (BioQuip, Rancho Dominguez, CA) covered with black paper on the bottom half that forced insects to move to the top half of the trap where they remained until emergence was complete in early June. Each sample branch, including both current and previous years growth was inserted in a wet Floral Foam Micro Brick (Oasis® Floral Products, Kent OH) in the bottom of the 11.4 x 20 cm cylindrical container. The Floral Foam was soaked in water periodically throughout the rearing process to keep the branches alive. Numbers of emerged adult gall wasps were recorded per branch after emergence had ended in June 2015.
4.2.3.3 Branch Mortality

To assess branch mortality across the three treatments, branches were collected in March 2015 at sites from both the fall and spring injection trials. Collection in March prevented any selection bias, as leaf-out did not begin until mid-April at any sites. For both the fall and spring trial, three branches were removed from the top of the crown of 10 trees in each treatment. Branches were classified as dead-infested, alive-infested or not infested. Infested branches were considered to be any branch with cavity presence in the current or previous year’s growth. Mortality was determined based on the status of the branch, not the presence of gall wasps.

4.2.3.4 Tree Canopy Condition

Tree condition was determined for all trees in both the fall and spring injection trials, both immediately before injection and approximately two years after injection in August 2015 to evaluate changes in canopy condition over time. The canopy of each tree was rated on a scale from 1-5, 1 being excellent with minimal gall presence and canopy damage and 5 being poor, with over 80% canopy dieback and gall presence (Doccola et al. 2009, Bhandari and Zhiqiang 2016).

4.2.4 Statistical Methods

Data were analyzed using RStudio Version 0.99.491 (R CoreTeam). Differences between the fall and spring injection trials were evaluated using Chi-square tests for gall wasp cavity counts, branch mortality and canopy condition. To compare the mean
number of cavities and the number of emerged gall wasps among treatments, we used a Poisson model and a Tukey and Kramer (Nemenyi) test for pairwise comparisons. To evaluate the average probability of branch mortality across the three treatments, a 2 x 2 contingency table and a Chi-squared test were calculated. A pairwise t-test, coupled with a Bonferroni correction for multiple comparisons, evaluated differences between treatments. Difference in canopy condition between the three treatments for the both spring and fall injection trials was compared with an ANOVA. To evaluate differences in canopy condition for each treatment in both of the injection trials, we used a Tukey and Kramer (Nemenyi) multiple comparison test.

4.3 Results

4.3.1 Measurements of Efficacy

4.3.1.1 Estimation of Gall Wasp Establishment

Pre-treatment cavity counts were significantly different between the fall and spring injection trials ($z = 11.65$; df = 1; $P < 0.001$, Table 1). This difference is likely due to the fact that trees in the spring trial had a higher level of infestation of *Z. davisae* compared to trees in the fall injections. There was not a significant difference between treatments in the pre-treatment sample for either the fall or spring trials (Fall: $z = 1.858$; df = 2; $P = 0.395$; Spring: $z = 1.506$; df = 2; $P = 0.2774$, Table 1). Post-treatment, as expected, there was still an injection date effect ($z = -12.93$; df=1; $p < 0.001$) and there was an injection date and treatment interaction for the combined data set of fall and spring trials ($z = 8.554$; df=1; $P < 0.001$). In both experiments, there was a significant difference between treatments (Fall: $z= 16.81$; df = 2; $P < 0.001$; Spring: ($z = 6.04$; df = 2;
In the fall, emamectin benzoate and imidacloprid were statistically significant from each another ($P = 0.016$), but they were not significantly different from the controls ($P = 0.423$; $P = 0.644$, respectively). In the spring trial, the injection treatments were not significantly different from each other ($P = 0.848$), but they were significantly different from the controls (Emamectin: $P = 0.019$; Imidacloprid: $P = 0.023$).

**Table 4.1 Pesticide treatment application information and pre (2014) and post-treatment (2015) *Zapatella daviseae* cavity densities for each pesticide treatment for fall and spring injection, including average diameter at breast height (DBH) pesticide dose and rate and mean number of cavities ($\pm$ SE) before and after treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Application timing</th>
<th>Avg. DBH (cm)</th>
<th>Dose (ml) per cm</th>
<th>Rate</th>
<th>Pre Treatment Avg. No. Cavities</th>
<th>Post Treatment Avg. No. Cavities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emamectin Benzoate</td>
<td>Fall</td>
<td>17.5 ± 1.33</td>
<td>10</td>
<td>4%</td>
<td>3.3 ± 0.91</td>
<td>3.4 ± 0.76</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Fall</td>
<td>16.2 ± 1.06</td>
<td>8</td>
<td>5%</td>
<td>4.9 ± 0.82</td>
<td>8.4 ± 1.76</td>
</tr>
<tr>
<td>Control</td>
<td>Fall</td>
<td>16.2 ± 1.40</td>
<td>NA</td>
<td>NA</td>
<td>5.3 ± 0.89</td>
<td>6.1 ± 1.19</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>Spring</td>
<td>17.6 ± 0.95</td>
<td>10</td>
<td>4%</td>
<td>8.6 ± 1.03</td>
<td>9.6 ± 1.66</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Spring</td>
<td>18.3 ± 1.25</td>
<td>8</td>
<td>5%</td>
<td>9.9 ± 2.48</td>
<td>10.0 ± 1.70</td>
</tr>
<tr>
<td>Control</td>
<td>Spring</td>
<td>19.3 ± 1.89</td>
<td>NA</td>
<td>NA</td>
<td>9.7 ± 1.76</td>
<td>19.4 ± 3.75</td>
</tr>
</tbody>
</table>

**4.3.1.2 Adult Gall Wasp Emergence**

Gall wasp emergence was only assessed for the fall injection trial. There was a significant difference in the average number of emerged gall wasps per branch between the pesticide treatments and the controls ($z = -3.62$; df $= 1$; $p < 0.001$) (Fig. 1). Branches treated with either emamectin benzoate or imidacloprid had fewer emerging gall wasps than control branches ($P = 0.004$; $P = 0.046$, respectively). Gall wasp emergence was not different between the two pesticide treatments ($P = 0.534$).
4.3.1.3 Branch Mortality

The fall and spring injection trials were significantly different from each other ($\chi^2 = 41.497; df = 1; P < 0.001$) (Fig. 2). Branch mortality was much higher in the spring trial compared to the fall trial. There was no difference among the three treatments in the fall injection trial, due to low branch morality rates ($\chi^2 = 3.8663; df = 2; P = 0.1447$). In contrast, in the spring injection trial there were significant differences among the three treatments ($\chi^2 = 26.327; df = 2; P < 0.001$). When evaluating across treatment differences within the spring trial, pesticides, as a whole, lowered branch mortality compared to the controls ($\chi^2 = 15.338; df = 1; P < 0.001$) (Fig. 2) and they both were equally effective ($P = 0.071$).
4.3.1.4 Tree Canopy Condition

There was no significant difference between the fall and spring injection trials ($F = 1.51; df = 1; P = 0.221$) (Fig. 3). In both trials, systemic injection of pesticides improved canopy condition compared to the controls ($F = 5.48; df = 1; P = 0.021$). Emamectin benzoate significantly increased tree canopy condition compared to the control ($P = 0.038$). Imidacloprid was associated with a slight positive change in canopy condition compared to the control, but the difference was not significant ($P = 0.262$).
4.4 Discussion

Our results show that both imidacloprid and emamectin benzoate have the potential to be effective control agents of *Z. davisae*, as they have been for other species of stem gall wasps. Both insecticides successfully lowered *Z. davisae* adult emergence, thereby decreasing the population size. Emamectin benzoate prevented an increase in the number of *Z. davisae* cavities, but imidacloprid did not. Previous studies have shown that pesticides that do not lower the number of new galls can nonetheless lower gall wasp survival and adult emergence (Eliaison and Potter 2000). In other cases, pesticides can directly affect new gall development. Bhandari and Zhiqiang (2016) found that injections of either emamectin benzoate or imidacloprid reduced densities of *Josephiella* sp. stem galls on Chinese Banyan tree. It is evident that the application of either of these two pesticides has the capacity to lower *Z. davisae* populations and reduce host tree damage.
Further research, however, is needed to better understand the details of how and at what point in Z. davisae's lifecycle each pesticide affects Z. davisae survival.

Major symptoms of Z. davisae infestation include flagging, leaf clumping, and branch mortality (Buffington et al. 2016). An examination of branches collected two years after injection showed that both pesticide treatments reduced branch mortality caused by Z. davisae. There was considerable variability in branch mortality between the fall and spring trials. Sites used in the spring injection trial experienced significantly more branch mortality and cavity development compared to sites used in the fall trial. This resulted in a significant difference in branch mortality between the two trials. The spring injection trial showed a significant treatment effect, an indication that systemic injections of either pesticide could prevent branch mortality. In sites where there is low branch mortality and cavity development, such as the fall injection sites, pesticides will have a correspondingly lesser impact.

Canopy condition is a common indicator of tree health. When a tree’s vascular system is stressed, it will often limit the allocation of resources to specific branches (Ramen 2011). Both pesticide treatments prevented foliar damage, with emamectin benzoate being the most effective. Our results agree with other studies completed on the effect of insecticides on canopy condition. Doccola et al. (2009) found that imidacloprid preserved canopy density and quality in E. sandwicensis trees infested with Q. erythrinae. Smitley et al. (2010) found that emamectin benzoate maintained canopy condition in ash trees, whereas the controls experienced a severe decline in canopy cover. A positive change in tree condition indicates that the injection had a positive impact on tree health and resilience.
Injection date (fall/spring) had no effect on tree canopy condition measurements. However, there was a difference between trials on branch mortality and cavity counts. Cavity counts and branch mortality rates were lower at sites used in the fall trial compared to the site used in the spring trial, which may have caused the injection date effect for these variables. Trends were similar in both trials, with emamectin benzoate leading to lower cavity counts and branch mortality compared to the control. Besides slight discrepancies between the two trials, it appears that both spring and fall injections have positive impacts on tree condition, mortality and gall wasp density control.

These trials provide options for landowners who wish to protect their trees from *Z. davisae damage*. Since our study was only performed over a two-year period, we cannot say how long treatment effects might persist or how often they might need to be re-applied. We have, however, demonstrated control for at least 17 months using either pesticide, with emamectin benzoate working better by some measure. Since black oak is one of the most common landscape trees on Cape Cod, there is a strong need for localized, small scale management. A pesticide protocol will help arborists mitigate the damage, and economic impact of *Z. davisae*. 
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