PHENOLOGY, DENSITY AND DISTRIBUTION OF THE INVASIVE ASIAN CHESTNUT GALL WASP (*DRYOCOSMUS KURIPHILUS* YASUMATSU) AND EVALUATION OF TWO SYSTEMIC INSECTICIDES IN MICHIGAN CHESTNUT ORCHARDS

By

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ABSTRACT

PHENOLOGY, DENSITY AND DISTRIBUTION OF THE INVASIVE ASIAN CHESTNUT GALL WASP (*DRYOCOSMUS KURIPHILUS* YASUMATSU) AND EVALUATION OF TWO SYSTEMIC INSECTICIDES IN MICHIGAN CHESTNUT ORCHARDS

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This thesis, presented in three chapters, focused on evaluating the phenology, density, distribution and parasitism of Asian chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus* Yasumatsu), as well as the level and persistence of two systemic insecticides in chestnut orchards in southwest Michigan.

In chapter one, phenology, density and parasitism of ACGW were monitored in up to nine Michigan chestnut orchards from 2017 to 2019. Phenology was related to cumulative degrees days which provided precise timing recommendations for scouting activities or applying cover spray insecticide applications that target the adult wasp. After cold winter temperatures in 2019, the ACGW population was significantly reduced at all monitored locations. The parasitoid of ACGW is established in Michigan and naturally spreading with ACGW.

In chapter two, spread and distribution of ACGW was monitored at both the local and regional scale. Diffusion of ACGW through individual orchards progressed quickly and annual spread across Michigan showed large jump distances. Cold winter temperatures in 2019 halted ACGW spread across the state, suggesting ACGW may face a climatic barrier.

In chapter three, imidacloprid and emamectin benzoate residues were assessed in chestnut foliage, catkins and nuts. Both insecticides were occasionally found in catkin samples and rarely found in nut samples. Imidacloprid foliage residues were generally high, but variable. Emamectin benzoate foliage residues varied considerably between two treatment years.

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Introduction

Chestnuts (*Castanea* spp.) are grown across much of the world for fresh consumption and are used in a variety of other commodities including flour, creams, pasta, flakes, candies, beer and liquors (Bounous 2005, Hochmuth et al. 2018). Commercial chestnut production is dominated by countries in east Asia and Europe; South Korea and China together account for more than 40% of global chestnut production (Hochmuth et al. 2018). In the U.S., at least 20 million USD of chestnuts are imported and sold annually, but commercial orchards in the U.S. currently account for less than one percent of chestnut production worldwide (Hochmuth et al. 2018).

Michigan is the largest chestnut producer in North America, with at least 330 ha of commercial orchards (Fulbright et al. 2010, Hochmuth et al. 2018). Chestnuts are a high value commodity. Orchard startup costs were estimated at just under 19,266 USD per ha, but a mature chestnut orchard can produce 1,121 to 1,680 kg of chestnuts per ha with a retail value of four to ten USD per kg (Black et al. 2013, Hochmuth et al. 2018). On average, Michigan orchards produce 110,000 kg of chestnuts annually and 3,362 kg per ha, valued at approximately 5,558 USD per ha (Fulbright et al. 2003, Biaggi et al. 2019).

Asian chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus* Yasumatsu) (Hymenoptera: Cynipidae), a native of China, is a major invasive pest of chestnut (*Castanea* spp.) trees in Japan, Korea, much of Europe and at least 14 states in the U.S. (EFSA 2010, Haack et al. 2011, Haack 2015). Larvae feed within small galls, 5-20 mm in diameter, that develop on expanding, current-year shoots and leaves in early spring. High densities of galls can affect tree vigor and reduce nut

yields, causing costly damage to commercial chestnut orchards in Japan, Korea, the U.S. and Europe (Payne 1978, Dixon et al. 1986, Kato and Hijii 1997, EPPO 2005, Cooper and Rieske-Kinney 2007, Rieske 2007, EFSA 2010, Battisti et al. 2014, Ugolini et al. 2014, Sartor et al. 2015). Apical galls that form at the distal end of shoots may be especially damaging because they reduce shoot elongation and inhibit flower production, which reduces nut formation (Kato and Hijii 1997, Battisti et al. 2014). A report from China indicated that when 30% of chestnut buds were infested, nut yield was reduced by up to 80% (Zhi-Yong 2009). In Italy, galls caused by ACGW similarly caused up to 80% yield loss if trees averaged more than six galls per 50 cm of live shoot (Battisti et al. 2014). In the U.S., orchards in Georgia have reported 50-70% reductions in yield following ACGW invasion (Payne et al. 1983, Dixon et al. 1986). Branch dieback and even tree mortality have been observed in China, Japan, Korea, the U.S. and Italy when ACGW densities are high (Cho and Lee 1963, Payne 1978, Moriya et al. 2003, Zhi-Yong 2009, Ugolini et al. 2014).

At least 25 countries across Asia and Europe, as well as North America, have been invaded by ACGW (EFSA 2010, Avtzis et al. 2019). Japan first detected ACGW in 1941 followed by Korea in 1958 and the U.S. in 1974 (Payne et al. 1975, Murakami et al. 1980, Avtzis et al. 2019). Italy was the first European country to detect ACGW in 2002 and 18 countries in Europe were infested as of 2016 (Avtzis et al. 2019). Spread of ACGW has been attributed to both natural dispersal and artificial transport of infested plant material. Adult wasp dispersal, both active and wind-assisted, has been reported at up to 25 km per year in Japan, Italy and the U.S. (Rieske 2007, Graziosi and Santi 2008, EFSA 2010). Transport of infested chestnut seedlings and scion wood accounts for long distance spread, a problem facilitated by the cryptic nature of tiny first instar larvae, which overwinter within buds (EPPO 2005, EFSA 2010).

In the U.S., ACGW was first detected in Peach County, Georgia in 1974 when a private grower imported scion wood from a Japanese chestnut tree (*Castanea crenata* Siebold and Zuccarini) growing in Japan (Payne et al. 1975, Anagnostakis 2012). Since then, ACGW has spread across much of the historical range of American chestnut (*Castanea dentata* (Marsh.) Borkh.) and as of January 2020, had been detected in 14 states (Cooper and Rieske-Kinney 2007, Haack et al. 2011, Haack 2015). Spread within the U.S. partially reflects natural dispersal of adult wasps, but transport of infested nursery stock is known to have resulted in establishment of new populations, such as in northeast Ohio in 2002 (Rieske 2007). Given the proximity of satellite infestations in Ohio, the Michigan Department of Agriculture and Rural Development issued an external quarantine in 2010 which banned importation of live *Castanea* spp. material from all states with known ACGW infestations (Haack et al. 2011). Despite this quarantine, ACGW was detected in two chestnut orchards in southwest Michigan in July 2015 and subsequently found in eight more orchards by late August 2015 (Haack 2015).

An array of methods to control ACGW have been employed by commercial growers, with varying levels of success. Cover sprays of conventional insecticides can effectively control ovipositing ACGW adults, but timing and adequate coverage are critical. Insecticide sprays are ineffective against immature stages that are protected within galls (Cooper and Rieske 2007, Bosio et al. 2010, Anagnostakis 2012, Bernardo et al. 2013). Little research on the efficacy of systemic insecticides for ACGW control has been conducted to date, although some success with systemics has been reported for other gall-forming insects, including eastern spruce gall adelgid (*Adelges abietis*) (Hemiptera: Adelgidae) and Cooley's spruce gall adelgid (*Adelges cooleyi*) (Hemiptera: Adelgidae), as well as another gall wasp (*Callirhytis cornigera*) (Hymenoptera:

Cynipidae) that colonizes pin oaks (*Quercus palustris*) (Nielsen and Balderston 1977, Eliason and Potter 2000, Bhandari and Cheng 2016).

All *Castanea* species can be colonized by ACGW, but host resistance, which varies among species and cultivars, may play a role in ACGW management (Panzavolta et al. 2012, Sartor et al. 2015). In Japan, resistant cultivars of *C. crenata* were widely planted in the mid 1950's, but by the late 1960's, these cultivars were infested by an adapted, more virulent biotype of ACGW (Shimura 1972, Murakami 1981). Cultivars of European chestnut (*Castanea sativa* Miller) exhibit a range of susceptibility to ACGW. Some, such as Pugnenga and Savoye, appear to be resistant, while others, such as Torcione Nero and Marrone di Zocca, are considered highly susceptible (Sartor et al. 2015). Hybrids of *C. crenata* x *C. sativa* vary from resistant to very susceptible. Bouche de Bétizac (*C. crenata* x *C. sativa*), a cultivar grown commercially in some Michigan orchards, is reportedly resistant to ACGW, while observations suggest Colossal (*C. crenata* x *C. sativa*), a popular cultivar which produces an abundance of large nuts, may be moderately to highly susceptible to ACGW (Sartor et al. 2015, Fulbright et al. 2018). To date, however, quantitative evaluations of ACGW resistance by the Colossal cultivar are lacking, despite its increasing popularity.

Classical biological control has also been implemented to reduce ACGW damage in commercial chestnut orchards. A parasitoid wasp native to China, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), was imported, reared and released in Japanese chestnut orchards in 1975 and additional releases occurred in 1979, 1981 and 1982 (Murakami et al. 1980, Kamijo 1982, Moriya et al. 2003). Italy, the first European country to be invaded by ACGW, introduced *T. sinensis* in 2005 and 2006 by rearing out and releasing *T. sinensis* adults from parasitoid-bearing galls imported from Japan (Quacchia et al. 2008, Gibbs et al. 2011). After the initial

releases in Italy, annual increases in *T. sinensis* populations corresponded to decreases in ACGW infestation levels, indicating successful control (Ferracini et al. 2019). In 1977, *T. sinensis* imported from Japan were released in infested orchards in Byron, Georgia (Cooper and Rieske-Kinney 2007, Rieske 2007). Subsequent research indicated *T. sinensis* had become established, spread and was credited with noticeable declines in ACGW infestations in Georgia, Kentucky, Virginia and Ohio (Rieske 2007, Rieske and Cooper 2011).

Effectiveness of this parasitoid reflects its synchronized life cycle with ACGW and host specificity (Quacchia et al. 2008, 2014, Ferracini et al. 2015). In early spring, as ACGW galls are forming, *T. sinensis* adult females lay an egg into a gall chamber occupied by an ACGW larva (Piao and Moriya 1999). The ectoparasitoid larva feeds on the ACGW larva within the gall chamber throughout the summer (Matošević et al. 2014). Although ACGW pupate and emerge as adults in midsummer, parasitoid larvae overwinter inside galls, which wither and harden in late summer (EPPO 2005, Shiga 2009). Parasitoids pupate in late winter and, as chestnut buds break and new galls form in spring, adult parasitoids emerge from the dry, previous-year galls to oviposit within the succulent, current-year galls (Shiga 2009, Matošević et al. 2014). In Italy, *T. sinensis* can reportedly parasitize some native oak-galling insects, but rates are low and there is little evidence that native insect populations are affected (Ferracini et al. 2015, 2017). In the U.S., there is no evidence that *T. sinensis* attacks any native species (Cooper and Rieske 2011).

Detection of ACGW caused substantial concern within the Michigan chestnut industry and generated questions about impacts and potential management options to mitigate potential damage by this invader. Our objectives included monitoring ACGW phenological development and survival in recently infested Michigan orchards. These orchards represent the most northerly populations of ACGW in the U.S. and potential effects of winter temperatures on ACGW larval

survival are not known. Phenological stages of chestnut trees and corresponding ACGW life stages were tracked and related to cumulative growing degree days, partly to identify optimal timing for adult ACGW control but also to assess whether such cover sprays could potentially harm non-target insects attracted to pollen-bearing catkins. Gall densities on chestnut species and cultivars grown commercially in Michigan were also recorded. Susceptibility of Colossal (*C. crenata* x *C. sativa*) trees was of particular interest, given the lack of previous research on this cultivar and its increasing popularity. We took advantage of an opportunity to assess gall densities on Colossal trees and on Dunstan chestnut trees (*C. dentata* x *C. mollissima*) growing in the same orchard. Because Chinese chestnut shares an evolutionary history with ACGW, we hypothesized that the Chinese hybrid Dunstan trees should be less susceptible than Colossal trees, which are hybrids of Japanese and European chestnut species. Finally, we assessed *T. sinensis* presence and parasitism rates annually from 2017-2019.

Materials and Methods

Study Sites

In 2017, we identified five chestnut orchards in southwest Michigan with known ACGW infestations. One site, located at the Michigan State University (MSU) Southwest Research and Extension Center (SWMREC), includes 36 chestnut cultivars representing *C. mollissima* (22 cultivars), *C. crenata* (2 cultivars) and the *C. crenata* x *C. sativa* hybrid (4 cultivars) (Table 1.1). Most cultivars, however, are represented by only one or two individuals or are young saplings.

The second site, HS, was a privately owned, commercial chestnut orchard (Table 1.2) with two cultivars, the *C. crenata* x *C. sativa* variety Colossal and a *C. dentata x C. mollissima* variety Dunstan, growing in the same field. Trees of both cultivars were of similar size and age

(Table 1.2), providing an unusual opportunity to compare gall densities between two common varieties.

Three additional commercial orchards, all with *C. mollissima* trees that became infested in 2015 or 2016 (Table 1.2), were surveyed in 2017. Size of these operations ranged from the FL site, which consisted of only 36 chestnut trees, to sites with two to three ha of trees.

Trees in four of the five 2017 sites were sampled again in 2018, but one site (PD) was dropped after trees were heavily pruned in late June 2017, eliminating our ability to access shoots. We added three more commercial orchards to our study in 2018, all of which became infested in 2017. Two sites, CM and BK, have *C. mollissima* trees, while the CB site has both *C. mollissima* and *C. sativa* trees (Table 1.2).

In 2019, we monitored ACGW and trees in the same sites as in 2018 along with two additional commercial orchards, both of which had *C. mollissima* trees and became infested by ACGW in 2018 (Table 1.2). In 2019, however, current year gall abundance dropped sharply in all sites, probably as a result of unusually cold temperatures in late January 2019.

Chestnut Tree Phenology

Visual monitoring of chestnut tree phenological stages, including bud break, leaf expansion and production of catkins, pollen and burrs, began in early May each year and observations were recorded at one to two week intervals through nut harvest in early October. Phenology data was collected from the SWMREC, HS and ET sites, which were the only sites sampled all three years. Phenological stages were based on predetermined, qualitative categories and ranks were assigned to trees sampled on each date (see below). Phenological stages were also related to corresponding cumulative growing degree days acquired from three MSU Enviro-weather stations in southwest Michigan, including Benton Harbor, Berrien Springs and Grand

Junction (www.enviroweather.msu.edu). Degree day accumulation was calculated using the Baskerville-Emin method and began on 1 January of each year with a threshold base temperature of 10°C. All sites were within 17 km of an MSU weather station.

Tree Selection for Sampling

In 2017, shoots were collected to evaluate galls from mid-May through July in each of the five sites. Because of the considerable distance from the MSU campus to the sites in southwest Michigan, we sampled trees at two or three sites in week 1, then sampled trees in the other sites the following week, continuing to alternate weeks until all or nearly all ACGW adults had emerged. Number of trees sampled on each visit ranged from nine to 18 trees per site, depending on field size and accessibility (Table 1.2). A grid was overlaid on site maps and one tree per grid cell was randomly selected, without replacement, for sampling on each visit. At the HS site, nine Colossal and nine Dunstan chestnut trees were sampled on each visit. Trees at all sites were sampled four times throughout the summer except for the PD site, which was not sampled after the heavy pruning in late June.

In 2018, we sampled trees at three or four sites on alternate weeks from late May through mid July. As in 2017, each site was sampled four times during the summer. Due to the small size of the FL site, only 4 trees were sampled per visit. At the HS site, 20 Colossal and 20 Dunstan trees were sampled on each visit. Either eight or ten trees were sampled from the other sites per visit. The CB site, added in 2018, included *C. mollissima* and *C. sativa* trees and five trees of each species were sampled on each visit (Table 1.2).

In 2019, we identified nine infested sites for sampling. Because it was not logistically possible to sample trees in nine sites within a two-week period, three sites, HS, ET and SWMREC, were visited weekly to monitor chestnut and ACGW phenology. Sampling began at

the end of May and continued through the beginning of August. These sites were large enough to accommodate frequent shoot collections and had abundant galls. In the remaining six sites, shoots were collected from 12 to 30 randomly selected trees once between 18 June and 24 July to assess overall gall density.

Tree Selection for Sampling at the SWMREC Site

In 2017, at the SWMREC site, we sampled only cultivars which were represented by at least two trees (> 10 cm DBH). This included seven cultivars representing three species: *C. mollissima* (Benton Harbor, Everfresh, Gideon, Peach and Qing), *C. crenata* x *C. sativa* (Colossal) and *C. crenata* (Labor Day) (Table 1.1). We sampled shoots from the same trees on each visit, with the exception of Colossal trees; three of the five trees were randomly chosen for sampling on each occasion.

In 2018, we modified our methods to sample more trees and focused on differences among species rather than cultivars. We sampled one to five trees per cultivar from a total of 20 cultivars representing *C. crenata* (2 cultivars), *C. crenata* x *C. sativa* (4 cultivars) and *C. mollissima* (14 cultivars) (Table 1.1). Each visit, we randomly chose eight trees to sample from all possible sampling trees. No trees were sampled more than once. A total of 31 trees were sampled during the summer.

In 2019, the SWMREC site was sampled weekly to monitor ACGW phenology. We sampled five trees per week from 28 different cultivars, including two *C. crenata*, four *C. crenata* x *C. sativa* and 22 *C. mollissima* (Table 1.1). Trees were randomly selected and no trees were sampled more than once.

Shoot Collection

In 2017, we clipped two shoots, 50 to 210 cm long, from opposite aspects of the mid canopy of each tree selected for sampling on each visit. Shoots were returned to the MSU Forest Entomology laboratory, where we recorded the length of each shoot and number of current-year and previous-year galls. Galls produced by developing larvae during the current growing season were fleshy and green, while remaining galls from previous years were withered, dry and brown. Each gall was recorded as apical (at the end of a terminal or axillary shoot), lateral (along the side of shoots) or as a leaf gall on leaf tissue or petioles. Shoot lengths and gall numbers were summed for each tree and densities of total galls, and apical, lateral and leaf galls, were standardized by linear meter of shoot.

In 2018 and 2019, we removed one shoot from the mid canopy of each tree on each visit. During the 2019 collections, due to a substantial decrease in gall abundance, the randomly selected trees were scouted to locate and collect a shoot with at least one gall present. Shoots were returned to the MSU Forest Entomology laboratory and processed according to 2017 methods.

Gall Dissections

In 2017, all galls were removed from each shoot, then two of the galls were randomly selected and refrigerated until they could be dissected. Galls from different shoots clipped from the same tree were stored and processed separately. All galls were examined and dissected within three months of collection. We measured gall length and width, then sliced each gall into several cross sections along the entire length and examined sections under a microscope. Number of gall chambers, live and dead ACGW by life stage, empty chambers and larvae we suspected to

be *T. sinensis* were recorded. Ten ACGW adults were submitted as voucher specimen to the Alfred J. Cooke Arthropod Research Collection at Michigan State University (Table A.1).

Suspected *T. sinensis* larvae were removed from galls and analyzed with PCR in 2017 to confirm their identity, following methods of Yara (2006). We extracted DNA using a ZymoBIOMICS DNA Kit with one, five and ten larvae and assessed DNA concentration with the Qubit dsDNA broad range quantification assay following manufacturer's protocols. A detectable quantity of DNA was found in the five and ten larval samples, but not the one larva sample. A species-specific pair of primers for *T. sinensis*, TSITS2F-1 (5'-CAT AAC CAG ACT GCT CGC G-3') and TSITS2R-1 (5'-CGA TAT ACC CAC GCA CGA C-3'), were used to amplify the ITS2 region from extracted DNA products (Yara 2006). PCR was performed using a 25µl reaction volume in an ABI 2720 Thermal Cycler under the following conditions: 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 1 min, followed by 72°C for 5 min.

Following confirmation of *T. sinensis* larvae, we visually identified parasitoid larvae, which were readily distinguishable from ACGW larvae, and recorded parasitism in the dissected galls. Parasitism rates were calculated in two ways, first as the proportion of all galls with at least one parasitoid present, which we refer to as parasitized galls, and second, as the proportion of all ACGW larval chambers occupied by a parasitoid larva, which we refer to as parasitized larvae. In 2018, one randomly selected gall from each collected shoot was set aside for dissection and in 2019, due to a notable drop in current-year gall abundance, we dissected all galls collected from sampled shoots.

Statistical Analysis

Differences in current-year gall density were analyzed between chestnut cultivars (Colossal, Dunstan) or among gall types (apical, lateral, leaf) with a two-way ANOVA (Proc MIXED; SAS 9.4) followed by Tukey's multiple comparison adjustment if the ANOVA results were significant. Normality and heterogeneity of variance were assessed with residual plots and Levene's test. Gall densities from 2017 and 2018 were log transformed to normalize distribution and equalize variance. Untransformed data are presented in figures. Simple linear regression was applied to determine if previous-year gall density predicted current-year gall density at the SWMREC site (Proc REG; SAS 9.4).

Current-year and previous-year gall densities recorded on *C. mollissima* trees in five fields in 2017 and in seven fields in 2018 were compared to examine differences among sites. Current-year gall densities in 2017 and 2018 were log transformed and analyzed with a one-way ANOVA (Proc MIXED; SAS 9.4) followed by Tukey's multiple comparison adjustment when the ANOVA results were significant. Untransformed data are presented in figures. Densities of previous-year galls from 2017 and 2018 and the proportion of gall chambers (per tree) containing a parasitoid larva in 2017, 2018 and 2019 were not normalized with transformations. The proportion of gall chambers containing a parasitoid larvae by gall type in 2018 and 2019 was analyzed and data was not normalized with transformations. These variables were analyzed with Kruskal-Wallis one-way nonparametric ANOVA (Proc NPAR1WAY; SAS 9.4) followed by a Dwass, Steel, Critchlow-Fligner multiple comparison test if the Kruskal-Wallis results were significant (Critchlow et al. 1991). Gall densities were extremely low in 2019 in all sites and were not analyzed.

Results

Chestnut Tree Phenology

Phenological development of chestnut foliage and pollen-bearing catkins was generally consistent among sites and across the three years (Table 1.3). In 2017, bud break was already underway on our first visits to sample trees at the SWMREC and HS sites (5 May and 16 May, respectively). In 2018 and 2019, buds were swelling by mid May, corresponding to approximately 100-125 DD_{10C} (Table 1.3). Leaves continued growing through May and most were fully expanded by early or mid June, corresponding to approximately 315-375 $DD_{10\,C}$ (Table 1.3). Pollen was first observed on catkins between mid June and early July (465 to 590 DD_{10C}). At the HS site, immature catkins on Colossal trees, which do not produce pollen, were shed at roughly the same time pollen appeared on catkins on the Dunstan trees (525-540 DD_{10C}) (Table 1.3). Pollen production peaked between late June and early July (580 to 660 DD_{10C}) and the first small burrs appeared soon thereafter, in early to mid July (640 to 745 DD_{10C}). Mature kernels were dropping from dry, desiccated burrs by late September and early October (1555 to 1755 DD_{10C}).

ACGW Phenology

In 2017, galls with early stage larvae were first observed on 16 May (246 DD_{10C}), and larval development progressed through 27 June (628 DD_{10C}) (Figure 1.1, 1.4). In galls collected from the FL and HS sites on 27 June, mostly pupae were present in galls; only 11% and 3% of ACGW, respectively, were still larvae. Pupae were observed in galls from 6 June (383 DD_{10C}) through 6 July (741 DD_{10C}) (Figure 1.1). Development rates varied somewhat among sites. For example, on 6 June, 94% of ACGW in galls from the HS site were pupae, compared with only 5% of ACGW in galls from the FL site. By 13 June (464 DD_{10C}), 61% and 64% of ACGW in

galls from the ET and SWMREC sites, respectively, were pupae. On 27 June (628 DD_{10C}), at least 50% of ACGW in galls were adults and we continued to observe adults within galls until our final collection on 25 July (945 DD_{10C}) (Figure 1.1, 1.5). Adult wasps began emerging in early July and emergence continued through early August.

In 2018, larvae were present in galls collected when we first visited sites for sampling on 22 May (171 DD_{10C}) through 26 June (555 DD_{10C}) (Figure 1.2, 1.4). Pupae were first observed in galls on 5 June (333 DD_{10C}) and a few galls collected on 10 July (748 DD_{10C}) still contained apparently viable pupae (Figure 1.2). From 20 June (500 DD_{10C}) to 2 July (639 DD_{10C}), most ACGW were pupating. Adults were first observed in galls on 26 June (555 DD_{10C}) and by 10 July (748 DD_{10C}), 72% to 100% of ACGW in galls from all sites were adults (Figure 1.2, 1.5). Adult emergence began by early July and continued through early August.

In 2019, larvae were present in all galls collected between 29 May (180 DD_{10C}) and 11 June (283 DD_{10C}) and we continued to observe larvae in galls through 9 July (593 DD_{10C}) (Figure 1.3, 1.4). Pupae were present in galls from 18 June (332 DD_{10C}) through 23 July (789 DD_{10C}) and pupation peaked between 26 June (411 DD_{10C}) and 9 July (593 DD_{10C}) (Figure 1.3). Galls had adult wasps from 2 July (496 DD_{10C}) until our final collection on 8 August (948 DD_{10C}) (Figure 1.3, 1.5). In mid July (688 DD_{10C}), 89% to 91% of the ACGW present in galls were adults (Figure 1.3). Emergence of adults began in mid July and continued through early August 2019.

Yearly phenological development of ACGW was generally consistent between 2017 and 2019 in relation to cumulative degree days. Overall, ACGW larvae were observed in galls until 628 DD_{10C} (27 June), 555 DD_{10C} (26 June) and 593 DD_{10C} (9 July) in 2017, 2018 and 2019, respectively, a span of only 73 DD_{10C} (Figure 1.4). Proportion of ACGW pupae in galls peaked

on 383 DD_{10C} (6 June), 555 DD_{10C} (26 June) and 496 DD_{10C} (2 July) in 2017, 2018 and 2019, respectively, a slightly larger range of 172 DD_{10C}. Proportion of ACGW that were observed as adults when galls were dissected peaked on 628 DD_{10C} (27 June), 748 DD_{10C} (10 July) and 688 DD_{10C} (16 July) in 2017, 2018 and 2019, respectively, a span of 120 DD_{10C} (Figure 1.5).

Gall Density at the SWMREC Site

In 2017, average (±SE) density of current-year galls on trees of five *C. mollissima* cultivars was consistently low, ranging from 2.2 ± 0.4 to 6.1 ± 0.8 galls per m on Qing and Benton Harbor cultivars, respectively (Table 1.4). Labor Day (*C. crenata*) and Colossal (*C. crenata* x *C. sativa*) trees had 1.5 to 4.5 times more current-year galls than any *C. mollissima* trees. Average density of previous-year galls ranged from 1.7 ± 0.6 to 3.7 ± 1.0 galls per m across all trees sampled at SWMREC and was not related to current-year gall density (R^2=0.001; *P*=0.6737).

In 2018, density of current-year galls on the *C. mollissima* trees, excluding a G-142 tree, averaged 8.0 ± 1.1 galls per m. The single representative of the G-142 cultivar, however, had 46.2 galls per m, which was at least twice the density of galls on any other trees from any of the cultivars or species. Only three *C. crenata* trees, representing two cultivars, were sampled in 2018 and the overall density of current-year galls averaged 16.5 ± 3.6 galls per m (Table 1.4). Average density of current-year galls on the *C. crenata* x *C. sativa* trees was again fairly high in 2018, with the exception of a single tree representing the Nevada cultivar (Table 1.4). The absence of galls on the Nevada tree is notable, given that all adjacent and nearby trees had galls. Total current-year gall density for trees from the other three *C. crenata* x *C. sativa* cultivars averaged 10.6 ± 1.7 galls per m. Overall densities of previous-year galls were similar among species, ranging from 2.0 ± 0.5 galls per m for *C. mollissima* trees, 3.5 ± 1.1 galls per m for *C.*

crenata x *C. sativa* trees and 4.1 ± 0.4 galls per m for *C. crenata* trees. Density of previous-year galls ranged from 0 on Everfresh (*C. mollissima*), Dunstan Revival (*C. mollissima*) and Nevada (*C. crenata* x *C. sativa*) trees, to 9.0 galls per m, on G-142 (*C. mollissima*). Densities of previous-year and current-year galls were significantly related in 2018 (R^2=0.3515; *P*<0.0001).

In 2019, abundance of current-year galls plunged, presumably because of high mortality of overwintering larva. We collected 109 galls on 42 shoots totaling 68.4 m in length in 2019 (1.6 galls per m). In contrast, we collected 665 galls from 31 shoots totaling 66.9 m (9.9 galls per m) and 925 galls from 120 shoots totaling 193.1 m (4.8 galls per m) in 2018 and 2017, respectively. Density of previous-year galls, which would not have been affected by cold winter temperature, were more consistent; a total of 286, 162 and 426 previous-year galls were collected from shoots in 2019, 2018 and 2017 respectively. Densities of previous-year and current-year galls were not related in 2019 (R^2=0.044; *P*=0.1356).

Gall Density at the HS Site

Alternate rows of similarly sized *C. dentata* x *C. mollissima* (Dunstan) and *C. crenata* x *C. sativa* trees (Colossal) at the HS site provided an opportunity to compare gall densities on the two cultivars under the same environmental conditions. Total density of current-year galls was lower on Dunstan trees than on Colossal trees in 2017 (F=56.29; df=1,228; *P*<0.0001) and in 2018 (F=48.95; df=1,480; *P*<0.0001) (Figure 1.6). Densities among apical, lateral and leaf galls also varied between Dunstan and Colossal trees in 2017 (F=67.06; df=2,228; *P*<0.0001) and 2018 (F=135.02; df=2,480; *P*<0.0001) (Figure 1.7). Interactions between cultivar and gall type were not significant in 2017 (F=2.02; df=2,228; *P*=0.14) nor 2018 (F=0.54; df=2,480; *P*=0.58). In 2017, lateral galls comprised 58% of the total galls, followed by apical galls (29%), while leaf

galls were not common (13%). In 2018, lateral galls were again abundant, representing 60% of the total galls followed by apical galls (21%) and leaf galls (19%).

In 2019, current-year gall abundance on trees at the HS site was substantially reduced by the cold winter, as at the other sites. We collected only 237 galls on 186 shoots totaling 217.1 m (1.1 galls per m), compared to 1448 galls from 162 shoots totaling 241.0 m (6.8 galls per m) in 2018 and 1073 galls from 78 shoots totaling 211.5 m (5.1 galls per m) in 2017. Previous-year galls were abundant in 2019, when we collected 641 galls, compared to 237 and 72 galls in 2018 and 2017, respectively.

Gall Density on C. mollissima Trees

While Colossal (*C. crenata* x *C. sativa*) and other cultivars are increasingly popular for commercial chestnut production, most operations in Michigan and other states are dominated by Chinese chestnut (*C. mollissima*) or *C. mollissima* hybrids. We therefore assessed current-year and previous-year gall density on *C. mollissima* trees across all five sites sampled in 2017, including the Dunstan (*C. dentata* x *C. mollissima*) cultivar at the HS site. Density of current-year galls ranged from 0 to 19.1 galls per m and varied among sites ($F=16.12$; df=4,156; $P<0.0001$) (Table 1.5, Figure 1.8). Trees at the SWMREC, HS and FL sites had higher densities than trees at the PD site, while trees at the SWMREC, HS and ET sites had intermediate densities. Current-year gall densities also differed among sites for apical ($F=5.38$; df=4,152; $P=0.0004$), lateral ($F=17.47$; df=4,152; $P<0.0001$) and leaf galls ($\chi^2=58.30$; df=4,152; $P=0.0001$) in 2017 (Figure 1.9). Apical gall density of trees at the SWMREC site did not differ from trees at any other site, while trees at the FL and PD sites had lower apical gall densities than trees at the HS site. Trees at the PD site had the lowest lateral gall density and trees at the ET and HS sites had lower densities than trees at the FL site. Trees at the SWMREC and FL sites had the highest

densities leaf galls. Density of previous-year galls recorded in 2017 also varied among sites (χ^2=87.61; df=4,154; *P*<0.0001) (Table 1.5, Figure 1.10). Trees at SWMREC had the highest density of previous-year galls.

In 2018, current-year gall density on *C. mollissima* and *C. mollissima* hybrid trees ranged from 0 to 46.2 galls per m and varied among the seven sites we sampled (F=12.85; df=6,229; *P*<0.0001) (Table 1.5, Figure 1.8). Densities of current-year galls were highest at the SWMREC and FL sites, while the BK site had the lowest densities. Current-year gall densities also differed among sites for apical (χ^2=21.03; df=6,248; *P*=0.0018), lateral (F=9.54; df=6,248; *P*<0.0001) and leaf galls (χ^2=28.49; df=6,248; *P*<0.0001) (Figure 1.11). Trees at the ET and CB sites had higher apical gall densities than trees at the BK site. Lateral gall densities were higher at the SWMREC and FL sites than at the BK site and intermediate at the FL and other four sites. Leaf gall densities were higher at the FL site than at the HS, ET, CM and BK sites, while the SWMREC and CB sites were intermediate (Figure 1.11).

Density of previous-year galls on trees sampled in 2018 ranged from 0 to 9.5 galls per m and varied among sites (χ^2=56.50; df=6,229; *P*<0.0001) (Figure 1.10). The SWMREC and FL sites had higher densities of previous-year galls than trees at other sites. Densities of previous-year galls at the HS, ET and BK sites were similar and intermediate. Gall densities did not differ among trees at the BK, ET and CM sites, while no previous-year galls were recorded on trees at the CB site.

Parasitism

In 2017, *T. sinensis* parasitoid larvae were present in galls on trees in four of the five sites, which included three that became infested in 2015 (SWMREC, FL, HS) and one that was first infested in 2016 (ET) (Table 1.6). We did not observe parasitoids in any of the 33 galls we

dissected from trees sampled at the PD site. Out of the 419 galls collected in the four sites where *T. sinensis* was present, 15% contained at least one parasitoid. Gall parasitism rates ranged from 27.3% of galls at the FL site to 2.7% of galls at the HS site (Table 1.6). In 2018, we observed *T. sinensis* parasitoids in galls on trees in six of the seven sites, including two sites that became infested in 2017 (CM, CB). Out of the 369 galls collected at the six sites where *T. sinensis* was present, 14% contained at least one parasitoid. Parasitism rates ranged from 70% of galls from the SWMREC site to 2.5% of galls from the ET site (Table 1.6). In 2019, despite the substantial drop in current-year gall density, parasitoids were again present in galls from trees at the same six sites. We collected a total of 13 and 74 galls from the HR and FV sites, respectively, both of which were first infested by ACGW in 2018. Parasitoids were present in four of the 74 galls from trees at the FV site, but were not recovered in any galls from trees at the HR site.

Mean proportion of total ACGW gall chambers per tree that were parasitized varied among sites in 2017 (χ^2=53.59; df=3,162; P<0.0001), 2018 (χ^2=118.80; df=5,314; P<0.0001) and 2019 (χ^2=97.84; df=6,335; P<0.0001) (Figure 1.12). Galls in trees at the SWMREC site and the FL site, both originally infested by ACGW in 2015, consistently had higher parasitism rates than other sites across all three years (Figure 1.12). Parasitism rates of galls and ACGW larvae were at least twice as high in these sites compared with any other site (Table 1.6).

Mean proportion of ACGW gall chambers per gall that were parasitized did not vary among gall types in 2018 (χ^2=5.86; df=2,332; P=0.054) or 2019 (χ^2=1.13; df=2,695; P=0.57). In 2018, the proportion of apical, lateral and leaf gall chambers that contained a parasitoid larva averaged 0.04 ± 0.02, 0.09 ± 0.02 and 0.11 ± 0.03, respectively. In 2019, the proportion of apical, lateral and leaf gall chambers that contained a parasitoid larva averaged 0.19 ± 0.06, 0.26 ± 0.02 and 0.28 ± 0.03, respectively.

Discussion

Although calendar dates provide a general timeline for activity and development of insect life stages, annual variation in spring or summer weather can be substantial (Ascerno 1991). Previous observations of ACGW phenology that reported calendar dates associated with specific life stages (e.g. Cho and Lee 1963, Otake 1980, EPPO 2005 and Bernardo et al. 2013) are obviously useful. Providing only calendar dates, however, makes it difficult to predict ACGW phenological events in other regions with different climates or in years with unusual weather. Cumulative growing degree days provide a means to standardize phenological development of insects as well as trees, among years and locations (Wilson and Barnett 1983). Our study benefitted from the extensive Michigan State University Enviro-weather network, comprised of 96 weather stations distributed across the state (https://enviroweather.msu.edu/) (Bishop 2016, Mason 2019). Other weather-related websites, however, such as the National Oceanic and Atmospheric Administration, provide growing degree day information across most of the U.S. (https://www.noaa.gov/).

Phenological development of ACGW life stages, as well as chestnut tree development, were generally consistent among the sites we monitored and across years in terms of cumulative degree days, although calendar dates varied, particularly among years. We first observed galls forming in early to mid May (100-190 DD_{10C}), just after the onset of bud break on chestnut trees. This timing presumably facilitates the ability of overwintered ACGW larvae to access nutrients translocated into expanding shoots and leaves. We also noted that adult ACGW emergence consistently began between 125 and 170 $DD_{10C,}$ which occurred after peak pollen production, by which time most catkins had dropped from trees.

Both the onset of larval development and gall formation as along with adult wasp emergence have practical implications for commercial growers. For example, scouting to assess

ACGW infestation levels can begin soon after chestnuts break bud. Little overlap between ACGW adult activity and abundant pollen indicates cover sprays of insecticides targeting ACGW adults should have few impacts on pollinator populations. Moreover, scouting trees and monitoring pollen production, along with cumulative degree days, can provide commercial growers with a fairly precise window for applying insecticide sprays.

Our observations of ACGW activity are fairly similar to reports from areas of Asia and Europe, but differed from observations in Georgia. In eastern Asia, ACGW larvae were observed within galls until mid June, pupae were present from early June to mid July and adult wasps were present in galls from mid June to mid July (Cho and Lee 1963, Otake 1980). In southern Italy, ACGW larvae were present in galls until mid June, pupation occurred from mid May through early July and adult wasps were seen in galls from early June through late July (EPPO 2005, Bernardo et al. 2013). In the Georgia study, larvae were feeding in galls by late March, just before trees broke bud, while adult wasp emergence from galls began in late May and early June (Payne 1978). Activity and development of ACGW in the southwest Michigan orchards we studied was typically five to six weeks later than in Georgia, reflecting the shorter winters and warmer climate in Georgia. Southwest Michigan is categorized in a lower plant hardiness zone with a 6a rating, compared to Peach County, Georgia with an 8a rating (USDA 2012).

The notable decrease in abundance of ACGW galls from 2018 to 2019 was observed in all fields we monitored, regardless of infestation history or gall densities in preceding years. Overall, densities of current-year galls were 73% and 65% lower in 2019 than in 2018 and 2017, respectively. We suspect this dramatic drop resulted from mortality of overwintering ACGW larvae. Mean daily minimum temperatures, retrieved from nearby MSU Enviroweather stations, were similar across southwest Michigan from 1 December to 28 February in 2018 and 2019,

averaging -6.4°C and -6.0°C, respectively, but the timing of extreme minimum temperatures differed. During the 2017-2018 winter, the coldest days occurred between 31 December 2017 and 3 January 2018, when minimum temperatures ranged from -17.6°C to -22.0°C. In the 2018-2019 winter, however, the coldest days occurred a month later, between 29 January and 1 February 2019, when extreme minimum temperatures reached -19.0°C to -26.7°C. We cannot determine whether the high mortality resulted from the slightly colder temperatures in 2019 or if larvae become more vulnerable to cold in late January than in late December.

In a recent study, Bonsignore et al. (2020) collected ACGW galls weekly between early May and late July, then experimentally exposed galls to seven days of temperatures controlled at 8°C. They found that fewer ACGW adults emerged from the cold-stressed galls than galls held at room temperature (14 to 25°C) (Bonsignore et al. 2020). Several studies with hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) have evaluated relationships between insect mortality and cold winter temperatures in the eastern U.S. (e.g., Paradis et al. 2008, Elkinton et al. 2017, McAvoy et al. 2017). Exposure to cold temperatures in late winter resulted in higher HWA mortality than exposure to the same temperatures earlier in the winter (Skinner et al. 2003). Evidence of genetic-based acclimation by HWA populations was reported in New England (Elkinton et al. 2017). While sudden drops in winter temperatures still caused high HWA mortality, frequent exposure to cold temperatures resulted in lower supercooling points and less winter mortality (Elkinton et al. 2017). Assuming the dramatic decline in ACGW galls we observed in 2019 was a result of extreme cold temperatures, it seems likely that stochastic winter weather in Michigan could periodically limit ACGW density and spread. Further monitoring of infested fields in southwest Michigan, which represents the most northerly distribution of

ACGW in the U.S., will be needed to more fully assess effects of winter temperatures on larval survival, population dynamics and potential distribution.

Our results show the ACGW larval parasitoid *T. sinensis* is established in Michigan and densities appear to be increasing within individual orchards. Spread of *T. sinensis* in Michigan is not far behind the progression of ACGW; parasitized ACGW larvae were observed only a year after ACGW became established in four sites and *T. sinensis* was present in seven of the nine sites we sampled in 2019. In contrast to ACGW larvae that overwinter in buds, *T. sinensis* larvae overwinter within galls, which may provide more protection from extremely cold temperatures. Parasitism rates increased notably from 2018 to 2019, presumably reflecting the low densities of current-year galls available for oviposition by adult *T. sinensis*.

Establishment and ongoing natural dispersal of *T. sinensis* indicates efforts to rear and introduce this parasitoid into Michigan chestnut orchards are unlikely to be necessary. Rearing involves collecting withered galls in fall from locations where *T. sinensis* is present, then ensuring development and release of adult parasitoids in spring is synchronized with ACGW larval development, a process that can be challenging (Quacchia et al. 2008). Natural dispersal of *T. sinensis* was recently observed in several European countries, including Slovenia, Hungary and Bosnia and Herzegovina, providing further reason to expect the parasitoid will continue spreading in Michigan (Avtzis et al. 2019). In the U.S., there was little effort to track *T. sinensis* after its initial release in 1977 in Georgia. However, it was widely distributed in eastern states by 2005, presumably reflecting either its natural dispersal or transport of parasitized galls on chestnut saplings (Cooper and Rieske 2007, Cooper and Rieske-Kinney 2007).

Declines in ACGW populations following establishment of *T. sinensis* were reported in Georgia, as well as in Italy and at least four other European countries (Rieske 2007, Avtzis et al.

2019, Ferracini et al. 2019). In Japan, after release of *T. sinensis*, ACGW infestation levels were reduced to less than one tenth of the initial infestation levels in just five years (Moriya et al. 1989). In northern Italy, ACGW populations noticeably decreased seven to eight years after *T. sinensis* establishment and parasitism rates exceeded 90% approximately 13 years following establishment, allowing chestnut production to steadily recover (Avtzis et al. 2019, Ferracini et al. 2019). In Japan and Italy, however, host-parasitoid dynamics may exhibit a boom-bust cycle (Shiga 2009, Ferracini et al. 2019). Since ACGW has only been in Michigan since 2015, continued monitoring will be necessary to understand the dynamics of this host-parasitoid interaction. Substantial increases in *T. sinensis* parasitism between 2017 and 2018 were observed in two of four sites, which is promising. High parasitism rates in 2019, however, are confounded with the dramatic decrease in the density of current-year galls. Further evaluation of *T. sinensis* parasitism rates in relation to ACGW density will be needed to determine whether the parasitoid will effectively maintain ACGW populations below damaging levels.

We originally hypothesized that in the HS site, Dunstan chestnut (*C. mollissima* x *C. dentata*) trees would have lower gall densities than Colossal (*C. crenata* x *C. sativa*) trees, reflecting the coevolutionary history between ACGW and *C. mollissima* trees in China. Results from the HS site where the two cultivars were planted in alternating rows and experienced the same environmental conditions, supported our hypothesis. On average, densities of current-year galls were at least 3.2 galls per meter higher on Colossal trees than on Dunstan trees in both 2017 and 2018 and maximum gall densities on Colossal trees were at least 1.4 times greater than on Dunstan trees.

Densities of apical, lateral and leaf galls also differed between Dunstan and Colossal trees at the HS site. Lateral galls, which could occur anywhere along the shoots, were consistently

more abundant than apical galls, which were limited by the number of branch tips on the shoot, and leaf galls. Lateral galls on branches can repress bud production and shoot growth, cause branch dieback and make a tree more vulnerable to secondary infection by pathogens (Gehring et al. 2018). Apical galls are especially damaging, however, as they can inhibit flower production and subsequent burr formation (Panzavolta et al. 2012, Battisti et al. 2014). Leaf galls do not directly limit burr production, but high densities can reduce leaf area and photosynthesis rates, leading to an overall reduction in tree vigor (Ugolini et al. 2014).

Varying levels of ACGW resistance among chestnut species and hybrids have been previously observed (Shimura 1972, Anagnostakis et al. 2009, 2011, Panzavolta et al. 2012, Metaxas 2013, Sartor et al. 2015). Studies from China, Japan and Italy have addressed ACGW resistance of *C. mollissima* cultivars, including Chushugong and Mifengqiu, *C. crenata* cultivars, including Ishizuchi and Tsukuba, and *C. sativa* cultivars, including Pugnenga and Savoye (Shimura 1972, Metaxas 2013, Sartor et al. 2015). Some hybrid cultivars have reportedly demonstrated resistance to ACGW, including a *C. crenata* x *C. sativa* cultivar (Bouche de Betizac), along with a *C. crenata* x *C. mollissima* cultivar (Dabeo), and some experimental cultivars of *C. dentata* x (*C. ozarkensis* x *C. mollissima*) and *C. dentata* x (*C. pumila* x *C. mollissima*) (Anagnostakis et al. 2009, 2011, Metaxas 2013, Sartor et al. 2015). But none of those studies included Colossal (*C. crenata* x *C. sativa*) trees.

Resistance to ACGW has been attributed to various traits, including phenological development. In an Alabama study, susceptibility of a *C. mollissima* cultivar, AU-Leader, was attributed to the presence of mature buds in mid summer when newly emerged adult ACGW wasps were ovipositing, while a second cultivar which was considered resistant, AU-54-17, had only a few, mostly underdeveloped buds available to emerging ACGW adults (Huang et al.

1990). In addition to phenological development, Huang et al. (1990) also reported resistance to ACGW was associated with bud structure, along with specific bark volatiles produced during ACGW adult emergence. Other traits reportedly associated with varying ACGW resistance among chestnut species or cultivars include morphological characteristics, volatile compounds, differential gene expression, and a hypersensitive reaction in bud tissues (Shimura 1972, Huang et al. 1990, Dini et al. 2012, Acquadro et al. 2020).

While any conclusions based on our data from trees at the SWMREC site are limited by the lack of replication, resistance did vary substantially among cultivars of each species. This site, which was first infested in 2015, is relatively small with closely spaced trees (3-6m) and all but one (*C. crenata* x *sativa* 'Nevada') of the 42 trees we sampled had ACGW galls. Given these conditions, the variation in gall density among *C. mollissima* cultivars was somewhat surprising. Some *C. mollissima* trees had high ACGW gall densities relative to other trees in the field, such as the G-142 and Douglass cultivars with 46.2 and 22.1 galls per m, respectively, while others had low gall densities, such as Qing and Everfresh with 2.2 and 2.5 galls per m, respectively. Variation also occurred among cultivars of the hybrid *C. crenata* x *C. sativa*, ranging from on the single Nevada tree which had no galls to an average of 13.4 ± 2.1 galls per m on Colossal trees. Additional research in fields with higher numbers of potentially resistant and highly susceptible cultivars would be useful for screening ACGW impacts and how gall densities vary over time.

APPENDICES

APPENDIX A. Tables and Figures

Table 1.1. Number, species, cultivar, mean (±SE) DBH of sampled chestnut trees and length of shoots collected from those trees to assess gall density and Asian chestnut gall wasp life stages in 2017-2019 at the SWMREC site, which was first infested in 2015.

		2017			2018			2019		
Species	Cultivar	No. trees sampled	Tree DBH (cm)	Shoot length (cm)[1]	No. trees sampled	Tree DBH (cm)	Shoot length (cm)[1]	No. trees sampled	Tree DBH (cm)	Shoot length (cm)[1]
C. mollissima	Amy	-			1	22.2	304.0	1	23.4	96.5 ± 12.0
	Benton Harbor	2	48.4 ± 18.8	127.7 ± 12.4	2	49.3 ± 18.5	249.0 ± 15.6	2	39.5 ± 10.8	100.2 ± 8.7
	Bruce	-			-			1	13.6	100.0
	Douglas	-			1	36.0	190.0	1	36.9	167.0
	Dunston Revival	-			1	34.7	97.0	2	27.9 ± 5.9	103.0 ± 5.0
	Eaton	-			-			1	24.6	94.5
	Everfresh	2	32.2 ± 9.4	143.2 ± 13.1	2	32.4 ± 10.0	194.5 ± 32.2	2	33.9 ± 9.7	150.0 ± 37.1
	G-142	-			1	21.8	78.0	2	21.1 ± 0.8	102.8 ± 18.2
	Gideon	2	15.0 ± 2.2	136.6 ± 15.6	2	15.2 ± 1.6	175.5 ± 19.5	4	18.0 ± 2.7	100.3 ± 8.6
	H2	-			-			1	25.1	111.5
	Hong Kong	-			1	7.8	106.0	1	10.7	64.0
	J-177	-			-			1	27.9	178.0
	J-26	-			1	20.5	132.0	1	21.2	156.0
	Miller	-			-			1	24.9	151.0
	Mossbarger	-			1	29.5	353.0	2	30.9 ± 1.0	113.8 ± 3.4
	Orrin	-			-			1	26.1	310.5
	Payne	-			1	3.6	95.0	1	4.9	33.0

Table 1.1. (cont'd)

	Peach	2	31.9 ± 5.3	124.1 ± 16.1	2	26.8 ± 1.2	251.0 ± 70.7	2	29.2 ± 2.3	161.7 ± 23.0
	Qing	2	20.1 ± 2.1	138.7 ± 13.4	2	16.6 ± 1.1	111.5 ± 13.8	2	17.9 ± 1.1	111.8 ± 13.3
	Shing	-			-			1	9.9	91.0
	Sleeping Giant	-			1	8.8	164.0	1	9.5	49.0
	Willamette	-			-			1	28.6	176.5
C. crenata	Labor Day	2	13.6 ± 0.6	109.3 ± 12.5	2	13.6 ± 1.3	166.5 ± 17.3	2	13.9 ± 1.2	211.5 ± 96.5
	J-65	-			1	21.2	294.0	1	23.5	58.5
C. sativa x C. crenata	Colossal	5	29.3 ± 4.1	132.2 ± 15.4	5	31.9 ± 7.3	133.0 ± 23.3	4	36.2 ± 5.0	195.9 ± 34.6
	Marki	-			1	27.1	97.0	1	28.0	90.0
	Marsol	-			2	16.9 ± 7.4	145.8 ± 30.2	1	27.7	229.5
	Nevada	-			1	31.6	101.0	1	34.1	143.5

[1]Mean and SE values are presented if more than one tree of a specific cultivar was sampled and multiple shoots were collected.

Table 1.2. Year of initial Asian chestnut gall wasp infestation, field size, chestnut species and cultivar (when available) in fields sampled in 2017-2019 in southwest Michigan. Number and mean (±SE) DBH of trees sampled, length of shoots collected from sampled trees and total number of galls dissected per field are shown by year.

Site	First infested	Size (ha)	Species/ Cultivar	Years sampled	No. trees sampled	DBH (cm)	Shoot length (cm)	No. galls dissected
HS	2015	2.07	*C. dentata* x *mollissima* 'Dunstan'	2017	39	18.3 ± 0.8	105.7 ± 5.0	58
				2018	81	18.9 ± 0.7	136.2 ± 4.7	101
				2019	93	22.8 ± 0.7	119.2 ± 5.8	187
			C. crenata x *C. sativa* 'Colossal'	2017	39	28.1 ± 1.8	103.4 ± 4.9	88
				2018	81	25.7 ± 0.9	145.4 ± 5.2	98
				2019	93	26.8 ± 0.7	111.9 ± 4.6	110
FL	2015	0.28	*C. mollissima*	2017	36	15.3 ± 1.0	205.7 ± 10.4	110
				2018	16	16.9 ± 0.9	170.4 ± 9.9	16
				2019	12	20.5 ± 0.9	145.5 ± 12.4	32
ET	2016	0.64	*C. mollissima*	2017	46	28.3 ± 1.2	144.3 ± 6.7	55
				2018	40	29.8 ± 1.1	149.6 ± 11.9	40
				2019	89	33.0 ± 1.3	124.5 ± 5.7	123
PD	2016	3.34	*C. mollissima*	2017	26	26.8 ± 1.2	174.8 ± 17.8	32
CB	2017	2.53	*C. mollissima*	2018	20	16.1 ± 0.6	141.7 ± 9.1	20
				2019	10	18.1 ± 1.1	118.4 ± 9.0	46
			C. sativa	2018	20	16.5 ± 1.1	104.7 ± 8.9	20
				2019	10	17.4 ± 2.0	97.2 ± 8.3	16
CM	2017	2.03	*C. mollissima*	2018	32	28.3 ± 1.2	122.8 ± 9.5	34
				2019	30	30.6 ± 1.7	119.3 ± 11.7	62
BK	2017	1.17	*C. mollissima*	2018	32	27.5 ± 1.4	147.1 ± 8.2	26
				2019	12	29.0 ± 1.4	146.4 ± 23.2	10
FV	2018	0.72	*C. mollissima*	2019	16	30.6 ± 1.9	112.2 ± 10.5	74
HR	2018	0.40	*C. mollissima*	2019	12	39.3 ± 2.0	101.2 ± 17.3	13

Table 1.3. Date and growing degree day accumulation[1] (base 10°C) corresponding to phenological stages recorded during visits to three southwest Michigan orchards in 2017-2019. Degrees day values for each date were acquired from three weather stations[2]. Missing values indicate that observations were not collected on that date.

Phenological category	Stage	Criteria	Site	2017		2018		2019	
				Date	DD_{10C}	Date	DD_{10C}	Date	DD_{10C}
Bud break	0	Buds dormant; protected by scales	SWMREC	-	-	3 May	68	6 May	80
			HS	-	-	3 May	75	6 May	86
			ET	-	-	3 May	79	6 May	95
	1	Bud scales separating; interior scales visible	SWMREC	5 May	187	9 May	111	16 May	105
			HS	-	-	9 May	120	16 May	114
			ET	-	-	15 May	151	16 May	126
	2	Leaves emerging but tightly rolled	SWMREC	-	-	15 May	134	-	-
			HS	-	-	15 May	148	-	-
			ET	-	-	-	-	-	-
	3	One or more leaves fully emerged	SWMREC	16 May	232	23 May	177	22 May	135
			HS	16 May	192	23 May	196	22 May	146
			ET	-	-	23 May	200	22 May	160
Leaf development	0[3]	Not all leaves fully emerged	-	-	-	-	-	-	-
	1	Leaves emerged; < 2.5 cm long	SWMREC	-	-	15 May	134	29 May	180
			HS	-	-	23 May	196	29 May	195
			ET	-	-	23 May	200	29 May	210
	2	Leaves > 2.5 cm long; not fully expanded	SWMREC	24 May	282	23 May	177	12 June	291
			HS	24 May	244	-	-	4 June	240
			ET	-	-	5 June	365	4 June	251
	3	Leaves fully expanded	SWMREC	6 June	377	5 June	333	18 June	332
			HS	6 June	342	5 June	356	12 June	314
			ET	13 June	463	13 June	444	12 June	326
Catkin development	0[3]	No catkins present	-	-	-	-	-	-	-
	1	Immature catkins observed	SWMREC	16 May	232	23 May	177	29 May	180
			HS	24 May	244	23 May	196	29 May	195

Table 1.3. (cont'd)

			ET	-	-	23 May	200	4 June	251
	2	Pollen appears on catkins	SWMREC	13 June	464	26 June	555	2 July	496
			HS	21 June	527	21 June	537	2 July	527
			ET	21 June	564	26 June	588	2 July	540
	3	Pollen abundant	SWMREC	27 June	619	29 June	591	10 July	609
			HS	27 June	580	29 June	620	10 July	643
			ET	27 June	618	29 June	626	10 July	661
	4	Pollen shed; catkins desiccated and dropping	SWMREC	6 July	729	2 July	639	16 July	688
			HS	6 July	690	2 July	669	16 July	725
			ET	6 July	729	2 July	677	16 July	744
Burr development	0[3]	Burrs absent	-	-	-	-	-	-	-
	1	Small burrs appear	SWMREC	6 July	729	2 July	639	16 July	688
			HS	6 July	690	2 July	669	16 July	725
			ET	6 July	729	2 July	677	16 July	744
	2[4]	Burrs growing; still green	-	-	-	-	-	-	-
	3	Burrs brown and opening; nuts dropping	SWMREC	30 Sept	1597	2 Oct.	1670	9 Oct.	1555
			HS	3 Oct.	1566	6 Oct.	1728	9 Oct.	1563
			ET	3 Oct.	1665	2 Oct.	1753	1 Oct.	1618

[1]Cumulative degree days calculated with Baskerville-Emin method, with a base threshold of 10°C and starting date of 1 January.
[2]Data were acquired from three MSU Enviro-weather stations in Benton Harbor, Berrien Springs and Grand Junction Michigan.
[3]Dates for stage 0 are assumed to be all dates prior to stage 1.
[4]Dates for stage 2 of burr development are assumed to be all dates between stages 1 and 3 of burr development.

Table 1.4. Number of trees sampled and densities (±SE) of current-year and previous-year galls by species and cultivar at the SWMREC site in 2017 and 2018.

Species	Cultivar	No. trees sampled	2017 Current-year gall density (gall/m)[1]	2017 Previous-year gall density (gall/m)[1]	2018 Current-year gall density (gall/m)[1]	2018 Previous-year gall density (gall/m)[1]
C. mollissima	Amy	1	-	-	13.5	2.3
	Benton Harbor	2	6.1 ± 0.8	1.8 ± 0.5	-	-
		2	-	-	8.2 ± 2.9	0.7 ± 0.5
	Douglas	1	-	-	22.1	5.3
	Dunston Revival	1	-	-	7.2	0
	Everfresh	2	2.5 ± 0.4	1.7 ± 0.6	-	-
		2	-	-	5.5 ± 0.37	0 ± 0
	G-142	1	-	-	46.2	9.0
	Gideon	2	3.2 ± 0.6	1.8 ± 0.3	-	-
		2	-	-	9.3 ± 0.4	2.3 ± 1.2
	Hong Kong	1	-	-	3.8	0.9
	J-26	1	-	-	12.1	3.8
	Mossbarger	1	-	-	4.7	0.9
	Payne	1	-	-	5.3	3.2
	Peach	2	5.9 ± 1.3	3.7 ± 1.0	-	-
		2	-	-	4.4 ± 1.7	0.6 ± 0.4
	Qing	2	2.2 ± 0.4	2.9 ± 0.5	-	-
		2	-	-	6.5 ± 0.8	2.3 ± 1.6
	Sleeping Giant	1	-	-	7.3	0.6
	Total	10	4.0 ± 0.4	2.4 ± 0.3	-	-
	Total	19	-	-	10.0 ± 2.2	2.0 ± 0.5
C. crenata	Labor Day	2	9.7 ± 1.3	3.3 ± 1.2	-	-
		2	-	-	17.8 ± 5.2	4.6 ± 0.3
	J-65	1	-	-	13.9	3.1
	Total	2	9.7 ± 1.3	3.3 ± 1.2	-	-
	Total	3	-	-	16.5 ± 3.6	4.1 ± 0.4

Table 1.4. (cont'd)

C. sativa x C. crenata	Colossal	5	10.1 ± 1.3	2.4 ± 0.7	-	-
		5	-	-	13.4 ± 2.1	5.9 ± 1.7
	Marki	1	-	-	4.5	0.7
	Marsol	1	-	-	8.0	1.8
	Nevada	1	-	-	0	0
	Total	5	10.1 ± 1.3	2.4 ± 0.7	-	-
	Total	8	-	-	9.3 ± 1.7	3.5 ± 1.1

[1]Cultivars where multiple trees were sampled give mean densities, while cultivars where only one tree was sampled give a single density.

Table 1.5. Number of trees sampled and mean (±SE), minimum and maximum density of current-year and previous-year galls of trees sampled by species/cultivar at five sites in 2017 and seven sites in 2018.

				Current-year gall density (galls per m)			Previous-year gall density (galls per m)		
Year	Site	Species/ Cultivar	No. trees sampled	Mean (±SE)	Min	Max	Mean (±SE)	Min	Max
2017	HS	*C. mollissima* x *C. dentata* 'Dunstan'	39	3.2 ± 0.3	0.3	7.4	0.2 ± 0.1	0	2.2
		C. crenata x *C. sativa* 'Colossal'	39	7.7 ± 0.7	0.8	18.9	0.3 ± 0.1	0	4.0
	FL	*C. mollissima*	39	4.5 ± 0.5	1.1	17.0	0.9 ± 0.1	0	3.5
	ET	*C. mollissima*	47	2.6 ± 0.4	0.2	19.1	0.02 ± 0.01	0	0.6
	PD	*C. mollissima*	26	1.2 ± 0.2	0	4.4	0.03 ± 0.02	0	0.6
2018	HS	*C. mollissima* x *C. dentata* 'Dunstan'	81	4.6 ± 0.3	0.8	13.0	0.5 ± 0.1	0	4.0
		C. crenata x *C. sativa* 'Colossal'	81	7.8 ± 0.4	0	18.2	1.3 ± 0.2	0	5.9

Table 1.5. (cont'd)

	FL	*C. mollissima*	16	9.3 ± 1.8	1.9	27.4	1.8 ± 0.4	0	4.8
	ET	*C. mollissima*	40	5.7 ± 0.6	0.4	15.2	0.2 ± 0.1	0	2.1
	CM	*C. mollissima*	32	4.9 ± 0.5	0.7	11.6	0.1 ± 0.1	0	1.7
	CB	*C. mollissima*	20	4.7 ± 0.6	1.0	10.4	0 ± 0	0	0
		C. sativa	20	8.2 ± 1.4	1.2	27.1	0.3 ± 0.2	0	2.9
	BK	*C. mollissima*	31	2.1 ± 0.3	0	6.3	0.2 ± 0.1	0	1.9

Table 1.6. Total number of galls dissected and parasitism rates of galls and ACGW larvae in fields sampled in 2017-2019. Percentage of galls parasitized indicates galls with at least one *T. sinensis* larva. Percentage of larvae parasitized represents individual ACGW larvae within galls that were parasitized.

	No. of galls dissected			**Percentage of galls parasitized**			**Percentage of larvae parasitized**		
Site	**2017**	**2018**	**2019**	**2017**	**2018**	**2019**	**2017**	**2018**	**2019**
SWMREC	108	40	108	23.7	70.0	77.8	10.2	39.2	71.0
HS	146	199	297	2.7	5.0	15.1	0.8	1.6	15.4
FL	110	16	32	27.3	50.0	65.6	16.7	12.8	66.7
ET	55	40	123	7.1	2.5	30.1	4.5	0.8	25.5
CM	-	34	62	-	2.9	12.9	-	1.2	12.3
CB	-	40	62	-	5.0	21.3	-	1.7	20.8
FV	-	-	74	-	-	5.5	-	-	3.3
HR	-	-	13	-	-	0	-	-	0

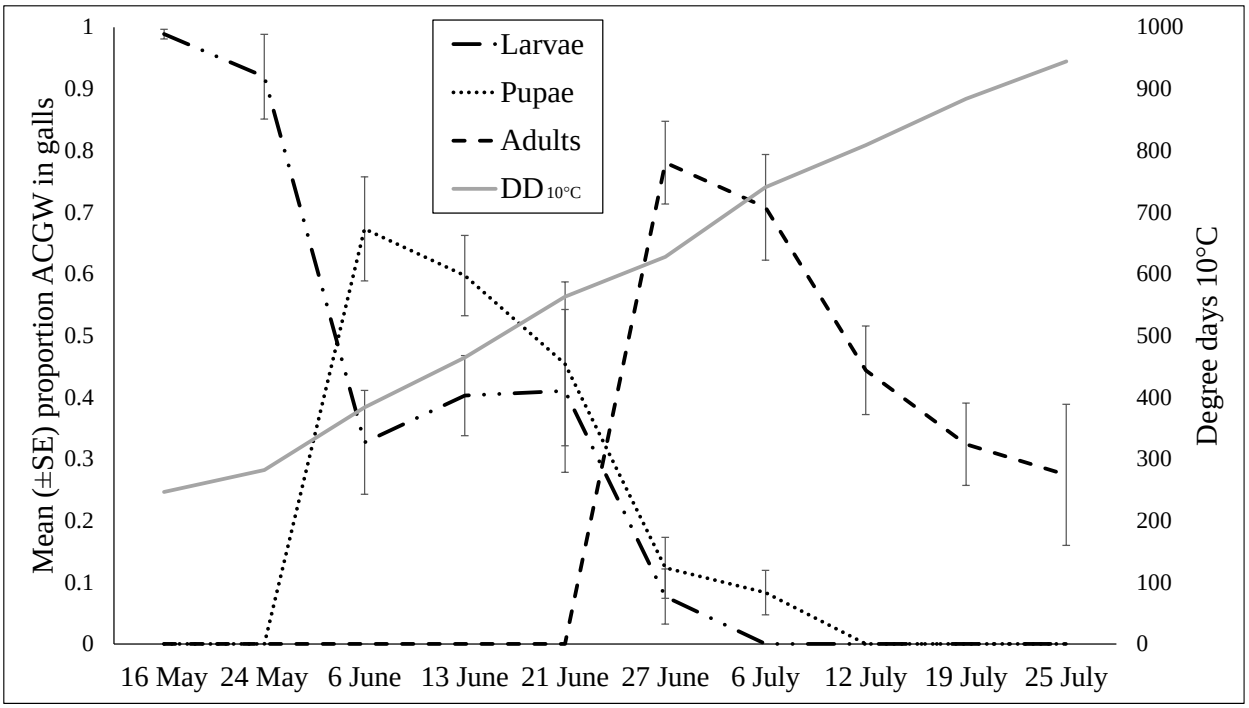

Figure 1.1. Date and cumulative growing degree days (base 10°C) associated with mean (±SE) proportions of ACGW by life stage in 308 dissected galls collected from 213 trees across five sites in 2017.

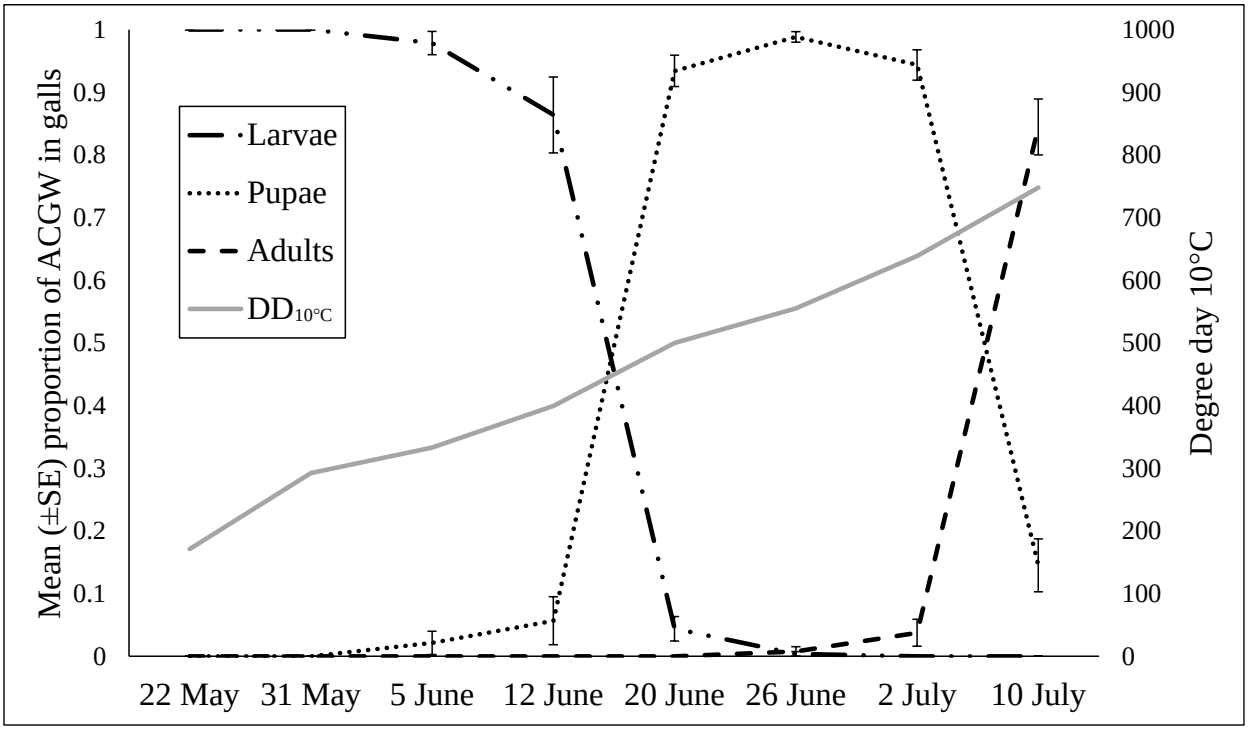

Figure 1.2. Date and cumulative growing degree days (base 10°C) associated with mean (±SE) proportions of ACGW by life stage in 395 dissected galls collected from 352 trees across seven sites in 2018.

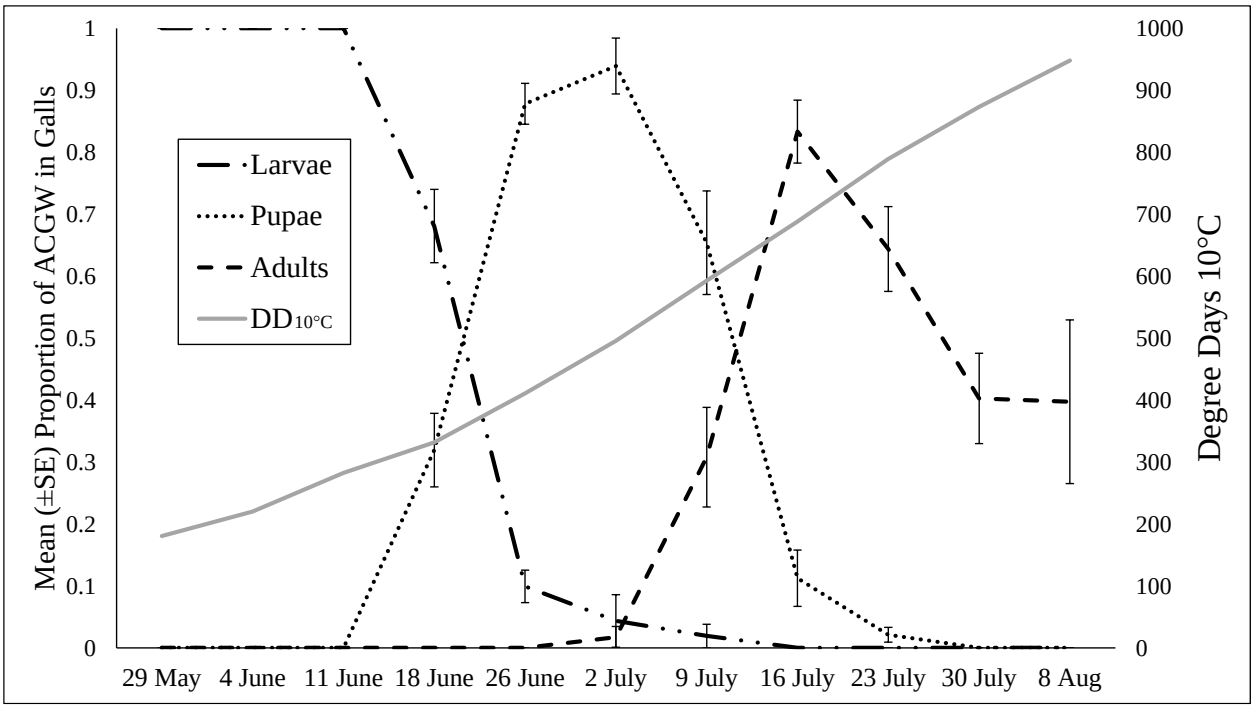

Figure 1.3. Date and cumulative growing degree days (base 10°C) associated with mean (±SE) proportions of ACGW by life stage in 779 dissected galls collected from 322 trees across nine sites in 2019.

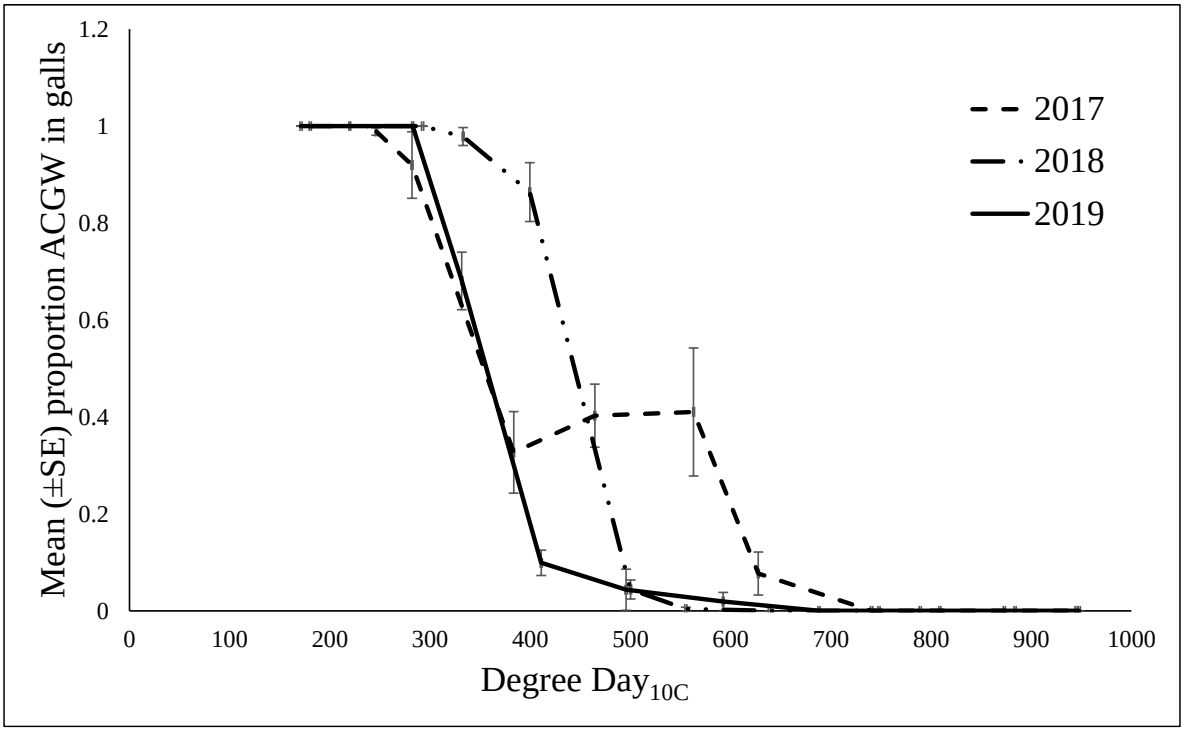

Figure 1.4. Mean (±SE) proportions of ACGW that were larvae in 308, 395 and 779 dissected galls collected in 2017, 2018 and 2019, respectively, and associated cumulative degree days (base 10°C). No larvae were observed in galls after 27 June 2017, 26 June 2018 and 9 July 2019.

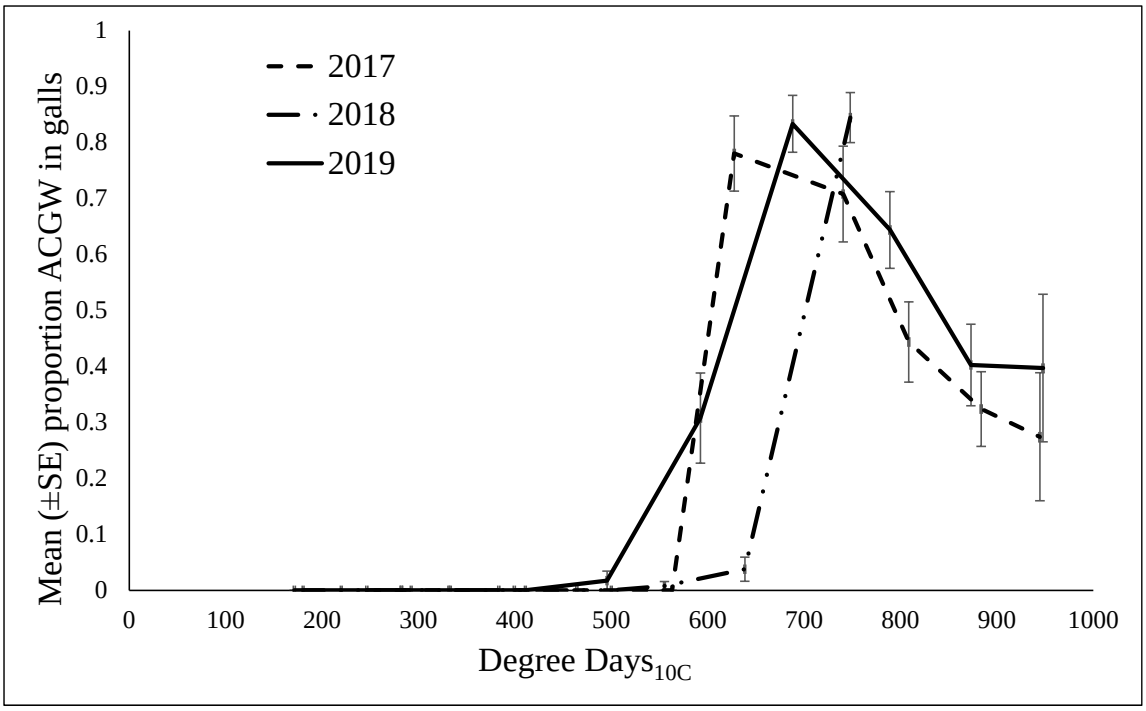

Figure 1.5. Mean (±SE) proportions of ACGW that were adults in 308, 395 and 779 dissected galls collected in 2017, 2018 and 2019, respectively, and associated cumulative degree days (base 10°C). Proportion of ACGW that were adults in galls peaked on 27 June 2017, 10 July 2018 and 16 July 2019.

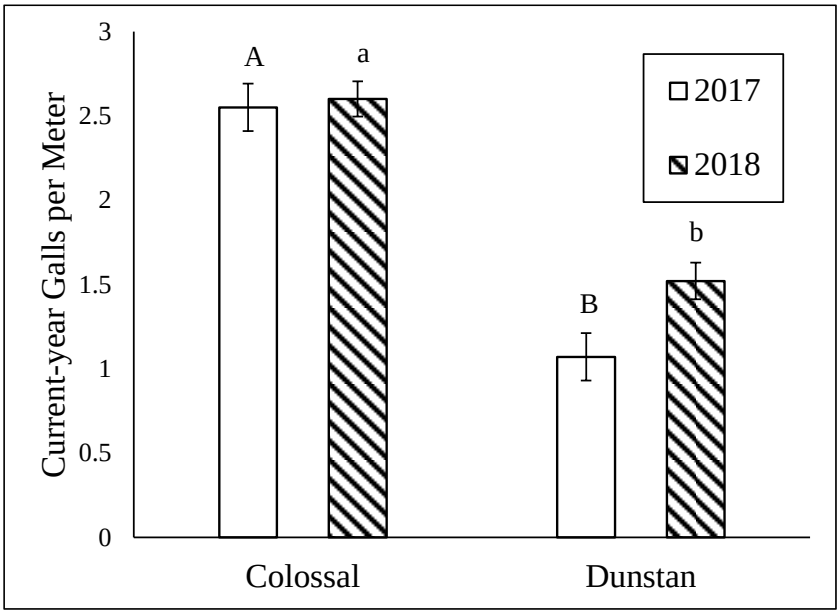

Figure 1.6. Mean (±SE) density of current-year galls collected from *C. mollissima* x *C. dentata* 'Dunstan' and *C. crenata* x *C. sativa* 'Colossal' chestnut trees at the HS site in 2017 and 2018. Galls were collected from 39 Colossal and Dunstan trees each in 2017 and 81 Colossal and Dunstan trees each in 2018. Capital letters indicate significant differences between 2017 densities and lower-case letters indicate significant differences between 2018 densities.

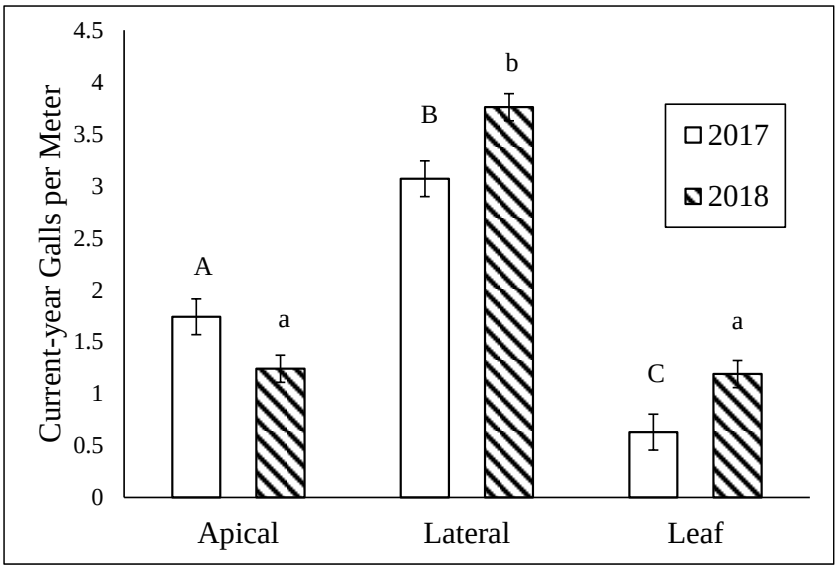

Figure 1.7. Mean (±SE) density of apical, lateral and leaf galls collected at the HS site from 39 *C. mollissima* x *C. dentata* 'Dunstan' and 39 *C. crenata* x *C. sativa* 'Colossal' chestnut trees in 2017 and 81 *C. mollissima* x *C. dentata* 'Dunstan' and 81 *C. crenata* x *C. sativa* 'Colossal' trees in 2018. Capital letters indicate significant differences among 2017 densities and lower-case letters indicate significant differences among 2018 densities.

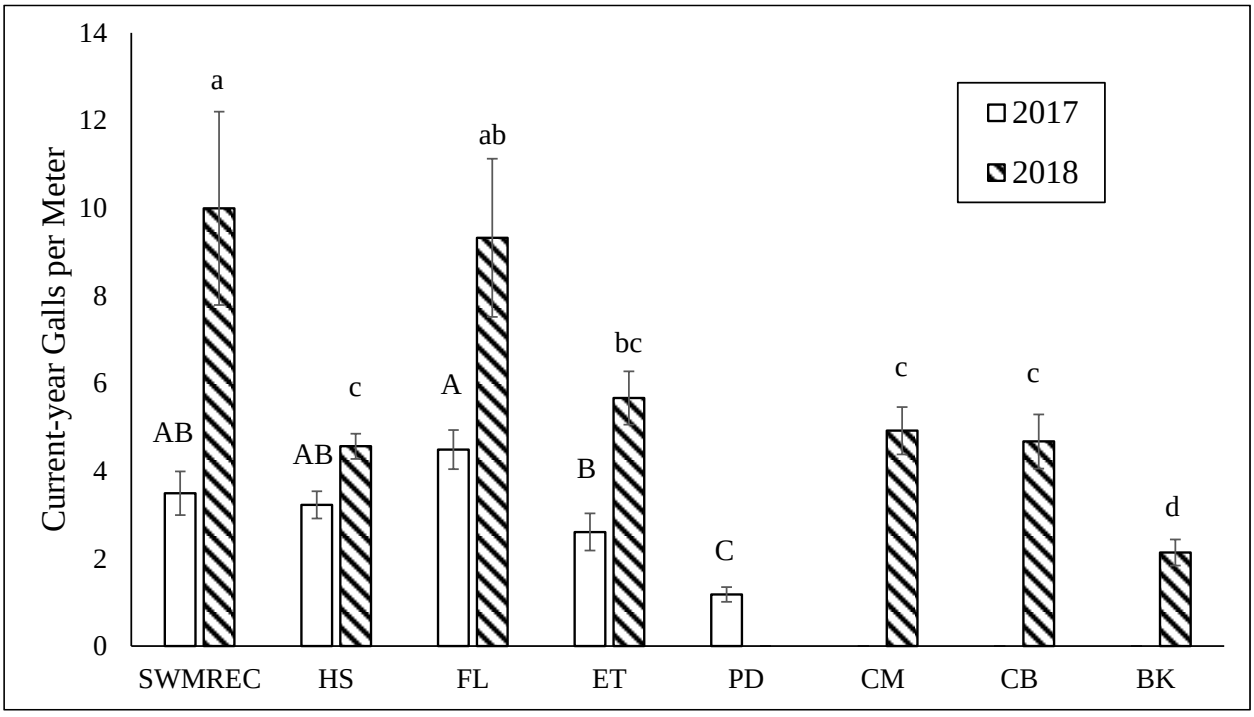

Figure 1.8. Mean (±SE) density of current-year galls collected from Chinese and Chinese hybrid chestnut trees at five sites in 2017 and seven sites in 2018. Galls were collected from 10, 39, 36, 47 and 26 trees in 2017 sites and 19, 78, 16, 40, 32, 20 and 31 trees in 2018 sites. Capital letters indicate significant differences among 2017 sites and lower-case letters indicate significant differences among 2018 sites.

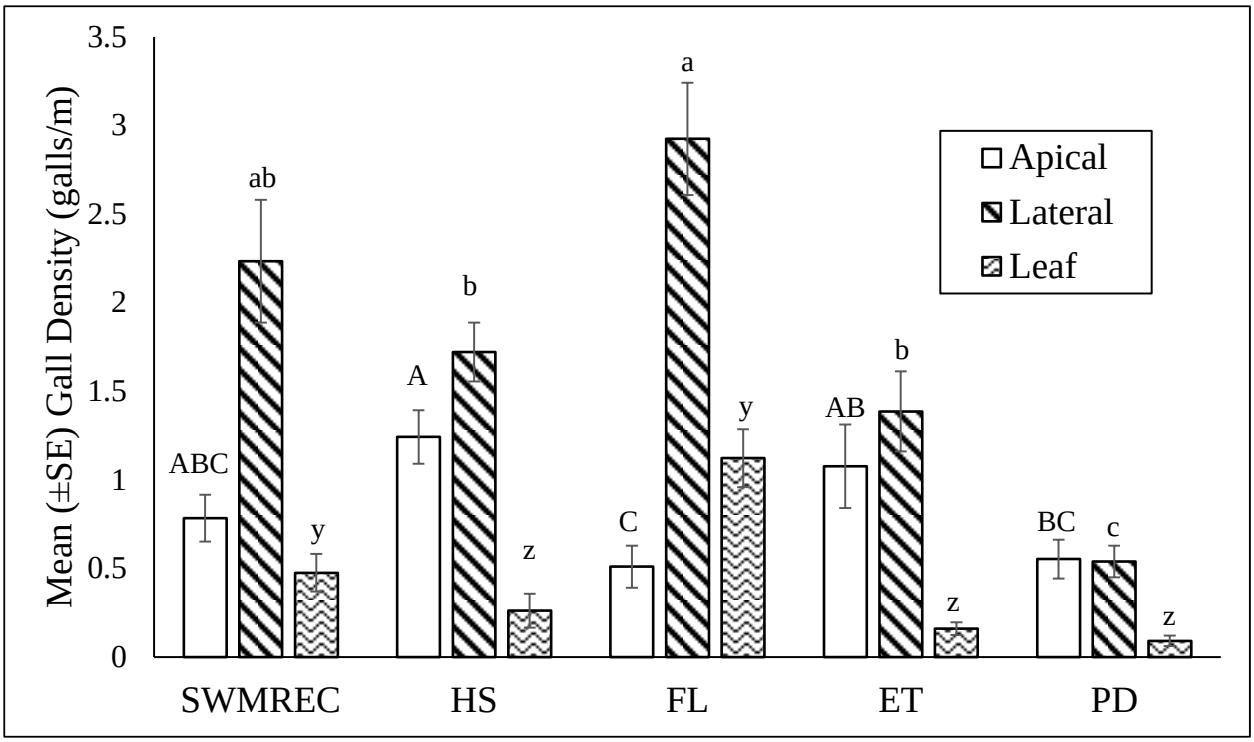

Figure 1.9. Mean (±SE) density of apical, lateral and leaf galls collected from 10, 39, 36, 47 and 26 Chinese and Chinese hybrid chestnut trees at the SWMREC, HS, FL, ET and PD sites in 2017, respectively. Capital a, b and c indicate significant differences among apical galls, lower case a, b and c indicate significant differences among lateral galls and lower-case y and z indicate significant differences among leaf galls.

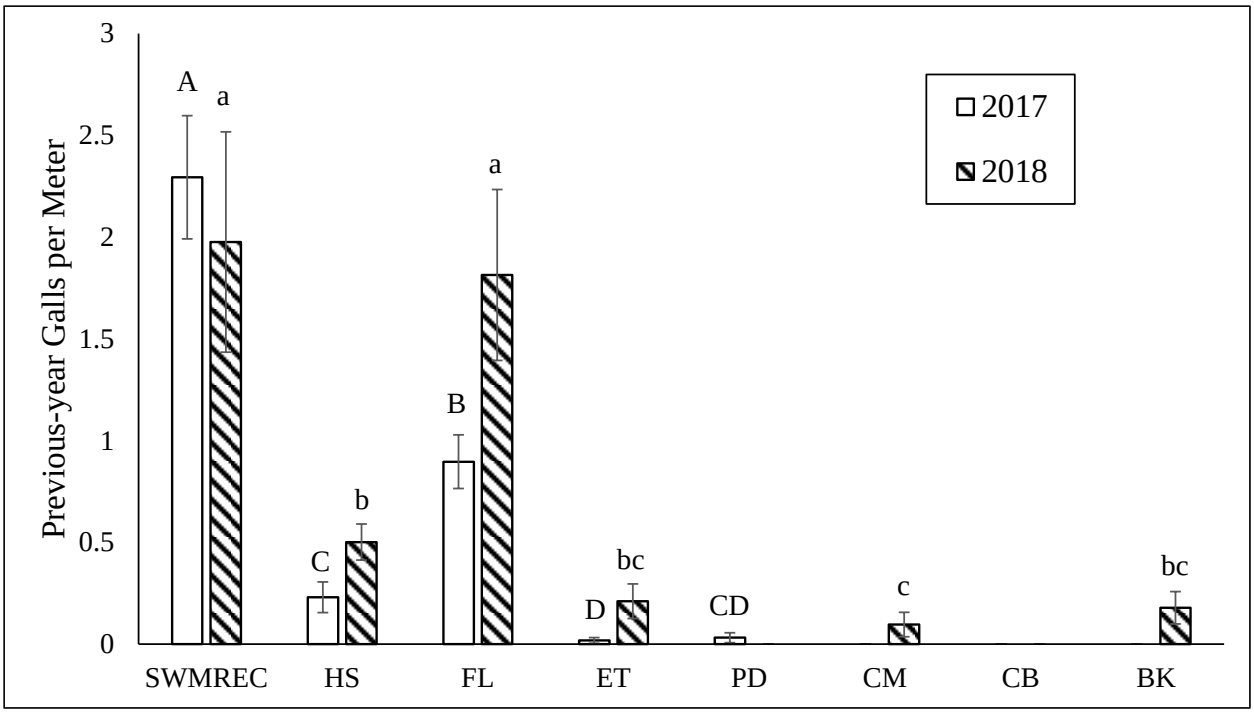

Figure 1.10. Mean (±SE) density of previous-year galls collected from Chinese and Chinese hybrid chestnut trees at five sites in 2017 and seven sites 2018. Galls were collected from 10, 39, 36, 47 and 26 trees at the SWMREC, HS, FL, ET and PD sites, respectively, in 2017 and 19, 78, 16, 40, 32, 20 and 31 trees at the SWMREC, HS, FL, ET, CM, CB and BK sites, respectively, in 2018. Capital letters indicate significant differences among 2017 sites and lower-case letters indicate significant differences among 2018 sites.

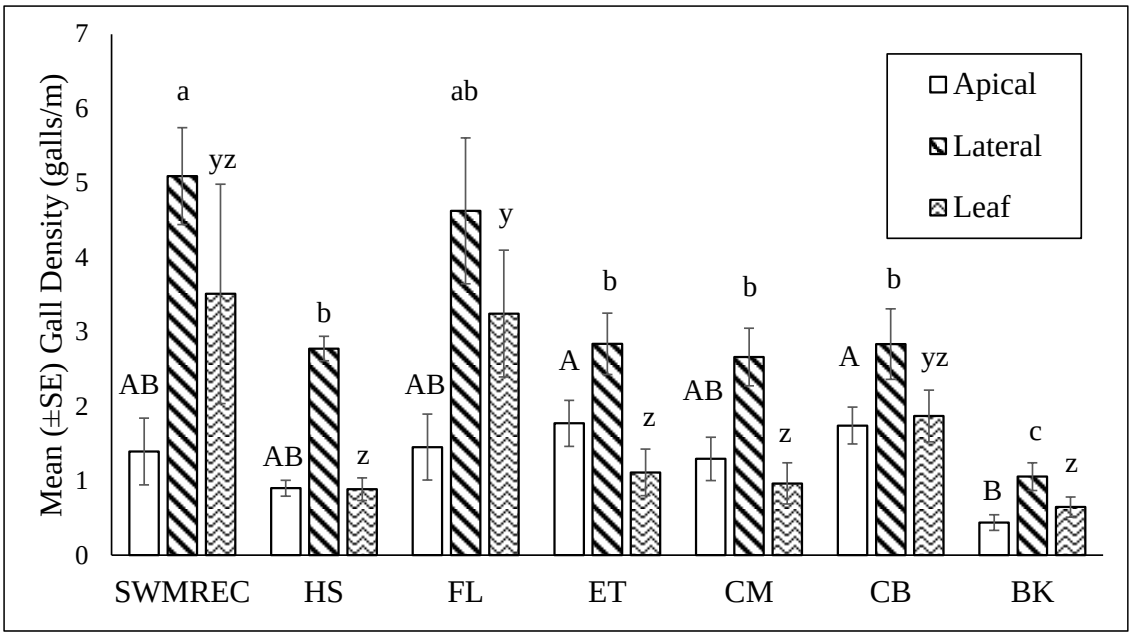

Figure 1.11. Mean (±SE) density of apical, lateral and leaf galls collected from Chinese and Chinese hybrid chestnut trees at seven sites in 2018. Galls were collected from 19, 78, 16, 40, 32, 20 and 31 trees in these sites. Capital a and b indicate significant differences among apical galls, lower case a, b and c indicate significant differences among lateral galls and lower-case y and z indicate significant differences among leaf galls.

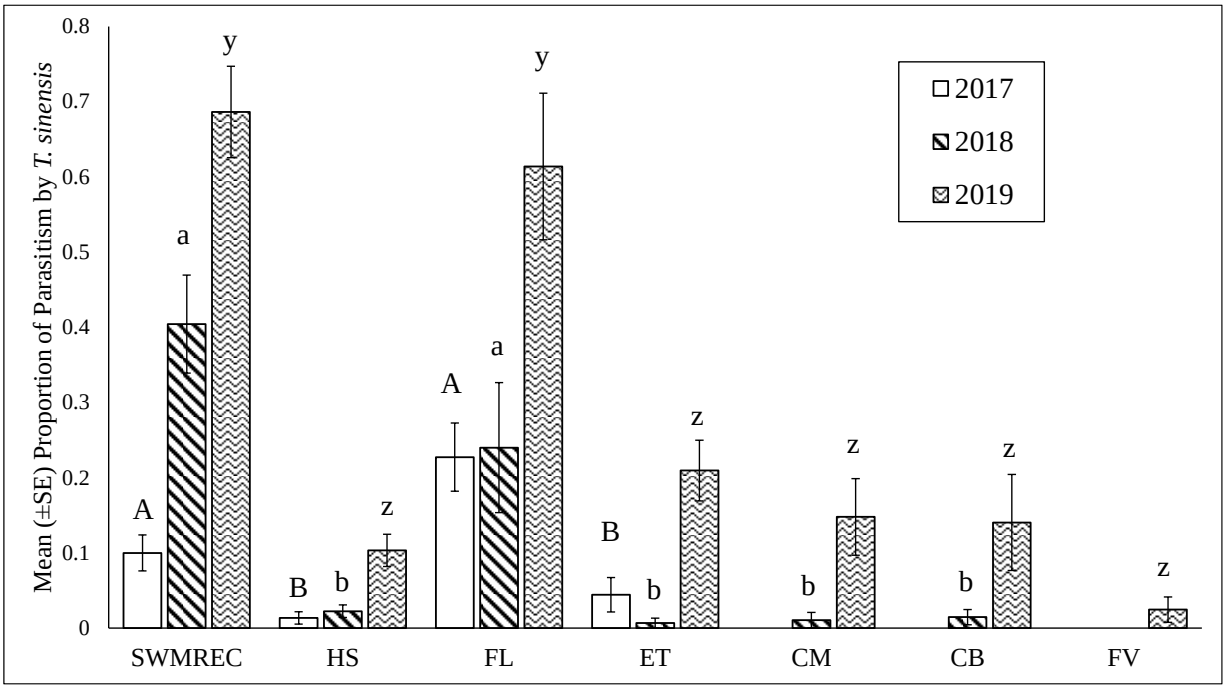

Figure 1.12. Mean (±SE) proportion of gall chambers per tree that contained the parasitoid *T. sinensis* from 2017 through 2019 in sites where it was present. Capital a and b indicate significant differences among 2017 sites, lower-case a and b indicate significant differences among 2018 sites and lower-case y and z indicate significant differences among 2019 sites.

APPENDIX B. Record of Deposition of Voucher Specimens

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2020-10
Author: Louise Labbate
Title of Thesis: Phenology, Density and Distribution of the Invasive Asian Chestnut Gall Wasp (*Dryocosmus kuriphilus* Yasumatsu) and Evaluation of Two Systemic Insecticides in Michigan Chestnut Orchards
Museum(s) where deposited: Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)
Specimens:

Table A.1 Quantity and preservation method of Asian chestnut gall wasp turned in as voucher specimens to the Albert J. Cook Arthropod Research Collection

Family	Genus-Species	Life Stage	Quantity	Preservation
Cynipidae	*Dryocosmus kuriphilus*	Adult	10	Pinned

CHAPTER 2: SPATIAL DISTRIBUTION AND SPREAD OF ASIAN CHESTNUT GALL WASP (HYMENOPTERA: CYNIPIDAE) (*DRYOCOSMUS KURIPHILUS* YASUMATSU) IN SOUTHWEST MICHIGAN

Introduction

Invasive species are a major threat to global food security and cause significant crop losses worldwide (Cook et al. 2011). The U.S., one of the largest agricultural producers in the world, sustains high economic costs from damage caused by invasive species (Paini et al. 2016). Invasive insects, including phloem and wood borers, sap feeders and foliage feeders, result in an estimated 3.5 billion USD annually in the U.S. in damages and pest control (Aukema et al. 2011). Introductions of non-native species to countries worldwide is predicted to continually rise with the consistent increases in global trade and demand for commodities, plus advancements in transportation (Levine and D'Antonio 2003, Hulme 2009).

Understanding the process and rate of spread of a new invasive species is an essential part of projecting distribution of the pest and developing a management strategy to mitigate damages. In general, there are three types of range expansion after invasion. Range expansion can occur outward from the edges of the primary population using solely short-distance dispersal, which usually results in a steady, linear rate of spread (Shigesada and Kawasaki 1997). It can also occur by employing long-distance migrants, in addition to short-range dispersal, to establish satellite colonies outside the primary population, but close enough that the colonies coalesce after a short period of time (Shigesada and Kawasaki 1997). This method starts with slow initial spread, but later increases to a higher rate of spread (Shigesada and Kawasaki 1997). Finally, expansion can occur again using short and long-distance dispersal, but satellite colonies are established far from the primary population and expand in isolation for a long period

(Shigesada and Kawasaki 1997). This results in a rate of spread that is continually increasing over time (Shigesada and Kawasaki 1997).

Asian chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus* Yasumatsu) (Hymenoptera: Cynipidae), a native of China, is a major invasive pest of chestnut (*Castanea* spp.) trees in Japan, Korea, much of Europe and is currently established in 14 states in the U.S. (EFSA 2010, Haack et al. 2011, Haack 2015). Larvae feed within small galls, 5-20 mm in diameter, that develop on expanding, current-year shoots and leaves in early spring. High densities of galls can affect tree vigor and reduce nut yields, causing costly damage to commercial chestnut orchards in Japan, Korea, the U.S. and Europe (Payne 1978, Dixon et al. 1986, Kato and Hijii 1997, EPPO 2005, Cooper and Rieske-Kinney 2007, Rieske 2007, EFSA 2010, Battisti et al. 2014, Ugolini et al. 2014, Sartor et al. 2015). Nut yield has been reported to be reduced by up to 80% in China and Italy, and by 50-70% in the U.S. (Payne et al. 1983, Dixon et al. 1986, Zhi-Yong 2009, Battisti et al. 2014). Branch dieback and even tree mortality have been observed in China, Japan, Korea, the U.S. and Italy when ACGW densities are high (Cho and Lee 1963, Payne 1978, Moriya et al. 2003, Zhi-Yong 2009, Ugolini et al. 2014).

In the U.S., ACGW was first detected in Peach County, Georgia in 1974 when a private grower imported infested scion wood from Japan (Payne et al. 1975, Anagnostakis 2012). Since then, ACGW has spread across much of the historical range of American chestnut (*Castanea dentata* (Marsh.) Borkh.) and as of January 2020, had been detected in 14 states (Cooper and Rieske-Kinney 2007, Haack et al. 2011, Haack 2015). Initial spread of ACGW was relatively slow, with detection in Tennessee in the mid 1980s and detection in North and South Carolina in the late 1990s (Haack et al. 2011). After 2000, rate of spread increased and by 2012, ACGW was detected in Massachusetts (Haack 2015).

Natural dispersal of ACGW, results from flight of adult wasps and is generally short range (EPPO 2005, Rieske 2007, Graziosi and Santi 2008, EFSA 2010). Studies from Japan showed that although the tiny ACGW are poor fliers, they passively disperse through wind assisted flight (EFSA 2010). Wind speed affects dispersal; winds of 0.15 to 0.45 m per second were identified as the optimal speed for transporting ACGW while wind speed over 0.73 m per second deter flight (EFSA 2010). Research in the U.S., Italy and Japan consistently found that ACGW could spread up to 25 km per year via wind assisted flight, consistently following prevailing winds (Rieske 2007, Graziosi and Santi 2008, EFSA 2010). Other invasive insects have also been known to utilize the wind for dispersal. First instar gypsy moth (*Lymantria dispar* Linnaeus) larvae suspend on silk threads can be blown by wind currents (Jankovic and Petrovskii 2013). Hemlock woolly adelgid (*Adelges tsugae* Annand) eggs, crawlers and winged adults can be blown by wind at least 1,350 m (McClure 1990). Beech scale (*Cryptococcus fagisugs* Lindinger) crawlers have dorsoventrally flatted bodies which increases their surface area facilitating efficient dispersal in wind (Wainhouse 1980).

Artificial dispersal, usually through the movement of infested plant material, can result in long distance spread of ACGW (EPPO 2005, Rieske 2007, Graziosi and Santi 2008, EFSA 2010). Chestnut nursery stock, seedlings and scion wood can bear ACGW life stages including eggs and larvae. The cryptic nature of the tiny first instar, which overwinter within buds, contributes to the difficulty of identifying infested material (EPPO 2005, EFSA 2010). Rate of spread of ACGW was more rapid in Italy than in the U.S., likely due to extensive exchange of infested nursery stock along with the continuity of chestnut trees in forests, orchards and landscapes (Graziosi and Santi 2008).

In the U.S., American chestnut distribution is fragmented, reflecting the impact of chestnut blight (*Cryphonectria parasitica*), an invasive fungus that invaded the eastern region of the U.S. and nearly eradicated native chestnut trees (Griffin 2000). Prior to chestnut blight, American chestnut was a major and often dominant forest species in the east, comprising an estimated 25% of the forest canopy in the Appalachians (Griffin 2000). While chestnut sprouts persist in many areas, most trees are infected and die before reaching maturity (Griffin 2000). Today, mature chestnut trees can more readily be found in commercial orchards. Although the commercial chestnut industry is relatively new in the U.S., many states across the country grow chestnut orchards, which primarily consist of the blight resistant Chinese chestnuts (*C. mollissima*) (Fulbright et al. 2010, Hochmuth et al. 2018). Major chestnut producing states include California, Washington, Oregon, Florida, Virginia, Ohio and Michigan (Hochmuth et al. 2018).

Natural dispersal of ACGW has been relatively slow, presumably reflecting the patchy and scattered distribution of chestnut trees in the U.S. For examples, ACGW was not observed in the Chattahoochee National Forest in northern Georgia, approximately 300 km from its origin in Peach County, until 1993 (Rieske 2007). Artificial dispersal has played a much larger role in the spread of ACGW. At least three distinct populations of ACGW in the U.S. have resulted from human transport of infested plant material (Rieske 2007, Lizotte and Fulbright 2015). In 2002, an ACGW infestation was identified in northern Ohio, first on three Chinese chestnut (*C. mollissima*) trees planted as ornamentals, then 41 km east in a commercial Chinese chestnut orchard (Rieske 2007). In 2006, a separate infestation, also in a commercial Chinese chestnut orchard, was detected on the border between Pennsylvania and Maryland (Rieske 2007). Most

recently, in 2015, a new ACGW infestation was identified in southwest Michigan in two commercial chestnut orchards (Lizotte and Fulbright 2015).

We monitored adult ACGW activity and distribution to assess spread of this invasive pest within commercial chestnut orchards across a 12,100 km^2 area in southwest Michigan from 2017 to 2019. We tracked onset and duration of adult ACGW activity in multiple orchards to determine the temporal period where natural dispersal occurs. Based on reports from other states and Europe, we expected annual spread rates to be approximately 25 km per year (Rieske 2007, Graziosi and Santi 2008, EFSA 2010). In addition, we monitored infestation levels of individual trees in two orchards with known invasion histories over the same three year period to assess localized dispersal of the insect. We were interested in determining whether an ACGW infestation diffused through an orchard due to localized movement of wasps from one tree to adjacent trees, or if scattered trees across the orchard became equally infested within one to two years.

Materials and Methods

Study Sites

In 2017, we identified five chestnut orchards in southwest Michigan where trees had ACGW galls along with six other sites where there was no evidence of ACGW. Four of the five infested sites were commercial chestnut orchards (HS, FL, ET, PD), ranging from 0.28 to 3.34 ha, while the fifth site was at the Michigan State University (MSU) Southwest Research and Extension Center (SWMREC) (Table 2.1). Five of the six uninfested sites were also commercial orchards (JN, CM, CB, BK, BY), ranging from 1.17 to 12.72 ha, while the sixth uninfested site (TT) consisted of a small group of 12 chestnut trees along a private road (Table 2.1).

In 2018, nine of the 2017 sites were again monitored and six new uninfested sites were added for a total of 15 sites. Two 2017 sites were excluded, TT because of its small size and JN because of the low number of ACGW adults captured in 2017. Five of the six new uninfested sites were commercial chestnut orchards (FV, HR, WN, RK, DK) ranging from 0.40 to 7.06 ha, while the fifth site was at the MSU Clarksville Research Center (CL) (Table 2.1).

In 2019, we monitored three sites (HS, ET, SWMREC) with known ACGW infestations and five of the 2018 sites (BY, WN, CL, RK, DK) which remained free of ACGW (Table 2.1). In 2019, however, ACGW abundance dropped sharply in all sites, as a result of unusually cold temperatures in late January 2019.

ACGW Adult Trapping

On 21 June 2017, six to 16 yellow sticky traps were deployed to monitor emergence of adult ACGW in the five sites with known ACGW infestations (Table 2.1). Number of traps was based on orchard size. Between 27 June and 12 July 2017, three to four yellow sticky traps were deployed in the six uninfested sites to determine if ACGW adults had invaded the site (Table 2.1). Timing of trap installation varied, depending on when growers provided access to orchards. Traps at all sites were collected and replaced weekly through 15 August. Collected traps were placed into individual plastic bags and returned to the MSU Forest Entomology laboratory. Each trap was examined under a magnifying glass to identify and count adult ACGW.

On 26 June 2018, four to six yellow sticky traps were deployed in eight sites with known ACGW infestations, three of which had become infested in 2017, to monitor the emergence of adult ACGW (Table 2.1). On 2 July, three to four yellow sticky traps were deployed in seven uninfested sites to monitor ACGW adult presence (Table 2.1). Traps at infested sites were

collected and replaced weekly while traps at uninfested sites were collected and replaced biweekly through 14 August.

On 2 July 2019, four to six yellow sticky traps were deployed in eight sites, including three known to be infested and five uninfested sites (Table 2.1). Traps at the three infested sites were collected and replaced weekly while traps at the uninfested sites were collected and replaced biweekly. Traps were monitored through 22 August.

Within Field Gall Distribution

In addition to trapping ACGW adults to monitor spread of this invader across the state, we also monitored two orchards with different invasion histories. We selected two relatively small sites where all trees were readily accessible. Our goal was to track localized ACGW distribution, spread and density following establishment within an orchard. In late May from 2017 to 2019, we recorded ACGW gall abundance on individual trees at the FL site, originally invaded in 2015, and at the ET site, invaded in 2016. At the FL site, four rows of *C. mollissima* trees were nine m apart. Within each row, nine trees were spaced 12 m apart (total of 36 trees). At the ET site, nine rows of *C. mollissima* trees spaced eight m apart, each included 13 trees also spaced eight m apart. Five trees were missing for a total of 112 trees.

At both sites, each tree was qualitatively evaluated and given a gall abundance rating of zero to five. Individual trees were examined on all sides for a total of two minutes. Trees were assigned a rating of 1 if ten or fewer galls had formed on them. Trees were assigned a rating of 2 if over ten galls had formed on them, but galls were sparsely scattered throughout the canopy of the tree. Trees were assigned a rating of 3 if approximately half of the tree branches had clusters of abundant galls. Trees were assigned a rating of 4 if most tree branches had galls formed on

them, with some branches having abundant galls and others having few galls. Trees were assigned a rating of 5 if most tree branches had an abundant number of galls formed on them.

Data Analysis

Minimum and maximum linear distances between previously and newly infested sites were determined per year from 2015 to 2019. Minimum distances were measured between the newly infested sites and the closest previously infested sites, while maximum distances were measured between the newly infested sites and the furthest previously infested sites.

To determine the overall spatial autocorrelation of the individual trees at the FL and ET sites, a spatial weight matrix was generated with distance-based weights assigned to each off-diagonal entry and Moran's I was calculated (Proc VARIOGRAM; SAS 9.4). Separate analyses were run for each site and each year.

Results

Adult ACGW Emergence

In 2017, ACGW adults were first captured on traps collected on 6 July (729 DD_{10C}), indicating wasp emergence began after the previous trap collection on 27 June (619 DD_{10C}). Adults continued to be trapped through 8 August (1096 DD_{10C}) (Figure 2.1). Wasps were collected from ten of the 11 sites where traps were monitored. Total captures per site ranged from four to 549 ACGW (Table 2.1). Peak captures occurred between 12 and 19 July (796 to 870 DD_{10C}), when 53% of the total wasps were collected (Figure 2.1). Traps at the FL, HS and SWMREC sites, which were originally infested in 2015, had the highest number of captures during that week, averaging 17.3 ± 2.9, 16.9 ± 2.4 and 11.5 ± 4.0 ACGW per trap, respectively. At the other seven infested sites, captures ranged from 0.5 ± 0.25 to 5.0 ± 0.7 ACGW per trap.

In 2018, ACGW adults were first captured on traps collected on 10 July (748 DD_{10C}), indicating emergence began after the previous trap collection on 2 July (639 DD_{10C}). Emergence continued for five weeks; the last wasps were on traps collected on 8 August (1103 DD_{10C}) (Figure 2.1). Wasps were collected from ten of the 15 sites where traps were monitored. Total captures per site ranged from six to 649 ACGW (Table 2.1). Peak captures occurred between 17 and 24 July (846 to 928 DD_{10C}), when 68% of the total wasps were collected (Figure 2.1). Seven sites with previously known ACGW infestations had higher numbers of ACGW captures in 2018 than any of the 2017 sites, ranging from 29.5 ± 5.3 to 82.0 ± 18.3 ACGW per trap. At the remaining three sites, one (BK) was initially infested in 2017 while the other two were newly discovered infestations; captures averaged 0.5 ± 0.4 to 5.7 ± 2.6 ACGW per trap.

In 2019, a total of five adult ACGW were captured on traps at the HS, ET and SWMREC sites. Traps captured ACGW adults for three weeks from 16 July to 6 August (688 to 948 DD_{10C}).

ACGW Distribution in Southwest Michigan

Orchards initially infested in 2015 and 2016 were identified and confirmed by university researchers. New infestations were distinguished by the presence of current-year galls and the lack of previous-year galls (Table 2.1). Linear distance between sites infested in 2015 and the ET and PD sites, which became infested in 2016, ranged from 43.0 to 58.2 km and 42.1 to 57.3 km, respectively. (Figure 2.2).

In 2017, ACGW adults were captured at five of the six previously uninfested sites (Table 2.1). Traps at the BY site did not capture any ACGW adults nor were galls observed in this site (Table 2.1). Infestations were relatively light at the five newly invaded sites; total captures per site ranged from 4 to 26 ACGW (Table 2.1). Minimum linear distances between previously

infested sites and the five sites infested in 2017 ranged from 6.0 (CM site) to 63.4 km (BK site) (Figure 2.2). Maximum linear distances between previously infested sites and the five sites infested in 2017 ranged from 41.9 (JN site) to 83.1 km (BK site) (Figure 2.2).

In 2018, we captured ACGW adults in two of the six previously uninfested sites (Table 2.1). A total of 6 and 18 ACGW adults were captured at the HR and FV sites, respectively, which were 36.4 to 119.5 km and 12.5 to 78.4 km, respectively, from previously infested sites (Table 2.1, Figure 2.2).

In 2019, no ACGW adults were captured at any of the five uninfested sites where traps were monitored.

Within Field Gall Distribution

Visual rankings of gall abundance conducted at the ET site in 2017, showed only five of the 112 trees (4%) were heavily infested and assigned a rating of 4 and none of the trees were rated as a 5 (Figure 2.3a). Nine trees remained uninfested while 55 trees had only a few galls and were given a rating of 1. Gall abundance on trees was spatially autocorrelated ($z=2.14$; $P=0.032$), reflecting the pockets of moderately or heavily infested trees (Figure 2.3a). Gall abundance and numbers of infested trees increased in 2018 (Figure 2.3b). All trees were infested, only eight trees were classed as 1, while 31 trees (28%) were assigned a rating of 4. Spatial autocorrelation was again significant ($z=12.32$; $P<0.0001$) (Figure 2.3b).

When gall abundance was ranked in May 2017, all 36 trees in the FL site were infested. Half of the trees were heavily infested, indicated by a 4 or 5 rating, while three trees were assigned a rating of 3, indicating a patchy distribution for galls within the canopies (Figure 2.4a). When trees were evaluated in May 2018, current-year gall density increased and 34 of the 36 trees were assigned a rating of 4 or 5 (Figure 2.4b). There was no evidence of spatial

autocorrelation in gall densities among trees in 2017 (z=0.221; *P*=0.8253), nor in 2018 (z=0.854; *P*=0.3932).

In 2019, abundance of current-year galls was substantially reduced in all sites. At the ET site, 20 trees were assigned to a rating of 2, eight trees had no gall abundance and the remaining trees were rated as 1 (Figure 2.3c). At the FL site, six trees were assigned a rating of 3, indicative of moderate gall abundance, while other trees were rated as 1 or 2 (Figure 2.4c). Given the reduction in ACGW gall abundance presumably caused by cold winter temperatures, spatial autocorrelation was not analyzed for the 2019 data.

Discussion

Timing of ACGW adult captures on sticky traps in orchards in southwest Michigan was surprisingly consistent in 2017 and 2018. Adult ACGW were active and dispersing from late June to early August each year, corresponding to approximately 620 to 1100 DD_{10C}. The first and last captures of wasps differed by roughly 20 DD_{10C} and less than ten DD_{10C}, respectively, between the two years. Peak captures occurred slightly earlier in 2017 than in 2018, differing by 58 DD_{10C}. In other studies conducted in the U.S. state of Georgia, Japan and Italy, ACGW adult activity reportedly occurred between late May and late June, mid June to late July and late May to late July, respectively (Payne 1978, Otake 1989, Bernardo et al. 2013). None of these studies reported cumulative degree days, however, limiting our ability to make direct comparisons given that ACGW adult emergence is influenced by temperature (Otake 1980, 1989). Onset and duration of adult wasp emergence has practical implications for commercial growers who need to track ACGW spread and detect early infestations. Cumulative degree days, rather than calendar dates, are especially useful because they account for fluctuations in seasonal temperatures from year to year. Monitoring activity in relation to cumulative degree days also

facilitates comparisons among locations, providing opportunities for growers to project and prepare for ACGW activity. Further monitoring of ACGW emergence in relation to cumulative degrees days in additional locations will help confirm our findings.

Annual spread of ACGW, over the three years of trapping in southwest Michigan exceeded the 25 km maximum annual spread previously reported in Georgia and Italy (Rieske 2007, Graziosi and Santi 2008, EFSA 2010). New infestations detected in the ET, BK and HR sites in 2016, 2017 and 2018, respectively, ranged from 36.4 to 63.4 km from the nearest infested site. Growers with infested fields did not provide chestnut trees, saplings, seedlings or scion wood to the newly infested orchards. While we cannot eliminate accidental transport of hitchhiking wasps on vehicles, it seems unlikely. We suspect the large jump distances we observed reflect strong summer winds that commonly blow from the southwest (Booth et al. 2006). Wind-aided dispersal is also consistent with the expansion of the infestation primarily to the north and east of the previous infestations.

In 2019, the dramatic reduction in ACGW abundance across all sites lead to no newly discovered infestations in sites we monitored during the growing season. The decrease in ACGW abundance was likely caused by extremely low temperatures from 29 January to 1 February 2019, which ranged from -19.0°C to -26.7°C (see chapter 1). This raises questions about a possible climatic barrier to ACGW expansion. Most commercial chestnut orchards in Michigan are planted along Lake Michigan on the west side of the state, because lake effect weather keeps temperatures in this area warmer and less variable (Fulbright et al. 2010). There are, however, commercial orchards planted further north and inland in the state where temperatures are less affected by the lake. Future cold winters, like the 2018-2019 winter, could result in a slowing of ACGW expansion and the colder temperatures in the north could completely limit their

inhabitable range. Further monitoring of the effects winter temperatures have on ACGW populations will help determine the role Michigan climate has on expansion.

Michigan is currently the largest chestnut producer in North America, with at least 330 ha of commercial orchards and increasing interest in this commodity suggests expansion will continue (Fulbright et al. 2010, Hochmuth et al. 2018). American chestnut trees have also been planted in localized areas across much of Michigan's lower peninsula, often to provide hard mast for wildlife (Fulbright et al. 2010). The fragmented distribution of chestnut trees seems likely to affect dispersal and spread of ACGW adults, particularly to the east where few commercial orchards occur. Additional efforts to monitor ACGW infestations and spread over time would be useful in evaluating dispersal of these tiny wasps.

We originally hypothesized that gall abundance among trees within individual orchards would vary, representing the process of ACGW invasion and establishment. The small FL orchard was invaded in 2015 and by the time we evaluated in 2017, all trees had moderate to high levels of galls. In contrast, significant spatial autocorrelation among trees at the ET site was observed in both 2017 and 2018, consistent with our original hypothesis. This orchard was first invaded in 2016 and in 2017, galls were notably abundant on trees growing along the eastern and western edges of the orchard. This could suggest dispersing ACGW adults, which we suspect were carried by winds, colonized the first trees they encountered. The substantial increase in gall abundance and the proportion of trees colonized by ACGW in 2018 point to the importance of early detection and intervention to minimize impacts of this invader. Further monitoring to track ACGW infestation in newly invaded sites would be useful to understand host colonization and dispersal behavior of adult wasps.

APPENDIX

Table 2.1. Year of initial Asian chestnut gall wasp (ACGW) infestation, field size, date and number of ACGW traps deployed, and total number of ACGW adults captured on traps in commercial chestnut orchards in southwest Michigan monitored in 2017-2019.

Site	Year infested	Size (ha)	Year trapped	Traps deployed	No. traps	No. ACGW
TT	2017	0.07	2017	27 June	3	15
JN	2017	3.54	2017	6 July	4	4
SWMREC	2015	0.32	2017	21 June	7	139
			2018	26 June	4	226
			2019	2 July	4	1
HS	2015	2.07	2017	21 June	16	549
			2018	26 June	6	234
			2019	2 July	6	1
FL	2015	0.28	2017	21 June	6	146
			2018	26 June	4	649
ET	2016	0.64	2017	21 June	12	56
			2018	26 June	4	231
			2019	2 July	4	3
PD	2016	3.34	2017	21 June	10	60
			2018	26 June	4	163
CM	2017	2.03	2017	12 July	4	12
			2018	26 June	4	431
CB	2017	2.53	2017	27 June	4	26
			2018	26 June	4	387
BK	2017	1.17	2017	27 June	4	12
			2018	26 June	5	28
BY	NA	12.72	2017	6 July	4	0
			2018	2 July	4	0
			2019	2 July	4	0
FV	2018	0.72	2018	2 July	3	18
HR	2018	0.40	2018	2 July	4	6
WN	NA	2.11	2018	2 July	4	0
			2019	2 July	4	0
CL	NA	0.38	2018	2 July	4	0
			2019	2 July	4	0
RK	NA	2.42	2018	2 July	4	0
			2019	2 July	4	0
DK	NA	7.06	2018	2 July	4	0
			2019	2 July	4	0

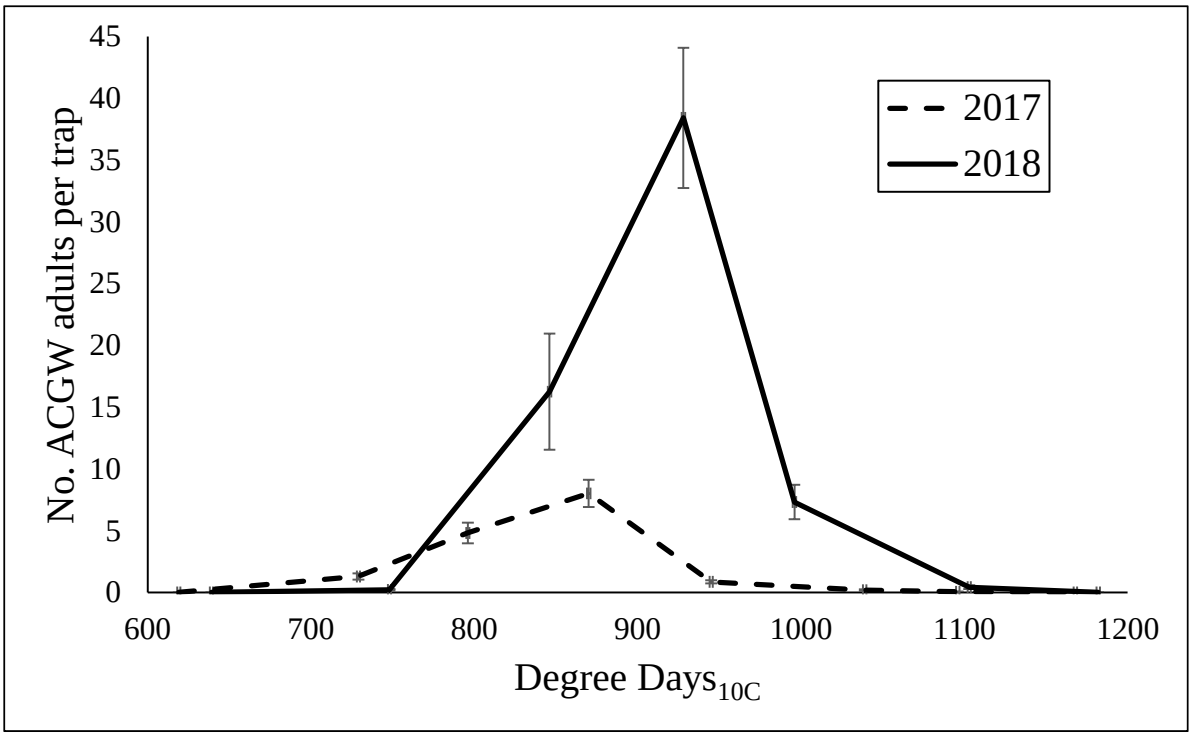

Figure 2.1. Mean (±SE) density of Asian chestnut gall wasp adults captured on traps and corresponding cumulative growing degree days (base 10°C) at ten commercial orchards monitored in 2017 and 2018.

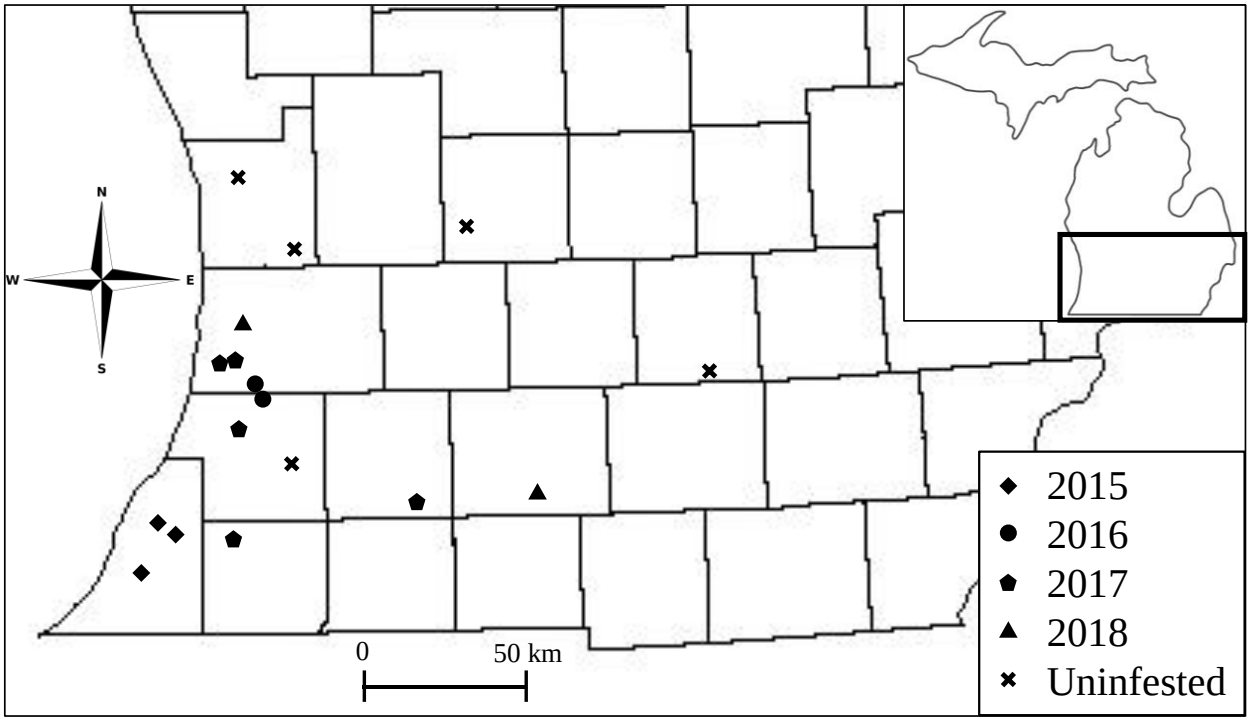

Figure 2.2. Location and first year of Asian chestnut gall wasp invasion in 17 sites in southwest Michigan monitored with sticky traps from 2017-2019.

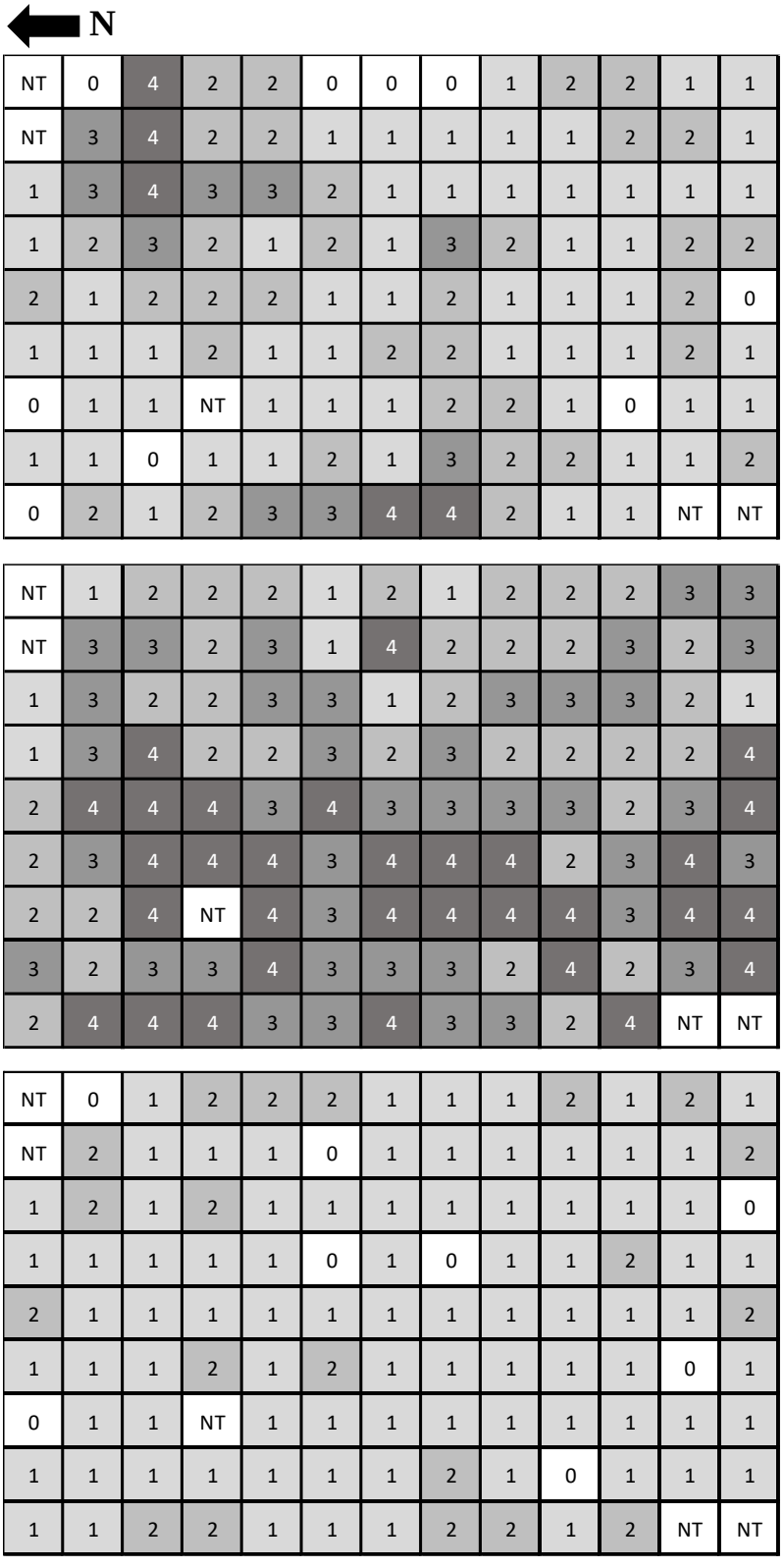

Figure 2.3. Qualitative abundance of current-year galls caused by Asian chestnut gall wasp on individual trees visually ranked at the ET site in May (a) 2017, (b) 2018 and (c) 2019. Each cell represents a single tree. Cells containing NT represent missing trees.

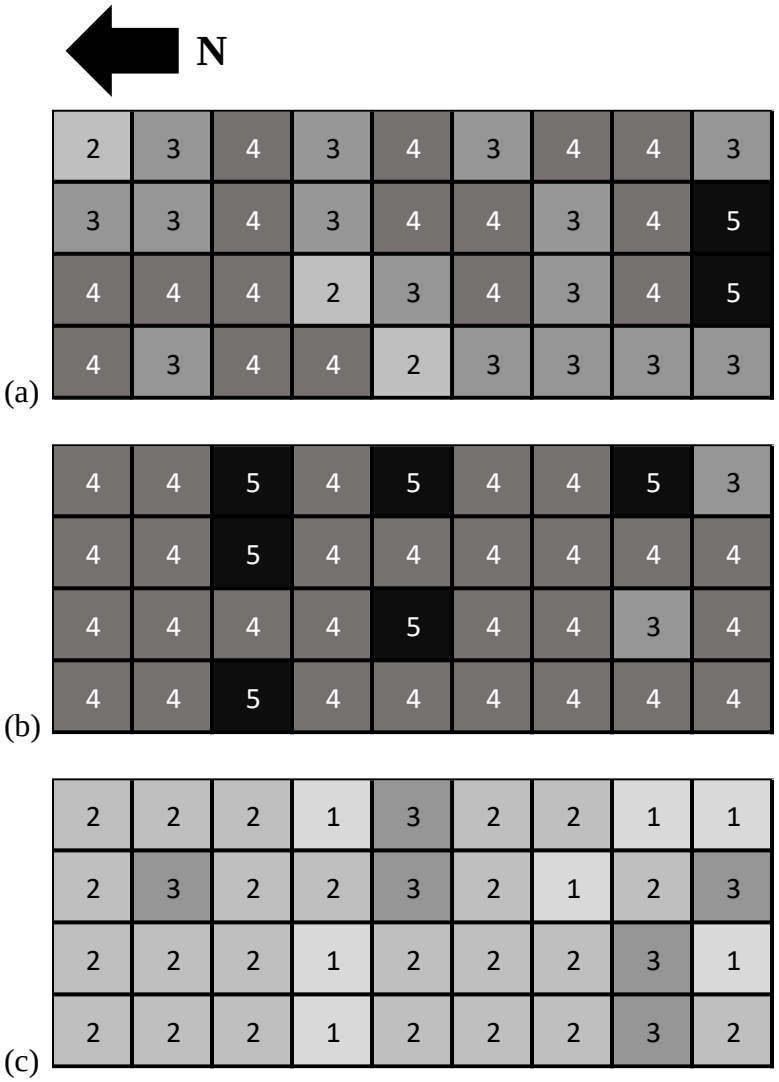

Figure 2.4. Qualitative abundance of current-year galls caused by Asian chestnut gall wasp on individual trees visually ranked at the FL site in May (a) 2017, (b) 2018 and (c) 2019. Each cell represents a single tree.

CHAPTER 3: LEVEL AND PERSISTENCE OF IMIDACLOPRID AND EMAMECTIN BENZOATE IN CHESTNUT (*CASTANEA* SPP.) TREES

Introduction

Systemic insecticides are increasingly replacing cover sprays in urban landscapes to control pests on high value amenity trees (Gill et al. 1999, Mota-Sanchez et al. 2009, Frank 2012, McCullough 2019). Systemics largely eliminate problems with drift, minimized exposure for applicators or residents, reduce potential effects on non-target insects and environmental contamination and can effectively control sheltered or protected insects, particularly in tall trees. Systemic insecticides are commonly used to protect trees from destructive pests in ecologically important forests and in area-wide eradication or suppression programs for major invasive forest pests, including Asian longhorned beetle (A*noplophora glabripennis* Motschulsky), emerald ash borer (*Agrilus planipennis* Fairmaire), hemlock woolly adelgid (*Adelges tsugae* Annand) and spotted lanternfly (*Lycorma delicatula* White) (Fidgen et al. 2002, Wang et al. 2004, Cowles et al. 2006, Mercader et al. 2015, Day et al. 2019).

Commonly used systemic insecticides include neonicotinoids such as imidacloprid and dinotefuran, emamectin benzoate, an avermectin compound, and products with azadirachtin as the active ingredient. Imidacloprid, which binds to nicotinic acetylcholine receptors, is widely used on many agricultural crops, in part because of its low toxicity to vertebrates, strong binding to organic matter reducing risks of the chemical leaching into groundwater, and its effective control of many Coleoptera (e.g., *A. glabripennis*, *Popillia japonica* Newman, *Chaetocnema pulicaria* Melsheimer) and Hemiptera (e.g., *A. tsugae*, *Pseudacysta perseae* Heidemann, *Bemisia tabaci* Gennadius) pests (Kuhar et al. 2002, Doccola et al. 2007, Wise et al. 2007, Byrne et al. 2010, Sheets 2010, Sohrabi et al. 2011, Ugine et al. 2012). When C^{14} labelled imidacloprid was applied via trunk injection to small ash (*Fraxinus* spp.) trees, residues were detectable for one

year (Mota-Sanchez et al. 2009). Emamectin benzoate, applied via trunk injection or foliar spray, is used to control a wide range of pests including emerald ash borer and some other Coleopterans (e.g., Curculionidae, Buprestidae and Cerambycidae), Lepidoptera (e.g., *Plutella xylostella* Linnaeus, *Trichoplusia ni* Hubner, *Spodoptera exigua* Hubner), and pinewood nematode (*Bursaphelenchus xylophilis*) (Jansson et al. 1997, Takai et al. 2003, Grosman and Upton 2006, McCullough et al. 2011, 2019). Emamectin benzoate, which binds to multiple sites within chloride channels causing irreversible paralysis, has low mammalian toxicity and in the case of emerald ash borer, a low dose provided highly effective control for up to three years (Smitley et al. 2010, McCullough et al. 2011, 2019, Bick et al. 2017).

Injecting systemic insecticides into active xylem at the base of the trunk ensures the product is contained within tree tissues, minimizing risks of soil or water contamination (Gill et al. 1999, Aćimović et al. 2014, Wise et al. 2014). Imidacloprid and dinotefuran can also be applied as a soil drench by pouring the formulated product around the base of the tree trunk for uptake by roots (Gill et al. 1999, Mota-Sanchez et al. 2009, Smitley, Rebek, et al. 2010, Tanis et al. 2012). Dinotefuran is often applied as a basal trunk spray, i.e., by spraying the bark around the circumference of the lower 1.5 m of the trunk (Cowles 2010, McCullough et al. 2011, 2019, Herms et al. 2014). This compound, which is highly water soluble, moves through outer bark and into xylem tissue (McCullough et al. 2011, 2019, Herms et al. 2014, Bick et al. 2018).

Systemic insecticides are translocated from the roots or base of the trunk to the canopy in xylem tissue. Expanding buds and leaves function as a major sink for xylem and insecticide residues are typically detectable in foliage for weeks, months or even years following application (Sánchez-Zamora and Fernández-Escobar 2004, Cowles et al. 2006, Doccola et al. 2007, Mota-Sanchez et al. 2009, McCullough et al. 2011, 2019, Tanis et al. 2012).

Uptake, translocation rates and persistence can vary substantially among tree species, insecticides, formulations and application timing. For example, in ash trees, imidacloprid is translocated to foliage within a few weeks, while research with hemlocks has shown imidacloprid residues do not peak until 9-15 months post treatment (Mota-Sanchez et al. 2009, McCullough et al. 2011, Tanis et al. 2012, Coots et al. 2013, McCullough 2019). Imidacloprid, however, may persist substantially longer. Foliar imidacloprid residues were present in hemlocks 4-7 years after treatment, while dinotefuran typically persists for one to two years (Joseph et al. 2011, Faulkenberry et al. 2012, Benton et al. 2015).

Tissues that do not conduct photosynthesis, such as developing fruits and seeds, are primarily sinks for carbohydrates transported in phloem (VanWoerkom 2012, Ryan and Asao 2014). For example, in a preliminary study, emamectin benzoate residues were not found in pollen of ash trees (Johnson 2017). In apple trees, emamectin benzoate residues were detectable in pollen, but levels were extremely low (VanWoerkom et al. 2014). Other systemics, including imidacloprid, have been identified in pollen of apple, orange and horse-chestnut trees, generating concern about exposure of insects needed for pollination (Škerl et al. 2009, Kobza et al. 2011, Byrne et al. 2014, Coslor et al. 2019a, Heller et al. 2020).

Use of systemic insecticides to manage pests affecting chestnut and other nut-producing trees has been limited to date. Commercial chestnut growers rely on cover sprays, typically with conventional chemical insecticides, to prevent injury or yield loss caused by insect pests such as chestnut weevils (*Curculio sayi* Gyllenhal and *C. caryatrypes* Boheman), potato leafhopper (*Empoasca fabae* Harris), Japanese beetle (*Popillia japonica* Newman) and the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu) (Speranza and Paparatti 2010, Warmund 2011, Anagnostakis 2012, Youngsteadt and Gurney 2013). Multiple sprays can be necessary, especially

for pests with multiple generations or an extended activity period and incorrect timing or incomplete coverage can result in poor control (Speranza and Paparatti 2010, Youngsteadt and Gurney 2013). Conventional cover sprays can have unintended negative effects on many non-target species, including beneficial pollinators, predators and parasitoids, as well as fish, amphibians, birds and even mammals, and can lead to secondary pest problems such as spider mites (Raupp et al. 2001, Sanchez-Bayo 2012, Douglas et al. 2015, Pisa et al. 2017, Ricupero et al. 2020).

Although systemics minimize many negative effects of insecticides, concerns about residues in nuts has prevented their use on chestnut and other nut-bearing crops. Additionally, while chestnut trees are wind pollinated, abundant pollen on catkins often attracts insect pollinators (Rutter et al. 1991). Previous research to quantify residues of systemic insecticides in nuts is scarce. When nutmeat of black walnuts (*Juglans nigra* L.) was evaluated, imidacloprid residues reportedly exceeded acceptable levels established by the U.S. Environmental Protection Agency, while dinotefuran was not detected (Nix et al. 2013).

We applied two commonly used systemic insecticides, imidacloprid and emamectin benzoate, to chestnut trees in fall or spring, then sampled foliage, pollen-bearing catkins and nuts to assess insecticide distribution and persistence. We were particularly interested in whether insecticide residues were present in chestnuts and if so, how those residue levels compared to residues in foliage and pollen. Results from studies with other hardwood trees showed systemic products were undetectable or present at very low levels in fruit or seeds, despite relatively high residues in leaves (VanWoerkom et al. 2014, Coslor et al. 2019a). If systemic insecticides do not move into chestnuts, these products could eventually become a viable option for commercial growers. We also compared residue levels in tissues of trees treated in either fall or spring to

assess whether application timing affected translocation or persistence of either imidacloprid or emamectin benzoate.

Materials and Methods

Study Sites

We selected chestnut trees at three sites, MSUCL, BCC and MSUPLP, for this study. The MSUCL site was located at the Michigan State University (MSU) Research Center near Clarksville, Ionia County, Michigan. This orchard includes approximately 130 trees representing a variety of species and cultivars. Three mature Chinese chestnut (*C. mollissima*) trees (dbh 21.0 ± 1.5 cm) were used for the study.

The second site, BCC, was a larger (approx. 520 trees), privately owned commercial chestnut orchard, located near Berrien Springs, Berrien County, Michigan. Similarly-aged trees of two chestnut cultivars, Colossal (*C. crenata* x *C. sativa*) and Dunstan (*C. dentata* x *C. mollissima*), were planted in alternating rows in the same field. Nine Colossal (dbh 28.6 ± 2.6 cm) and nine Dunstan (dbh 22.8 ± 1.6 cm) trees were used in the study.

The third site, MSUPLP, was located at the MSU Plant Pathology Farm in East Lansing, Ingham County, Michigan. This site includes approximately 200 trees representing *C. dentata, C. mollissima, C. crenata* and *C. crenata* x *C. sativa*. We used 48 trees for this study (dbh 13.5 ± 0.8 cm), which included 32 *C. dentata* and 16 trees of other species or cultivars.

Insecticide Treatments

On 12 October 2017, after burrs had dropped, we applied imidacloprid as a trunk injection to three trees at the MSUCL site and to six trees (3 Dunstan; 3 Colossal) at the BCC site (Table 3.1). Imidacloprid was applied as ImaJet® (5%) with the ArborJet Quick-Jet Air® tool and #4 plugs at a rate of 3 ml (0.04 grams a.i.) per 2.5 cm dbh.

On 23 May 2018, we treated six additional trees at the BCC site with a trunk injection of imidacloprid, using the rate and methods described above (Table 3.1). Six other trees at the BCC site were treated on the same day with a trunk injection of emamectin benzoate applied as TREE-age® (4%), again using the Quick-Jet Air® tool with #4 plugs at a rate of 2.5 ml (0.11 grams a.i.) per 2.5 cm dbh. All 12 trees were retreated in spring 2019 with the same insecticides. Trees were re-injected with imidacloprid on 24 April 2019 and with emamectin benzoate, which is typically translocated more rapidly, on 10 May 2019 (Table 3.1).

At the MSUPLP site we treated 12 trees with trunk injections of either imidacloprid or emamectin benzoate (12 imidacloprid; 12 emamectin benzoate) on 22-23 October 2018, using the application methods and rates as above (Table 3.1). An additional 24 trees at this site were injected on 30 April 2019 (12 imidacloprid; 12 emamectin benzoate) using the same methods as before (Table 3.1).

Chestnut Tissue Sampling

2018: Insecticide residues in chestnut trees treated with trunk-injected imidacloprid or emamectin benzoate at the MSUCL and BCC sites were assessed in samples of leaves, pollen-bearing catkins and nuts collected in 2018. On each sampling date, the same tissues were collected from two to four additional untreated trees, which served as controls. Composite foliage samples consisting of 6-12 mid canopy leaves from each aspect of the canopy were collected in mid-June (Leaves 1) and again in late July (Leaves 2) in 2018 (Table 3.1). Pole pruner blades or clippers were cleaned with a bleach solution between trees. Samples were returned to the MSU Forest Entomology Laboratory and frozen until they could be processed. This involved removing leaves from shoots and petioles, which were discarded.

Small shoots with five to ten pollen-bearing catkins were collected from mid canopy branches on all four aspects of the trees during peak pollen production in late June 2018 (Table 3.1). Catkins from each tree were consolidated into a single composite sample, then frozen. At the BCC site, catkins were only collected from Dunstan trees because Colossal trees do not produce pollen.

One burr, which typically contains three to four nuts, was clipped from mid canopy branches on all four aspects of the trees in late September 2018, just as burrs were beginning to drop (Table 3.1). Individual burrs were bagged separately and returned to the MSU lab. Nuts from each burr were removed, placed into individual bags and frozen. Frozen foliage, catkins and nut samples were shipped overnight to collaborators at the USDA APHIS PPQ laboratory in Buzzards Bay, MA for insecticide residue analysis, led by Dr. Phillip Lewis.

Once at the lab, samples were thawed, dried for approximately two weeks, then each sample was finely ground in a blender to a powdery consistency. A solution was created by adding 0.5 grams of the ground sample to 10 mL of methanol then shaking, centrifuging and decanting the mixture. The resulting solution was assayed for insecticide residue using ELISA kits for either imidacloprid (Envirologix Inc., Portland, ME) or emamectin benzoate (Horiba Ltd., Kyoto, Japan).

2019: Two composite samples of chestnut leaves, along with composite samples of pollen-bearing catkins, and burrs were collected from trees at the BCC and MSUPLP sites, as in 2018 (Table 3.1). Burrs were collected from trees in mid October 2019, a few weeks later than in 2018 (Table 3.1). Nuts from the four burrs collected from each tree were consolidated into a single sample in 2019 to ensure adequate material was available for residue analysis. Sample processing, freezing and shipping followed 2018 methods. However, collaborators at the APHIS

lab removed pericarps from the 2019 chestnuts before analyzing insecticide residues in the nutmeat.

Statistical Analysis

Normality and heterogeneity of variance of residue data were assessed with residual plots and Levene's test, then transformed if necessary to meet assumptions of statistical tests. Untransformed data are presented in figures. Imidacloprid residue levels in both foliage samples, catkins and nuts collected in 2018 from trees treated in fall 2017 at the MSUCL site were compared using one-way ANOVA (Proc MIXED; SAS 9.4), followed by Tukey's multiple comparison adjustment when ANOVA results were significant. Imidacloprid residues in foliage collected in early and late summer 2018 from Colossal and Dunstan trees treated in either fall 2017 or spring 2018 at the BCC site were analyzed using two-way ANOVA with repeated measures (Proc MIXED; SAS 9.4). Residue data were log transformed for analysis. Catkin residues from the BCC trees were not normalized with transformations so differences between Dunstan trees treated in fall 2017 and spring 2018 were analyzed using one-way nonparametric ANOVA (Proc NPAR1WAY; SAS 9.4). Because Colossal (*C. crenata* x *C. sativa*) trees do not produce pollen, cultivars were not compared.

One-way ANOVA with repeated measures (Proc MIXED; SAS 9.4) was conducted to assess differences in foliar imidacloprid residues in the two samples collected in summer 2019 from Colossal and Dunstan trees that were treated in spring 2018 and again in spring 2019 at the BCC site. Emamectin benzoate residues collected in summer 2018 and 2019 were similarly analyzed. All foliar residue data were log transformed for analysis.

Differences in 2019 imidacloprid residues among foliage and catkin samples and between trees treated in fall 2018 and spring 2019 at the MSUPLP site were analyzed using two-way

ANOVA (Proc MIXED; SAS 9.4) followed by Tukey's multiple comparison adjustment when ANOVA results were significant. Emamectin benzoate residues in the two 2019 foliage samples collected from trees treated in fall 2018 or spring 2019 were also analyzed using two-way ANOVA. Residues in catkins, which were very low or undetectable, were excluded from analysis. All imidacloprid and emamectin benzoate residues were log transformed to normalize data.

Results

MSUCL Site

Imidacloprid residues collected in 2018 from the three trees treated in October 2017 at the MSUCL site varied among tissues (F=9.89; df=3,8; *P*=0.005) (Figure 3.1). Residues in leaves collected in June 2018 (Leaves 1) were slightly higher on average than residues in leaves collected in July (Leaves 2), but differences between June and July foliage samples and between foliar and catkin samples were not significant. Imidacloprid residues were present in catkins collected from all three trees in late June, ranging from 0.76 to 2.68 ppm (Figure 3.1). No imidacloprid was detected in nuts from any of the treated trees (Figure 3.1).

BCC Site

Imidacloprid residues in June (Leaves 1) and July (Leaves 2) foliage samples collected in 2018 from the BCC site did not differ between trees treated in fall 2017 and spring 2018 (F=1.18; df=1,8; *P*=0.31), between the Colossal and Dunstan cultivars (F=1.78; df=1,8; *P*=0.22), nor was the interaction significant (F=0.43; df=1,8; *P*=0.53) (Table 3.2, Figure 3.2). Residues in leaves sampled in June 2018 were highly variable, ranging from 1.89 to 21.37 ppm for Colossal trees and 0.15 to 17.23 ppm for Dunstan trees (Figure 3.2). One foliage sample collected in July from a Dunstan tree treated in spring 2018 had no detectable residues. Residues in June (Leaves

1) and July (Leaves 2) foliage samples collected in 2019 from trees re-treated with imidacloprid in April 2019 at the BCC site did not differ between the Colossal and Dunstan cultivars (F=0.01; df=1,4; *P*=0.95) (Figure 3.3). Residues in leaves sampled in 2019 were lower than in 2018 foliage, ranging from 1.68 to 7.17 ppm for Colossal trees and 1.94 to 3.83 ppm for Dunstan trees.

Four of the six catkin samples collected from Dunstan trees had detectable albeit very low imidacloprid residues, including two trees treated in fall 2017 and two trees treated in spring 2018. Catkin residues ranged from 0.42 to 1.41 ppm and differences between trees treated in fall 2017 and spring 2018 were not statistically significant (F=1.23; df=1,4; *P*=0.33) (Table 3.2, Figure 3.2). Low levels of imidacloprid were also found in catkin samples collected in 2019 from the three trees re-treated in April 2019. Catkin residues ranged from 0.48 to 1.15 ppm, averaging 0.88 ± 0.17 ppm.

Traces of imidacloprid, ranging from 0.15-0.16 ppm, were detected in nuts collected in September 2018 from four of the six trees treated in October 2017 at the BCC site. Nuts from one of four burrs collected from three trees and nuts from two of four burrs from another tree had detectable residues. No residues were detected in nuts from any of the four burrs collected from the two remaining trees. Traces of imidacloprid (0.15-0.19 ppm) were also detected in nuts from one, two or three of the four burrs collected in September 2018 from the six trees treated with imidacloprid in May 2018, while nuts from two trees had no detectable residues in any nut samples. No detectable residues were present in any samples of nuts collected in October 2019 from the six trees re-treated with imidacloprid in April 2019.

Emamectin benzoate residues in foliage samples collected in June (Leaves 1) and July (Leaves 2) 2018 from the BCC site trees injected in May 2018 were unusually high and did not differ between the Colossal and Dunstan cultivars (F=0.05; df=1,4; *P*=0.83) (Figure 3.4).

Residues from leaves collected in June 2018 averaged 30.8 ± 1.1 ppm and ranged from 25.5 to 33.6 ppm. Although residues dropped in all trees by the July collection, levels remained relatively high, averaging 20.1 ± 1.5 ppm and ranging from 18.2 to 23.2 ppm.

In contrast, despite re-treatment with emamectin benzoate in May 2019, residue levels in foliage samples collected a few weeks later in June (Leaves 1) and July (Leaves 2) 2019 from trees at the BCC site were extremely low or even absent (Figure 3.5). Not surprisingly, residues did not differ between Colossal and Dunstan cultivars ($F=0.49$; $df=1,4$; $P=0.52$) (Figure 3.5). In foliage samples from June, one tree had no detectable residues while the other five trees had very low levels, ranging from 0.08 to 0.27 ppm. Only one tree had detectable foliar residues (0.28 ppm) in July.

Pollen bearing catkin samples were collected in June 2018 from the three Chinese chestnut trees injected with emamectin benzoate in May 2018 at the BCC site, but only two trees yielded enough pollen to analyze. A trace amount of emamectin benzoate. 0.06 ppm, which could have been a result of contamination, was present in one of the two samples. When catkins were collected again in June 2019 from all three trees re-treated with emamectin benzoate in May 2019 no residues were detected in any samples.

In chestnuts collected in September 2018 from the six trees injected with emamectin benzoate in May 2018 at the BCC site, nuts from one of the four burrs collected from one tree had 0.07 ppm of residue. Samples of nuts from the other 23 burrs had no emamectin benzoate residues. No residues were detected in any nut sample collected in October 2019 from the six trees re-treated with emamectin benzoate in May 2019.

MSUPLP Site

Imidacloprid residues in the 2019 June (Leaves 1) and August (Leaves 2) leaf samples and catkin samples differed between trees treated in October 2018 and April 2019 (F=52.76; df=1,66; *P*<0.0001) and among the various tissues (F=25.81; df=2,66; *P*<0.0001) at the MSUPLP site (Table 3.2, Figure 3.6). The interaction of treatment timing and tissue type was not significant (F=0.48, df=2,66, *P*=0.62). June and August foliar residues were similarly high in trees treated in October 2018, although residues in the August samples varied considerably (Table 3.2, Figure 3.6). Foliar residues from trees treated in fall 2018 were at least two to three times higher than residues in trees treated in spring 2019 (Figure 3.6). Foliar residues in both the June and August samples were higher than residues in catkin samples from trees treated in either October 2018 or April 2019 (Figure 3.6).

At the MSUPLP site, we were able to collect adequate amounts of chestnuts in October 2019 from only 16 of the 24 trees treated with imidacloprid in either fall 2018 or spring 2019. Only two of the 16 samples, had a trace residue of imidacloprid (0.15 ppm), while samples from the other 14 trees had no detectable residues.

Foliar emamectin benzoate residues did not differ between the June (Leaves 1) and August (Leaves 2) 2019 leaf samples (F=2.51; df=1,44; *P*=0.12), nor between foliage from trees treated in October 2018 versus April 2019 (F=0.16; df=1,44; *P*=0.69) (Table 3.2, Figure 3.7). The interaction of treatment timing and tissue type was also not significant (F<0.00, df=1,44, *P*=0.97). Residues were consistently very low, averaging 0.4 ± 0.06 ppm and 0.3 ± 0.05 ppm for June and August samples, respectively. The highest residue levels, 1.1 ppm in June and 0.7 ppm in August, were recorded on one and two trees, while leaf samples from four trees in June and seven trees in August had no detectable emamectin benzoate. Residues were also absent in most

of the catkin samples, which were collected in July 2019 from 12 chestnut trees injected with emamectin benzoate in fall 2018 and 12 trees injected in spring 2019 (Table 3.2). Only 11 samples had any residue detections and all were very low, ranging from 0.06 to 0.17 ppm.

We collected chestnuts in October 2019 from only 12 of the 24 trees treated with emamectin benzoate in either fall 2018 or spring 2019 at the MSUPLP site. No detectable residues were found in any nut samples.

Discussion

Our research indicates the potential of both imidacloprid and emamectin benzoate to provide protection to chestnut trees against insect pests, such as defoliators and sap feeders, as both were consistently found in the foliage. Both insecticides were also found in some pollen-bearing catkin samples and a few nut samples, however, generating a need for further research to fully understand the translocation of these insecticides in chestnut trees.

Evaluating presence and levels of the two systemic insecticides in samples of nuts were major goals of this project. Imidacloprid residues were detected in only 15 of the 90 nut samples (17%) collected in 2018 and 2019 from trees at the BCC and MSUPLP sites, while nut samples collected in 2018 from trees at the MSUCL site had no detectable residues. When residues were detected, they were much lower than levels in foliage, ranging from 0.1 to 0.19 ppm. While these levels are low, they still exceed the 0.05 ppm tolerance level for food items, established by the Environmental Protection Agency (EPA) (EPA 2014). Nearly all (87%) of the nut samples with detectable residues were collected and analyzed in 2018, when the pericarp (husk) were left intact. In contrast, in 2019, when husks were removed and only the edible nutmeat was analyzed, only two of the 16 samples (12.5%) had detectable imidacloprid. The trace levels of imidacloprid could indicate residues may sometimes accumulate in the non-edible husks or reflect

contamination of nut samples, despite our best efforts. Data on imidacloprid residues in fruits and nuts of other tree crops is limited and most studies involved fleshy fruits. In a study with apple trees injected with 0.2 or 0.4 g a.i. of imidacloprid per 2.5 cm dbh in spring, residues in apples harvested the same year ranged from 0.02 to 1.37 ppm in trees and increased with dose (VanWoerkom et al. 2014). Residues in apples harvested the following year ranged from zero to 0.03 ppm in trees treated with 0.2 or 0.4 g a.i. per 2.5 cm dbh (VanWoerkom et al. 2014). Coslor et al. (2019a) reported application rates for apple trees 17 to 20 cm dbh as 0.1 or 0.2 g a.i. per tree and found maximum residues of 0.1 ppm in apples for both rates (Coslor et al. 2019a). When avocado trees were treated with a soil drench of imidacloprid at 1.02 L per ha or trunk injections at 0.04 and 0.08 g a.i. per 2.5 cm dbh, residues were not detected in any fruit collected the summer following spring treatments (Byrne et al. 2012). In a 2011 study, imidacloprid was applied as soil tablets at 0.5 g a.i. per 2.5 cm dbh to black walnut trees and residues in the nutmeat and nut husk, which were analyzed separately, exceeded the 0.05 ppm tolerance level (Nix et al. 2013). Given the sometimes contrasting results of residue analyses, further research to assess imidacloprid translocation into nutmeat, pericarp and even burrs of chestnuts is clearly needed to evaluate safety of nuts for consumption. A broader understanding about translocation of systemic insecticides into nuts and other reproductive structures would be valuable for an array of tree crops.

Emamectin benzoate residues were detected in only one of the 42 samples (2.4%) of nuts collected in 2018 or 2019 at the BCC and MSUPLP sites. The 0.07 ppm recorded from this 2018 sample exceeds the EPA tolerance level of 0.02 ppm for emamectin benzoate (EPA 2014). Whether this positive sample reflects contamination or insecticide moving into nutmeats or pericarp (analyzed together in 2018), cannot be determined. Since emamectin benzoate was

registered for trunk injection in trees fairly recently (2009), very few studies on post-treatment residues in tree crops are available. In apple trees, maximum residues of 0.004, 0.005 and 0.006 ppm were recorded in apples from trees injected earlier in the year at rates of 0.04, 0.08 and 0.8 g a.i. per tree (17-20 cm dbh) (Coslor et al. 2019a). In apples trees injected with 0.2 or 0.4 g a.i. per 2.5 cm dbh, residues in apples harvested the same year ranged from 0.0004 to 0.003 ppm, and in apples harvested the following year, residues ranged from 0.0001 to 0.0039 ppm (VanWoerkom et al. 2014). More research on emamectin benzoate translocation in nut producing tree crops would be valuable since data from apples and our chestnut study indicate this insecticide can control foliage feeding insects but perhaps will not move into nuts.

Imidacloprid residues were consistently detected in pollen-bearing catkins from chestnut trees in samples collected in 2018 and 2019 from all sites. Our results showed pollen residues that were much higher than residues detected in other studies on citrus, ash and apple trees as well as a variety of herbaceous plants (Byrne et al. 2014, VanWoerkom et al. 2014, Tong et al. 2016, Johnson 2017, Coslor et al. 2019a). Whereas most other studies that test for residues in pollen, separate out the pollen grains from the rest of the flower, our residue measurements were taken from the entire catkin, which likely caused our atypically high pollen residues. In apple trees, some studies have found no detectable imidacloprid residues in pollen samples collected up to one year after either spring or fall trunk injections of 0.2 g a.i. per tree (17-20 cm dbh) or 0.2 to 0.4 g a.i. per 2.5 cm dbh (VanWoerkom et al. 2014, Coslor et al. 2019a). In a study with trunk-injected ash trees imidacloprid residues in pollen collected from trees treated the previous summer averaged 0.06 ppm, but when the study was conducted the following year, residues dropped to less than 0.01 ppm (Johnson 2017). When a cover spray of imidacloprid was applied to a citrus orchard (280 g a.i. per ha), residues ranging from 0.0058 to 0.0066 ppm were detected

in pollen collected from apiaries (Byrne et al. 2014). Most studies on imidacloprid residues in pollen from trunk injected trees have reported residues below the 0.1 ppm threshold for toxicity to bees (Cowles and Eitzer 2017). These studies, along with our concern that the catkin tissue inflates pollen residue levels, suggest imidacloprid residues in chestnut pollen are likely much lower than the levels determined in our study.

Emamectin benzoate residues were detected in less than half of the pollen-bearing catkins from chestnut trees in samples collected in 2018 and 2019. As with the imidacloprid values, emamectin benzoate residues associated with our samples were higher than in other studies (VanWoerkom et al. 2014, Johnson 2017, Coslor et al. 2019a). In apples trees treated at either 0.08 g a.i. per tree (17-20 cm dbh) or 0.2 and 0.4 g a.i. per 2.5 cm dbh, residues in pollen averaged 0.00115 ± 0.00069 and 0.002 ppm, respectively approximately one year after treatment (VanWoerkom et al. 2014, Coslor et al. 2019a). Pollen from trunk injected ash trees, however, had zero emamectin benzoate residues (Johnson 2017). Residue levels detected in the studies with apple, as well as ash trees, are well below the LD_{50} of 0.4 ppm for bees in pollen (Johnson 2017). Due to the fairly recent registration of trunk injected emamectin benzoate for use in controlling pests of trees, research on pollen residues is scarce. Further testing to assess emamectin benzoate in pollen extracted from chestnut catkins would be helpful.

Residues of both insecticides in foliage samples collected early and late summer in 2018 and 2019 were evaluated. Imidacloprid residues did not differ between leaf samples collected in mid June and samples collected later in the summer. Research on ash trees similarly showed that imidacloprid residues did not differ among four collections of foliage over a span of two months following spring treatments (McCullough et al. 2011). In contrast, a study with small containerized ash, and studies with apple and avocado trees showed residues in foliage increased

over the summer as translocation progressed (Mota-Sanchez et al. 2009, Byrne et al. 2012, Aćimović et al. 2014, VanWoerkom et al. 2014).

Imidacloprid foliar residues varied considerably among individual trees from samples collected at the BCC site in 2018 and the MSUPLP site in 2019. The residues from foliage collected at the BCC site in 2019 and the MSUCL site in 2018 were less variable. In apple trees, foliar residues ranged from 0.33 to 32.85 ppm from trees trunk injected with either 0.2 or 0.4 g a.i. per 2.5 cm dbh (VanWoerkom et al. 2014). Another study with maple trees also reported large variation in foliar residues, ranging from 0 to 49.17 ppm, from trees trunk injected in late April or late May with either 0.22 or 0.44 g a.i. per 2.5 cm dbh if trees were larger than 61 cm (Ugine et al. 2013). Variability among individual trees could be at least partly accredited to translocation and transpiration rates. Tanis et al. (2012) showed that in small ash trees, the orientation of the injection points relative to the branches affected imidacloprid translocation to foliage. Additionally, since transpiration is largely controlled by sun exposure and atmospheric pressure, both of which can vary at the microclimate scale, transpiration rates can vary among individual trees within the same stand (Wullschleger et al. 2001).

Emamectin benzoate residues did not differ between early and late summer foliage collections. Some studies on ash and apple trees found that emamectin benzoate reaches foliage quickly and residue levels remain fairly stable throughout the growing season (McCullough et al. 2011, VanWoerkom et al. 2014, Coslor et al. 2019a). One study with apple trees reported emamectin benzoate residues were lower in foliage sampled 71 days after treatment than in samples collected 43 days after treatment one year, but residues did not differ in another year (Coslor et al. 2019b).

Foliage from the BCC trees treated with emamectin benzoate in spring 2018 had very high residues, reaching 33.6 ppm. In one study on apple trees, spring treatments with 0.04, 0.08 or 0.8 g a.i. per tree (17-20 cm dbh) resulted in foliage residues of up to 65 ppm (Coslor et al. 2019a). Spring trunk injections of 0.1, 0.15 or 0.2 g a.i. per 2.5 cm dbh resulted in foliar residues up to 11.1 ppm in ash trees (McCullough et al. 2011). However, the extremely low emamectin benzoate residues in 2019 foliage from the BCC trees and the MSUPLP trees, including trees re-treated with emamectin benzoate in spring 2019, were unexpected. The 2019 residues were approximately 30 times lower than in the 2018 samples. Studies with apple trees found similarly low residues in foliage (0 to 0.06 ppm) despite using higher doses of insecticide (up to 0.4 g a.i. per 2.5 cm dbh) (VanWoerkom et al. 2014, Coslor et al. 2019b). Finally, one study with apple trees recorded the exact same phenomena we observed with foliar residues of emamectin benzoate over a two year study (Coslor et al. 2019a). This study treated trees using either 0.04, 0.08 or 0.8 g a.i. per tree (17-20 cm dbh) in the spring of 2013 and 2014 and found residues up to 65 ppm in 2013, but only up to 0.2 ppm in 2014 (Coslor et al. 2019a). Mechanisms driving the dramatic difference in residues in our chestnut trees between 2018 and 2019 are unclear. Injection methods and rates were the same in both years, data records have been double-checked and the same methods were used for sampling and processing foliage. Also, imidacloprid residues did not show the same pattern, indicating weather and horticultural practices are not likely to be a cause. Given the limited research on trunk injected emamectin benzoate and the unusual results observed here and by Coslor et al (2019a), more research is clearly necessary to understand why such patterns occur.

We compared residues of both insecticides from foliage samples between the Colossal and Dunstan cultivars at the BCC site and found no significant differences. Other studies found

that foliar residues did not vary among species of ash trees (Mota-Sanchez et al. 2009, Tanis et al. 2012). This indicates that the Colossal and Dunstan chestnut species have similar enough physiologies to not substantially impact insecticide uptake or translocation.

Understanding differences in translocation of the two insecticides between fall and spring treatments has potential implications for pest management practices. When comparing imidacloprid residues from the BCC site between fall 2017 and spring 2018 treatments, we found no differences in any tissues sampled in 2018, which suggests that application timing did not affect translocation. These results were contrary to other studies on apples and avocados which have reported that treatment timing has a significant effect on translocation of imidacloprid after trunk injection (Byrne et al. 2014, Coslor et al. 2019). Although no statistical difference was found, the means for foliage and catkin samples were consistently higher in trees treated in spring 2018 than trees treated in fall 2017 and the lack of significance likely reflects our limited sample size of 12 trees. Transpiration rates in oaks (*Quercus* spp.), which are in the same family as chestnut trees (Fagaceae) and also ring-porous, are moderate in early October, which was when we treated the BCC site trees (Wullschleger et al. 2001). If spring treated trees do result in higher residues, this could indicate that some insecticide was translocated into foliage prior to leaf fall, then ultimately lost before the following growing season.

At the MSUPLP site, we found that imidacloprid residues were higher in fall 2018 than in spring 2019 treated trees in both foliage samples collected in summer 2019, perhaps indicating relatively slow translocation of imidacloprid. In apple trees, following imidacloprid applications in spring and fall of the same year, residues in foliage collected after the fall treatment were higher in the spring treated trees (Coslor et al. 2019a). When imidacloprid was applied to avocado trees in spring, leaves expanded too rapidly for the relatively slow-moving insecticide to

keep up (Byrne et al. 2010). Our fall trunk injections at the MSUPLP site occurred in late October after leaf fall was underway and transpiration was minimal (Wullschleger et al. 2001). It seems unlikely, therefore, that much of the imidacloprid was lost before the following growing season. In ash trees, a reservoir of imidacloprid near the injection sites in the trunk moved into foliage as leaves expanded and transpiration began in spring (Mota-Sanchez et al. 2009, Tanis et al. 2012). Such results are important to growers who must adequately control pests while integrating insecticide applications with nut harvesting in fall and other activities.

Emamectin benzoate residues in chestnut foliage collected in 2019 from the MSUPLP site were similar in trees injected in fall and spring. One study with ash trees showed that foliar residues were detected within one month of trunk injection and levels remained similarly high throughout the summer (McCullough et al. 2011). Another study on avocado trees found no differences among emamectin benzoate residues in increment cores from three different heights of the trunk or between samples taken three and six months after injection (Byrne et al. 2020). Given the lack of previous research on this topic and our fairly limited sample size, continued research is necessary for more definitive results.

APPENDIX

Table 3.1. Date of insecticide application via trunk injection, insecticide product, species, number and mean (±SE) dbh of treated trees, and dates when samples were collected for residue analysis at three sites in Michigan.

Site	Treatment date	Product	Species	No. trees treated	Mean (±SE) dbh (cm)	Sample date	Chestnut tissue
MSUCL	12 October 2017	Imidacloprid	*C. mollissima*	3	21.0 ± 1.5		
						13 June 2018	Leaves 1
						25 June 2018	Pollen
						25 July 2018	Leaves 2
						27 Sept. 2018	Nuts
BCC	12 October 2017	Imidacloprid	*C. dentata* x *C. mollissima*	3	19.6 ± 3.0		
			C. crenata x *C. sativa*	3	36.3 ± 4.1		
	23 May 2018	Imidacloprid	*C. dentata* x *C. mollissima*	3[2]	25.2 ± 2.9		
			C. crenata x *C. sativa*	3[2]	28.3 ± 1.0		
	23 May 2018	Emamectin benzoate	*C. dentata* x *C. mollissima*	3[3]	25.1 ± 0.1		
			C. crenata x *C. sativa*	3[3]	25.8 ± 3.3		
						13 June 2018	Leaves 1
						27 June 2018	Pollen
						24 July 2018	Leaves 2
						26 Sept. 2018	Nuts
	24 April 2019	Imidacloprid	*C. dentata* x *C. mollissima*	3[2]	25.2 ± 2.9		
			C. crenata x *C. sativa*	3[2]	28.3 ± 1.0		
	10 May 2019	Emamectin benzoate	*C. dentata* x *C. mollissima*	3[3]	25.1 ± 0.1		
			C. crenata x *C. sativa*	3[3]	25.8 ± 3.3		
						27 June 2019	Leaves 1
						9 July 2019	Pollen
						30 July 2019	Leaves 2
						17 Oct. 2019	Nuts
MSUPLP	22 October 2018	Imidacloprid	*C. dentata*	8	12.6 ± 1.9		
			Various[1]	4	13.8 ± 3.0		

Table 3.1. (cont'd)

	23 October 2018	Emamectin benzoate	*C. dentata*	8	11.7 ± 1.0		
			Various[1]	4	16.8 ± 3.8		
	30 April 2019	Imidacloprid	*C. dentata*	8	12.2 ± 0.6		
			Various[1]	4	17.7 ± 3.6		
		Emamectin benzoate	*C. dentata*	8	11.5 ± 0.7		
			Various[1]	4	18.0 ± 3.0		
						25 June 2019	Leaves 1
						11 July 2019	Pollen
						2 August 2019	Leaves 2
						14 Oct. 2019	Nuts

[1]Various chestnut species included *C. mollissima*, *C. crenata* and *C. crenata* x *C. sativa.*

[2]These trees were originally treated with imidacloprid in May 2018, then re-treated with imidacloprid in April 2019.

[3]These trees were originally treated with emamectin benzoate in May 2018, then re-treated with emamectin benzoate in May 2019.

Table 3.2. Mean (±SE) imidacloprid residue levels (ppm) in samples of chestnut leaves, pollen and nuts and number of trees sampled from trees treated on 12 October 2017 or 23 May 2018 at the BCC site. Mean (±SE) imidacloprid and emamectin benzoate residue levels (ppm) in samples of chestnut leaves, pollen and nuts and number of trees sampled from trees treated on 22-23 October 2018 or 30 April 2019 at the MSUPLP site.

Site	Product	Tissue	No. trees	Fall 2017 Mean ± SE (ppm)	No. trees	Spring 2018 Mean ± SE (ppm)	No. trees	Fall 2018 Mean ± SE (ppm)	No. trees	Spring 2019 Mean ± SE (ppm)
BCC	Imidacloprid	Leaves 1	6	2.86 ± 0.99	6	9.53 ± 3.47				
		Leaves 2	6	1.96 ± 0.54	6	3.49 ± 0.98				
		Pollen	3	0.35 ± 0.15	3	0.88 ± 0.36				
		Nuts	6	0.03 ± 0.01	6	0.05 ± 0.01				
MSUPLP	Imidacloprid	Leaves 1					12	12.53 ± 1.96	12	3.48 ± 0.49
		Leaves 2					12	15.51 ± 4.72	12	4.77 ± 0.92
		Pollen					12	3.63 ± 0.67	12	1.33 ± 0.15
		Nuts					11	0.03 ± 0.02	5	0.0 ± 0.0
	Emamectin benzoate	Leaves 1					12	0.39 ± 0.07	12	0.39 ± 0.10
		Leaves 2					12	0.27 ± 0.06	12	0.24 ± 0.07
		Pollen					12	0.05 ± 0.02	12	0.05 ± 0.02
		Nuts					6	0.0 ± 0.0	6	0.0 ± 0.0

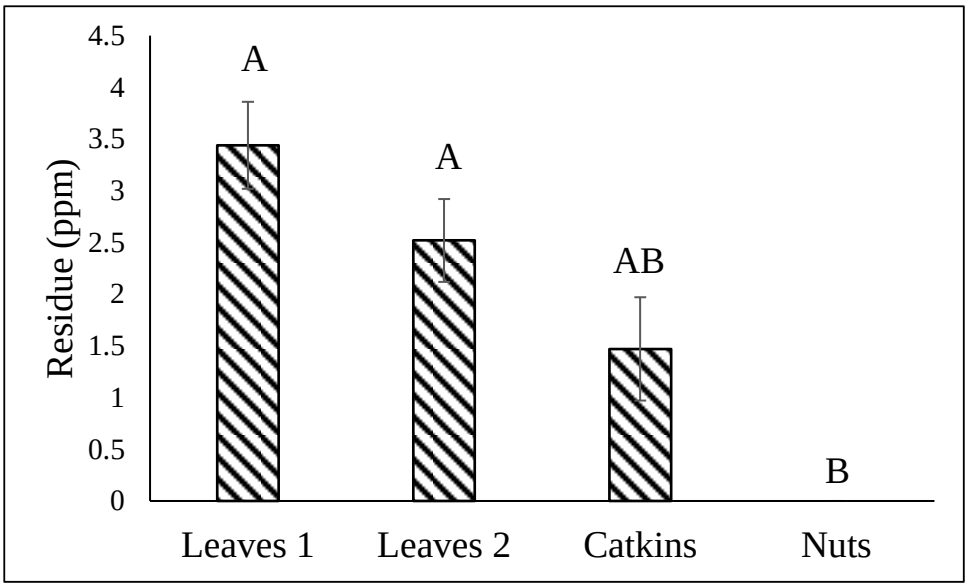

Figure 3.1. Mean (±SE) imidacloprid residues (ppm) in composite samples collected in 2018 from chestnut trees treated in fall 2017 at the MSUCL site (n=3 trees). Letters indicate significant differences among chestnut tissues.

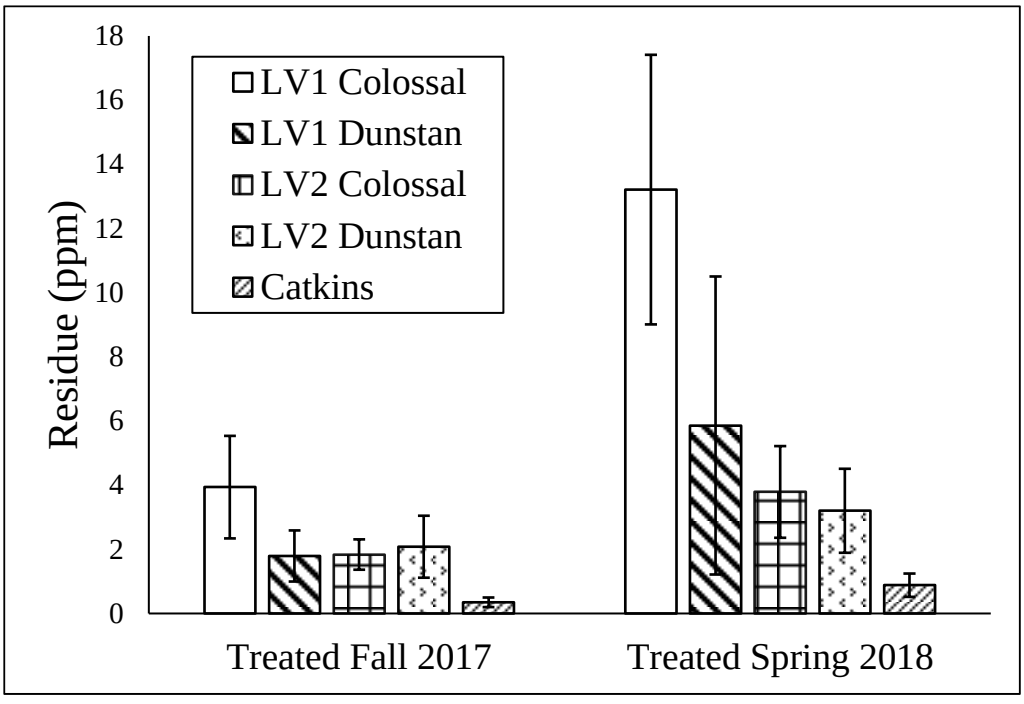

Figure 3.2. Mean (±SE) imidacloprid residues in foliage samples collected in June (LV1) or July (LV2) 2018 from Colossal (*C. crenata* x *C. sativa*) and Dunstan (*C. dentata* x *C. mollissima*) chestnut trees treated in either fall 2017 or spring 2018 (n=3 trees per treatment per cultivar) at the BCC site. Pollen samples were collected from Dunstan trees; Colossal trees do not produce pollen. Differences among foliage samples and between catkin samples were not significant.

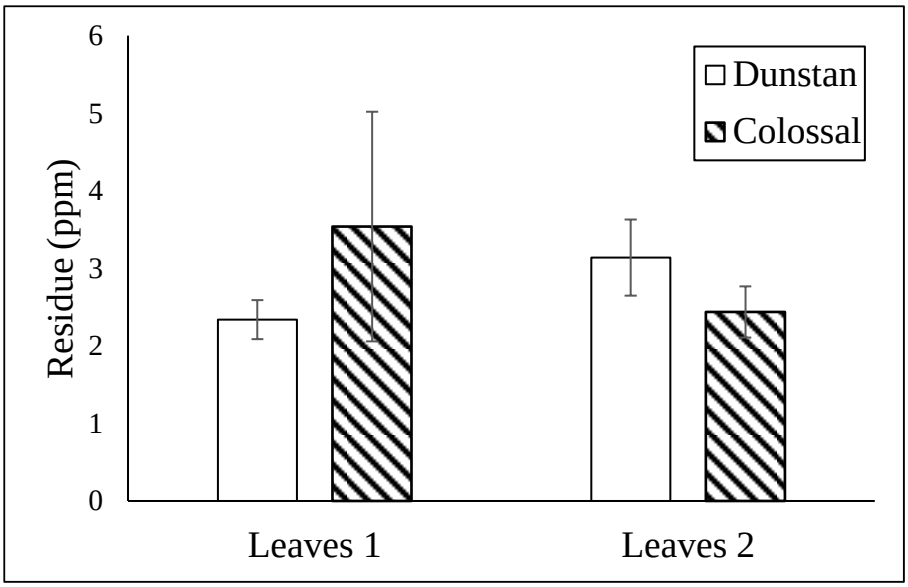

Figure 3.3. Mean (±SE) imidacloprid residues (ppm) in foliage samples collected in June (Leaves 1) and July (Leaves 2) 2019 from three Colossal (*C. crenata* x *C. sativa*) and three Dunstan (*C. dentata* x *C. mollissima*) chestnut trees treated in spring 2019 at the BCC site. Differences between cultivars and foliage samples were not significant.

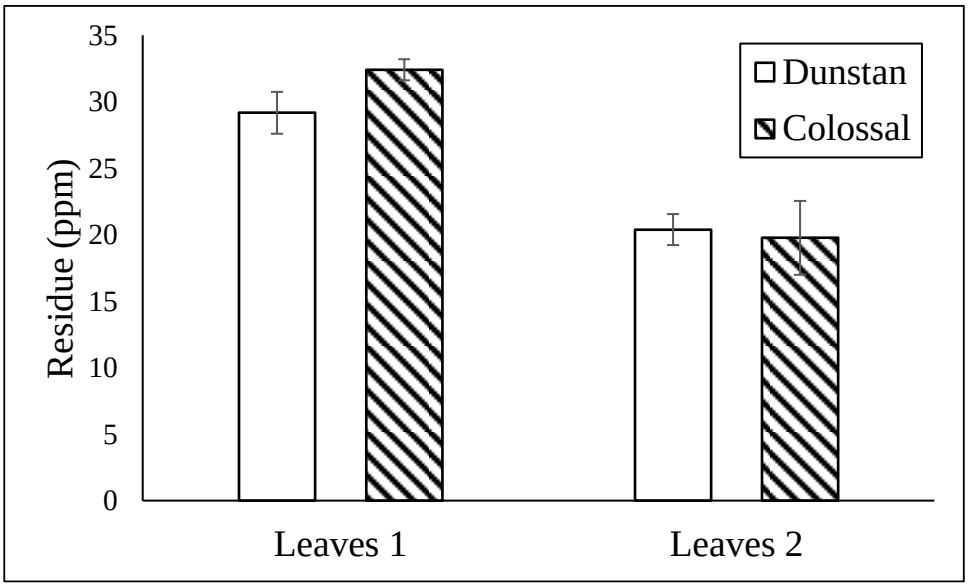

Figure 3.4. Mean (±SE) emamectin benzoate residues (ppm) in foliage samples collected in June (Leaves 1) and July (Leaves 2) 2018 from three Colossal (*C. crenata* x *C. sativa*) and three Dunstan (*C. dentata* x *C. mollissima*) chestnut trees treated in spring 2018 at the BCC site. Differences between cultivars and foliage samples were not significant.

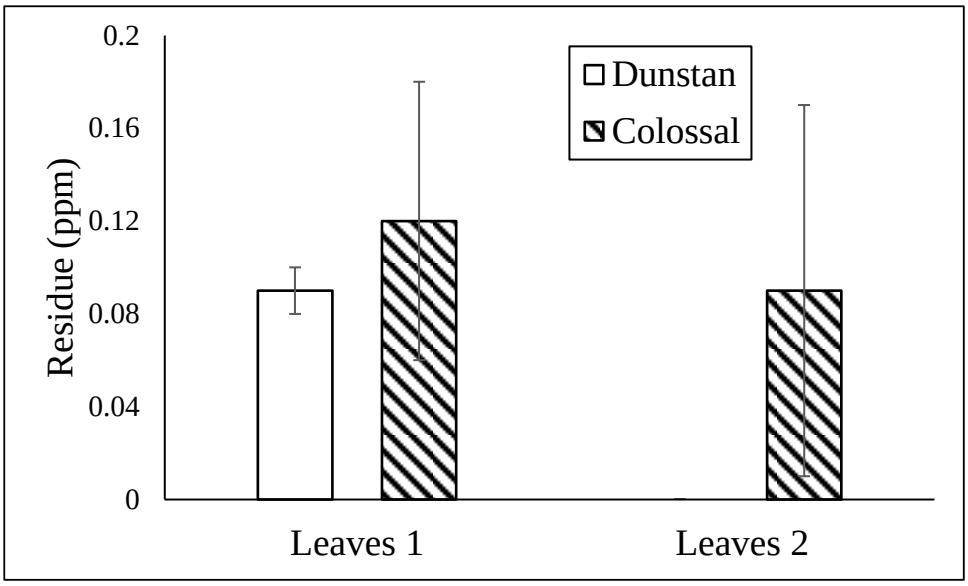

Figure 3.5. Mean (±SE) emamectin benzoate residues in foliage samples collected in June (Leaves 1) and July (Leaves 2) 2019 from three Colossal (*C. crenata* x *C. sativa*) and three Dunstan (*C. dentata* x *C. mollissima*) chestnut trees treated in spring 2019 at the BCC site. Differences between cultivars and foliage samples were not significant.

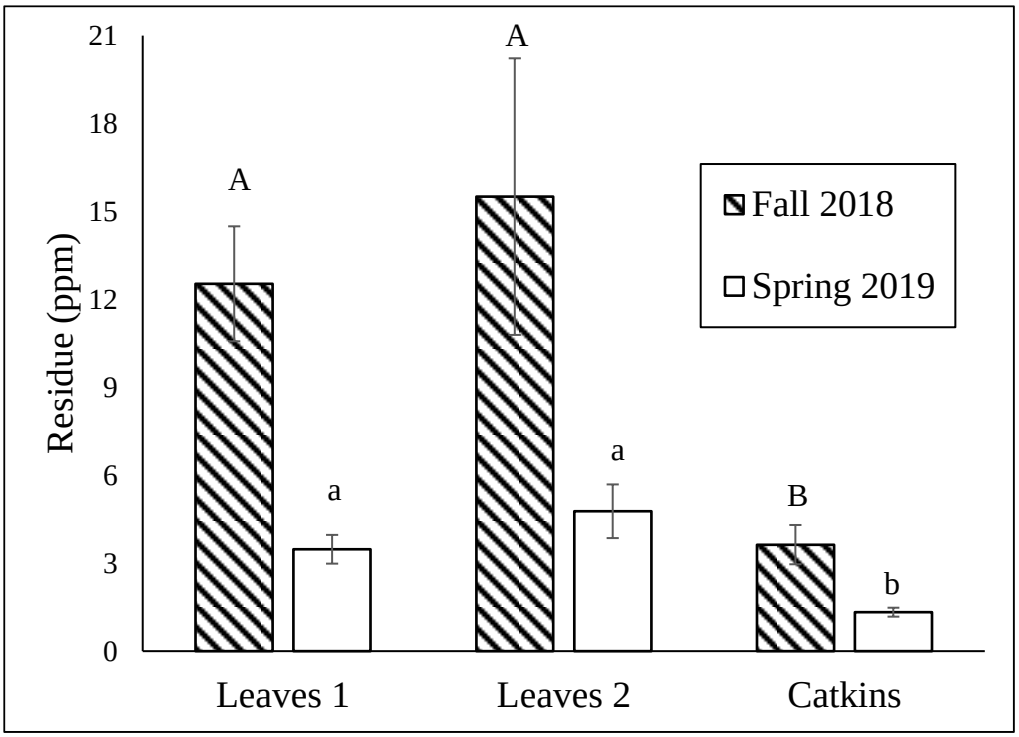

Figure 3.6. Mean (±SE) imidacloprid residues in June (Leaves 1) and August (Leaves 2) foliage samples and in catkin samples collected in 2019 from chestnut trees treated in either fall 2018 or spring 2019 (n=12 trees in fall; n=12 trees in spring) at the MSUPLP site. Capital letters indicate differences among chestnut tissues from trees treated in fall 2018 and lower-case letters refer to differences among chestnut tissues from trees treated in spring 2019.

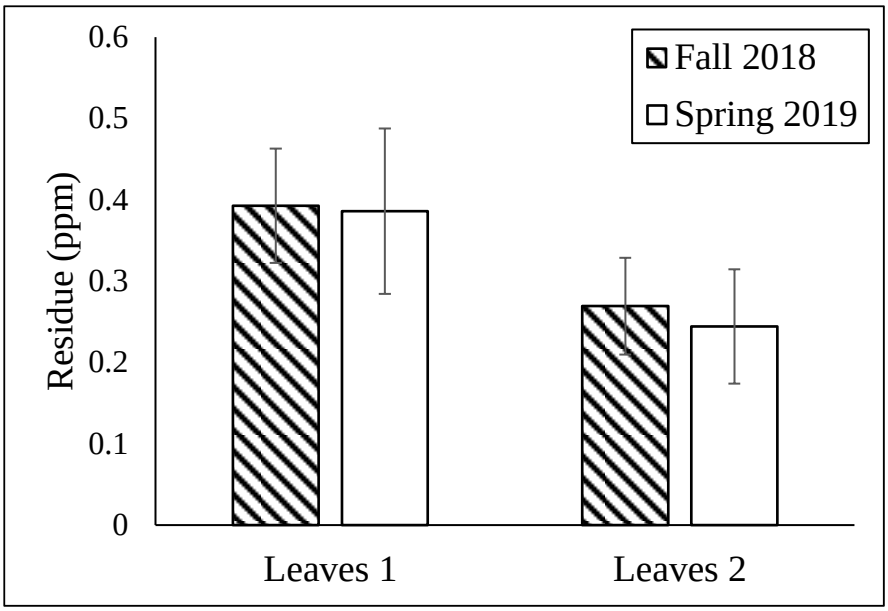

Figure 3.7. Mean (±SE) emamectin benzoate residues in June (Leaves 1) and August (Leaves 2) foliage samples collected in 2019 from chestnut trees treated in either fall 2018 or spring 2019 (n=12 trees in fall; n=12 trees in spring) at the MSUPLP site. Differences between foliage samples and treatment timings were not significant.

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