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THE GREAT LAKES ENTOMOLOGIST

Vol. 56, Nos. 1-2

Synopsis of Biological Control for European Fruit Lecanium (Parthenolecanium corni) by Parasitoids in North America and Preliminary Findings in Hybrid Hazelnut Orchards

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> > Abstract

The European fruit lecanium, Parthenolecanium corni (Bouché) (Hemiptera: Coccidae) is a native insect to North America that causes significant damage to a large variety of fruit and ornamental trees worldwide. Here we provide a summary of the insect's worldwide distribution and synopsis of all the Hymenopteran parasitoid wasps found to parasitize P. corni in North America from past literature. Additionally, a preliminary parasitoid survey of *P. corni* was carried out in two hybrid hazel (*Corylus avellana* \times *C. americana*) plantings as hazelnuts represent a potential new crop for the region. Parthenolecanium corni, were collected over two sample days in July 2022 from two hybrid hazelnut plantings and their parasitoid fauna recorded. Parasitism rates of P. corni were estimated for hymenopterous parasitoids as well as the entomophagous fungus Ophiocordyceps clavulata (Ascomycota: Hypocreales: Ophiocordycipitaceae). Hymenopterous parasitoids were identified to the lowest taxonomic level possible. Relationships between parasitism rates (a binomial response) and P. corni density (the predictor variable) were analyzed using binomial generalized linear models. Parthenolecanium corni experienced high parasitism rates: 24.0% by Hymenopteran parasitoids, and 63.4% by entomophagous fungi, giving a combined parasitism rate of 87.4%. Hymenopteran and fungal parasitism exhibited contrasting density-dependent relationships. Plants with higher densities of scales experienced lower parasitism rates from Hymenopteran parasitoids, but higher scale densities experienced higher parasitism rates from O. clavulata. Further research is needed over the whole adult female life stage of *P. corni* to learn more about these ecological relationships that could be of great benefit to hybrid hazelnut growers if *P. corni* becomes a significant pest.

Keywords: scale insects; soft scales; Coccidae; biocontrol

European fruit lecanium, Parthenolecanium corni (Bouché) (Hemiptera: Coccidae) (synonymous with 90 other scientific names), is a univoltine scale insect that attacks and causes significant economic damage to a large variety of fruit and ornamental trees globally (Sanders 1909, DeBach 1939, Asquith 1949, Madsen and Barnes 1959, Williams and Kosztarab 1972, Kosztarab 1996, García et al. 2016; Fig. 1A). The species wide geographic distribution, along with its host-induced morphological variation, has caused confusion regarding its taxonomy and native range, but is generally thought to be native to North America and introduced to Europe; being first recorded as abundant in Hungary in 1883 on black locust (Robinia pseudoacacia L.) (Ebeling 1938, Clausen 1956, Flanders 1970, Bartlett 1978, Hamon

and Williams 1984, Miller and Miller 2003, Hodges and Braman 2004, Camacho 2015, Miller et al. 2005; Fig. 2). Heavy infestations of *P. corni* can build up quickly and control is difficult once females mature (Williams and Kosztarab 1972). When populations are high, P. corni can cause wilting, stunting and dieback of their host plants (Gill 1988, Davidson and Raupp 2009). Additionally, there is evidence that P. corni is a vector for assorted viruses, such as among grapes in vineyards (Hommay et al. 2020). Parthenolecanium corni also excrete honeydew while feeding that can form sooty mold on fruit and foliage, consequently reducing photosynthesis and growth, and are commonly tended and protected by ants (Hymenoptera: Formicidae) from natural enemies (Gill 1988, Davidson and Raupp 2009, Flanders 1970; Fig. 1A).



Figure 1. Adult female European fruit lecanium, *Parthenolecanium corni* (Bouché) A) tended by ants (Formicidae) on the branches of hybrid hazel (*Corylus avellana* L. × *C. americana* Walter) in an orchard in Rosemount, Minnesota in July 2022. *Ophiocordyceps clavulata* is characterized by their club-like, warty stromata ranging from brown to black in color and B) infested with *Ophiocordyceps clavulata* (Schwein.) Petch. Photos by SGT.

Interspecific hybrid hazels, crosses between European hazel (Corylus avellana L.) and American hazel (Corylus americana Walter), are of growing interest to producers in the Midwestern US and Canada due to their ability to both survive the harsh climate of this region and produce profitable hazelnut yields (Bohnhoff et al. 2019, Braun et al. 2019). Hazels (*Corylus* spp. L.) are host plants of *P. corni* on which it has been recorded as a harmful pest in the US state of Oregon, as well as in Canada, Greece, and Turkey (Santas 1985, Ecevit and Yanilmaz 1987, Dale et al. 2012, Chernoh and Wiman 2022). The insect was first formally recorded on wild American hazel in the US state of New York in 1857 (Fitch 1857), and recently, Chediack et al. (2022) recorded all life stages of *P. corni* feeding on hybrid hazelnut at multiple orchards in Wisconsin, eliciting concern.

In European hazel orchards in Oregon, chemical treatments directed at the juvenile stages of *P. corni*, called "crawlers", that appear in June or early July, include acetamiprid, buprofezin, clothianidin, imidacloprid, pyriproxyfen, and spirotetramat (Chernoh and Wiman 2022). However, many parasitoid wasps (Hymenoptera: Chalcidoidea) are known to attack *P. corni* at various life stages in North America, and outbreaks of *P. corni* may be the result of broad-spectrum

insecticide use that reduce numbers of natural enemies (Flanders 1970, MacPhee and MacLellan 1971). To our knowledge, parasitoid communities, and parasitism rates of P. corni in North America have only been formally studied and recorded in the states of Georgia, Indiana, North Carolina, and Virginia to date (Hodges and Braman 2004, Camacho 2015, Dawadi and Sadof 2023). Additionally, an entomophagous fungus commonly found in North America and rarely in Europe, Ophiocordyceps clavulata (Schwein.) Petch (Ascomycota: Hypocreales: Ophiocordycipitaceae), may play an important role in the control of P. corni worldwide (Mains 1958, Kryukov et al. 2011; Fig. 1B, 2). Ophiocordyceps clavulata is thought to be the only recorded North American species of an ophiocordycypid fungus that utilizes the Coccidae family as hosts (Mains 1958, Shrestha et al. 2017; Fig. 1B). Therefore, our goals for this study were multifaceted: first, to compile literature documenting P. corni parasitized by Hymenopteran parasitoids within localities of North America and create a map of the known worldwide distribution of the entomophagous fungus associated with P. corni, O. clavulata; and second, to conduct a preliminary field experiment in two hybrid hazel orchards in Minnesota in the summer of 2022 in order to 1) record parasitism rates of P. corni by Hymenopteran parasitoids and

THE GREAT LAKES ENTOMOLOGIST



Figure 2. Worldwide distribution of European fruit lecanium, *Parthenolecanium corni* (Bouché), as of 2022 depicted in red overlayed with known occurrences of *Ophiocordyceps clavulata* (Schwein.) Petch as black dots. Geographic distribution data of *P. corni* and *O. clavulata* taken from ScaleNet (García et al. 2016) and GBIF (2022) respectively on 18 October 2022. Map created by HNS.

O. clavulata and examine their host-density relationships, and 2) to rear and record species of Hymenopteran parasitoids that emerged from field-collected *P. corni*.

Materials and Methods

Table 1 summarizes the known indigenous and introduced parasitoid and hyperparasitoid wasp species (Hymenoptera: Chalcidoidea) of North America that utilize P. corni or parasitoids of P. corni as hosts and the respective studies in which they were documented. Additional distribution localities in North America listed in Table 1 were added from ScaleNet and valid names of species were confirmed with the Natural History Museum's Universal Chalcidoidea Database (García et al. 2016, Noyes 2019). A distribution map was constructed to visualize the worldwide distribution of *P*. corni overlayed with recorded occurrences of O. clavulata (package, function: ggplot2, geom_polygon and geom_jitter; Wickham 2016; Fig. 2).

The preliminary field experiment was conducted at two experimental hybrid hazel plantings in Rosemount, Minnesota. Full details of study sites can be found in the Supplementary Materials. Maximum

and minimum daily temperatures were taken from the nearest weather station and accumulated growing degree days (AGDD) for P. corni were calculated using a base temperature of 12.8 °C and a start date of 1 January 2022 (Hodges and Braman 2004; Menne et al. 2012a, 2012b; Camacho et al. 2017). On 11 July 2022 seven infested hazel plants were chosen to sample for scale insects from one planting (n = 7), and then on 22 July 2022 from both plantings (n = 4, n =3), based on previous observations by HNS of *P. corni* infestation at the plantings, for a total of 14 plants. On each date, sampling of each plant was done for 15 person-minutes (i.e., three observers searching each plant simultaneously for scale insects for five minutes), during which branches observed to contain one or more scale insects, no matter the status (i.e., showing signs of parasitism or not), were collected via hand pruners.

Collected branches were immediately brought back to the lab and thoroughly examined to determine the number and status of every scale insect, broken into three categories: showing signs of parasitism by fungus (i.e., fungal stromata protruding from scale insect), showing signs of parasitism by Hymenopteran parasitoids (i.e., containing a parasitoid exit hole in shell), or showing no



THE GREAT LAKES ENTOMOLOGIST



Figure 3. Relationships between European fruit lecanium (*Parthenolecanium corni*) density and A) the percent *P. corni* infected by *Ophiocordyceps clavulata* and B) the percent of *P. corni* parasitized by wasp parasitoids found across two sampling dates in late summer 2022 in two hybrid hazelnut (*Corylus avellana* L. × *C. americana* Walter) orchards in Rosemount, Minnesota.

signs of parasitism. Scale insects collected in this study were identified by HNS using information from Gill (1988), Ben-Dov (1993), Hodgson (1994), and Kosztarab (1996). Fungal stromata from scale specimens were measured and identified to species by SGT using information from Mains (1958), White Jr. et al. (2003), and Shrestha et al. (2017).

Branches containing scale insects that showed no signs of parasitism were preserved in florist foam or rose picks and put into plastic grip jars modified for air-exchange at 26 °C, 50% RH, and 24:0 LD for a duration of three weeks for purposes of parasitoid rearing. Jars were inspected every other day and any emerged parasitoids were collected with an aspirator or paintbrush into a microcentrifuge tube and frozen at -20 °C until they could be identified. On 8 August 2022, all scale insects contained in jars were inspected for parasitoid exit holes, dissected to check for parasitoid larvae, pupae, or adults, and then discarded. Reared adult Hymenopteran parasitoids were determined by JCL using keys from Gibson et al. (1997) and Krombein et al. (1979). Several of the reared parasitoids were sent to Dr. Jason L. Mottern (USDA-APHIS, PPQ, NIS) for confirmation of determinations. To assess the density relationships between the proportion of P. corni that were parasitized by Hymenoptera and O. clavulata fungus (binomial responses) and P. corni density (a continuous predictor variable), we used generalized linear models with a binomial data family (package,

function: stats, *glm*; R Core Team 2022). All statistical analyses were conducted with R version 4.2.1 (R Core Team 2022) in Rstudio version 2022.07.0 (RStudio Team 2022). Detailed methods for recording parasitism and rearing parasitoids can be found in the Supplementary Materials.

Results and Discussion

A total of 549 scale insects were collected from the 14 plants over the two sampling dates for this study. Scale insects collected were verified to be *P. corni* and fungal bodies occurring on P. corni scales were confirmed to be O. clavulata. We found stromata of fungal specimens on P. corni measured between 0.55 mm and 2.06 mm in length with ovalshaped, warty, brownish-black heads that emerge from the sclerotium which matched the description given by Mains (1958) (Fig. 1B). Combining the parasitism rates of O. clavulata and the Hymenopteran parasitoids gave a combined parasitism rate of 87.4%, leaving only 12.6% of the collected scales that were not parasitized. Most scales were collected on the 11 July sampling date (478 AGDD), when 514 scales yielded parasitism rates of 22.0% for Hymenopteran parasitoids, 65.9% for O. clavulata, and a combined parasitism rate of 87.9%. On 22 July (598 AGDD), only 35 scales were collected, with 54.3% being parasitized by parasitoid wasps and 25.7% being parasitized by O. clavulata for a combined parasitism rate of 80.0%.

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Species	Localities found	Studies
Aphelinidae		
Coccophagus cinguliventris Girault	MD, IL, WI	Muesebeck et al. 1951
Coccophagus cowperi Girault	CA, SK, TX	Bartlett 1978
Coccophagus lycimnia (Walker)	AL, AR, BC, CA, CO, CT, DC, FL, GA, IA, II, IN, KS, LS, MD, MS, MN, NJ, NM, NY, NC, OH, ON, PA, QC, SC, SK, TX, VA	Bailey 1964, Camacho 2015, Dawadi and Sadof 2023
Coccophagus ochraceus Girault	CA, LA	Muesebeck et al. 1951
Coccophagus perflavus Girault	QC, WI	Muesebeck et al. 1951
Coccophagus pulvinariae Comp.	CA	Peck 1963, Flanders 1959
Coccophagus scutellaris (Dalman)	CA	Muesebeck et al. 1951
Encarsia spp. Foerster	IN	Dawadi and Sadof 2023
Encvrtidae		
Diversinervus elegans Silvestri*	CA	Krombein et al.1958, Peck 1963
Encyrtus spp. Latreille	IN	Dawadi and Sadof 2023
Encyrtus fuscus (How.)	AL, CA, CT, KS, MA, NJ, NY, NC, OH, ON, QC, TN, VI, WI	Smith 1929, Peck 1963, Bailey 1964, Flanders 1970
Gahaniella californica Timberlake	CA	Muesebeck et al. 1951, Peck 1963
Metablastothrix claripennis Comp.	CA, NC, PA	Peck 1963
Metaphycus spp. Mayr	IN	Dawadi and Sadof 2023
Metaphycus annulipes (Ashmead)	ON, FL	Muesebeck et al. 1951, Peck 1963
Metaphycus californicus (How.)	CA, MD, NC, PA	Peck 1963, Flanders 1970
<i>Metaphycus fuscipennis</i> How.	CA	Muesebeck et al. 1951, Peck 1963
Metaphycus helvolus (Comp.)*	CA	Flanders 1959, Bartlett 1978
Metaphycus johnsoni (How.)	IL, NC, ON, PA, QC, VA	Muesebeck et al. 1951, Krombein et al.
Metaphycus maculipennis (Timberlake)* Metaphycus rilevi (Timberlake)	CA NY. OH. ON. TX	1958, Peck 1963 Clausen 1956, Peck 1963 Clausen 1956, Peck 1963

THE GREAT LAKES ENTOMOLOGIST

Table 1. Known indigenous and introduced parasitoid and hyperparasitoid wasp species (Hymenoptera: Chalcidoidea) of North America

Vol. 56, Nos. 1-2

<i>Metaphycus</i> spp. Mercet <i>Microterys sylvius</i> (Dal.)	GA Canada, CA	Hodges and Braman 2004 DeBach 1939, Peck 1963, Flanders 1970, Docom 1076
Blastothrix sericea (Dal.)* Blastothrix longipennis How.	BC, CA, CT, LS, NS, NW, PA, QC, SK BC, CA, DC, GA, IA, MA, MI, MN, NM, NY, ON, QC, TX, WI	Graham 1970 Graham and Prebble 1953, Flanders 1970 Hodges and Braman 2004
Eulophidae <i>Aprostocetus</i> spp. Westwood <i>Pnigalio</i> spp. Schrank	IN NI	Dawadi and Sadof 2023 Dawadi and Sadof 2023
Ceraphronidae <i>Aphanogmus</i> spp. Thomson	IN	Dawadi and Sadof 2023
Diaspididae Plagiomerus sp. Crawford	GA, SC	Camacho 2015
Mymaridae Anagrus armatus (Ashmead)	CA, CO, CT, DC, FL, IL, IA, MD, NY, OH, ON, OR, PA, WA	Thompson 1955, Peck 1963
Platygastridae <i>Synopeas</i> spp. Förster	IN	Dawadi and Sadof 2023
Pteromalidae Pachyneuron spp. Walker Pachyneuron eros Girault	IN, VA BC, CA, HI, OR, UT, WA	Camacho 2015, Dawadi and Sadof 2023 Girault 1917, Peck 1963

*Indicates introduced species to North America

THE GREAT LAKES ENTOMOLOGIST

6

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THE GREAT LAKES ENTOMOLOGIST

Vol. 56, Nos. 1-2

Table 2. Parasitoid wasps (Hymenoptera) reared or dissected from adult female *Parthenolecanium corni* (Bouche) individuals on hybrid hazel (*Corylus avellana* L. × *C. americana* Walter) branches collected on 11 July 2022 from an orchard in Rosemount, Minnesota. Notes taken from Muesebeck et al. 1951, and Gibson et al. 1997, and Burks 2003.

Taxa	Method collected	Notes
Aphelinidae		
Aphytis sp.* Howard or Centrodora sp. Förster	Rearing (1)	<i>Aphytis</i> are typically parasitoids of armored scales (Diaspididae). <i>Centrodora</i> commonly parasitoids of Hemipteran eggs, though some have been reared as hyperparasitoids, and biology completely unknown for many spp. Could not distinguish between these two genera due to damage to the specimen's head.
Coccophagus sp.* Westwood	Rearing (2)	Parasitoids of soft scale insects; several spp. use <i>P. corni</i> as a host in the USA with two being found in the Upper Midwest
<i>Marietta</i> sp.* Motschulsky	Rearing (1)	External hyperparasites on scale in- sects, emerging from primary parasites in multiple Aphelinid genera
Encyrtidae Encyrtus sp.* Latreille	Dissection (1)	Parasitizes multiple Coccidae genera, especially <i>Lecanium</i>
Eulophidae Closterocerus sp.*	Rearing (1)	Generally, parasitoids of Lepidoptera but also reported from sawfly eggs (Ar- gidae, Diprionidae) and armored scales (Diaspididae)
Eupelmidae Eupelmus sp.* Dalman	Dissection (1), Rearing (1)	Two specimens of a short-winged species. Some Eupelmines are solitary endoparasitoids of Coccoidea. There are also some that are hyperparasit- oids.
Pteromalidae <i>Hypopteromalus</i> sp. Ashmead	Rearing (1)	Hyperparasites of Ichneumonidae and Braconidae wasps

*Indicates scale insects are documented as a hosts (i.e., Coccidae or Diaspididae)

Plants with higher densities of scales experienced higher parasitism rates from the fungus *O. clavulata*, indicating a positive density-dependent relationship ($X^2 = 64.84$; df = 1; P < 0.0001; Fig. 3A). Although not an aim of our preliminary study, we observed that two hybrid hazel plants out of the 14 total hybrid hazel plants sampled, contained 340 *P. corni*, with 297 (i.e., 87.4%) of the scales being parasitized by *O. clavulata*; coincidently, these two plants were the

same hybrid hazel genotype (Supplementary Materials).

Ophiocordyceps clavulata parasitized 63.4% of all scales insects collected in our study and we found a negative density-dependent relationship between infection escape rate from O. clavulata and density of P. corni (Fig. 3A). Existing literature supports a density-dependent relationship between cordycypid and ophiocordycypid fungi and their hosts. Kryukov et al. (2011) recorded species of Cordyceps fungus causing epizootic episodes in lepidopteran and sawfly larvae (Hymenoptera: Symphyta) in Siberia and concluded that these outbreaks occurred when the host insects were in optimal conditions for development. According to Borisov et al. (2006) cited in Kryukov et al. (2011), similar outbreaks have been observed in Krasnodar Krai, Russia with O. clavulata parasitizing P. corni in high proportions. Mains (1934) also noted an apparent outbreak of O. clavulata on scale insects near Munising, Michigan in 1933.

We found 24.04% of all scale insects collected, 132 out of 549, were parasitized by Hymenopteran parasitoids. Plants with higher densities of scales experienced lower parasitism rates, therefore exhibiting an inverse density-dependent relationship (X^2 = 55.71; df = 1; P < 0.0001; Fig. 3B). This parasitism rate is relatively lower than rates found in other studies of *P. corni* in North America but could likely be due to competition for hosts from O. clavulata (Camacho 2015, Dawadi and Sadof 2023). Camacho (2015) found that the rates of Hymenopteran parasitism on a complex of adult female *P*. *corni* and *Parthenolecanium quercifex* (Fitch) (Hemiptera: Coccidae), sampled between February and June from 2010 to 2013, were between 27-92% for scale populations on willow and oak trees in the US state of South Carolina. Dawadi and Sadof (2023) found Hymenopteran parasitism rates of ~30% on populations of adult female *P. corni* on honey locust (Gleditsia triacanthos L.) (Fabales: Fabaceae) in early June 2019 and 2020 in the US state of Indiana. Camacho (2015) states that the phenology of *P. corni* is earlier in southern US states than in northern states, and hence, justifies the comparatively later sampling dates used in our study. Additionally, Dawadi and Sadof (2023) reported a positive density-dependent relationship overall, except for on trees with the highest population densities in which there was a density-independent relationship. Surprisingly, neither of these studies reported presence of O. clavulata although it has been observed in North Carolina and Indiana (Fig. 2). Nonetheless, the possibility that the Hymenopteran and fungal parasitoids experience resource competition is an intriguing question that should be explored in further research. It is unclear at this time whether a high rate of fungal parasitism can cause a decrease in the amount of Hymenopteran parasitism, or vice versa.

A total of nine Hymenopteran parasitoid specimens were collected from rearing jars or dissections of the scale insects and identified to the genus level (Table 2). All nine specimens recovered came from scales collected from the first planting on 11 July 2022 and none from scales collected

at the second planting or on 22 July 2022. Seven of the nine specimens were found to be associated with either armored scales (Hemiptera: Diaspididae) or soft scales in the literature (Table 1, 2). A potential additional scale-associated specimen could not be distinguished between the genera Aphytis Howard (Aphelinidae) or Centrodora Förster (Aphelinidae). Aphytis spp. are endoparasites of armored scales (Diaspididae), while *Centrodora* are commonly parasitoids of Hemipteran eggs though some have been reared as hyperparasitoids and the biology is completely unknown for other Centrodora spp. (personal communication to HNS from Dr. Jason Motten [USDA-APHIS, PPQ, NIS] on 28 November 2022; Rosen 1973). The genera recovered that are associated with soft scales hosts are Coccophagus Westwood (Aphelinidae), Encyrtus Latreille (Encyrtidae), Eupelmus Dalman (Eupelmidae), and Marietta Motschulsky (Aphelinidae) (Table 2). Several Coccophagus species and one Encyrtus sp. are known to use P. corni as hosts in the US with several being recorded in the Upper Midwest (Muesebeck et al. 1951, Bailey 1964, Gibson et al. 1997, Camacho 2015; Table 1). Additionally, the recovery of the hyperparasitoid *Marietta* specimen from a rearing jar indicated the presence of one of its primary host genera which include Comperiella Howard (Encyrtidae), Anagrus Haliday (Mymaridae), Microterys Thomson (Encyrtidae), Signiphora Ashmead (Signiphoridae), Metaphycus Mercet (Encyrtidae), Aphelinus Dalman (Aphelinidae), Plagiomerus Crawford (Encyrtidae), and Coccophagus. With only *Coccophagus* being reared in our study, this may indicate its likely host genus as such (Gibson et al. 1997). Lastly, one of the wasps found via dissection of a scale was a species of *Eupelmus*, which are mostly known as a primary and secondary parasitoids of other insect families, however, some *Eupelmus* spp. are solitary endoparasitoids of Coccoidea (Krombein et al. 1979). This specimen provides evidence that P. corni scales are also part of prey fauna of Eu*pelmus* spp. which had not been previously recorded (personal communication to HNS from Dr. Jason Motten [USDA-APHIS, PPQ, NIS] on 21 December 2022).

Our preliminary results indicate that natural enemies such as the entomophagous fungus *O. clavulata* and Hymenopteran parasitoids may have the potential to provide high levels of control in hybrid hazel orchards. However, we recognize several limitations of our study. First, collected scales infested with *O. clavulata* introduced a possible source of sampling bias. The scales that were parasitized by *O.clavulata* were held fast to the branches, but scales that were not parasitized by *O. clavulata* were easily

THE GREAT LAKES ENTOMOLOGIST

Vol. 56, Nos. 1-2

knocked off. During sampling, the branches were transported as carefully as possible back to the laboratory to be processed. Even so, it is likely that fungal-parasitized scales were overestimated compared to scales parasitized by Hymenopteran parasitoids and non-parasitized scales because of sample loss during transport. Second, this sampling took place near the end of the life cycle of the scales sampled. In the weeks before sampling began, higher rates of adult female scales had been observed at the field site. Because the second sampling event took place eleven days after the first, fewer scales were collected on that day, and therefore, very few of them came from the second experimental planting. It is known that parasitoid-host relationships between Lecanium scales (i.e., various soft scales pests of multiple trees and ornamental species in the genera Eulecanium Cockerell, Parthenolecanium Sulc, and Sphaerolecanium Sulc) and wasp fauna change drastically throughout the scales' phenology (Peterson 1960, Camacho 2015). This study could draw more conclusive results if higher sampling had taken place at different points along the scales' growth curve but does provide meaningful data for the latter part of the life stage of the adult female scales.

These preliminary findings support the consideration of natural enemies in developing pest control in hybrid hazelnut plantings. Bentley and Day (2010), cited in Camacho 2015, suggested that insecticidal treatment against \underline{P} . corni on fruit trees in the Central Valley of California could be omitted if a large (but unspecified) number of scale insects are parasitized in the summer. It has also been demonstrated that excessive use of insecticides can increase scale densities by killing Hymenopteran parasitoids that regulate their populations. In a case study documented by MacPhee and MacLellan (1971), DDT treatment in a mature apple orchard caused a documented decrease in both Coccophagus spp. and Blastothrix sericea (Dalman) (Encyrtidae) and a corresponding outbreak of the related coccid Eulecanium *tiliae* (L.) (Coccidae). It is important moving forward that knowledge of these tiny but ecologically important parasitoid wasps be made available to growers and researchers working with hybrid hazelnuts since it is a novel crop for the region.

Increased understanding of *O. clavulata* as a biological control agent will also be important going forward. The implications of *Cordyceps* and *Ophiocordyceps* fungi as biological control agents have been studied in several experiments, but few or none have focused on *O. clavulata* specifically. Ou et al. (2019) examined *Cordyceps javanica* (Frieder, & Bally) Kepler, B. Shrestha & Spatafora

(Hypocreales: Cordycipitaceae) in tandem with the parasitoid wasp Eretmocerus hayati (Rose and Zolnerowich) (Hymenoptera: Aphelinidae) and their individual and combined effects on the whitefly Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae). The study found that while there was some mortality of the Hymenopteran parasitoid due to the fungus (mostly in the nymphal stage); treatments that combined fungal sprays with parasitism by the wasp were more effective at controlling the whitefly than either of the parasitoids on their own (Ou et al. 2019). More interdisciplinary study is needed to understand the relationships between O. clavulata and the Hymenopteran parasitoids that attack the P. corni. Nonetheless, given the density-dependent relationship of O. clavulata's with scale insects, it has the potential to be an important source of biological control. This finding is especially relevant given the risks of chemical sprays in causing scale outbreaks.

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Vol. 56, Nos. 1-2

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118

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Supplementary Materials for "Synopsis of biological control for European fruit lecanium (*Parthenolecanium corni*) by parasitoids in North America and preliminary findings in hybrid hazelnut orchards"

Field sites

The field experiment was conducted at two experimental hybrid hazel plantings: the first planting (Rosemount, Minnesota; 44°43'36" N, 93°05'59" W; established 2011-2013) represents 270 plants comprising 18 replicated hybrid hazelnut genotypes in a randomized complete block design, and the second planting (Rosemount, Minnesota; 44°41'44.5"N 93°05 01.0"W; established 2013) represents 77 plants comprising seven replicated hybrid hazelnut genotypes in a random complete block design. Maximum and minimum daily temperatures were taken from the nearest weather station (located at 44° 43' 0.2274" N, 93° 5' 52.728" W; a distance of 1.11 km from the first planting and 2.62 km from the second planting) in the United States National Climatic Data Center's "Global Historical Climatology Network-Daily Database" (Menne et al. 2012a, 2012b). Accumulated growing degree days (AGDD) for *P. corni* were calculated using a base temperature of 12.8 °C and a start date of 1 January 2022 (Hodges and Braman 2004; Camacho et al. 2017).

Scale insect sampling

Hybrid hazel plants were not chosen randomly to sample, but instead based on previous observation of *P. corni* infestation at the field sites and the goal of sampling from a diverse pool of hybrid hazel genotypes as to avoid potentially compounding plant genotype-specific effects. Seven hybrid hazel plants were sampled for *P. corni* at the first site on 11 July 2022 (478 accumulated GDD). The genotypes sampled from this day included Rose 11-8 (n=4), Arb 7-1 (n=1), and Marie (n=2). On 22 July 2022 (598 accumulated GDD), four different hybrid hazel plants from the first planting (genotypes 'Rose 11-12' (n=1), Rose '10-5' (n=1), Arb 7-1 (n=2) and 'Gibs 6-23' (n=1)) and three trees from the second planting (genotypes 'Rose 17-4' (n=2) and 'SpC-2C7' (n=1)) were sampled for *P. corni* as. Sampling of each tree was done for 15 person-minutes (i.e., three observers searching each plant for scales for five minutes), during which time every branch observed to contain one or more

Vol. 56, Nos. 1-2

scale insects, no matter the status (i.e., showed signs of parasitism or not) was collected via hand pruners. Branch samples ranged from short twigs (i.e., approximately 15 cm) up to about one meter in length.

Following each sampling day, trimmed branches collected during sampling were brought back to the lab to isolate individual scales or groups of scales that appeared alive on the branches. Scale insects collected in this study were identified by HNS using keys, diagrams, and information from Gill (1988), Ben-Dov (1993), Hodgson (1994), and Kosztarab (1996) which are cited in the main text. Each branch sample was inspected under a microscope, and each scale that occurred on branches was counted and color-coded in which a small dot of acrylic paint was placed on the branch near the scale to indicate its status as follows: yellow for scales with no signs of parasitism, blue for scales with parasitoid exit holes, and orange for scales with signs of fungal disease (i.e., possessing stromata). Scales that exhibited no signs of parasitism were not confirmed to be alive, which would have involved destructively removing them from the branches, for the purpose of rearing out parasitoid adults. Once the status of the scales had been recorded, branch trimmings from the same plant containing scales that did not appear to be parasitized (i.e., marked with yellow) were preserved in water picks or wetted floral foam and put into a 3.8-liter (i.e., one gallon) plastic grip jars (ULINE, Pleasant Prairie, WI, USA) with a modified cloth top to provide air exchange for monitoring of parasitoid emergence. Eight fungal stomata from each specimen of fungal-parasitized scales (i.e., those marked with orange) were measured using a Leica photographic microscope in order to confirm the identity of the fungus by SGT using diagrams and information from Mains (1958), White Jr. et. al. (2003), and Shrestha et. al. (2017). Branches that did not contain any scales without signs of parasitism (i.e., marked with yellow) were discarded after the status of scales was recorded.

Jars were kept in the laboratory at 26 °C, 50% RH, and 24:0 LD. Any spots of mold that developed on branches or scales were carefully, manually removed via a wetted paintbrush. Jars were monitored every other day for a total of three weeks for parasitoid emergence, but the branches inside were only kept alive for one week via watering. Wasps were collected with an aspirator or a paintbrush into a 2 ml microcentrifuge tube and then frozen at -20 °C until they could be identified. On 8 August 2022 the scale specimens contained in jars were closely inspected for signs of parasitism (i.e., parasitoid exit holes), dissected to check for parasitoid larvae, pupae or adults and then discarded. One adult wasp was found in good condition for identification, while six others were not in good condition for identification, while six others were not in good condition for identification, while six others were adult Hymenopteran parasitoids were determined by JCL using keys from Gibson et al. (1997) and Krombein et al. (1979). Several of the reared parasitoids were sent to Dr. Jason L. Mottern (USDA-APHIS, PPQ, NIS) for confirmation of determinations.