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Gut Content Analysis of Arthropod Predators of Codling Moth in Washington Apple Orchards

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Abstract

Codling moth, *Cydia pomonella* (L.), is the key pest of pome fruits in many temperate areas of North America, Eurasia, South Africa, South America and Australia. Many predatory arthropods species are found in organic apple orchards of central Washington; here we use PCR-based gut content analysis of arthropod predators to identify predators that attack codling moth. Predators were sampled from tree canopies, tree trunks and from the understory and were homogenized in a lysis buffer to provide a template for direct PCR. PCR showed 8.9% of 2591 predators had preyed on *C. pomonella*. Spiders, including 25 genera from 15 families, two carabid beetle species (*Pterostichus melanarius* (Illiger) and *Harpalus pennsylvanicus* DeGeer) and the European earwig (*Forficula auricularia* [L.]) represented 87% of predator specimens analyzed and were 8.2%, 8.3% and 14.7% positive for *C. pomonella*. PCR products from 38% of predators that appeared positive for *C. pomonella* COI were sequenced; all showed 99% or more similarity to *C. pomonella* COI sequences in GenBank. Digestion rates of adult earwigs fed on mature codling moth larvae showed a detection half-life of 3.7 days; half-life from the fecal pellets from the same earwigs was 3.6 days. When fed mature codling moth larvae, the carabid *P. melanarius* showed a digestion half-life of 3.14 days. Identification of the key predators of *C. pomonella* can guide the use of selective insecticides and the conservation of these natural enemies, enhancing biological control and supporting stable IPM programs in pome fruit orchards in the western USA.

Keywords: *Cydia pomonella*, predatory arthropods, Polymerase Chain Reaction, Conservation
Biological Control

1. Introduction

Codling moth, *Cydia pomonella* (L.), (Lepidoptera: Tortricidae) (CM) is a key pest of apples (*Malus domestica* Borkh.), pears (*Pyrus communis* L.) and walnuts (*Juglans regia* L.) in the Western U.S. (Barnes, 1991). Traditionally, apples in central Washington were protected from CM and many secondary pests with applications of organophosphate pesticides as the basis of an Integrated Pest Management (IPM) program. The use of broad-spectrum insecticides did not allow for conservation biological control because of significant suppression of natural enemies, with the exception of predatory mites (Hoyt, 1969; Croft and Hoyt, 1983). In the last decade, most organophosphates have been removed from use in apple, and replaced by newer insecticides with alternative modes of action. Some of the newer insecticides will allow conservation of natural enemies, but their use will require revision of our IPM programs (Jones et al., 2009, 2010b).

Conservation biological control is a critical component of integrated pest management (IPM) and the key approach is to reduce use of disruptive insecticides (Stern et al., 1959; Kogan, 1998; Jones et al., 2009). When broad-spectrum pesticides are removed, natural enemies become more abundant and show greater diversity and evenness (Crowder et al., 2010). CM can be suppressed by new insecticides, but some of these materials can suppress natural enemies (Mills et al., 2016; Beers et al., 2016, this issue). Pheromone-based mating disruption (Vickers et al., 1991; Witzgall et al., 2008) and CM granulovirus (Lacey and Unruh, 2005; Lacey et al., 2008) are selective and are organic certified. In the last 20 years, organic apple production in Washington has increased 14 fold, due largely to mating disruption and granulovirus. Suppression of CM and leafrollers (Knight, 1994, 2008; Arthurs et al., 2007; Lacey et al., 2008; Monteiro et al., 2013) and the

woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Nicholas et al., 2005; Gontijo et al., 2015) are due to the use of those selective products.

Multiple studies have demonstrated the importance of generalist predators in pest suppression in agro-ecosystems (Symondson et al., 2002). A great diversity of predatory arthropods has been collected in organic orchards in central Washington (Miliczky et al., 2000; Miliczky and Horton, 2005; Horton et al., 2012). A Leslie matrix model simulating larval survival of CM with 25% mortality from predation resulted in 68% reduction of female CM over the season (Jones et al., 2009). A question we ask is which predators would best help attain 25% or more predation of CM as well as secondary pests in apple orchards. Our goal was to identify predators collected from Washington apple orchards that showed evidence of feeding on CM based on molecular gut content analyses.

A recent study in France (Boreau de Roincé et al., 2012) analyzed gut contents to detect CM and Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) in predators collected from the understory of nine organically-managed apple orchards. In our study, we collected predators from abandoned, organic, research and conventionally-managed apple orchards for gut content analysis to detect the presence of CM. We also examined digestion patterns of CM in the gut contents of a carabid beetle and the European earwig. For the earwig, we compared the use of fecal pellets versus whole body extracts for PCR analysis to discover whether fecal pellets can be an effective, non-destructive, approach for detecting digestion rates and evaluating the inclusion of specific prey species within a predator's diet. We discuss the influence of sampling methods on the probability of detection of feeding on CM for different predator taxa, and finish

with a discussion of the need for mark-release-recapture methods to estimate predator abundance allowing for rational methods to identify the relative importance of predator species.

2. Materials and Methods

2.1. Specimen collection

Predatory arthropods were collected from seven apple orchards in Yakima Co., WA. The geographic location, area, management approach, year(s) sampled and which strata were sampled, are summarized for each orchard in Table 1. Three strata were potentially sampled: the tree canopy, tree trunk area and the orchard floor. Beat trays were used to dislodge predators from the canopy (Miliczky and Horton, 2005). When visible spiders were not dislodged onto beat trays, they were collected by hand and were classified as ‘trays’. Predators active on the tree trunk and the base of scaffold branches were captured in cardboard bands and rolls. Bands consisted of two layers of 10-cm-wide, single-faced, cardboard (B flute; Weyerhaeuser, Tacoma, WA) (Unruh and Lacey, 2001; Miliczky et al., 2008) secured around the tree trunk with staples. Cardboard rolls of 5-6 cm diameter were tied to scaffold branches (Epstein et al., 2001) oriented such that the flutes were parallel to the trunk or branch; these were grouped as ‘bands’. Ground dwelling predators were captured in dry pitfall traps (Greenslade, 1964) consisting of two 0.75 l clear plastic cups, one within the other, allowing easy specimen removal. Small perforations were made through the bottom of both cups to allow water drainage. Traps were covered/shaded with green plastic dessert plates (17.8 cm dia., Chinex, Hutamaki, De Soto KS,) suspended 8 cm above ground using aluminum fence ties (ChainlinkFittings.com) to form tripod legs affixed to the plates with heavy-duty tape. Pitfall traps were deployed for 24 h, after which predators were

removed. Specimens retrieved by each method were immediately placed in vials or zip lock plastic bags, placed under ice packs in a cooler and frozen at -20 °C within 4 h.

Beat trays and cardboard retreats allow predators to come and go and forage until they are collected. However, predators that fall into pitfall traps are retained up to 24 h (median of 12 h) before being removed which can reduce gut content detection rates.

2.2. Specimen identification

Specimens were identified by morphological traits; however, identifications of Carabidae, Formicidae and Staphylinidae were assisted by DNA barcoding (Hebert et al., 2003). DNA was extracted from three legs of each of two beetles from each of 12 morphotypes collected. DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA) were used to extract DNA and 2 ul of the template was used in 25 ul PCR reactions. Whole bodies of four ant and four staphylinid individuals were homogenized in a lysis buffer which was used directly as the template in Direct PCR (see section 2.4 below); only 1 ul of lysis buffer was used as template in 25 ul reactions. Universal insect primers for cytochrome oxidase 1 (COI) (Folmer et al., 1994) were used in the PCR and also for subsequent DNA sequencing. PCR product was treated with ExoSAP-IT (Affymetrix, Santa Clara, California) and sequenced in an ABI 3730XL sequencer at Molecular Cloning Laboratories (MCLAB; South San Francisco, CA). DNA sequences from the carabids and ants and staphylinids were compared to corresponding families in GenBank using BLAST (Altschul et al., 1990). Once identified by barcoding, specimens of similar morphotype were used as vouchers.

2.3. Preparation for gut content analysis by PCR

Prior to PCR, specimens were surface sterilized in 70% EtOH for 1 min, then in 1% sodium hypochlorite and 0.05% sodium dodecyl sulfate (SDS) solution for 2 min, rinsed under running tap water for 30 s and blotted dry (modified from Lacey et al., 2007). For carabids and earwigs, wings and elytra were removed prior to sterilization; specimens were then directly homogenized or frozen at -20°C. Specimens were placed in a sterile 1.5 ml microcentrifuge tube with 1:40 ratio (w:v) of specimen and lysis buffer (0.025 N NaOH and 0.0125% SDS; Kiatpathomchai et al., 2001). Five 2.5 mm dia. glass beads were added and specimens homogenized for 55 s by shaking in a BioSpec Mini-Bead Beater 24 (BioSpec Products, Inc., Bartlesville, OK). The tube was boiled for 10 min, chilled on ice, spun at 5,000 RPM and the clear supernatant was used as PCR template (=Direct PCR; Templeton et al., 2015; see Discussion).

2.4 Primer design, PCR/TAQ selection and PCR sensitivity

Primers created for the mitochondrial COI region of CM were developed in the primer design module in Geneious 6 (Kearse et al., 2012) and candidate primer pairs were tested against Insecta using primer BLAST (Ye et al., 2012). A high stringency (67.5°C) primer pair was used: forward F2 5' TTGAGCCGGAATAGTAGGAACTTCTC 3' and reverse R324 5' GTTCCTGCTCCATTTTCAACGATT 3'. The PCR program used was 30 s at 95°C, 45 cycles of [15 s at 95°C, 30 s at 67.5°C, 30 s at 72°C] and 2 min at 72°C. The master mix was 0.2 μ M of each primer, 0.1 mM of each dNTP, 0.25 X Sybr Green1, 1 unit of Titanium® Taq DNA Polymerase (Clontech, Inc. , Mountain View, CA, USA) and 1X buffer provided by vendor. One μ l of template (lysate) in the 25 μ l reaction was used for dPCR. To ensure sensitivity in reactions, positive controls were used for each run. Representative predators, free of CM DNA and 5th instar CM larvae were each homogenized in lysis buffer in 1:40 ratios as above. Dilutions of 10⁻³,

10^{-4} and 10^{-5} of CM homogenate in predator homogenate as diluent were used as controls for PCR runs; runs were repeated if 10^{-4} or 10^{-5} CM dilutions were not positive.

2.5. Verification of CM detection

Direct real time PCR was the first stage of verification that a product was CM DNA. Melt curves showing a temperature (T_m) of 79.7 ± 1.5 °C, or overlapping that range, triggered agarose electrophoresis. If band size was 300 bp, the product was deemed CM; this reduced gel electrophoresis by more than 70%.

The specificity of the primer set was tested against 37 non target species in duplicate PCRs (Appendix A, Table S1). One non-target species was amplified with the CM primer set, hence we conducted further validation with 38% of predators nominally positive for CM. PCR products were sequenced in both directions and BLAST of sequences were conducted as described in section 2.2.

2.6. Detection half-lives for *Forficula auricularia* and *Pterostichus melanarius*

Feeding trials were conducted to measure the half-lives of detection of CM DNA (Greenstone et al., 2010, 2014) in the gut contents of *F. auricularia* and *P. melanarius* that had been fed with CM larvae. A colony of adult *F. auricularia* was collected from the USDA-ARS farm in Moxee WA (orchard M). Earwigs were maintained in 30 L plastic storage boxes with crumpled brown paper towels for aggregation of these gregarious, nocturnal insects. Dry cat food (Whiskas® brand; Mars Inc., McLean VA), fresh rose-petals and water were provided in 9 cm glass petri dish bottoms. Food and water was replenished weekly and earwigs were twice transferred to cleaned boxes.

After cleansing their guts of CM, 132 earwigs (66 male and 66 female) were individually moved to 59 ml plastic cups (P200 and PL2; Solo Cup Co., Urbana, IL). Earwigs were starved the day prior to the feeding trial. Eleven treatment groups of 12 earwigs (six of each sex) were used. One treatment group, the positive control, were provided with a freshly killed (damaged head) fifth instar CM larvae and allowed to feed for 24 h (day zero), then frozen at -20 °C. A second treatment group was the negative control; earwigs were starved for 24 h (day zero) and then frozen. To determine if meal size influenced detectability of the CM signal, half of each of the remaining nine treatment groups was fed a large meal and half were provided a smaller meal. Specifically, three of the six males and three of the six females were fed one half of a 5th instar CM larva (average weight and SE: 20.2 ± 0.7 mg); the remaining three males and females were fed three halves of CM larvae (48.8 ± 0.2 mg). The expected 3-fold greater weight of the latter group was only 2.4-fold greater, likely due to greater loss of liquid during the bisections. Each earwig was weighed before and after feeding for 24 h (day zero) to determine weight gain as a crude measure of prey consumed. From day one through nine, the treatment groups of earwigs were provided an eight mm cube of apple pulp as a chaser food (Weber and Lundgren, 2009). Each day fecal pellets were collected from all treatment groups and one treatment group was terminated. The adults from terminated treatment groups were placed in 1.5 ml microcentrifuge tubes and frozen at -20 °C. Prior to homogenization and dissection of the adults, wings were removed and earwigs were washed and homogenized in the bead beater as described above (section 2.3). Three male and three female earwigs from each group were homogenized as whole bodies; the remaining three males and females had their guts excised and homogenized. Fecal pellets were collected using sterile toothpicks and placed into 0.5 ml microfuge tubes; they were

homogenized using plastic pestles with 10 μ l of lysis buffer for one to two pellets and 20 μ l for more pellets.

Pterostichus melanarius were collected by pitfall trapping in a garden area distant from apple orchards and were maintained individually in 59 ml plastic cups for two weeks on a diet of diapausing mature larvae of Oriental fruit moth (*G. molesta*) and water to clear their guts of CM. Each beetle was starved for two days and then provided a killed 5th instar CM larva and allowed to feed for 24 h. One treatment group of 15 beetles was not fed (negative control), a second treatment group was fed (positive control) and both were frozen after day zero. In a pilot test, we found the beetles to be erratic in eating either apple cubes or Oriental fruit moth larvae each day after day zero as chaser food. Hence, no chaser food was provided, but beetles were provided a 25 μ l droplet of water daily. Subsequently, one treatment group of 15 beetles was frozen each day on days one to five. Fecal production was erratic and was not used for PCR. Wings, elytra and legs were removed from the carabids, they were surface sterilized as described above, and whole guts were excised, homogenized in the bead beater, and used as templates in dPCR.

2.6. Data summary and statistical analyses

The percent positive for CM DNA detection in the gut content analysis of predators collected from apple orchards was summarized both graphically and in tables. Analysis of the percentage of predators testing positive for CM was based on χ^2 test as implemented in PopTools (Hood 2010). For the feeding trials, the decay in detection rates of CM DNA for both earwigs and carabids through time was fitted to a probit model with a logistic cumulative distribution using

SAS (Probit Procedure; SAS ver. 9.4, 2013) providing predicted probability of detection (including half-life) with 95% confidence intervals. For earwigs, probit models were based on CM detection from homogenates of whole bodies and excised guts (pooled for the analysis), and from homogenates of fecal pellets. The analyses were repeated using meal size, sex, and weight gain as covariates. For *P. melanarius*, the probit model was based on CM detection from homogenates of excised guts with no covariates.

3. Results

3.1. PCR selectivity and validation by DNA sequencing

Specificity of the primer set for CM was tested against 37 non-target herbivore and predator arthropods (Appendix A, Table S1). One species, *Lacanobia subjuncta* (G & R) (Lepidoptera: Noctuidae), was amplified in two replicate PCR runs. The *L. subjuncta* PCR products exhibited a 300 bp band and T_m of 80.2 and 80.4 °C, overlapping the T_m of CM (79.7 ± 1.5 °C). The COI sequence of *L. subjuncta* in GenBank was three base-pairs different for CM in the reverse primer region. *L. subjuncta* was not observed in the orchards sampled, but was abundant in some orchards in the late 1990s (Landolt, 1998, 2000). To ensure putative CM-positive PCR products from predators were not from the non-target *L. subjuncta*, or other non-targets, PCR products from 87 (38%) nominally CM positive specimens were sequenced including: 34 earwigs, 26 spiders, 20 carabids, 3 staphylinids, 2 ants and 2 assassin bugs. All sequences returned from BLAST with 99-100% identity to CM. In contrast, *L. subjuncta* sequences showed 89% similarity to CM, providing strong inference that our positive PCR products were CM DNA.

3.2. Predators analyzed for CM signal from apple orchards

The number of predator specimens analyzed by PCR from the seven apple orchards varied from 48 to 718 per orchard for a total of 2591 specimens (Table 1). Nearly half of the predator specimens analyzed (1278) were collected from five orchards in 2006. Pitfall trapping was the main collection method employed across all years and orchards, resulting in the capture of 1856 predator specimens.

CM DNA was detected in $8.9 \pm 0.6\%$ (SE) of the 2591 predator specimens, including the earwig *F. auricularia*, the carabids *Pterostichus melanarius* (Illiger) and *Harpalus pennsylvanicus* DeGeer, 25 genera from 15 spider families, two staphylinids *Xantholinus linearis* (Olivier) and *Philonthus carbonarius* (Gravenhorst), two ant taxa *Formica* nr. *sanguinea* group and *Formica* nr. *truncorum*, and the harvestman *Phalangium opilio* L. Additional carabids collected and identified by DNA sequencing included *Carabus* spp., *Calosoma scrutator* (Fabricius), *Calathus ruficollis* Dejean, *Harpalus affinis* (Schrank), *Bembidion rapidum* (LeConte) and *Amara* spp. , but only *P. melanarius* and *H. pennsylvanicus* were analyzed by PCR because they represented more than 90% of carabids captured. Similarly, eight unidentified assassin bugs were collected from one orchard in one year; two of the bugs tested positive for CM DNA (25%), but were not included in the larger analyses due to small numbers.

The six predator taxa represented in Figure 1 showed significant variation in their percentage positive CM signal ($\chi^2 = 42.2$, $df = 5$, $P < 0.001$) which is also evident by the bars of 2 standard errors. The earwig, carabids, and spiders accounted for 86% of the predators analyzed and, as a group, their percent positives for CM detection were significantly higher than the ants,

harvestmen and staphylinids when grouped ($\chi^2 = 19.7$, $df = 1$, $P < 0.001$) (Fig. 1). The two carabid species, *P. melanarius* ($n = 567$) and *H. pennsylvanicus* ($n = 327$), showed similar positive signal for CM: 8.1 and 8.6 respectively.

The 776 spiders analyzed represented a diverse set of families, genera and species (Appendix A, Table S2) and for 15 of the most abundant spider taxa (733 spiders) combined were 8.5% positive for CM (Fig. 2). The percent positive for CM detection for spiders was 11.5% for trays ($n=253$), 5.4% for pitfall traps ($n = 445$) and 14.1% for bands ($n = 68$), showing significant variation among collection methods ($\chi^2 = 12.0$, $df = 2$, $P < 0.002$). All insect predators combined were 9.5% CM positive ($n = 1722$) and all spiders combined were 8.2% CM positive, suggesting that these two arthropod groups have equal representation as CM predators ($\chi^2 = 0.9$, $df = 1$, $P = 0.34$).

3.3. Detection half-lives for *Forficula auricularia* and *Pterostichus melanarius*

The digestion patterns and half-lives of the CM signal derived from earwig whole bodies, including excised guts, (3.7 ± 1.4 d, Fig. 3) was congruent with that from fecal pellets (3.6 ± 0.2 , Fig. 4). Goodness of fit tests for both fecal pellets and adult bodies could not be rejected ($P > 0.4$). The Type III test of the probit model for adult earwigs was significant (Wald χ^2 for time effect = 24.1, $P < 0.0001$) and the Type III test for fecal pellets was highly significant (Wald $\chi^2 = 226.7$; $P < 0.0001$). Earwigs fed one half of a CM larva gained 11 ± 1.2 mg while those fed three halves of a larvae gained 17 ± 0.9 mg (mean $\pm$ SE; $n = 59$ in each feeding group; two weight-gain outliers were not used). When sex, meal size and weight gain were used as covariates in the probit model for the fecal pellets, they proved to be insignificant effects. The

type III analyses of effects with covariate was similar to that without covariates (Wald χ^2 for time effect = 226.7, $P < 0.0001$; χ^2 for sex effect = 3.9, $P = 0.07$; χ^2 for meal size = 1.0, $P = 0.31$; χ^2 for weight gain = 0.34, $P = 0.56$).

The narrow confidence intervals for the fecal pellet model were due to a steady decline in PCR sample size through time by 12 each day from 98 on day 1 to 12 on day 8 (Fig. 4). Hence, PCR assays from fecal pellets collected daily resulted in higher sample size than whole body assays until the final day. In contrast, 12 PCR samples were used each day for eight days for whole bodies.

The digestion pattern for *P. melanarius* showed a half-life of 3.14 (± 0.74) with broader confidence intervals due to a daily sample size of 15 (Fig. 5). Nonetheless, the goodness of fit test for the probit model could not be rejected ($P = 0.14$) and the Type III test was highly significant (Wald $\chi^2 = 19.08$, $P < 0.0001$).

DISCUSSION

Risk of predation for CM is low for larvae feeding inside the fruit. However, eggs on the fruit surface, neonates entering the fruit, mature larvae seeking cocooning sites and cocoons lasting from late summer until spring are at a greater risk of predation (Geier, 1963). Heteroptera, ants and earwigs have been observed to eat CM eggs (Glen, 1977; Glen and Milson, 1978), carabids readily attack tethered mature CM larvae on the ground (Riddick and Mills, 1994), naked and cocooned larvae in petri dishes are rapidly attacked by adult *P. melanarius*, a wolf spider (*Schizocosa mccooki* [Montgomery]), earwigs and harvestmen (Unruh, unpublished

observations). In addition, adult CM in flight can be captured by bats (Long et al., 2014) and in spider webs. Orb weavers making webs across orchard rows have become common in central Washington orchards (Unruh, Jones, Horton and Miliczky, unpublished observations) suggesting they should be evaluated as predators of adult CM by gut content analysis or observation.

The CM DNA signals detected in predators in this study were likely due to direct predation, but intra guild predation (IGP) and scavenging on CM carcasses cannot be rejected. Frequent IGP is known among ladybeetles and lacewings in habitats with high densities of aphids (Rosenheim et al., 1999; Gagnon et al., 2011) and among hemipteran predators attracted to abundant whitefly prey (Hagler and Blackmer, 2015). While meta-analyses indicate that IGP is less in diverse habitats (Janssen et al., 2007), such as organic and abandoned apple orchards, we remain uninformed about the extent of IGP and scavenging (Juen and Traugott, 2005; Von Berg et al., 2012). Additional knowledge of the feeding behavior of the predator taxa that we found to be CM positive in this study may help to resolve this.

Direct PCR uses homogenate of a tissue (predator) as a template for PCR instead of extraction of DNA. It has the advantages of more rapid preparation, economy of supplies and often more DNA product than PCR using extracted DNA. Direct PCR is becoming commonly used in medical and forensic applications and shows similar accuracy to standard PCR (Fumanal et al., 2005; Queipo-Ortuno et al., 2008; Swaran and Welch, 2012; Wong et al., 2014; Templeton et al., 2015). We used direct PCR successfully for gut content analysis with all 2591 predator specimens in this study, and for whole bodies and the fecal pellets used in the detection half-life trials. We saved more than \$4,500 in materials and many hours of sample preparation time. Also,

DNA was stable in the homogenate when stored at room temperature for 2 months and for three years at -20 °C.

Predators captured in dry pitfall traps are both starved and continue to digest their prey for up to 24 h before collection (12 h median), greatly reducing prey DNA. In contrast, beat trays and cardboard bands do not confine predators, but cardboard bands may enhance predation rates of some predators, such as spiders and earwigs, because of closer proximity to mature CM larvae which also use cardboard as a pupation site (Mizell and Schiffhauer, 1987). Preferences for canopy versus ground and cryptic sites on the tree trunk are evident among several of the spider genera from the different methods used to collect them (Appendix A, Table S2), which is consistent with known searching modes among spider families (Foelix, 2010; Cardoso et al., 2011).

The European earwig is a well-known predator of woolly apple aphid (Mueller et al., 1988; Nicholas et al., 2005), pear psylla (*Cacopsylla pyricola* Foerster) (Solomon et al., 1999) and green apple aphid (*Aphis spiraecola* Patch) (Romeu-Dalmau et al., 2012). Suppression of CM eggs has been associated with earwigs (Glenn, 1977; Glen and Milson, 1978, Falcon and Huber, 1991; Knight et al., 1997) and Causse (1976) observed cocooned larval or pupae of CM being preyed upon by earwigs. Earwigs collected from the canopy may have fed on CM eggs and neonate larvae. Also, since they are gregarious, thigmotactic, nocturnally active and able to find cryptic sites such as bark crevices, crotches of branches, and various sites in the understory (Burnip et al., 2002), they may find and feed on mature larvae and cocoons of CM. Surprisingly, earwigs were not abundant in the recent study of predation of codling moth in France (Boreau de Roince et al., 2012).

Digestion rates (detection half-life) of predatory arthropods from laboratory feeding trials have been proposed as a way to rank the relative importance of predator species in pest suppression (Greenstone et al., 2010, 2012, 2014). Many factors influence digestion rates (Hagler and Blackmer, 2013) including chaser food (Weber and Lundgren, 2009), size of the DNA region amplified (Hoogendoorn and Heimpel, 2001; Agustí et al., 2003), feeding mode (Greenstone et al., 2007; Lundgren et al., 2009), meal size (Hagler and Naranjo, 1997), temperature and other factors (King et al., 2008). Most studies have used short feeding times (minutes to a few hours) and small meals (Greenstone et al., 2014). Our use of a longer feeding time (24 h) with a larger meal (mature CM larva) for *F. auricularia* and *P. melanarius*, allowed these predators to eat until satiated, perhaps more similar to field behavior. This resulted in digestion half-lives two to three fold longer than seen in other studies with the same predator species. For example, a small number of aphids as prey were fed to earwigs (Romeu-Dalmau et al., 2012) and to *Pterostichus melanarius* (Shepard et al., 2015), both resulting in half-lives threefold smaller than when the same predators species were feed whole CM larvae in our study. Larger meals take longer to digest (Hagler and Naranjo, 1997; Naranjo and Hagler, 1998), but other factors, particularly prey composition, may also influence digestion rates. For example, the abundance of mitochondria is high in muscle tissues and consequently any preference for muscle tissue could influence a predator's digestion rate. Different digestion rates for the same predator species can also arise from variable sensitivities of primers used for different prey species (Ye et al., 2012). Boreau de Roince et al. (2012) provided a carabid predator a 50:50 mixture of CM and Oriental fruit moth (OFM) and found detection half-lives of 24 h for CM (PCR product size of 153 bases) and 39 h for OFM (PCR product size of 186 bases). As digestion half-lives can differ for predators due to

sensitivity of primers, PCR product size, meal quality, meal size and prey preference, this questions the utility of half-lives for ranking the relative importance of predators.

A more direct approach to identify key predators in agricultural systems is to measure their abundance and that of their target prey coupled with gut content analysis and diet breadth. In simplified habitats such as soil, where both predator and prey abundance can be estimated by taking a volume sample (e.g. Lundgren et al., 2009) identification of key predators is feasible. However, in the diverse habitat of an apple orchard, estimation of both CM and predator abundance is not feasible using a single sampling technique. Hence, for both Boreau de Roince et al. (2012) and our study there was no attempt to measure CM abundance or that of the diverse predator fauna.

A viable, but challenging, approach to rank the relative importance of multiple predators would be to estimate their abundance using mark-release-recapture and to couple this with gut content analysis and digestion rate studies as presented here. Predators could be captured using beat trays, dry pitfall traps and cardboard retreats, which are nondestructive methods, and marked with protein powders, antibodies, fluorescent dyes or other markers (Hagler and Jackson, 2001). Marked predators could then be released in the area of capture and recaptured to measure the percentage marked. Suitable experimental designs and methods of analysis can be found in Krebs (1999) and the software program MARK (www.phidot.org/software/mark).

Recent studies have shown that several new insecticides used in pome fruits and walnuts can cause acute or sub lethal effects on predatory mites, lacewings, predatory Heteroptera and parasitoids (Amarasekare and Shearer, 2013; Amarasekare et al., 2016; Beers et al., 2016; Mills et al., 2016, this issue). Similarly, *F. auricularia* is negatively affected by several insecticides

(Shaw and Wallis, 2010; Logan et al., 2011). Thus the use of biorational pesticide practices to protect key natural enemies, several of which have been identified in this study, represents a logical path to enhance conservation biological control in pome fruits (Jones et al., 2009; Mills et al., 2016, this issue).

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Table Captions

Table 1

Orchard names are presented as first letter only, area is in hectares, years sampled, longitude and latitudes, pest management method used, collection methods used; n= numbers of predators analyzed by PCR; % = percent positive for predation on CM.

Appendix A, Table S1

Herbivore and predator non-targets tested by PCR with codling moth PCR primers (* = PCR proved positive).

Appendix A, Table S2

Spiders analyzed by PCR for detection of codling moth (CM) DNA by method of collection. n = number of specimens analyzed; Prop. = proportion of specimens testing positive for CM signal.

Figure Captions

Fig. 1. Percent positive for codling moth (CM) DNA detected in gut content analysis of arthropod predators representing six taxa collected from seven apple orchards. Black bars = percent of specimens in each taxa group that were CM positive; grey bars = the percent of the CM positive specimens in each group relative to the total community of CM positive predators. Numbers above the bars are the sample size for each taxon.

Fig. 2. Percent positive for codling moth (CM) DNA detected in gut content analysis of 12 spider genera and two spider families from seven apple orchards. Black bars = percent of specimens in each taxa group that were CM positive; grey bars = the percent of the CM positive specimens in each group relative to the total community of CM positive predators. Numbers above the bars are the sample size for each taxon. Numbers above the bars are the sample size for each taxon. See Table S2 for complete collection details.

Fig. 3. Probit regression of the probability of detection of codling moth DNA from adult bodies of *Forficula auricularia* in relation to days since feeding to show detection half-life and 95% CI (grey band). Adults from day 1 were inadvertently not frozen until day 2 and are thus combined with day 2. Day zero represents the positive control.

Fig. 4. Probit regression of the probability of detection of codling moth DNA from fecal pellets of *Forficula auricularia* in relation to days since feeding to show detection half-life and 95% CI

(grey band). Day 9 is not shown because only three earwigs survived and provided no fecal pellets. Day zero represents the positive control.

Fig. 5. Probit regression of the probability of detection of codling moth DNA from excised guts of adult *Pterostichus melanarius* in relation to days since feeding to show detection half-life and 95% CI (grey band). The digestion study was curtailed at day 5 because only 6 of 15 carabids survived to day 6.

Table 1

Orchard	Size ha	Years sampled	Latitude	Longitude	Pest management	Collection method	n	% positive	SE
D	0.9	2011	46°29'25.49"N	120°25'40.70"W	Conventional	pitfall	161	0.6	0.6
G	141	2006	6°34'47.19"N	120°25'37.62"W	Organic	pitfall	48	14.6	5.1
L	64	2006, 2009	46°27'9.80"N	120°14'2.87"W	Organic	pitfall tray	10 56	10.0 8.9	10.0 3.8
M	2.5	2006, 2009- 2011	6°29'51.64"N	120°10'23.04"W	Research	bands pitfall tray	180 462 50	17.2 4.1 16.0	2.8 0.9 5.2
T	61	2010, 2011	6°23'41.23"N	120°19'35.03"W	Conventional	pitfall	695	5.8	0.9
W	0.77	2006, 2010, 2011	6°29'36.63"N	120°25'53.89"W	Abandoned	bands pitfall tray	30 462 226	20.0 13.0 15.5	7.4 1.6 2.4
Y	3.4	2006	6°28'51.84"N	120°21'47.99"W	Organic	pitfall tray	18 193	0.0 8.8	0.0 2.0

Figure 1

Figure 2

Figure 5

Figure 3

Figure 4