Biological Control 102 (2016) 85–92

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Gut content analysis of arthropod predators of codling moth in Washington apple orchards

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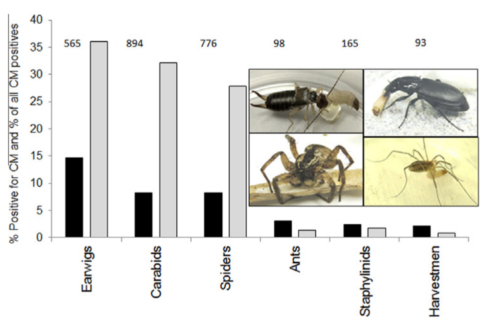
HIGHLIGHTS

- Codling moth (CM) DNA was in 8.9% of the 2591 predators analyzed by PCR.
- Spiders, insect predators and harvestmen tested 8.2, 9.5 and 2.2% CM positive.
- Detection half-life for CM in earwigs was 3.6 d and 3.7 d using fecal pellets and adult bodies as templates for PCR.
- Homogenates of predators in a lysis buffer were used as DNA templates for Direct PCR.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 April 2015
Revised 21 May 2016
Accepted 26 May 2016
Available online 1 June 2016

Keywords:
Cydia pomonella
Predatory arthropods
Polymerase chain reaction
Conservation biological control

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.

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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.

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http://dx.doi.org/10.1016/j.biocontrol.2016.05.014
1049-9644/Published by Elsevier Inc.

(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; Jones et al., 2010a,b). 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). Pheromone-based mating disruption (Vickers and Rothschild, 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 'tray'. 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.

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.

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	10	10.0	10.0
						Tray	56	8.9	3.8
M	2.5	2006, 2009–2011	6°29′51.64″N	120°10′23.04″W	Research	Bands	180	17.2	2.8
						Pitfall	462	4.1	0.9
						Tray	50	16.0	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	30	20.0	7.4
						Pitfall	462	13.0	1.6
						Tray	226	15.5	2.4
Y	3.4	2006	6°28′51.84″N	120°21′47.99″W	Organic	Pitfall	18	0.0	0.0
						Tray	193	8.8	2.0