Biological Control 102 (2016) 7–16

Contents lists available at ScienceDirect

Biological Control

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

Testing the selectivity of pesticide effects on natural enemies in laboratory bioassays

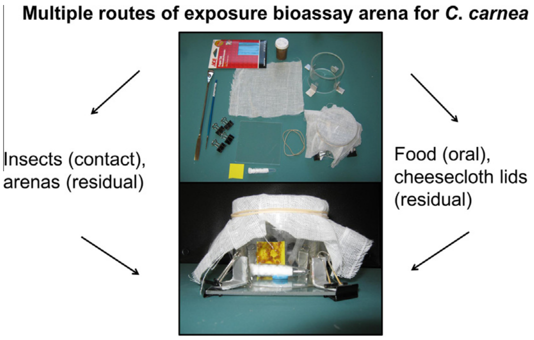
Kaushalya G. Amarasekare [a,*], Peter W. Shearer [b], Nicholas J. Mills [c]

[a] Department of Agricultural and Environmental Sciences, Tennessee State University, 3500 John A. Merritt Blvd, Nashville, TN 37209-1561, USA
[b] Oregon State University, Mid-Columbia Agricultural Research and Extension Center, 3005 Experiment Station Drive, Hood River, OR 97031, USA
[c] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720-3114, USA

HIGHLIGHTS

- Pesticide impact on *C. carnea* and *T. pallidus* was assessed using lethal and sublethal effects.
- Cyantraniliprole, spinetoram and lambda-cyhalothrin are toxic to *C. carnea* and *T. pallidus.*
- Chlorantraniliprole and novaluron are toxic to *C. carnea.*
- Sulfur is toxic to *T. pallidus.*
- Multiple routes of exposure assays yielded meaningful toxicological results for both species.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 January 2015
Revised 25 September 2015
Accepted 5 October 2015
Available online 15 December 2015

Keywords:
Chrysoperla carnea
Trioxys pallidus
Reduced risk insecticide
Intrinsic rate of increase
Sublethal effects
Multiple routes of exposure

ABSTRACT

The toxic effects of older classes of pesticides on natural enemies are typically acute and exposure usually occurs through direct contact with foliar residues. However, older chemistries are being replaced by newer classes of pesticides that can cause sublethal effects in addition to direct mortality. We developed a set of life table response protocols to quantify the effects of multiple routes of exposure to pesticides on individual-level life history parameters of predators and parasitoids. We then integrated the data into population-level endpoint estimates of population growth rates using stage-structured population models. For this study, we evaluated the impacts of five insecticides (cyantraniliprole, chlorantraniliprole, spinetoram, novaluron and lambda-cyhalothrin) and two fungicides (sulfur and a mixture of copper hydroxide and mancozeb) on a generalist insect predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and an aphid parasitoid *Trioxys pallidus* (Haliday) (Hymenoptera: Braconidae). Green lacewings and *T. pallidus* are key members of the natural enemy community in western USA orchards. The results of these laboratory studies demonstrate that both *C. carnea* and *T. pallidus* were negatively affected by cyantraniliprole, spinetoram and lambda-cyhalothrin while only one species was affected by chlorantraniliprole and novaluron (*C. carnea*) or sulfur (*T. pallidus*). The benefits of integrating acute, chronic, and sublethal effects from laboratory bioassays to assess the selectivity of pesticides with respect to natural enemies are discussed.

1. Introduction

From the introduction of organophosphorus (OP) insecticides in the late 1950s until mid-1990s, most integrated pest management (IPM) programs used in the orchards in the western United States

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[*] Corresponding author.
E-mail address: kaushalya2641@yahoo.com (K.G. Amarasekare).

http://dx.doi.org/10.1016/j.biocontrol.2015.10.015
1049-9644/

relied heavily on them (Jones et al., 2009). Significant findings from previous research that evaluated adverse effects of agricultural pesticides on the environment health (Pimentel, 1995; Pimentel et al., 1993; Pimentel and Levitan, 1988) plus the enactment of the Food Quality Protection Act of 1996 resulted in the removal of many OP insecticides (Jones et al., 2010; US EPA, 1996). This led to the introduction of newer pesticide chemistries with novel modes of action and lower mammalian toxicities (Agnello et al., 2009; Kim et al., 2011; Whalon et al., 1999).

Most of these newer reduced risk insecticides are target specific, but there is evidence that some of these insecticides could affect key natural enemies that regulate secondary insect and mite pests (Agnello et al., 2009; Amarasekare and Shearer, 2013a, 2013b; Brunner et al., 2001; Crampton et al., 2010; Kim et al., 2006; Myers et al., 2006; Villanueva and Walgenbach, 2005, 2006). In contrast to neurotoxic OP insecticides, some of the newer reduced risk insecticides have been shown to have sublethal rather than lethal effects on natural enemies (Amarasekare and Shearer, 2013a, 2013b; Beers and Schmidt, 2014; Desneux et al., 2007; Kim et al., 2006). Many systemic neonicotinoid insecticides have unintended side effects on bees and natural enemies including predators and parasitoids (Cloyd and Bethke, 2011; Cresswell, 2010; He et al., 2012; Laycock et al., 2012; Li et al., 2015; Rahmani and Bandani, 2013; Yao et al., 2015). In addition to reduced risk insecticides, some fungicides used in pest management may have insecticidal and miticidal properties that affect natural enemies (Amarasekare and Shearer, 2013a, 2013b; Hoyt, 1969; Jepsen et al., 2007; Stavrinides and Mills, 2009). Thus, additional information is needed to better understand the impacts of reduced risk pesticides on natural enemies including both lethal and sublethal effects (Jones et al., 2009).

Traditionally, measurement of acute toxicity of pesticides to natural enemies has relied largely on the determination of an acute median lethal dose (LD_{50}) or concentration (LC_{50}) (Desneux et al., 2007). The effects of pesticides on natural enemies were examined further by running selectivity tests (pests/natural enemies) to identify products with the lowest non-target activity. Because of the increasing economic importance of natural enemies in agriculture and the recognition of limitations associated with traditional methods for studying non-target pesticide effects, a growing number of studies have focused on the inclusion of sublethal effects during past several decades (Ahmadi 1983; Banken and Stark, 1998; Desneux et al., 2007; Longley and Stark, 1996; Stark et al., 1995, 2007; Stark and Banks, 2003; Theiling and Croft, 1989). Older classes of pesticides, such as OPs and carbamates, are acutely toxic and thus the analysis of sublethal effects is less straightforward (Wennergren and Stark, 2000). However, newer classes of pesticides are often less lethal to natural enemies than the older classes, and consequently a more comprehensive approach is needed for assessment of their non-target selectivity.

In evaluating a pesticide's potential for compatibility with natural enemies, the International Organization for Biological Control (IOBC) recommends a tiered approach whereby initial pesticide screening is done in the laboratory and depending upon the results obtained, semi-field or field tests may be conducted (Hassan, 1992; Vogt et al., 2000). The IOBC classifies pesticides into the following four categories depending on the extent of mortality or reduction in life history performance that they cause to natural enemies: 1 = harmless (<30%), 2 = slightly harmful (30–79%), 3 = moderately harmful (90–98%) and 4 = harmful (>99%) (Hassan, 1992; Vogt et al., 2000). Although the tiered approach advocated by the IOBC is admirable, there are limitations to this method for assessment of pesticide side effects (Stark et al., 2004). Laboratory life table response experiments (LTREs) and demographic analyses have proved to be an effective approach to evaluate the combined lethal and sublethal effects of pesticides (Stark and Banks, 2003; Stark et al., 2007; Theiling and Croft, 1989). In contrast to the standardized tests developed by IOBC to study pesticide effects of fresh pesticide residues on natural enemies in the laboratory (Hassan, 1985; Vogt et al., 2000), our objective was to develop a set of bioassays for arthropod predators and parasitoids that were designed as LTREs and that incorporated multiple routes of pesticide exposure including topical, residual and oral (Banken and Stark, 1998; Longley and Stark, 1996; Stark et al., 1995).

The current study was part of a large, multi-state project conducted in apple, pear and walnut orchards in Washington, Oregon and California, respectively (Jones et al., 2016). A major goal of the study was to enhance the sustainability of biological control in western USA orchard systems. A central theme was to investigate the secondary impacts of pesticides used against codling moth (*Cydia pomonella* (L.), Lepidoptera: Tortricidae), the common key pest found in these three orchard systems. This approach is illustrated here by presenting the methodology, impacts on life history parameters, and population endpoint estimates of the effects of five insecticides (cyantraniliprole, chlorantraniliprole, spinetoram, novaluron and lambda-cyhalothrin) and two fungicides (sulfur and a mixture of copper hydroxide and mancozeb) on a generalist insect predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and an aphid parasitoid *Trioxys pallidus* (Haliday) (Hymenoptera: Braconidae). The fungicide mixture of copper hydroxide and mancozeb is one of the most important fungicides used in walnuts to control walnut blight but not used in pears and apples. Sulfur is generally used in pears and apples but not in walnuts. We incorporated both fungicides (the mixture of copper hydroxide and mancozeb and sulfur) in our studies for all natural enemies tested across the three cropping systems because most of these natural enemies are commonly found in pear, apple and walnut orchards. In this study we followed Stark and Banks (2003) in using LTREs and population models to explore the demographic effects of pesticides on natural enemy populations.

Green lacewings (Neuroptera: Chrysopidae) are important predators of arthropod pests in many horticultural and agricultural cropping systems, including vegetables, fruits, nuts, fiber and forage crops, ornamentals, greenhouse crops and forests, both in the context of natural biological control as well as in augmentative release programs (Nordlund et al., 2001; Pappas et al., 2011; Ridgway and Kinzer, 1974; Ridgway and Murphy, 1984). *C. carnea* is a species native to Eurasia that has been used throughout the world in such programs (Henry et al., 2002). *T. pallidus* is an introduced solitary endoparasitoid of the walnut aphid *Chromaphis juglandicola* (Kaltenbach), a pest of walnuts in the U.S. and many walnut growing areas in the world (Hougardy and Mills, 2009). Thus green lacewings and *T. pallidus* are thus key members of the natural enemy community in western USA orchards and were selected here as examples of two different functional groups of natural enemies to illustrate the approach that we developed for testing the selectivity of pesticides in laboratory bioassays.

2. Materials and methods

2.1. *C. carnea* colony

A colony of *C. carnea* was maintained at 23 °C, 50–60% R.H. and a photoperiod of 16:8 h *L:D* in the laboratory using the methods described in Amarasekare and Shearer (2013b). Adults were reared in an open-top glass aquarium (26 × 30 × 50 cm) with a wire mesh screen lid (6 × 6 mm mesh). To facilitate egg laying, the opening at the top of the aquarium was covered with a piece of cheesecloth and secured with a wire mesh top. Artificial diet was prepared in the laboratory and used to feed the adults (Vogt et al., 2000). Adults were provided with new food, water and cheesecloth cover