



# The effects of insecticide dose and herbivore density on tri-trophic effects of thiamethoxam in a system involving wheat, aphids, and ladybeetles



Michael M. Bredeson<sup>a, b</sup>, R. Neil Reese<sup>a</sup>, Jonathan G. Lundgren<sup>b, \*</sup>

<sup>a</sup> Department of Biology and Microbiology, South Dakota State University, Brookings, SD, 57007, USA

<sup>b</sup> USDA-ARS, North Central Agricultural Research Laboratory, Brookings, SD, 57006, USA

## ARTICLE INFO

### Article history:

Received 16 July 2014

Received in revised form

16 December 2014

Accepted 19 December 2014

Available online 27 December 2014

### Keywords:

Non-target effects

Clothianidin

*Coleomegilla maculata*

Predators

*Rhopalosiphum padi*

Systemic insecticide

Tri-trophic interactions

Wheat

## ABSTRACT

We assess how herbivore density and insecticide dose affects the tri-trophic interactions among thiamethoxam-treated wheat (*Triticum* sp.), *Rhopalosiphum padi* and the predatory *Coleomegilla maculata* DeGeer. In the first experiment 2nd and 4th instar *C. maculata* were fed aphids reared for 24 h on wheat plants treated with sub-lethal thiamethoxam soil drenches to assess the effect of insecticidal dose on the predator. In the second experiment a constant, sub-lethal thiamethoxam soil drench was used on wheat plants, but the plants were infested with different densities of aphids. Aphids from each density treatment were then fed to *C. maculata* 2nd instars. Insecticide levels within wheat and aphid tissues were quantified using a competitive ELISA. In experiment one, 4th instars of *C. maculata* displayed no negative effects after consuming aphids from treated plants. However, 2nd instars of *C. maculata* ate more treated aphids than untreated aphids and larval development times varied between the untreated larvae and those receiving the low dose of the insecticide. In experiment two, the 2nd instar *C. maculata* displayed slower walking speeds after consuming aphids from low aphid density plants. Thiamethoxam within wheat tissue was found at higher levels with increasing dose. This insecticide was also found at higher levels in wheat plants with the lowest aphid density. Clothianidin, a toxic metabolite of thiamethoxam, was found in aphids that had fed on thiamethoxam-treated wheat, but no differences between treatments were observed. The neonicotinoid treatment altered the quality of *R. padi* as prey for 2nd instar *C. maculata*, but this depended on the aphid infestation level on the plants. In addition to revealing a potential deleterious effect of thiamethoxam systemic insecticides in wheat, our assays provide insight into the design and interpretation of toxicity assays involving systemic neonicotinoids and herbivores.

Published by Elsevier Ltd.

## 1. Introduction

Since their introduction in 1991, neonicotinoid insecticides have become commonly used in many agricultural settings to control pest insects (Nauen and Denholm, 2005). Neonicotinoids act as agonists to the acetylcholine receptors within the nervous system of an animal, mimicking the action of naturally produced acetylcholine (Jeschke et al., 2013), a neurotransmitter which allows action potentials to move from nerve cell to nerve cell. The

\* Corresponding author. USDA-ARS, NCARL, 2923 Medary Avenue, Brookings, SD, 57006, USA.

E-mail addresses: [jonathan.lundgren@ars.usda.gov](mailto:jonathan.lundgren@ars.usda.gov), [jgl.entomology@gmail.com](mailto:jgl.entomology@gmail.com) (J.G. Lundgren).

overstimulation of nerve cells due to neonicotinoid insecticides can cause paralysis (Girolami et al., 2009) and death (Iwasa et al., 2004) in insects. The popularity of neonicotinoid use has risen in part because of the diverse means by which the chemicals can be applied. This class of insecticides can be applied as granules in the soil (Lanka et al., 2014), foliar sprays (Juraska et al., 2009), soil drenches (Stoner and Eitzer, 2012), tree injections (Cowles et al., 2006) and topically to animals (Dryden et al., 2011). However, the most widespread application method of neonicotinoids has been as seed treatments that protect plants from herbivory (Jeschke et al., 2010).

Although the threat against beneficial insects is reduced by using systemic insecticides compared to broad spectrum insecticidal sprays, there is evidence that systemically treated plants do in

fact harm beneficial insects through a number of pathways (Lundgren, 2009). For example, Moser and Obrycki (2009) exposed Asian ladybeetle (*Harmonia axyridis* Pallas (Coleoptera: Coccinellidae)) larvae to corn seedlings grown from seed treated with neonicotinoids. The ladybeetle larvae expressed neurotoxic symptoms such as trembling, paralysis and loss of coordination in 72% of cases, presumably the result of the observed and quantified facultative herbivory (Moser and Obrycki, 2009). In another toxicology study, Rogers et al. (2007) confined *Chrysoperla carnea* (Neuroptera: Chrysopidae) adults to the flowers of neonicotinoid treated *Fagopyrum esculentum* and *Asclepias curassavica* where they consumed the nectar. Survival of *C. carnea* was significantly reduced in the insecticide treatment after 10 d compared to an untreated control group; the authors concluded that survival was diminished due to the ingestion of nectar containing neonicotinoid insecticide (Rogers et al., 2007).

Besides direct mortality of beneficial insects resulting from the use of systemic neonicotinoids, sub-lethal concentrations of insecticide within plants can act as one of a suite of stressors that result in diminished fitness of non-target species. Consequences of ingesting sub-lethal concentrations of insecticides can include reduced fecundity (Shi et al., 2011), lowered mobility (Scholer and Krischik, 2014), loss of orientation abilities (Fischer et al., 2014), limited overwintering survivability (Grewal et al., 2001) and reduced foraging capacity (Schneider et al., 2012). The sub-lethal effects of neonicotinoids can potentially reduce the efficiency of predator and parasitoid communities, threatening biotic resistance to pest proliferation (Seagraves and Lundgren, 2012).

Predation on crop pests by predators and parasitoids provides not only environmental benefits, but also has tangible economic value to land owners, and is an important component of Integrated Pest Management (IPM) (Zhang and Swinton, 2009). Plant incorporated systemic insecticides may affect non-target predators through various trophic pathways, primarily considered bi-trophic (Lundgren, 2009), or tri-trophic (prey-mediated) effects (Birch et al., 1999). Beneficial insects are exposed to systemic insecticides through omnivory on non-prey foods such as vegetative plant tissue (Moser and Obrycki, 2009), pollen (Cresswell, 2011; Dively and Kamel, 2012), guttation fluid (Girolami et al., 2009), floral (Krischik et al., 2007) or extra-floral nectar (Stapel et al., 2000). Predators can also be adversely affected by an insecticide if their herbivorous prey contains the toxin or the quality of the prey is lessened by ingesting insecticide-containing plant tissues (Dutton et al., 2002). Documenting the trophic relationships between systemically treated-plants, pests and beneficial insects is important for understanding the compatibility of systemic insecticides within IPM systems.

Wheat is a crop of significant importance worldwide; 18.5 million ha of wheat was harvested in the United States in 2013 alone (NASS, 2014). *Rhopalosiphum padi* (L.) (bird cherry-oat aphid, Hemiptera: Aphididae) is a common pest of wheat in the U.S. and worldwide, causing significant damage resulting from feeding and transmitting viruses (Jiménez-Martínez et al., 2004). Neonicotinoid seed treatments are highly water soluble (Maienfisch et al., 2001) and are readily transported through both xylem and phloem, making this class of insecticides and method of application well adapted for the control of phloem feeding insects, like aphids (Magalhaes et al., 2009), in a variety of crops including wheat (Gray et al., 1996).

Members of the Coccinellidae family have historically been used frequently and effectively as a useful tool in biological control programs to limit pest arthropod herbivory (Biddinger et al., 2009; Weber and Lundgren, 2009), especially from aphids (Obrycki et al., 2009). One species of Coccinellidae, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), is a predator of *R. padi* in wheat (Elliott

et al., 1999) and in other crops including corn (Lundgren and Wiedenmann, 2005), thus *C. maculata* could potentially be affected tri-trophically by aphids feeding on systemically treated wheat. Sublethal levels of neonicotinoid insecticides can be found in crops either as the seed treatment dissipates over the season (McCornack and Ragsdale, 2006; Seagraves and Lundgren, 2012), or through uptake of residual insecticides found in the soil from previous insecticide applications. Here we examine the tri-trophic interactions among wheat plants containing sublethal doses of thiamethoxam, aphids, and the predatory *C. maculata*. Specifically, we examined how insecticide dose in the wheat plants and aphid densities on the wheat plants interact to affect *C. maculata* larvae.

## 2. Methods

### 2.1. Wheat, ladybeetle and aphid bioassays

#### 2.1.1. Insects and plants

A *C. maculata* DeGeer colony was established from individuals collected from a corn field near Brookings, SD, USA (44.3114° N, 96.7981° W), and was raised for three generations (approximately 15 mo) in the laboratory. The colony was maintained on a semi-artificial diet (Lundgren's Super *C. mac* Diet, (Lundgren et al., 2011)) and honey. The beetles were raised at 26.5 °C with 16:8 h (L:D; light: dark) photoperiod. The *R. padi* colony was established from individuals collected in Hays, Kansas. Aphids were reared on barley plants (*Hordeum vulgare* L. var. Robust, Poales: Poaceae) at 19 °C and 16:8 h L:D. Wheat (*Triticum aestivum* L., Poales: Poaceae) was produced individually in planting cones (Ray Leach Container cells, SC10 Super, Tangent, OR, USA, 97389) filled with potting soil mix (Miracle-Gro® MicroMax®, Marysville, OH. 43041). Each plant was given 20 mL of water daily and grown in a greenhouse set at 24 °C and 16:8 L:D (Greenhouse lights: GE® Lucalox LU1000, and GE® MVR1000/U, General Electric Company®, Fairfield, Ct, USA, 06828).

#### 2.1.2. Experiment 1. Effect of insecticide-fed aphids on 2nd and 4th instar *C. maculata* fitness

The tri-trophic effects of systemic insecticides on predators were evaluated in separate assays involving 2nd and 4th instar *C. maculata*. For each assay, *C. maculata* larvae were fed one of three treatments; 1) aphids reared on plants treated with a high-sublethal dose of insecticide (2.5 mg/L thiamethoxam in water), 2) aphids reared on plants treated with a low-sublethal dose of insecticide (1.5 mg/L thiamethoxam in water), and 3) aphids reared on untreated plants (water only). These doses of thiamethoxam (Thiamethoxam PESTANAL®, Sigma–Aldrich®, Product number: 37924, St. Louis, MO, USA, 63103) were determined to be sublethal using the methods described in Daniels et al. (2009).

To establish these treatments, 300 wheat plants were grown to the two-leaf stage (about 10 d). At this point, each individual plant ( $n = 100$  plants per treatment) received a 10 mL soil drench of an experimental solution at the base of the plant. The soil surface was then covered with 1 cm deep white sand (Industrial Quartz, Granusil® Lillca Fillers, Le Sueur, MN. 56058). Four hours after the soil drench, *R. padi* were placed at the base of each of the plants, and were exposed to the treatments for 24 h (19 °C, 16:8 L:D).

After 24 h, living *R. padi* of all life stages were removed from the plants and fed to the *C. maculata* larvae; 25 aphids were fed to each 2nd instar and 50 to each 4th instar ( $n = 15$  larvae per treatment). In addition to the aphids, each larva was given a water-saturated cotton wick, and larvae were kept at 26.5 °C with a 16:8 L:D photoperiod. After 24 h, the number of aphids remaining in each cup was counted. Fitness assessments of the larvae are described below.

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