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Carolina Camargo
University of Florida, ccamargogil@ufl.edu

Thomas Hunt
University of Nebraska-Lincoln, thunt2@unl.edu

Loren J. Giesler
University of Nebraska-Lincoln, lgiesler1@unl.edu

Blair D. Siegfried
University of Florida, bsiegfried1@ufl.edu

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Thiamethoxam Toxicity and Effects on Consumption Behavior in *Orius insidiosus* (Hemiptera: Anthocoridae) on Soybean

Carolina Camargo,[1] Thomas E. Hunt,[2] Loren J. Giesler,[3] and Blair D. Siegfried[1]

1 Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611 (ccamargogil@ufl.edu; bsiegfried1@ufl.edu)
2 Department of Entomology, University of Nebraska-Lincoln, Haskell Agricultural Laboratory, Concord, NE 68728 (thunt2@unl.edu)
3 Department of Plant Pathology, University of Nebraska- Lincoln, Plant Sciences hall Lincoln, NE 68583 (loren.giesler@unl.edu)

Corresponding author — C. Camargo, ccamargogil@ufl.edu

Abstract
Neonicotinoid residues can be present in soybean vegetative tissue, prey insects, and flower tissues, possibly making them toxic to pollinators and natural enemies. Baseline information on the toxicity of neonicotinoids to beneficial insects other than pollinators through multiple routes of insecticide exposure is limited. The objectives of this study were 1) to evaluate the toxicity of thiamethoxam to the hemipteran predator, *Orius insidiosus* Say, exposed to residues through treated vegetative tissue and insect prey, and 2) to evaluate the effect of thiamethoxam on the abundance of this predator species in soybean fields. Predators were exposed to thiamethoxam in soybean leaves and *Aphis glycines* Matsumura using a systemic bioassay. Abundance of the predator was evaluated in thiamethoxam seed-treated fields during two different soybean seasons. Our results indicate that concentrations required to kill >50% of the evaluated insects were higher than the concentrations that the insects are likely to encounter in the field. Consumption of *A. glycines* by *O. insidiosus* was affected at 10ng/ml and 5 ng/ml of thiamethoxam at 24h of evaluation. There was significant mortality for *O. insidiosus* at 24h after exposure to thiamethoxam-treated aphids at these concentrations. In soybean fields, there were no significant differences in *O. insidiosus* number between the plots treated with thiamethoxam and the control. Thiamethoxam may have significant effects on the predators if *O. insidiosus* feeds on early soybean vegetative tissue or contaminated prey. These results suggest that the compatibility of thiamethoxam with IPM programs for *A. glycines* needs further evaluation.

Keywords: thiamethoxam, toxicity, consumption, Aphis glycines, Orius insidiosus

Predators are one of the most important groups of natural enemies that provide biological control of soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) within an IPM (integrated pest management) framework (Mignault et al. 2006, Schmidt et al. 2008, Costamagna et al. 2013). Under high infestation pressure, soybean aphids can significantly affect soybean yield by plant feeding and reduction of photosynthesis due to mold growth on honeydew excretions (Tilmon et al. 2011). Thus, the *A. glycines* is considered the most important pest of soybean in the North Central Region of the United States (Ragsdale et al. 2011, Tilmon et al. 2011). In absence of predation, soybean aphid populations have been reported to grow from 2 to 7 times faster than when predators are present (Costamagna and Landis 2007). Based on the importance of predatory insects in the management of *A. glycines*, there has been increased interest in the implementation of pest control practices that allow the conservation of these species (Tilmon et al. 2011).

Orius insidiosus (Say) (Hemiptera: Anthocoridae) is one of the key predators of *A. glycines* in the United States (Rutledge et al. 2004, Rutledge and O'Neil 2005, Butler and O'Neil 2007a, Butler and O'Neil 2007b, Ragsdale et al. 2007). Consumption of *A. glycines* by nymphs and adults of *O. insidiosus* can effectively suppress population growth of this pest in soybean fields (Rutledge and O'Neil 2005). The efficiency of this predator to control soybean aphid populations has been related with its time of migration and establishment in soybean fields (Rutledge and O'Neil 2005). Rutledge et al (2004) found an inverse relationship between the number of weeks that *O. insidiosus* colonized the field and the severity of soybean aphid infestation. Therefore, to receive the ecological service provided by this predator in soybean fields, pest management practices need to be compatible with the early establishment of this predator in soybean fields.

Seed treatments with neonicotinoid insecticides have been promoted to be relatively nontoxic to natural enemies due to the lack of direct exposure to chemical residues (Seagraves and Lundgren 2012). Exposure of predatory insects can occur by ingestion of residues in prey and plant material or through contact with guttation drops or dust particles that are generated during planting (Gentz et al. 2010, Pisa et al. 2015). Residues of neonicotinoids in insect prey and vegetative material can affect the compatibility of these insecticides with biological control through direct mortality to natural enemies or indirectly through reduction in consumption rates of the insect pests (Gentz et al. 2010, Seagraves and Lundgren 2012).

Predators of soybean aphids, such as *O. insidiosus*, also consume plant tissues such as pollen, nectar, guttation drops, and leaf tissue in the absence of prey, a behavior which is referred to as zoophytophagy (Albajes and Alomar 1999, Canard et al. 2001, Moser and Obrycki 2009). Consumption of plant material can be detrimental for predatory species if soybean plant tissues or vegetation near treated areas contain lethal concentrations of systemic pesticides (Seagraves and Lundgren 2012). Neonicotinoid toxicity to zoophytophagous predators has been reported for *Harmonia axyridis* Pallas (Coleoptera: Coccinelidae) feeding on seed treated corn seedlings under laboratory conditions using a systemic plant bioassay (Moser and Obrycki 2009). If predators exhibit leaf-feeding behavior during the early growth stages of the crop, there is increased likelihood of direct toxicity of neonicotinoids to zoophytophagous species. However, baseline information on the toxicity of neonicotinoids in vegetative tissue to predators is still limited (Moser and Obrycki 2009, Pisa et al. 2015).

On the other hand, residues of neonicotinoids in insect prey may affect consumption behavior of predatory insects through repellency, antifeedant effects, or a decrease of attack rate and handling time due to toxicity to the predators (Desneux et al. 2007). For *A. glycines* predatory species, there is limited information on how consumption behavior may be affected by neonicotinoid residues in soybean vegetative tissue and insect prey (Varenhorst and O'Neal 2012). Perturbation of host feeding behavior by neonicotinoids may drastically influence the efficiency of predators in controlling *A. glycines* populations and therefore, the compatibility of this class of insecticides with biological control is uncertain (Varenhorst and O'Neal 2012). The risk of neonicotinoids to predatory insects has been difficult to quantify, as few studies have determined the acute and chronic toxicity of neonicotinoids among different beneficial species other than pollinators (Pisa et al. 2015). Studies on the effect of neonicotinoids on consumption behavior of predatory species are also limited (Desneux et al. 2007, Pisa et al. 2015). To our knowledge, there are no studies available on the effect of neonicotinoids on the consumption behavior of *O. insidiosus* on soybean aphid.

This research was conducted to evaluate the toxicity of thiamethoxam on *O. insidiosus* exposed to residues in soybean vegetative material and soybean aphids. We evaluated the effect on the consumption rate of *O. insidiosus* when feeding on aphids exposed to thiamethoxam-treated plants. We also evaluated the effect of thiamethoxam alone and in combination with the conventional fungicide used in seed treatments on the abundance of *O. insidiosus* in different soybean fields in Nebraska.

Materials and Methods

Insecticide, Plant and Insect Material

Technical grade thiamethoxam (99.5%) was purchased from Chem. Services (West Chester, PA). Chemicals were maintained at –4 °C. Stock solutions were diluted in acetone at 5 µg μl^{-1} and stored at 20°C prior the experiments.

Soybean plants were grown in a greenhouse (24± 5 °C and a photoperiod of 16:8 [L:D] h) using potting medium composed of peat moss, perlite, pine bark, and vermiculite (Fafard 3B Mix). Three plants were grown in plastic pots (15 cm diameter by 17 cm deep). Plants were watered daily and fertilized weekly with NPK fertilizer of 20:10:20 ratio.

A colony of soybean aphid was initiated in 2011 from individuals collected from infested fields near the University of Nebraska Northeast Research and Extension Haskell Agricultural laboratory in Concord, NE. The colony was maintained under continuous supply of V3 soybean plants of the susceptible variety SD76R. The colony was maintained in an environmental chamber at 24 °C and 70% RH and a photoperiod of 16:8 (L:D) h conditions.

Predators were purchased from a laboratory colony established by Rincon-Vitova Insectaries (Ventura, CA). Adult predators of *O. insidiosus* were shipped overnight to Lincoln, NE. Adults were placed in an environmental chamber at 24 °C and 70 RH% for 24 h before the experiments. To increase genetic diversity of *O. insidiosus* in the toxicity studies, a field population of adults was collected in soybean fields at Lindsay, NE (41° 44′22.9″ N, 97° 41′59.4″ W). The collected population was transferred in groups of 20 individuals to petri dishes (100mm in diameter) with plastic beads in the dishes to avoid cannibalism. Predators were maintained for 48 h until initiation of the experiments by feeding them with eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Predators from the field and colony were randomly combined for the experiments.

Toxicity of Thiamethoxam Residues in Soybean Vegetative Tissue

To evaluate the toxicity of thiamethoxam in treated soybean leaves, a systemic bioassay was used following the methodology of Magalhaes et al. (2009). The concentration range was 500, 100, 50, 10 and 5 ng/ml. The control treatment consisted of 0.01% acetone in distilled water. Stock solutions were prepared in acetone and diluted in distilled water. The solution of acetone and the insecticide was 0.01% of the total solution in water. Cut petioles of the first trifoliate of V3 soybean were immersed in each insecticide solution and distilled water. The leaves were exposed to the insecticide solution for 24 h prior the introduction of the predators to allow the uptake of the insecticide. No alternative food source was introduced in the experiment to maximize zoophytophagy by the predators. A total of 10 insects were introduced per experimental unit. A total of 10 experimental units per treatment were evaluated in two time blocks.

Toxicity of Thiamethoxam Residues in A. glycines

A completely randomized design was used to evaluate the toxicity of thiamethoxam residues in soybean aphids and the effect of this insecticide on the aphid consumption rate of *O. insidiosus*. The treatments consisted of four concentrations of the insecticide and a control: 10, 5, 2.5, 1.25, 0 ng/ml with seven replications per treatment. This concentration range corresponds to those that aphids are likely to encounter in soybean fields and that allow survivorship of the aphids (Magalhaes et al. 2009). Based on the consumption behavior of *O. insidiosus* reported by Rutledge and O'Neil (2005), a prey density of 20 aphids was placed in each experimental unit.

A systemic bioassay was used following methods described by Magalhaes et al. (2008). Briefly, cut petioles of soybean leaves were immersed in each insecticide solution and distilled water. After exposing the petiole to the chemical for 24 h, third- to fourth-instar apterous aphids were placed on the soybean leaves using a camel hair paintbrush. A week prior the experiment aphid instar was synchronized by placing adults on a single caged plant during 48 h. Adults were removed after the 48 h to obtained homogenous instars for consumption experiments. A total of 25–26 aphids were kept on the leaves for 24 h to allow feeding at the different concentrations of the insecticide. A total of 20 aphids were counted prior the introduction of the predator and the excess number removed. One *O. insidiosus* female was placed in each experimental unit. The number of aphids consumed was recorded 24 h after the predator introduction. Consumed aphids were identified by the feeding puncture left on the aphid body. Mortality of the predator was also recorded at 24 h after exposure.

Abundance of O. insidiosus in Thiamethoxam Seed-Treated Soybean

The toxicity to *O. insidiosus* of thiamethoxam alone and in combination with fungicides used in commercial seed treatments was evaluated under field conditions during 2014 and 2015 in two different fields in Nebraska. Research plots were located at the UNL Agricultural Research and Development Center (ARDC) near Ithaca, NE, and at the UNL East Campus in Lincoln, NE. In 2014, the plots at ARDC were located at latitude 41° 9′54.49″ N, longitude 96° 24′50.45″ W, and the plots in Lincoln at latitude 40° 50′9.93″ N, longitude 96° 39′44.95″ W. In 2015, fields were located in the same research stations with different coordinates; ARDC plots were located at latitude 41° 9′26.50″ N, longitude 96° 25′26.04″ W, and Lincoln plots were located at latitude 40° 50′11.40″ N, longitude 96° 39′41.85″ W.

The experimental design for all fields and years was a randomized complete block with four replicates of four treatments. Treated seeds were provided by Syngenta (Stanton, MN) at the rate applied in commercially available products. The treatments included 1) Untreated seeds as the control treatment, 2) Fungicide mixture: mefenoxam, fludioxinil, and sedaxane at 0.0113, 0.0038, 0.0038mg ai/seed, respectively, 3) Fungicide mixture + Insecticide: mefenoxam, fludioxinil, sedaxane, and thiamethoxam at 0.0113, 0.0038, 0.0038, and 0.0756mg A.I/seed, respectively, and 4) Insecticide alone: thiamethoxam at 0.0756mg A.I/seed. Plots were 8 rows at 76.2 cm apart and 5.18 m long planted at 350,000 seeds/ha.

Sampling was conducted during soybean flowering (reproductive stages R1–R2) 47 d after planting in 2014 and 50 d after planting in 2015. Insects were collected using a 80- by 30-cm plastic tray with a white bottom and shaking the plants from a 1 m length of a 4.57-m-long row into the tray. The foliage of one row was slowly bent to the tray and beaten vigorously for 5 s and predators were counted and removed to avoid recounting. Three different foliage sections were taken from each plot. Because bean leaf beetle, *Cerotoma trifurcata* (Foster) (Coleoptera: Chrysomelidae), were present at the time of sampling, abundance of this pest insect was also recorded as an indicator of the effect of the seed treatments on bean leaf beetle in soybean at early reproductive soybean stages.

Statistical Analysis

A generalized linear mixed model fitting a linear regression using a normal distribution was used to analyze the effect of the concentrations of thiamethoxam on the consumption rate and mortality percentage of the *O. insidiosus*. An analysis of covariance (ANCOVA) was used to analyze the effect of the interaction between the number of consumed aphids and the concentration of thiamethoxam on the percentage mortality of the predator. The number of aphids was used as a covariate in the model, the concentrations of thiamethoxam as a continuous fixed variable, and the mortality as the response variable.

Differences in the susceptibility of *O. insidiosus* between times of evaluation in the contact and systemic toxicity bioassays were evaluated through an ANOVA using a generalized linear model with a binomial distribution. The ante-dependence Ante (1) covariance structure was used to take into account the correlation of repeated measurements of the experimental units over time. Lethal concentrations for *O. insidiosus* of thiamethoxam in vegetative tissue were assessed only at 24 h. Natural mortality unrelated to the insecticide treatment was corrected through the Abbot's formula. Probit analysis for LC estimations was conducted using the PROC PROBIT package in SAS/STAT Software.

The abundance of *O. insidiosus* and *C. trifurcata* in soybean fields was analyzed using an ANOVA with a generalized linear mixed model with a normal distribution for each insect species. Year, location, and treatment were used as fixed variables. Random variables include the blocks per location per year. Means between treatments were compared by the Fisher's least significant difference test.

All statistical analyses were performed using the statistical package SAS/STAT software version 9.1.3 (SAS Institute Inc., Cary NC 2004).

Results and Discussion

Toxicity of Thiamethoxam Residues in Soybean Vegetative Tissue

There was a significant difference in the mortality of *O. insidiosus* between 24, 48, and 72 h in the systemic bioassays (F=4.76; df=2, 18; P<0.0001). Because natural mortality of *O. insidiosus* increased to over 20% after 48 h (Fig. 1), acute systemic toxicity is more reliability assessed before 48 h in the absence of prey. There were significant increases in mortality of the predators across the evaluated concentrations of thiamethoxam (F=4.76; df=5, 18; P=0.006).

The LC_{50} at 24 h after exposure for adults of *O. insidiosus* through systemic exposure was 227.35 ng/ml with 95% confidence limits of 133.52 ± 389.61 (Table 1). Previous studies by Piitz (2012) report that an average concentration of 250 ng/ml

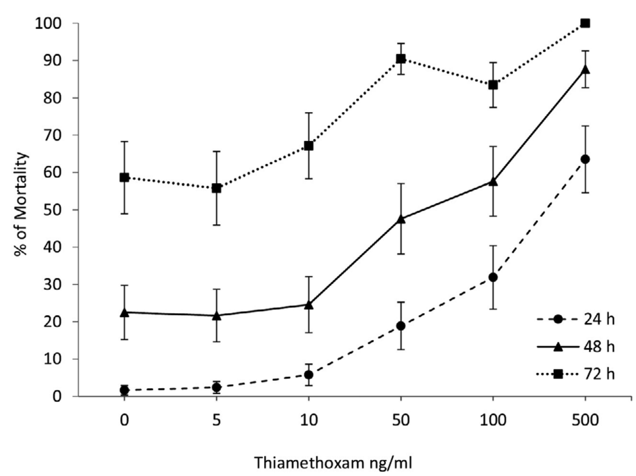

Fig. 1. Means of the adult mortality of *O. insidiosus* at different hours after exposure in response to a concentration range of thiamethoxam in water solution under laboratory conditions. Soybean leaves were placed in the solution for insecticide uptake during 24 h. Predators were exposed only to soybean leave tissue without an alternative prey. Error bars correspond to the standard errors of the mean.

Table 1. Susceptibility of *O. insidiosus* to thiamethoxam exposed through soybean vegetative tissue after 24 h of exposure

Lethal concn ng/ml	95% Confidence limits ng/ml Lower CL	Upper CL	Slope	SE	Pearson Chi-Square	Goodness of Fit P>Chi-Sq
LC_{10} = 19.61	2.32	46.62	1.2	0.27	3.49	0.32
LC_{50} = 227.35	133.52	389.61				
LC_{90} = 2635	1106	22708				

thiamethoxam in solution using similar bioassay methods corresponds to a concentration of 844 ng/g of thiamethoxam per soybean leaf. Using this conversion, the LC_{50} in vegetative tissue for *O. insidiosus* can be estimated to 767 ng/g with 95% confidence limits of 450.76 ± 1315.32 ng/g.

Direct toxicity in the field through exposure to soybean vegetative tissue is unlikely because the LC_{50} of thiamethoxam for *O. insidiosus* in plant tissue found in this study does not overlap with the highest reported levels of thiamethoxam in soybean fields at early vegetative stages. Concentrations of thiamethoxam in soybean at early vegetative stages (V1-V2) have been reported at 105 ng/g at 17 d after planting (Magalhaes et al. 2009) and 150 ng/g thiamethoxam at 15 d after planting (Camargo et al. unpublished data). Although there is limited information on the level of thiamethoxam at VC and VE stages where high concentrations of the insecticide are more likely to be found in vegetative tissue, it is unlikely that *O. insidious* migration to soybean fields will overlap with these early vegetative stages. Migration and early establishment of *O. insidiosus* in soybean fields has been suggested to correlate with the establishment of thrips populations in soybean fields (Rutledge and O'Neil 2005, Yoo and O'Neil 2009). Thrips typically appear in soybean fields after VE stages increasing its population from early vegetative (V1-V2) to early reproductive stages of soybean (R1- R2) (Price 1976, Yoo and O'Neil 2009). In consequence, predator arrival is more likely to overlap with thiamethoxam concentrations below the LC_{50} of vegetative tissue, and the effects of thiamethoxam residues in leaves are more likely to be sublethal in nature.

Effect of Thiamethoxam Residues in A. glycines

Mortality of *O. insidiosus* was positively correlated with the concentration of thiamethoxam that soybean aphids were exposed to in treated soybean trifoliates (F=9.58; df=1, 31; P=0.0041, Fig. 2). Mean mortality of *O. insidiosus* was higher when consuming soybean aphids feeding on leaves with 5 and 10 ng/ml of thiamethoxam in solution, which corresponds approximately to 16.88 and 33.76 ng/g in vegetative tissue (Fig. 2). These concentrations are likely to be present in soybean fields during early vegetative stages and exposure to soybean aphid is likely dependent on timing of migration. Generally, *A. glycines* arrives to soybean fields between June and July (Tilmon et al. 2011) when concentrations in the plant are between 1 to 88 ng/g of vegetative tissue (Magalhaes et al. 2009). Concentrations below 33.76 ng/g in vegetative tissue using the systemic bioassay are considered sublethal for soybean aphid (Magalhaes et al. 2009). Consequently, there is a potential for continuous uptake of the insecticide by aphids exposed to thiamethoxam below these field concentrations. While the median lethal concentration for soybean aphid exposed to thiamethoxam in soybean leaves has been estimated at 16.9 ng/ml in solution (Magalhaes et al. 2008), the actual concentration that aphids can uptake or accumulate before mortality is uncertain. Residues of thiamethoxam in soybean aphid are likely related to consumption of the insecticide during plant feeding and the level of tolerance of the aphids to the insecticide.

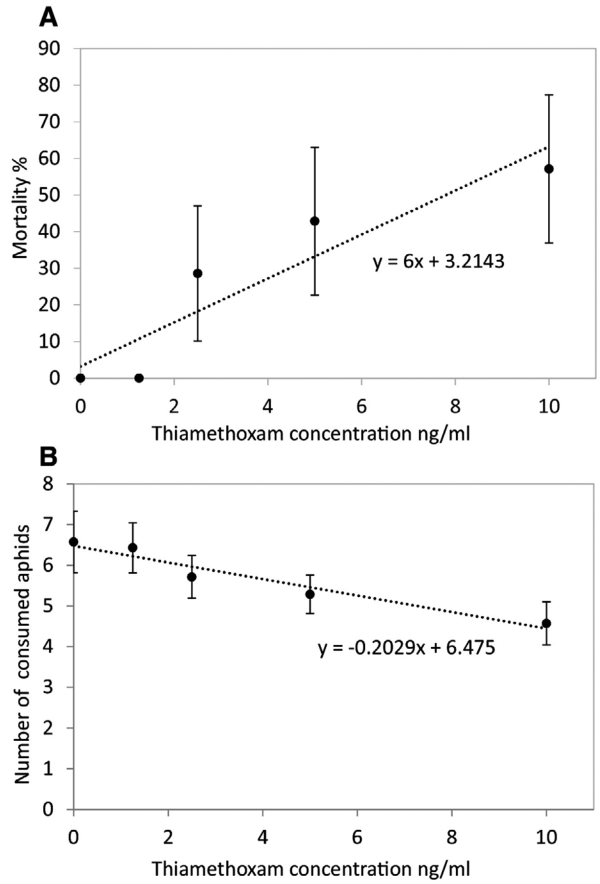

Fig. 2. Mean (±SE) of percent mortality of *O. insidiosus* and number of aphids consumed on soybean foliage treated with increasing concentrations of thiamethoxam. Dotted lines represent the fitted linear regression for the correlation of (A) the mortality of the predator at the different concentration of thiamethoxam and (B) the number of aphids consumed by *O. insidiosus* at the different concentrations of thiamethoxam.

The mortality of the predator at 16.88 and 33.76 ng/g in vegetative tissue suggests that the residues of thiamethoxam in *A. glycines* in conjunction with residues in vegetation could cause significant mortality of *O. insidiosus*. Lethal concentrations of *O. insidiosus* exposed to vegetative tissue ranged from the LC10 at 66.20 to the LC_{50} of 767.53 ng/g, which are higher than the concentrations used in the aphid consumption experiment. These estimates suggest that mortality of the predator is more likely to occur as a result of the residues encountered in *A. glycines* than exposure to residues in soybean vegetative tissue. We used this experimental design because it represents the scenario of how predators will encounter residues in insect prey under field conditions although it does not determine whether the toxicity came exclusively from ingestion of treated soybean aphids.

Residues of neonicotinoids in aphids have been reported for the bird cherry oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) (Bredeson and Lundgren 2015). These authors reported residues of the metabolite of thiamethoxam, clothianidin, at a concentration of 0.5860.0037 pg/aphid on wheat plants where 10 ml of 1.5 mg/liter was applied by soil drench. Clothianidin has shown higher toxicity than thiamethoxam in a number of different insect species (Nauen et al. 2003, Bredeson et al. 2015) and therefore, it is not unexpected for it to cause higher mortality of predatory insects. Thiamethoxam is quickly metabolized to clothianidin in the insect body (Nauen et al. 2003). Thus, it is likely that the mortality of the predators that consume aphid tissues is at least partially due to the metabolite, clothianidin. Residues of clothianidin reported by Bredeson and Lundgren (2015) in the bird cherry oat aphid displayed significant effects on fitness parameters of second-instar larvae of *Coleomegilla maculata (Degeer)* (*Coleoptera: Coccinelidae*). In contrast to this study, no significant mortality was observed in *R. padi* predators consuming aphids with traces of clothianidin. It is also possible that some unmetabolized thiamethoxam present in *A. glycines* is uptake by the predator and then converted to clothianidin. Characterization of the level of residues that *A. glycines* can hold after feeding can give a good understanding on the level of exposure that beneficial insects might have in the field.

In the present study, the average mortality of the *O. insidiosus* was variable in response to the evaluated concentrations (Fig. 2) which may be due to inherent variability in the amount of insecticide consumed and residing in the aphids. We also observed that the mortality of *O. insidiosus* did not increase when more aphids were consumed (F=0.02 df=1, 31; P=0.87). This again may be related to the amount of insecticide that each aphid takes up which is likely to be variable, and therefore mortality of the predator is independent of the number the aphids consumed by a single predator.

At the higher thiamethoxam concentrations, the number of aphids consumed by *O. insidiosus* was reduced (F=96.83; df=5,30; P<0.0001; Fig. 1), and there was a significant effect of predator mortality on the consumption rate (F=55.94; df=8, 27, P<0.0001). Lower consumption was recorded when higher mortality of the predator was observed (Fig. 2). This suggests that consumption was directly affected by toxicity of the predator and not by an effect on other behavioral parameters. Negative effects on consumption caused by neonicotinoids have been reported in other predator species including the coccinellid predator, *Serangium japonicum*, and the predatory mites, *Neoseiulus californicus* and *Phytoseiulus macropilis* (Poletti et al. 2007, He et al. 2012). These reports document deleterious effects on predatory species during the process of prey identification, attack, consumption and digestion when exposed to sublethal concentrations. Negative effects of thiamethoxam on consumption behavior can compromise the compatibility of this insecticide with biological control services provided by *O. insidiosus* and the use of this insecticide in IPM programs.

Abundance of O. insidiosus in Conventional Seed- Treated Soybean

There were no significant differences between locations or year in the abundance of *O. insidiosus* and bean leaf beetle (P>0.05), and no significant differences in abundance of *O. insidiosus* were observed among any of the treatments (F=0.22; df=3, 32; P=0.882; Fig. 3). However, significant differences in bean leaf beetle abundance between the treatments were observed during early soybean reproductive stages (F=5.41; df=3, 32; P=0.0040). Higher abundance of adult bean leaf beetles were observed in the control and fungicide treatments than in the insecticide alone and the insecticide mixture treatment (Fig. 3). There were no significant differences between thiamethoxam alone and the treatment of thiamethoxam in combination with multiple fungicides in the abundance of both insect species (Fig. 3), and it is unlikely that the effect of thiamethoxam on target and nontarget insects varies when the insecticide is used alone or in combination with fungicides commonly used in seed treatments.

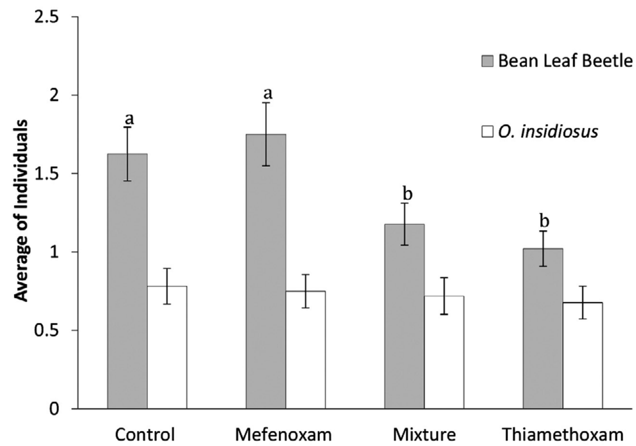

Fig. 3. Abundance of adults of the minute pirate bug, *O. insidiosus*, and bean leaf beetle, *C. trifurcata*, in the field during soybean flowering (reproductive stages R1-R2) 47–50 d after planting in 2014–2015. Results pooled from four different fields located in Ithaca and Lincoln, NE, during 2014 and 2015. Control treatment corresponds to untreated seeds. Mefenoxam corresponds to the fungicide treatment mixture alone: mefenoxam, fludioxinil, and sedaxane at 0.0113, 0.0038, and 0.0038mg A.I./seed, respectively. Mixture corresponds to the fungicide mixture+insecticide: mefenoxam, fludioxinil, sedaxane, and thiamethoxam at 0.0113, 0.0038, 0.0038, and 0.0756mg A.I/ seed, respectively, and thiamethoxam to the insecticide alone at 0.0756mg A.I/seed. Different letters correspond to significant differences at the 95% CL. Error bars correspond to the standard error of the mean.

Orius insidiosus was not observed in the field at vegetative stages from VC to V5, therefore abundance was reported only for early reproductive stages (R1-R2, V6-V8). The arrival of *O. insidiosus* to the fields in northern states has been reported to occur mainly in June through July (Rutledge and O'Neil 2005). During these months, soybean crops in the north central United States can develop from early to mid-vegetative stages (VC-V5) through early reproductive stages (R1-R2; Pedersen and Lauer 2004). Levels of thiamethoxam in soybean vegetative tissue can be relatively high in early vegetative stages but decreases over time during the growing season (Magalhaes et al. 2009). As previously discussed, the LC_{50} for *O. insidiosus* was 756.53 ng/g of vegetative tissue and is unlikely to overlap with concentrations in the plant at the time of arrival of the predator to soybean fields. Therefore, it is not surprising that abundance of the predator was unaffected. Mortality of the predator in soybean fields can also occur by ingestion of contaminated aphids at concentrations in the plant of 5 ppb. However, *A. glycines* was not observed in the fields evaluated during the two years of study. Yet, there is still very limited information on the level of residues in soybean plant tissue in relation to different planting dates and environmental conditions. Such information could allow a better characterization of the exposure levels to thiamethoxam in the field and a more accurate characterization of risk of neonicotinoids to beneficial species in soybean crops.

The results on the abundance of *O. insidiosus* in soybean fields with neonicotinoid seed treatments are consistent with the studies by Ohnesorg et al. (2009) and Seagraves and Lundgren (2012), where no significant differences in *O. insidiosus* abundance were observed between fields derived from neonicotinoid treated and untreated fields. *Orius insidiosus* is a generalist predator consuming thrips, pollen, and nectar from flowers, vegetative tissue, and aphids in soybean fields (Rutledge and O'Neil 2005). The diversity in food sources for *O. insidiosus* in soybean fields during early reproductive stages may reduce the level of exposure to thiamethoxam concentrations that could cause affect the survivorship of the predator in the field. Lethal effects are unlikely to occur under field conditions that would affect predator abundance. While there is potential overlap of sublethal concentrations of thiamethoxam with the predator migration and establishment in soybean fields, field effects may be more difficult to observe under the smaller spatial and temporal scales used in this and previous studies.

Continuous exposure over space and time could affect predator communities not only at local but also at regional and larger temporal scales, which should be considered in future studies. In a scenario with late planting dates and early arrival of the predator to soybean fields, there is a chance for *O. insidiosus* to encounter high concentrations of thiamethoxam in soybean leaves and lethal concentrations in aphid prey which might have a significant effect on predator abundance. In the second scenario, if there is early planting and late arrival of the predator, exposure to lethal concentrations is less likely. Thus, planting dates, arrival time and the availability of insect prey might affect the results on survivorship and abundance of the predator in fields with thiamethoxam seed treatments.

In conclusion, residues in prey and vegetative tissue can have significant effects on the survivorship of *O. insidiosus*. Effects of thiamethoxam on consumption behavior and mortality of *O. insidiosus* suggest possible antagonism between the use of thiamethoxam seed treatments and biological control services provided by this species. Additional research is necessary to determine if the effects on consumption of *A. glycines* is observed under field scenarios. No effect of neonicotinoids seed treatment on abundance of *O. insidiosus* have been reported at the scale that this and previous studies have used to determine the effect of seed treatments. There is a need to understand the impact of neonicotinoids on beneficial insects at larger temporal and spatial scales and their effects on biological control services. The use of thiamethoxam seed treatments may have negative effects on *O. insidiosus* if early season plant feeding occurs or if the predators are exposed to residues in the insect prey. Determination of the concentrations of neonicotinoids that *A. glycines* and other early season prey (e.g. thrips, spider mites) can accumulate is necessary to fully characterize exposure of *O. insidiosus* and other beneficial arthropods to neonicotinoids residues. In addition, the effects and combined effects of the various pest management practices used in soybean (e.g. seed treatments, contact and systemic foliar pesticide applications) must be investigated in order to fully understand the impact of pest management on beneficial species. This will lead to a better understanding of the impacts of current management practices on natural enemy species in soybean.

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