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ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

Microbial Degradation of Neonicotinoid Insecticides in the Soil and Potential Implication on Thrips (Thysanoptera: Thripidae) Control in Cotton

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ABSTRACT

An experiment was conducted in 2013 to determine the extent that soil microbes degrade neonicotinoid insecticides, commonly used as insecticide seed treatments, into secondary metabolites. Soil was collected from a field where efficacy problems against thrips (Thysanoptera: Thripidae) were observed in cotton during 2013. At the same time, soil was also collected from an area with no previous exposure to insecticides. Part of the soil from each location was sterilized by autoclaving. Both sterilized and unsterilized soil were treated with an identical dilution of either Gaucho 600 (imidacloprid) or Cruiser 5F (thiamethoxam). After 25 days, samples were tested to determine the concentrations of neonicotinoid insecticides, including metabolites. Thiamethoxam and two of its metabolites were detected in soil treated with the Cruiser dilution. Imidacloprid and three of its metabolites were detected in soil treated with Gaucho. Sterilizing the soil sample significantly reduced the concentrations of imidacloprid and thiamethoxam metabolites. These results suggested that soil microbes were present in the soil samples from both locations that can degrade insecticides. The levels of degradation to secondary metabolites were approximately 14% and 2% or less for imidacloprid and thiamethoxam, respectively. It is unlikely that these relatively low levels of microbial metabolism would substantially impact the efficacy of insecticide seed treatments, especially considering the primary metabolites found retain some insecticidal activity.

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Several species of thrips (Thysanoptera: Thripidae) are common pests of cotton that routinely rank among the top three insects reducing yield in the United States (Stewart et al. 2013, Williams 2013). Preventative at-planting treatments, either in-furrow granular or liquid insecticides or seed treatments, are often recommended to control thrips infestations in seedling cotton (Cook et al. 2011). In the last ten years, neonicotinoid seed treatments such as Gaucho (imidacloprid; Bayer CropScience, Raleigh, NC) or Cruiser (thiamethoxam; Syngenta, Greensboro, NC) have been used almost exclusively for thrips control in Tennessee and much of the Cotton Belt.

Under field conditions, neonicotinoid insecticides are known to persist in the soil for a year or longer, albeit at relatively low levels relative to initial concentrations (Stewart et al. 2014, Xu et al. 2016). Under laboratory conditions, Sharma and Singh (2014) found the half-life of imidacloprid in soil to be 32 – 43 days, depending upon soil type. Metabolites found in this study included chloronicotinic acid, imidacloprid-nitroguanidine, imidacloprid olefin and imidacloprid 5-hydroxy. Similarly, Karmakar et al. (2006) reported a half-life of thiamethoxam of 11 – 26 days in four soil types. Clothianidin is a recognized primary metabolite of thiamethoxam (Nauen et al. 2003, Tomizawa and Casida 2005).

Beginning roughly in 2011 in the Mid-South, thrips control failures with neonicotinoid seed treatments, particularly thiamethoxam, became more commonly observed (Stewart 2013). Continuous use of some pesticides has been known to speed up microbial degradation of certain herbicides, such as atrazine (Mueller et al. 2010, 2015), and insecticides such as aldicarb (Suett and Jukes 1988). Zhou et al. (2013) reported the bacterium *Ensifer adhaerens* degrades thiamethoxam from the rhizosphere soil, so metabolism of neonicotinoid insecticides by soil microorganisms is not unexpected. Therefore, an experiment was conducted in 2013 to determine the extent that soil microbes degrade thiamethoxam and imidacloprid into secondary metabolites, potentially reducing their efficacy to control insect pests.

MATERIALS AND METHODS

Soil Collection and Preparation. In early July of 2013, a Collins silt loam soil was collected from a field at the Milan Research and Education Center in Milan, TN. Neonicotinoid seed treatments had been used annually in this field for cotton or corn, since 2002, with the exception of two years (2006, 2009) where soybean was planted without an insecticide seed treatment. This field was selected because poor control of thrips by neonicotinoid seed treatments, especially thiamethoxam, had been observed during 2012 and 2013 as noted by Vineyard (2015) (Fig. 1). At the same time, a Bibb fine sandy loam soil was collected at the West Tennessee Research and Education Center in Jackson, TN from a mowed grassy area that was isolated from agricultural fields and had no previous exposure to insecticides. Approximately two 19.4-liter (5 gal) buckets of soil were collected at each location from the top 5 - 7.5 cm of the surface. The soil was thoroughly mixed, and about 50% of the soil from each location was sterilized by autoclaving for 60 minutes at 121°C and allowed to cool to ambient temperature.

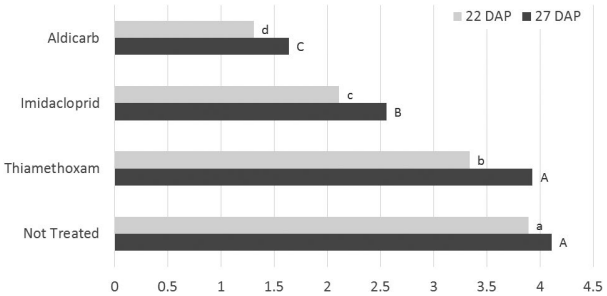

Figure 1. Thrips injury observed in cotton during 2013 where an in-furrow treatment of aldicarb (Temik 15G, 820 g ai/ha) or insecticide seed treatments of thiamethoxam or imidacloprid (Cruiser 5F or Gaucho 600, 0.375 mg ai/seed) was applied. Relative injury was rated on a 0 – 5 scale with 0 indicating no injury. Data are shown for 22 and 27 days after planting. Different letters indicate significant differences among treatments by rating date ($P < 0.05$).

Soil Insecticide Treatment. Both sterilized and unsterilized soil from each location were treated with an identical dilution of either Gaucho 600 (imidacloprid, Bayer CropScience) or Cruiser 5F (thiamethoxam, Syngenta). Because drying might affect the viability of soil microbes, there was no attempt to standardize differences in the moisture of soil collected from the two locations or resulting from autoclaving. A dilution was prepared of 0.5 ml of formulated product per 1,000 ml of water, and a syringe was used to add 10 ml of this solution to 341 g (12 oz) of soil. This was replicated four times for each combination of insecticide, autoclaving treatment, and soil-collection location.

Immediately after treating, the soil was mixed within self-sealing plastic bags, transferred to 250 ml plastic beakers, and stored in an open shed for 15 days at shaded, ambient outside temperatures. Daily maximum temperatures averaged 31.0°C (range = 28.9 – 33.3°C) during the duration of the study, with an average minimum temperature of 19.5°C (range = l5.0 – 22.8°C). Distilled water (30 ml) was added on day three and nine of the 15-day storage period to prevent desiccation. After 15 days, the beakers were transferred to a temperature-controlled room and held for ten days at 20-23°C to allow for additional drying. The soil was again mixed as described above, and a 57 g (2 oz) subsample was submitted for testing of neonicotinoid concentrations.

Chemical Analyses. Soil samples were analyzed to determine the levels of neonicotinoid residues by the United States Department of Agriculture Agricultural Marketing System (USDA AMS) Science and Technology Laboratory Approval and Testing Division, National Science Laboratories, Gastonia, NC. This laboratory is accredited to ISO/IEC 17025:2005 for specific tests in the fields of chemistry and microbiology, including testing for pesticide residues. The samples were extracted for analysis of agrochemicals using a refined methodology for the determination of neonicotinoid pesticides and their metabolites using an approach of the official pesticide extraction method (AOAC 2007.01), also known as the QuEChERS method, and analyzed by liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS) (Kamel 2010; Lehotay et al. 2005; Zhang et al. 2011). Quantification was performed using external calibration standards prepared from certified standard reference material. The analytical limit of detection (LOD) for each neonicotinoid insecticide and its metabolites are shown in Table 1.

Statistical Analyses. Within an insecticide treatment, data were analyzed as a two by two factorial of soil location and autoclaving treatment. The relative amount of total metabolites present as a percentage of the total neonicotinoid concentration was calculated for each sample. An arcsine transformation was used because percentages were analyzed and preliminary analysis indicated that transformation normalized the data. Proc GLIMMIX (SAS Institute Inc. 2013) was used to determine main effects of location, autoclaving treatment, and their interaction on the concentration of neonicotinoid metabolites ($\alpha = 0.05$, LSMEANS, DDFM=SATTERTHWAITE). The relative concentrations of individual metabolites were evaluated identically.

Table 1. Neonicotinoid residues of parent compounds, their metabolites, and the analytical limit of detection (LOD) that were screened for during analyses of soil samples

Pesticide Residue	LOD (ng/g)	Pesticide Residue	LOD (ng/g)
Thiamethoxam	1.0	Imidacloprid	1.0
Thiamethoxam metabolites		Imidacloprid metabolites	
Clothianidin [z]	1.0	6-Chloronicotinic acid	30
Clothianidin MNG	50	Imidacloprid 5-hydroxy	1.0
Clothianidin TMG	50	Imidacloprid des nitro hcl	2.0
Clothianidin TZMU [z]	50	Imidacloprid olefin [z]	10
Clothianidin TZNG	50	Imidacloprid olefin des nitro [z]	16
		Imidacloprid urea [z]	1.0

[z]Metabolites detected in this study

RESULTS

Thiamethoxam and two of its metabolites, clothianidin and clothianidin TZMU, were detected in soil treated with Cruiser. Clothianidin composed 89% of metabolites detected, but represented <1% of the total neonicotinoid concentration in the soils treated with thiamethoxam. Imidacloprid and three of its metabolites were detected in soil treated with Gaucho. Detectable imidacloprid metabolites included imidacloprid olefin, imidacloprid olefin des nitro, and imidacloprid urea. Across both locations and autoclaving treatments, these metabolites represented 6.9, 1.1, and 0.6% of the total neonicotinoid concentration, respectively. Average concentration levels (ng/g or ppb) for parent neonicotinoids and total metabolites are presented in Table 2.

For soil treated with imidacloprid, there was a significant main effect of autoclaving (F = 237; df = 1, 12; $P < 0.0001$). There was approximately a 10 – 12% reduction in metabolites of imidacloprid when the soil was autoclaved (Table 3). The source of the soil did not significantly affect this result (P = 0.5633) nor was there a significant interaction of soil source and the sterilization treatment (P = 0.0977).

Much lower percentages of metabolites were found in soil treated with thiamethoxam, ranging from 0.1 – 2.1% of the total neonicotinoid concentration (Table 3). However, soil source, the autoclaving treatment, and the interaction of these two factors were highly significant (F = 66.7, 46.3 and 33.2 respectively; df = 1, 12; $P < 0.0001$). In general, a higher percentage of thiamethoxam metabolites were found in soil collected from the agricultural field in Milan compared with the Jackson location (Table 3). For thiamethoxam-treated soil from the Milan location, metabolites as a percentage of total neonicotinoid concentrations were about five-fold higher in unsterilized soil compared with sterilized soil. In the non-agricultural field (Jackson), total thiamethoxam metabolites were reduced 2.2-fold by sterilizing the soil.

When analyzed across both locations, within the insecticide treatment, all individual metabolite concentrations were reduced by autoclaving the soil (data not shown; $F > 60$; df =1, 12; $P < 0.0001$ for all).

Table 2. Mean ± SE concentration levels (ng/g) of parent neonicotinoid insecticides and the total of their metabolites in soil treated with thiamethoxam (Cruiser) or imidacloprid (Gaucho)

Soil Treatment	Location	Autoclaved	Concentration (ng/g)	
			Thiamethoxam	Metabolites
Thiamethoxam	Jackson	Yes	6,070 ± 347	6.3 ± 4.5
	Jackson	No	5,878 ± 472	17.8 ± 17.8
	Milan	Yes	6,480 ± 218	27.8 ± 1.8
	Milan	No	7,828 ± 164	171 ± 6.9
			Imidacloprid	Metabolites
Imidacloprid	Jackson	Yes	4,660 ± 283	113 ± 67.1
	Jackson	No	3,900 ± 330	643 ± 44.6
	Milan	Yes	5,703 ± 83	230 ± 20.9
	Milan	No	5,860 ± 489	900 ± 45.2

Table 3. Percent of metabolites relative to total neonicotinoid concentrations for unsterilized and sterilized (autoclaved) soil from two locations that were treated with either thiamethoxam (Cruiser) or imidacloprid (Gaucho)

Location	Soil Treatment	% Metabolites	
		Unsterilized	Autoclaved
Jackson	Thiamethoxam	0.29	0.10
Milan	Thiamethoxam	2.14	0.43
Jackson	Imidacloprid	14.2	2.4
Milan	Imidacloprid	13.3	3.9

DISCUSSION

One possible reason for diminished performance of insecticide seed treatments could be microbial decay of parent compounds into less active metabolites within the soil. We only allowed 15 days for degradation of insecticides within the soil before processing the samples for analysis, although there was an additional ten-day drying period where some additional degradation might have occurred. However, any decay of the parent compounds would have to occur quickly to substantially affect the performance of insecticide seed treatments on thrips control. Cotton plants are primarily susceptible to thrips injury during the first 21 days after emergence and diminished activity of neonicotinoid seed treatments has often observed beginning 14 – 28 days after planting (Cook et al. 2011 and references therein).

Soil sterilization reduced the concentrations of imidacloprid and thiamethoxam metabolites observed in our study, suggesting that soil microbes could degrade both insecticides. However, the amounts of degradation to secondary metabolites were relatively low even in unsterilized soil (approximately 14% and 2% or less for imidacloprid and thiamethoxam, respectively). There was some indication of increased degradation in the soil collected from the agricultural field (Milan), particularly for thiamethoxam. These differences were minor given the overall low levels of degradation and, as likely as previous exposure to neonicotinoid insecticides, could have resulted from differences in soil textures, organic matter, or inherent differences in the microorganism community.

It is possible that the rate of insecticide metabolism by microorganisms may vary depending upon the concentration of the insecticide in the soil. Thiamethoxam and imidacloprid were applied in this study at a rate of 3 mg ai/341 g of soil. At a standard seed treatment rate of 0.375 mg ai/seed for either imidacloprid or thiamethoxam, this represents a dose equivalent to eight treated seed in the same volume of soil. This would appear to be a reasonable approximation of a field dose. It seems unlikely that the levels of microbial metabolism we observed would appreciably impact either insecticides ability to control insect pests when used as seed treatments. Also, the primary metabolites detected, clothianidin and imidacloprid olefin, retain some insecticidal activity. For example, both Surchail et al. (2001) and Nauen et al. (2001) reported that imidacloprid and imidacloprid olefin had similar oral LD50 values for adult honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), whereas imidacloprid urea is essentially non-toxic. Clothianidin is a commonly used seed treatment in many crops including corn, *Zea mays* L., although personal observations in field tests indicate less activity than thiamethoxam when used at equivalent rates for thrips control in cotton (unpublished data).

Tobacco thrips, *Frankliniella fusca* (Hinds), are the most common thrips species found on seedling cotton in the Mid-South and Southeast (Stewart et al. 2013). More recent research has shown that tobacco thrips have developed resistance to neonicotinoid insecticide in much of the Mid-South and Southeast (Darnell et al. 2015, 2016; Huseth et al. 2016), and insecticide resistance has been associated with the diminished performance of neonicotinoid insecticides in controlling thrips in cotton. Indeed, assays of tobacco thrips collected from the Jackson and Milan locations during 2014 indicated resistance to both thiamethoxam and imidacloprid (Huseth et al. 2016). In comparison to documented resistance, microbial degradation in soils from these locations appears to be considerably less important.

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