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CONCENTRATIONS OF IMIDACLOPRID AND OLEFIN-IMIDACLOPRID METABOLITE IN THE WALNUT HUSK MAGGOT (DIPTERA:TEPHRITIDAE)

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Black walnut, *Juglans nigra* L., is native to eastern North America and is valued for its economic, ornamental, and ecological importance throughout the United States (Williams 1990; Harlow & Harrar 1969; Smith & Follmer 1972). Although more than 300 insect species have been associated with this valuable tree (Weber et al. 1992), only a few of these species are considered pests (Reid et al. 2004). Because walnut trees are labeled as a tree crop, the type and method of insecticides applied are restricted (Protection of Environment, Part 180 2010). Therefore, different types of insecticides, such as imidacloprid, have been studied to track the movement and concentration levels of the chemical throughout the tree and into the nut tissue, which may be consumed by humans and wildlife (Nix et al. 2013).

The walnut husk maggot, *Rhagoletis suavis* (Loew), is considered a minor pest of black walnut (Brooks 1921). Adults emerge in mid-July, with peak abundance in mid to late Sep. Oviposition occurs from late Aug throughout Sep (Gibson & Kearby 1978). Larvae develop inside the husk of the nut, causing premature nut drop, preventing nut maturity and affecting productivity. Maggots also cause walnut husks to become slimy and sticky, which blackens the walnut shells, making them unmarketable and the nutmeat difficult to process (Poland et al. 2006). Imidacloprid is commonly used as a foliar spray on numerous fruit-producing tree species because of its low mammalian toxicity and ability to control insect pests (Leicht 1996). For example, foliar sprays of imidacloprid produced a decline in larval populations of the walnut husk fly, *R. completa* Cresson, and the western cherry fruit fly, *R. indifferens* Curran, on cherry trees in laboratory trials (Yee & Alston 2006; Van Steenwyk et al. 2010). Imidacloprid has also been demonstrated to be effective against the apple maggot, *R. pomonella* (Walsh) (Hu & Prokopy 1998).

Although work has been done using imidacloprid against fruit flies in walnuts as foliar sprays (Van Steenwyk et al. 2010), limited or no work has been done using systemic soil applications of imidacloprid against the larval stage of the walnut husk maggot. In the process of conducting a research project to evaluate the concentration levels of imidacloprid and its insecticidal metabolite, olefin-imidacloprid, in walnut tissues (Nix et al. 2013), we discovered infestations of walnut husk maggots within the walnuts. Because this systemic insecticide was documented in the walnut husks during this study, the impacts of these compounds on the walnut husk maggot larvae feeding inside the husk were assessed. Thus, our objective was to assess concentrations of imidacloprid and olefin-imidacloprid within the tissue of the walnut husk maggot. Data documenting the concentration levels of imidacloprid and olefin-imidacloprid in the various tissues in the lower and upper canopy of this valuable tree will provide more information on the effect of these chemicals on insects dwelling within this tree species, as well as the concentration level retained within the edible nutmeat of black walnuts (Nix et al. 2013).

Mature black walnut trees (n = 14) were selected at the Strong Stock Farm in Knoxville, TN (N 36° 3' 7.1526" W 83° 47' 23.3802") in Apr 2011. Selected trees averaged 17.8 m (8.5 - 27.4 m) in height and 51.6 cm (25.1 - 116.1 cm) in diam breast height (dbh). Trees (n = 14) were arranged in a complete randomized block design with split plot treatments and sampling. The test consisted of 7 blocks of 2 trees per block (consisting of one imidacloprid-treated tree and one untreated control tree) and walnut samples collected from the lower and upper strata of each tree's canopy. Imidacloprid was applied using CoreTect, a systemic soil pellet containing 20% imidacloprid applied to the soil at 1 pellet per 2.5 cm of tree dbh on 20 Apr 2011. Pellets were placed ca. 30.0 cm away from the trunk and buried ca. 5.0 cm deep in the soil encircling the tree. Temperature averaged 19.0 °C (5.0-29.4) and rainfall averaged 0.9 cm (0-9.0)/day for the immediate 2 weeks post-application.

Two mature nuts were collected from the lower stratum (below ca. 5 m) (n = 28) and 2 from the upper stratum (above ca. 5 m) (n = 28) 5 months post-treatment on 27 Sep 2011. This study was part of a companion study investigating imidacloprid concentrations in tree tissue (Nix et al. 2013). Mature walnuts (n = 56) were collected with a 10-m pole pruner, sealed in plastic bags, packed in ice, taken to the laboratory, and stored in a freezer at -18 °C until chemical extraction. Larvae were removed from the husk, dried, shredded, weighed, and placed into 1-g unit vials for chemical analysis. The number of larvae per walnut sample were counted and recorded. Imidacloprid and olefin-imidacloprid concentrations (ppb) in only the larvae were determined using high pressure liquid chromatography coupled with tandem mass spectrometry (LC/MS) (Schöning & Schmuck 2003). Concentrations in plant tissue types were earlier documented in a companion study by Nix et al. (2013) and were compared with concentrations in walnut husk maggot larval tissue to increase our understanding of insecticide translocation in this system. Data were analyzed using the Shapiro-Wilks W test for normality and Levene's test of homogeneity of variances to verify that chemical concentration data conformed to the assumptions of analysis of variance (ANOVA). ANOVA was performed to detect differences (P = 0.05) among imidacloprid and olefin-imidacloprid concentrations in the tree strata as well as differences among numbers of walnut husk maggots among strata and treatments. The least significant differences (LSD) procedure was used to determine significant differences (P = 0.05) among mean concentrations of imidacloprid and olefin-imidacloprid in larvae collected in the upper and lower tree strata, as well as differences among numbers of walnut husk maggot between treatments and tree strata (SAS Institute 2005).

Mean imidacloprid concentration levels in walnut husk maggot larvae differed significantly (F = 1.42; df 1, 3; $P < 0.05$) by stratum (Fig. 1). Imidacloprid concentrations were significantly higher (LSD test; $P < 0.05$) in larvae collected from the lower stratum (11.72 ppb) than larvae in the upper stratum (9.25 ppb). However, imidacloprid concentrations were lower in walnut husk maggot specimens (11.72 ppb) in the lower stratum than in the walnut husk (56.40 ppb) and nutmeat (84.06 ppb) as reported earlier in a companion study by Nix et al. (2013). In addition, imidacloprid concentrations were lower in larvae (9.25 ppb) collected from the upper stratum compared with concentration levels found in the upper walnut husk (11.79 ppb) and nutmeat (72.19 ppb) samples (Nix et al. 2013). The detection of these residues in the larvae infers movement of the insecticidal materials from plant tissue into the pest feeding on these tissues.

Concentration levels of olefin-imidacloprid in larvae were also significantly different between stratum (F = 1.42; df 1, 3; $P < 0.05$) (Fig. 1). However, and in contrast to imidacloprid, olefin-imidacloprid concentrations in larvae, were significantly lower (LSD test; $P < 0.05$) in the lower stratum

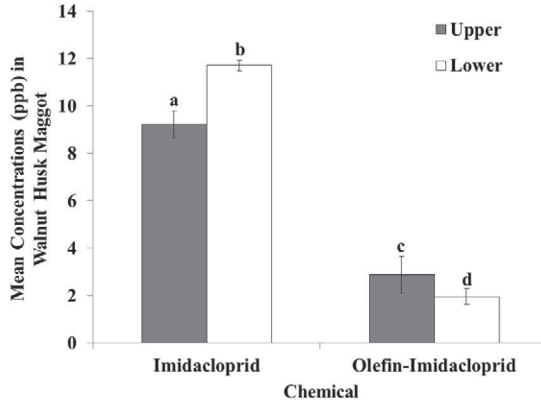

Fig. 1. Impact of insecticide treatment and tree stratum on mean (± SE) concentration (ppb) of imidacloprid and olefin-imidacloprid in walnut husk maggot. Means (n = 28 total nuts per stratum) followed by the same letter do not differ significantly (LSD test; $P > 0.05$).

than the upper stratum. This converse pattern is consistent with earlier studies on olefin-imidacloprid concentrations in eastern hemlock that were documented to increase as imidacloprid concentrations decrease (Dilling et al. 2010; Coots et al. 2013). They noted the possible breakdown of the insecticide over time as it translocated from the lower to upper canopy. Additionally, olefin-imidacloprid concentrations were significantly lower (LSD test; $P < 0.05$) than imidacloprid concentrations within each stratum. As with imidacloprid when compared with nutmeat and husk tissue data from a companion study (Nix et al. 2013), walnut husk maggot larvae had lower levels of olefin-imidacloprid in both strata. These data are in agreement with the progressive increase of olefin-imidacloprid concentrations over time as imidacloprid concentrations decrease over time as reported for eastern hemlock (Leicht 1996; Dilling et al. 2010; Nix et al. 2013). It is plausible that the higher olefin-imidacloprid levels in the upper stratum compared to lower stratum are a result of metabolism of imidacloprid in the upper stratum resulting in the observed decreased imidacloprid concentration in the upper stratum.

All walnuts sampled contained at least one walnut husk maggot larva. Imidacloprid treatments impacted walnut husk maggot populations throughout the canopy, as the mean number of larvae in walnuts was significantly lower (F = 68.00; df 3, 24; $P < 0.0001$) in nuts from the lower stratum of imidacloprid-treated trees compared to untreated controls (Fig. 2). There were 50% fewer larvae in walnuts in the upper stratum (average of 3.0, range of 2.0-4.0), and 67% fewer larvae in the lower stratum of imidacloprid-treated trees (average of 2.0, range of 1.0-3.0) when compared to control trees. Additionally, the abun-

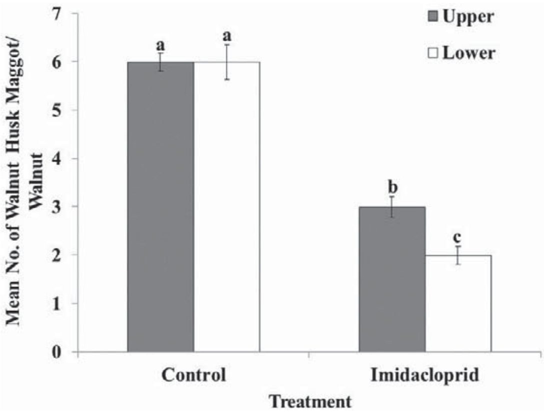

Fig. 2. Impact of insecticide treatment on the mean number (± SE) of individual walnut husk maggots within each stratum. Means (n = 28 total nuts per stratum) with the same letters are not significantly different (LSD test; $P > 0.05$).

dance of walnut husk maggot larvae (n = 106) in the lower stratum was significantly different from the abundance of larvae (n = 132) in the upper stratum of treated and untreated trees ($P <$ 0.05).

While the systemic use of imidacloprid in the form of a soil pellet is not recommended for control of any pest of black walnut, these data may be useful in understanding the movement of imidacloprid through other species of mast-bearing hardwood species and potential impact to insects feeding on those host trees.

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Summary

Several species of insects diminish the value of black walnut, which is considered a valuable economic and environmental tree species. Because imidacloprid has been used successfully against pests of other host plants, we investigated how it would impact larvae of the walnut husk maggot, *Rhagoletis suavis* (Loew), on black walnuts. Thus, the objective of this project was to document the concentration levels of imidacloprid and olefin-imidacloprid, an insecticidal metabolite of imidacloprid, within the walnut husk maggot larvae dwelling within the nut husk of fly-infested mature black walnut trees. CoreTect, a pellet formulation of imidacloprid, was applied to the soil surrounding mature black walnut trees in spring of 2011. Concentrations of both imidacloprid and olefin-imidacloprid were assessed in walnut husk maggot tissue using liquid chromatography coupled with tandem mass spectrometry (LC/MS). Both imidacloprid and olefin-imidacloprid were detected in walnut husk maggot larvae from both the lower (11.72 ppb) and upper (9.25 ppb) strata. Olefin-imidacloprid concentrations in larvae were significantly lower in the lower stratum compared with the upper stratum, while the opposite was true when assessing concentration levels of imidacloprid. Olefin-imidacloprid concentrations were significantly lower than imidacloprid concentrations within each stratum. Populations of walnut husk maggot were significantly lower in treated trees compared to control trees indicating that imidacloprid, applied as a soil pellet, reduced populations of the walnut husk maggot.

Key Words: black walnut, *Juglans nigra,* imidacloprid, olefin-imidacloprid, walnut husk maggot, *Rhagoletis suavis*

Resumen

Varias especies de insectos disminuyen el valor del nogal negro, que se considera una especie de árbol económicamente y ambientalmente valioso. Debido que imidacloprid ha sido utilizado con éxito contra las plagas de otras plantas hospederas, investigamos cómo impactaría larvas de la mosca de la cáscara del nogal, *Rhagoletis suavis* (Loew), en el nogal negro. Por lo tanto, el objetivo de este proyecto fue documentar el nivel de concentración de imidacloprid y olefina-imidacloprid, un metabolito de insecticida imidacloprid, dentro las larvas de la mosca de la cáscara del nogal viviendo dentro de la cáscara de nueces de árboles maduros de nogal negro infestados con moscas. CoreTect, una formulación de pellets (bolitas) de imidacloprid, se aplicó a la tierra alrededor de árboles maduros de nogal negro en la primavera del 2011. Se evaluó la concentración de ambos imidacloprid y imidacloprid-olefina en el tejido de las larvas de la mosca de la cáscara del nogal, usando una cromatografía líquida junta con una espectrometría de masas en tándem (LC/MS). Tanto imidacloprid y imidacloprid-olefina se detectó en las larvas de la mosca de la cáscara del nogal tanto en el estrato inferior (11.72 ppb) y superior (9.25 ppb). La concentración de imidacloprid-olefina en larvas fue significativamente menor en el estrato inferior en comparación con el estrato superior, mientras que lo contrario fue cierto en la evaluación de los niveles de concentración de imidacloprid. La concentración de imidacloprid-olefina fue significativamente menor que la concentración de imidacloprid dentro de cada estrato. La población de la mosca de la cáscara del nogal fue significativamente menor en los árboles tratados en comparación con los árboles de control que indica que el imidacloprid, aplicado como una bolita de suelo,

reduce las poblaciones de la mosca de la cáscara del nogal. Palabras clave: nogal negro, *Juglans nigra*, imidacloprid, olefina-imidacloprid, mosca de la cáscara de nogal, *Rhagoletis suavis*

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