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REPELLENCY OF THE BIOPESTICIDE, AZADIRACHTIN, TO WIREWORMS (COLEOPTERA: ELATERIDAE)

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ABSTRACT

The neem tree *Azadirachta indica* A. Juss produces numerous allelochemical compounds. The most effective active ingredient in *A. indica* based insecticides is azadirachtin. We found that azadirachtin did not cause mortality, antifeeding responses, or change growth rate of *Melanotus communis* (Gyllenhal) wireworms. However, azadirachtin treated soil was repellent to the wireworms. This is the first report of azadirachtin being repellent to any of the large and economically important family of Elateridae.

Key Words: Neem, azadirachtin, wireworms, *Melanotus communis*

RESUMEN

El árbol de neem, *Azadirachta indica* A. Juss produce compuestos alleloquímicos numerosos. El ingrediente activo mas efectivo en insecticidas de base *A. indica* es azadirachtin. Descubrimos que azadirachtin no causó la mortalidad o respuestas de no alimentarse, ni cambiar la tasa de crecimiento del gusano alambre, *Melanotus communis* (Gyllenhal). Sin embargo, el suelo tratado con azadirachtin fue repelente a los gusanos alambres. Este es el primer informe de que azadirachtin es un repelente a cualquiera de los Elateridae, una familia con numerosas especies y economicamente importante.

Wireworms are important insect pests of Florida sugarcane. Of the different wireworm species found in Florida sugarcane, *Melanotus communis* (Gyllenhal) is the most important pest (Cherry 2007). Hall (1985) noted that *M. communis* damaged sugarcane by feeding on root primordia, buds, and roots as well as directly on the stem of young plants. Hall (1990) later reported that tillering during the growing season compensated for early stand losses due to wireworm damage. Wireworms are primarily a pest in newly planted sugarcane although the insects are also found in ratoon sugarcane.

Phytochemicals have been used for many years to control insect pest damage on agricultural crops (Lee et al. 1997). Plants produce a wide range of secondary metabolites (e.g., terpenoids, alkaloids, and phenolics) that often possess insecticidal, fungicidal, bactericidal, antiviral, antifeeding, or growth retardant properties (Singh et al. 1989; Benner 1993; Wilson et al. 1997).

The neem tree *Azadirachta indica* A. Juss produces numerous allelochemical compounds. The most effective active ingredient in *A. indica* based insecticides is azadirachtin. This compound affects a large number of pest insects, acting as a repellent, feeding and oviposition deterrent, growth regulator, and reproduction inhibitor. Azadirachtin has been shown to exhibit biological activity against >400 insect species. However, little is known about the effects of this insecticidal plant on sugarcane pests (Garcia et al. 2006).

In Florida, organic farmers such as some sugarcane growers cannot use synthetic chemicals and have no effective alternative for wireworm management besides field flooding (Hall & Cherry 1993), which is not always readily available. Our objective was to determine if azadirachtin had any effect on wireworms that attack Florida sugarcane. This information may provide an organic control option for Florida sugarcane growers. Moreover, these data will provide insight into the effect of azadirachtin on soil insect pests for which there is little information. For example, in spite of the numerous publications on azadirachtin-insect relations, no publication exists on wireworms (Family Elateridae), which are major soil insect pests on many crops.

MATERIALS AND METHODS

Melanotus communis wireworms (larvae) were collected by digging under sugarcane stools in commercial sugarcane fields. After collection, wireworms were stored in moist muck soil with sliced carrots for food at 18°C until used in tests.

Mortality Tests

Mortality to wireworms by azadirachtin was tested in buckets. Buckets were 23 cm high by 23 cm diameter. Moist muck soil (80% organic matter) was collected from sugarcane fields and insects and debris removed to insure homogeneity. Thereafter the soil was placed 15 cm deep into each bucket. Five small carrot cubes (1 cm^2/cube) were put in the soil in each bucket to provide food for the wireworms.

Soil insecticides are typically applied over sugarcane seedpieces in rows at sugarcane planting for wireworm protection. Three treatments, the untreated control, Thimet 20-G, and Aza-Direct were used. Rates were calculated based on a 0.3-m band application in row and 1.5 m between sugarcane rows, which is standard in Florida sugarcane. Thimet 20-G (AI = 20% phorate) is a soil insecticide commonly used for wireworm control in Florida sugarcane and was applied at full field rate of 22.3 kg/ha. Aza-Direct® (Gowan Co. Yuma, AZ) is an all natural azadirachtin-based insecticide derived from the Neem tree *A. indica.* The Aza-Direct (AI = 1.2% azadirachtin) was applied at 7.3 L/ha and mixed with water for a total application rate of 663 L/ha. This latter rate was selected by the manufacturer (Gowan Company, Yuma, AZ) as a feasible rate for testing against the wireworms in sugarcane under muck soil conditions.

Thimet and Aza-Direct were applied evenly on the soil surface and then stirred throughout the soil. This procedure simulates insecticide application in sugarcane furrows at planting and the subsequent covering with soil. Thereafter, 6 medium size wireworms were dropped into each bucket. The mean weight of wireworms was 0.07g (range 0.05 to 0.12) and wireworms were selected to insure no significant weight differences between treatments. Buckets were then covered with clear plastic to maintain soil moisture and stored for 14 d at 25°C. Thereafter, buckets were emptied and live wireworms counted. Each replication consisted of 1 bucket for each of the 3 treatments. Five replications were conducted during Feb-Mar 2007 and Tukey's test was used to separate means (SAS 2009).

Antifeeding Tests

Antifeeding tests were conducted with the azadirachtin rate previously described. Medium size wireworms were starved 1 week previous to testing to stimulate a feeding response. Testing was conducted in metal circular pans. Metal was used because it is odorless. Each pan was 24 cm diameter and 4 cm deep. Moist muck was placed 3 cm deep in the pan so that wireworms could not escape by climbing out. The pan was marked into 3 equal size sectors, i.e., 120° apart. Three treatments were tested in each pan. The first was a 4-g piece of fresh carrot. This carrot consisted of the hard outer part of a carrot to reduce decomposition and show feeding damage rather than softer carrot interior. The second was the same as the first treatment except the carrot piece was dipped into a solution of the application rate of azadirachtin noted earlier and allowed to drain dry on a metal screen. The third was the same as the first treatment except that the carrot piece was wrapped in a fine mesh metal screen. This screen allowed air, moisture, mites, and nematodes to pass, but excluded wireworms from feeding on the carrot. Each carrot piece was placed in soil 5 cm from the pan edge in the middle of 1 of the 3 pan sectors and covered with soil. Thereafter, 25 medium size wireworms (approx. 0.07g each) were released in the pan center. Hence, the wireworms had free access to feed on carrots in treatments one and two, but were excluded from the carrot in treatment three. The pan was then covered with aluminum foil and stored at 25°C for 4 d. At the end of 4 d, carrots were recovered, loose soil brushed off, feeding damage noted (i.e., holes), and weighed. Ten replicates were conducted during Jan-Feb, 2009 and Tukey's test used to separate means (SAS 2009).

Growth Rate Tests

Growth rate tests were conducted with the azadirachtin soil mixture described previously. Wireworms were starved 1 week previous to testing to enhance a feeding response. Soil (control or azadirachtin treated) was put into metal cans that were 6 cm diameter × 2 cm high. A 0.5-g carrot slice was also placed in the soil in each can for food. Wireworms were selected within the range of 0.060 to 0.090 g/wireworm for testing and were selected for similar weights between the 2 treatments. Cans were stored at 25°C and opened after 7 d to add new carrot if necessary. Cans were opened again 14 d after test initiation and wireworms weighed. Ten replicates were conducted and *t*-test analysis (SAS 2009) performed to compare mean weight changes in the 2 treatments.

Repellency Tests

Repellency tests were conducted with the azadirachtin soil mixture described previously, again to simulate field application. Testing was conducted in a glass container measuring 86 × 10 × 12 cm deep with a glass top. A glass container was used because glass is odorless. Untreated soil was put 10 cm deep into one half of the container and azadirachtin treated soil into the other half. Ten medium size wireworms were placed on the top of the soil in the middle of each of the 2 soils. Wireworms are mobile and the soil was not compacted, hence allowing the wireworms to move freely between the 2 soils. The container was fully covered with aluminum foil for total darkness and then held 72 h at 25°C. Thereafter, the container was opened and the location of the wireworms in the 2 soils noted. The azadirachtin soil was repellent to wireworms during the 0 to 72 h period after azadirachtin application. Hence, to determine if azadirachtin soil had any residual repellency, additional tests were conducted. In these tests, wireworms were placed into the container 14 d or 28 d after treatment and again recovered after 3 d and their location noted. Ten replicates were conducted for each of the 3 time periods and *t*-test analysis (SAS 2009) made to compare mean number of wireworms in the 2 soil treatments at each time period.

Results

Mortality tests

Wireworm survival after soil treatment is shown in Table 1. Control survival was 100%, which is unusual for an insect. However, wireworms are typically hard to collect, but have high survival after collection so that the high survival in this test is not abnormal. The Aza-Direct treatment was not significantly different from the control indicating the neem product caused little, if any, mortality to the wireworms. In contrast, the Thimet treatment had significantly lower wireworm survival than the control and Aza-Direct treatment. This latter statement shows the experimental design was correct for showing wireworm mortality in the presence of an insecticidal compound.

Antifeeding Tests

Carrot weight (g) in different carrot treatments after exposure to wireworm feeding is shown in Table 2. Untreated carrots protected by screen weighed significantly more than untreated unprotected carrots or Aza-Direct treated unprotected carrot pieces. All carrots in all replications except screened carrots showed visual feeding damage, including wireworms in carrots. There was no significant difference in carrot weight between untreated carrots versus Aza-Direct treated carrots. In total, these data show that the wireworms were actively feeding and that Aza-Direct did not reduce feeding on treated carrot pieces.

Growth Rate Tests

In the test to determine if Aza-Direct treated soil affected growth rates of wireworms, wireworms of comparable weights had been selected for use in the Aza-Direct treatment versus the controls. The mean ± SD weights of wireworms at the start of the test were 0.078 g ± 0.009 and 0.078 ± 0.010 for controls and Aza-Direct treatments, respectively. Obviously, these means were not significantly different (t = 0.13, 18 df, P = 0.89). After 14 d, wireworms in both treatments grew as indicated by a positive weight change. The mean ± SD weight change in wireworms was 0.004 ± 0.004 and 0.006 ± 0.003 for controls and Aza-Direct treatments, respectively. The means were not significantly different (t = -1.15, 18 df, P = 0.27) showing that the Aza-Direct soil treatment had no significant effect on wireworm growth.

Table 1. Wireworm survival after 14 d in different soil treatments.

Treatment	Mean[1]	SD	N[2]	Range
Aza-Direct	5.0 A	1.0	5	4-6
Control	6.0 A	0	5	6-6
Thimet	1.0 B	0.7	5	0-2

[1]Means followed by the same letter are not significantly different (alpha = 0.05) using Tukey's test.
[2]Replications. Six wireworms were used initially per replication.

Table 2. Carrot weight (g) in different carrot treatments after exposure to wireworm feeding.

Treatment	Mean[1]	SD	*N*[2]	Range
Aza-Direct	2.6 B	1.1	10	1.3-3.9
Untreated	2.7 B	1.1	10	1.3-3.7
Screened	4.1 A	0.1	10	4.0-4.1

[1]Means followed by the same letter are not significantly different (alpha = 0.05) using Tukey's test.
[2]Replications.

Repellency Tests

Repellency of azadirachtin to wireworms at different times after soil application is shown in Table 3. An analysis with a *t*-test showed there were significantly more wireworms in controls than azadirachtin treated soil from 0-3 d, and 14-17 d after treatment, but not 28-31 d after treatment. The percentage of wireworms in the treated soil increased over time showing the loss of repellency over time.

Discussion

Wireworms (Elateridae) consist of >800 species distributed worldwide. They are significant pests wherever they occur infesting a wide variety of crops. Control strategies are primarily based on the use of synthetic soil insecticides. However, these chemicals may have adverse environmental effects and/or pose health hazards. In addition, organic farmers are looking for alternatives to synthetic pesticides that meet organic production guidelines (Waliwitiya et al. 2005).

Few studies have assessed the efficacy of botanical formulations against subterranean root herbivores (Ranger et al. 2009). Examples are evaluations of botanical products against scarab pests in sugarcane (Abdullah et al. 2006) and nurseries (Ranger et al. 2009). Weathersbee and Tang (2002)

TABLE 3. REPELLENCY OF AZADIRACHTIN (AZ) TO WIREWORMS AT DIFFERENT TIMES AFTER SOIL APPLICATION.

	0-3 d[1]		14-17 d[2]		28-31 d[3]	
	control	AZ	control	AZ	control	AZ
Mean ± SD	13.3 ± 2.1	6.5 ± 2.4	11.4 ± 1.9	8.6 ± 1.9	9.2 ± 3.4	10.2 ± 5.1
Range	10-16	3-10	9-15	5-11	5-13	9-16
% Total	67	33	57	43	47	53

[1]A *t*-test showed that the control mean was significantly greater ($P < 0.0001$) than that of AZ.
[2]A *t*-test showed that the control mean was significantly greater ($P < 0.0005$) than that of AZ.
[3]A *t*-test showed that there was no significant difference ($P > 0.05$) between means.

reported on the effect of neem seed extract on feeding, growth, survival, and reproduction of the curculionid, *Diaprepes abbreviatus* (L). There are hundreds of publications on the effects of neem products on insects. However, no studies have been conducted on the use of neem for wireworms.

We found that azadirachtin did not cause mortality, antifeeding responses, or change growth rate of wireworms at the rate used in this study. However, azadirachtin treated soil was repellent to wireworms at up to 17 d after application. This repellency was gone by 28-31 d after application. Earlier studies have shown that some insecticides such as aldrin and lindane are repellent to wireworms (Vernon et al. 2008). However, this is the first report of azadirachtin being repellent to any of the large and economically important family of Elateridae. Moreover, this is important because repellency in itself may be important in organic farming practices. For example, when corn is protected from wireworm damage during the first 3 weeks of growth, economic damage may be minimized. Therefore, acceptable qualities of a biopesticide include not only direct toxicity but also repellency effects on the pest (Waliwitiya et al. 2005). Wireworms are pests in a wide range of agricultural crops, which have a wide range of economic damage levels. Future research should be conducted to determine if repellency of azadirachtin at different rates may be useful in reducing wireworm damage in both sugarcane and other agroecosystems, especially those involved in organic farming.

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