Response of Sugarcane Wireworms (Coleoptera: Elateridae) and White Grubs (Coleoptera: Scarabaeidae) to Ethanol in Soil[1]

Ron Cherry[2] and Jahangir Bhadha

Everglades Research and Education Center, University of Florida, 3200 E. Palm Beach Road, Belle Glade, FL 33430 USA

J. Entomol. Sci. 54(1): 54–60 (January 2019)

Abstract The wireworm, *Melanotus communis* (Gyllenhal), and the white grub, *Cyclocephala parallela* Casey, are important pests of Florida sugarcane. The objective of this study was to determine the orientation of fed and starved *M. communis* and *C. parallela* larvae to ethanol concentrations in soil. Tests were conducted in rectangular glass containers and circular aluminum containers containing sandy soil and marked off into zones with and without ethanol. Insects were held 48 h in containers and insect movement among zones recorded. At a high ethanol concentration (10 ml ethanol/860 cm^3 soil), both species became comatose, negating any measurement of movement. However, at a low ethanol concentration (2.5 ml ethanol/860 cm^3 soil), both species were attracted to ethanol in the soil.

Key Words sugarcane, ethanol, wireworms, white grubs

Wireworms (Coleoptera: Elateridae) are important soil 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 caused by wireworm damage. Wireworms are primarily a pest in newly planted sugarcane, although the insects are also found in ratoon sugarcane. In a recent survey, Cherry et al. (2017) reported that *M. communis* were found in 100% of Florida sugarcane fields and were 88% of all wireworms found.

Besides wireworms, white grubs (Coleoptera: Scarabaeidae) have also been reported to be soil insect pests of Florida sugarcane (Gordon and Anderson 1981). These authors reported that six species were associated with Florida sugarcane, with three species considered pests. Of these three species, *Cyclocephala parallela* Casey was noted to be a serious pest in areas of sand-muck. Most recently, Cherry et al. (2017) reported on changes in the relative abundance of soil-dwelling insect pests in sugarcane grown in Florida. In this latter study, *C. parallela* was found in 70% of fields sampled and was 29% of all Scarabaeidae found in fields.

[1]Received 31 March 2018; accepted for publication 25 June 2018.
[2]Corresponding author (rcherry@ufl.edu).

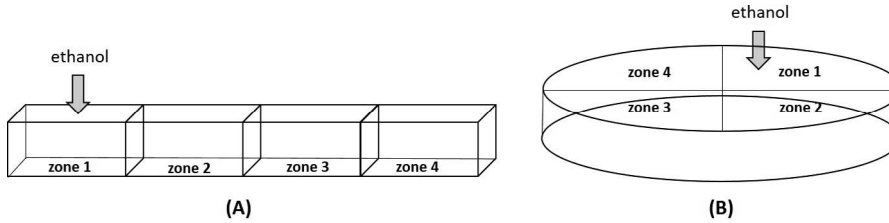

Fig. 1. (A) Zones in glass rectangular container used for wireworm and grub tests. (B) Zones in circular aluminum pans used for grub tests.

Soil-dwelling insects are known to react to chemical cues they encounter in the rhizosphere. Whether wireworms use root-emitted volatile organic chemicals to localize their host plant remains, however, poorly understood (Barsics et al. 2017). The objective of this study is to report on orientation of fed and starved *M. communis* and *C. parallela* larvae to ethanol concentrations in soil.

Materials and Methods

Wireworm assays. Wireworms (larvae) were collected for testing during November 2016–March 2017 and November 2017–March 2018. Wireworms were collected by digging under Florida sugarcane stools where they are aggregated (Cherry 2007). Collection was made in commercial sugarcane fields after harvest for easy access to fields for collection. After collection, wireworms were stored in moist soil with sliced carrots for food at 18°C until used in tests.

Wireworm attraction/repellency tests were conducted in clear-glass rectangular containers measuring 86 × 10 × 12 cm with a clear-glass top. These containers have proved useful in previous wireworm orientation tests (Cherry and Nuessly 2010). Glass containers were used because glass is odorless, reducing possible odor contamination. Moist (3% soil moisture) sandy soil was sieved to remove plant material and insects and then placed 4 cm deep throughout a container. Containers were covered on their outside bottom and sides with aluminum foil. Thus, light only entered from a clear-glass top to simulate field conditions. Containers also were marked to designate four, equal-sized zones within each container (Fig. 1A). Zone 1 was at one end where the ethanol was placed followed by Zones 2, 3, and then 4 at the other end of the container. This zone sequence was used in order to determine if any gradation in wireworm response to or from Zone 1 occurred. During tests, containers were held at 26°C and a 12:12 h L:D photo regime in an insectary.

The first test (Test 1) was conducted to determine the distribution of fed wireworms over time in the glass container in the absence of ethanol. Five fed (carrots previously available) wireworms were placed on top of the soil in the center of each of the four zones. These wireworms averaged 0.07 g/wireworm and were previously weighed and selected so that there was no significant difference in wireworm weight among zones in a container. Wireworms quickly dug into the soil, a normal behavior. Containers were held 48 h, then opened, and live wireworms in

each zone counted. Live wireworms were defined as active and/or moving when prodded. Each container was one replicate with a total of eight replicates.

The second test (Test 2) was conducted to determine the distribution of fed wireworms over time in the presence of a low concentration of ethanol in the soil. Low ethanol concentration is defined here as 2.5 ml ethanol/860 cm^3 soil. This test was essentially a repeat of methods of Test 1 except that ethanol was added to Zone 1. Preliminary data obtained in preliminary ethanol solvent tests indicated that 2.5 ml pure ethanol mixed in the soil of a zone should be attractive to wireworms. Hence, 2.5 ml of pure ethanol was mixed with the soil in Zone 1. The other three zones were also mixed, but contained no ethanol. Each replicate was again stopped after 48 h, and wireworms in each zone counted.

The third test (Test 3) was a repeat of Test 2 except that a higher concentration of ethanol was tested. The high ethanol concentration is defined here as 10 ml ethanol/860 cm^3 soil. This test was terminated after four replications because some wireworms recovered in Zones 1–3 were comatose (e.g., larvae appearing alive, but not moving). The percentages of comatose wireworms in Zones 1–4 were 89, 92, 9, and 0%, respectively. Being comatose obviously made it impossible to measure wireworm movement to or from different zones. After being held 48 h in fresh soil, 90% of comatose wireworms regained movement.

The fourth test (Test 4) was conducted to determine the distribution of starved wireworms in the glass container in the absence of ethanol. Wireworms used in this test were held without carrots for 2 weeks previous to testing. Methods were simply a repeat of Test 1 except that starved, rather than fed, wireworms were used in this test.

The fifth test (Test 5) was conducted to determine the distribution of starved wireworms in the presence of the low ethanol concentration. Methods were a repeat of Test 2 except that starved, rather than fed, wireworms were used in this test.

White grub assays. Grubs (third instar larvae) were collected and stored until testing as described previously for wireworms. Like *M. communis* wireworms, *C. parallela* grubs are also found aggregated under Florida sugarcane stools (Cherry 1984).

Test 6 was conducted to determine the distribution of the fed grubs in the glass container in the absence of ethanol. Methods were essentially the same as Test 1 for wireworms except that the grubs are larger than the wireworms, averaging 0.5 g/grub versus 0.07 g/wireworm. Each test of one glass container was a replication and eight replications were conducted.

Test 7 was conducted to determine the distribution of fed grubs in the presence of the low concentration of ethanol in the soil. This test originally was intended to correspond with the wireworm Test 2 in the glass container. However, Test 6 showed that, unlike the wireworms, the grubs tended to aggregate at ends of the glass rectangular container. Hence, to eliminate this problem, aluminum circular deep pizza pans were used. Aluminum was used because, like glass, it has no odor. Each pan was 36 cm in diameter and 3 cm deep. Each pan was divided into four, equal-size quadrats (4 zones) labeled 1–4 clockwise (Fig. 1B). This configuration approximated the zonation of the glass container in that one zone (Zone 1) would have ethanol, two zones (Zones 2 and 4) were proximal to Zone 1 (being adjacent to Zone 1), and one zone (Zone 3) would be most distal from Zone 1. Soil was placed 2 cm deep in each pan. Because all four quadrats were exactly

the same without corners or ends, testing for grub distribution in the absence of ethanol in a zone was not conducted. Total soil volume in a pan was 60% of the soil volume used in the glass containers. Hence, 1.5 ml of pure ethanol was mixed with the soil in Zone 1, corresponding to the low ethanol concentration used in previous wireworm tests in glass containers. Five grubs were again placed in the middle of each zone. Thereafter, a clear-glass top was placed over each pan to prevent the soil from drying. Pans were held 48 h as previously described. Each test with one pan was a replication and eight replications were used.

Test 8 tested the response of fed grubs to the high ethanol concentration as conducted in Test 3 with the wireworms. In this test, 6 ml of pure ethanol were mixed into Zone 1 of aluminum pans. This high ethanol concentration corresponds to the high ethanol concentration used in the wireworm test (Test 3) in glass containers which had a larger soil volume. This test was stopped after four replications because many grubs, like the smaller wireworms, became comatose, preventing measurement of movement.

Test 9 was conducted to determine the distribution of starved grubs in the glass container in the absence of ethanol. Methods were the same as Test 6 except that grubs starved 2 weeks prior to testing were used rather than fed grubs.

Test 10 was conducted to determine the distribution of starved grubs in the presence of the low concentration of ethanol. Test 9 showed that, like fed grubs, starved grubs also aggregated at the ends of the glass rectangular container with no ethanol present. Hence, Test 10 was essentially a repeat of Test 7, using circular aluminum pans, with the exception that starved grubs were used rather than fed grubs.

Means analysis in all tests was conducted using the least significant difference (LSD) test at the alpha $= 0.05$ level of significance.

Results and Discussion

Numbers of wireworms in different zones of the glass rectangular containers are shown in Table 1. There were no significant differences ($P > 0.05$) in numbers of fed or starved wireworms recovered in the four zones without ethanol after 48 h. Recovery of wireworms after 48 h was greater than 95%, and these wireworms were alive and active. The even distribution of the wireworms in the containers was not due to the even placement of wireworms at the test start and their inactivity in zones. This statement is corroborated by noting that wireworms recovered were active, and there was a large range of 2–9 wireworms/zone recovered that showed clear movement among zones.

The distribution of the wireworms in the containers changed when exposed to the low ethanol concentration in zone 1. Significantly ($P > 0.05$) more fed and starved wireworms were found in Zone 1 than other zones. These wireworms were alive and active and not comatose, as observed at the high ethanol concentration of Test 3. There was a gradient of response in that Zone 1 had the most wireworms, intermediate Zones 2 and 3 had intermediate numbers of wireworms, and the most-distant zone from the ethanol (Zone 4) had the fewest wireworms. Starvation did not increase the wireworm response to ethanol, although we suspected it might. Overall, these data are very consistent in showing that both fed and starved

Table 1. Mean (±SD) numbers of wireworms in different zones at 48 h of exposure to soil with and without ethanol.

	Fed Wireworms/Zone**			
Ethanol*	1	2	3	4
0	5.1 ± 1.6 A	4.6 ± 1.8 A	5.0 ± 2.1 A	4.6 ± 2.3 A
2.5 ml	8.3 ± 1.0 A	4.4 ± 0.9 B	4.8 ± 2.5 B	2.3 ± 2.0 C
	Starved Wireworms/Zone**			
Ethanol*	1	2	3	4
0	4.7 ± 2.3 A	5.1 ± 2.5 A	4.8 ± 1.8 A	4.8 ± 2.8 A
2.5 ml	8.1 ± 1.1 A	4.4 ± 0.9 B	4.9 ± 2.5 B	2.0 ± 1.6 C

*100% ethanol only in Zone 1.
**Means ± SD in a row followed by different capital letters are significantly different (alpha = 0.05) using the least significant difference test.

wireworms were attracted to the low concentration of ethanol in the soil, as we had previously observed in preliminary testing (Table 1).

White grubs in different zones of glass and aluminum containers are shown in Table 2. Interestingly, there were behavioral differences in grubs from wireworms in how the grubs distributed themselves in the glass rectangular containers after 48 h. In contrast to wireworms, there were significant ($P > 0.05$) differences in number of fed and starved grubs recovered in the four zones without ethanol after 48 h. Quite simply, grubs had a tendency to aggregate at the ends of the rectangular container (Zones 1 and 4) with significantly fewer in the middle Zones 2 and 3. Recovery of grubs was greater than 95% being alive and active. Because of significant differences in grubs among zones, it is obvious that the grubs were active and mobile among zones. Reasons for behavioral differences between the wireworms and grubs resulting in differences in their spatial distribution in the rectangular containers without ethanol are not known.

White grub testing with ethanol exposure was switched to circular aluminum pans to avoid grub aggregation at container ends. Both fed and starved grubs were found in higher numbers in Zone 1, containing the low concentration of ethanol, than in the other three zones. These grubs were alive and active and not comatose as observed at the higher ethanol concentration of Test 8. For both fed and starved grubs, significantly more grubs were found in Zone 1 than one other zone, but not the other two zones. As with wireworms, starvation did not increase the response to ethanol.

In comparing data from wireworms in Table 1 and white grubs in Table 2, two differences are apparent. The spatial distributions of the wireworms versus white grubs in the glass container with no alcohol differed. Also, the attraction to ethanol of the wireworms versus white grubs differed. In Table 1, the mean number of wireworms in Zone 1 with the low concentration of ethanol was consistently and

Table 2. Mean (±SD) number of white grubs in different zones at 48 h after exposure to soil with or without ethanol in glass and aluminum test arenas.

	Fed Grubs/Zone**			
Ethanol*	1	2	3	4
0	6.5 ± 1.2 A	2.3 ± 1.9 B	3.0 ± 1.2 B	7.3 ± 1.4 A
1.5 ml	6.5 ± 3.1 A	4.8 ± 1.9 AB	3.0 ± 2.7 B	5.3 ± 3.4 AB
	Starved Grubs/Zone**			
Ethanol*	1	2	3	4
0	7.1 ± 1.8 A	2.8 ± 1.5 B	2.5 ± 1.8 B	6.6 ± 1.2 A
1.5 ml	6.4 ± 2.9 A	4.3 ± 1.3 AB	4.3 ± 1.9 AB	4.0 ± 2.4 B

*Tests with no alcohol were conducted in glass rectangular containers (see text). Tests with 1.5 ml ethanol only in Zone 1 were conducted in aluminum circular pans (see text).
**Means ± SD in a row followed by different letters are significantly different (alpha = 0.05) using the least significant difference test.

significantly greater than the other three zones. In Table 2, more white grubs were found in Zone 1 with the low ethanol concentration than the other three zones, but Zone 1 had significantly greater numbers than the other zones only twice. In Table 1, 43% of all wireworms in the ethanol tests were found in Zone 1. In Table 2, 33% of all white grubs in the ethanol test were found in Zone 1. Lastly, the coefficients of variation (standard deviation [SD]/mean) were 0.12 and 0.13 for fed and starved wireworms, respectively, in Zone 1 with ethanol. In contrast, the coefficients of variation were 0.48 and 0.45 for fed and starved grubs, respectively, in Zone 1 with ethanol. The lower coefficients of variation for the wireworms showed the wireworms were more consistent and less variable in their responses to ethanol versus the grubs, which showed more variability among replications. In all, our data show that white grubs were attracted to the low concentration of ethanol in the soil but not as strongly as were the wireworms.

Root-feeding insects are key components in many terrestrial ecosystems. Like shoot-feeding insect herbivores, they exploit a range of chemical cues to locate host plants. Respiratory emission of carbon dioxide from the roots is widely reported as the main attractant; however, there is conflicting evidence about its exact role. At least 74 other compounds elicit behavioral responses in root-feeding insects (Johnson and Nielsen 2012).

More specifically, Villani and Gould (1985) conducted screening of crude plant extracts as feeding deterrents against *M. communis* wireworms (larvae). And, most recently, Cherry and Nuessly (2010) reported on repellency of the biopesticide azadirachtin to *M. communis* wireworms. We know of no reports on the behavior of *C. parallela* grubs in response to chemicals in the soil. This is the first report to show that larvae of *M. communis* and *C. parallela* are attracted to ethanol in soil.

Finally, while studying aquatic plant compounds extracted with ethanol, we discovered that the ethanol itself was attractive to *M. communis*. Those earlier unpublished observations provided the stimulus for this study. The use of plant material extracted with ethanol to test insects is common as shown by recent studies (Alim et al. 2017; Rijal and Bergh 2016; Wagan et al. 2017). Our study shows that when using plant material extracted with ethanol, attention must be paid to possible behavior effects of the ethanol itself on insects being tested.

References Cited

Alim, M., J. Song, U. Lim, S. Choi and M Hossain. 2017. Bioassay of plant extracts against *Aleurodicus dispersus* (Hemiptera: Aleyrodidae). Florida Entomol. 100: 350–357.

Barsics, F., B. Delory. P. Delaplace, F. Francis, M. Fauconnier, E. Haubruge and F. Verheggen. 2017. Foraging wireworms are attracted to root-produced volatile aldehydes. J. Pest Science. 90: 69–76.

Cherry, R. 1984. Spatial distribution of white grubs (Coleoptera: Scarabaeidae) in Florida sugarcane. J. Econ. Entomol. 77: 1341–1342.

Cherry, R. 2007. Seasonal population dynamics of wireworms (Coleoptera: Elateridae) in Florida sugarcane fields. Florida Entomol. 90: 426–430.

Cherry, R. and G. Nuessly. 2010. Repellency of the biopesticide, azadirachtin, to wireworms (Coleoptera: Elateridae). Florida Entomol. 93: 52–55.

Cherry, R., M. McCray and H. Sandhu. 2017. Changes in the relative abundance of soil-dwelling insect pests in sugarcane grown in Florida. J. Entomol. Sci. 52: 169–176.

Gordon, R. and D. Anderson. 1981. The species of Scarabaeidae (Coleoptera) associated with sugarcane in south Florida. Florida Entomol. 64: 119–138.

Hall, D. 1985. Damage by the corn wireworm, *Melanotus communis* (Gyll.) to plant cane during germination and early growth. J. Am. Soc. Sugar Cane Tech. 4: 13–17.

Hall, D. 1990. Stand and yield losses in sugarcane caused by the wireworm *Melanotus communis* (Coleoptera: Elateridae) infesting plant cane in Florida. Florida Entomol. 73: 298–302.

Johnson, S. and U. Nielsen. **2012.** Foraging in the dark - chemically mediated host plant location by below ground insect herbivores. J. Chem. Ecol. 38: 604–614.

Rijal, J. and J. Bergh. 2016. Food-finding capability of grape root borer (Lepidoptera: Sesiidae) neonates in soil column bioassays. J. Entomol. Sci. 51: 54–68.

Villani, M. and F. Gould. 1985. Screening of crude plant extracts as feeding deterrents of the wireworm, *Melanotus communis*. Entomol. Exp. Appl. 37: 69–75.

Wagan, T., W. Wang, H. Hua and W. Cai. 2017. Chemical constituents and toxic, repellent, and oviposition-deterrent effects of ethanol extracted *Myristica fragrans* (Myristcaceae) oil on *Bemisia tabaci* (Hemiptera: Aleyrodidae). Florida Entomol. 100: 594–601.