

ASSESSING THE FORAGING BEHAVIOR OF AGRIOTES SORDIDUS WIREWORMS IN DUAL-CHOICE OLFACTOMETERS

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SUMMARY

The different steps of the foraging process of wireworms (Coleoptera: Elateridae) would be better understood if accurate and holistic information regarding the role of plant-produced chemicals constituting their environment were available. Volatile organic compounds (VOC) play important roles in the interactions between plants and insects in many ecosystems, whether they take place aboveground or belowground. The roles of VOC are still relatively unknown for wireworms, and deserve attention. Here, we performed three experimentations with barley roots as baits. In the two first, we assessed the effect of chopped roots and fungus infected roots on the orientation of wireworms. In the third experiment, the larvae were confronted to both healthy and fungus infected roots. We discuss the results in terms of suitability of the olfactometers we designed for the investigation of olfaction in wireworms, and we provide suggestions to improve their use.

Key words: chemical ecology, belowground pests, insect-plant interactions, olfactometry, integrated pest management.

INTRODUCTION

The soil is a complex matrix sheltering many organisms that can be beneficial or noxious to agriculture. They interact with each other, with plants, and aboveground organisms. Pest management strategies arise from the increasing knowledge concerning mechanisms underpinning these interactions (Agelopoulos et al., 1999, Cook et al., 2007). While foraging in the dark, phytophagous insects must locate and identify suitable resources. They rely on plant chemical signals which potential in pest control is admitted (Johnson and Nielsen, 2012, Hiltbold et al., 2013).

The semiochemical roles of VOC in aboveground interactions have been studied on various models for many years, but the recent advances on multi-trophic models focusing on the Western Corn Rootworm (*Diabrotica virgifera virgifera* Leconte) highlighted equally complex interactions in the soil (Rasmann et al., 2005, Erb et al., 2008, Hiltbold et al., 2011, Robert et al., 2012). Many other pests develop underground, but the VOC impact on their chemical ecology is far less advanced, while increasing that knowledge could lead to new alternatives for their management (Johnson and Nielsen, 2012, Hiltbold et al., 2013). Wireworms, the larvae of click beetles (Coleoptera: Elateridae), represent a typical example thereof. These pests develop belowground for many years, and feed on a variety of crops to reach maturity (cereals, potatoes, sugarcane, lettuce, strawberries...), inducing considerable economic losses in wide areas of the Palearctic Region. As

other pest species, they require efficient management, with decreasing resort to synthetic pesticides. Investigating the role of olfaction in their foraging behavior constitutes an important step that could lead to interesting management alternatives (Johnson and Nielsen, 2012, Barsics et al., 2013).

Behavioral experimentations with VOC require the use of olfactometers in which insects are confronted with a cue originating from plants themselves or reconstituted synthetic blends. These devices allow highlighting the attractive or repellent effect of volatile compounds. Olfactometry requires conditions as close to reality as possible. For soil-dwelling insects, tests must take place in a substrate. Defining experimental parameters that are suitable to observe significant behavioral responses is a key step to that approach. In this work, we present three different experimentations performed in dual-choice olfactometers on *Agriotes sordidus* Illiger wireworms. These bioassays are complementary to those reported in Gfeller et al. (2013) and Barsics et al. (2012), where experimental parameters were set in both presence and absence of CO₂, a known key semiochemical in wireworm foraging (Doane et al., 1975). Firstly, we exposed wireworms to chopped barley roots (*Hordeum distichon* L.), in order to determine the effect of cues from decaying plant material. Secondly, wireworms were exposed to roots infected with a phytopathogenic fungus, *Cochliobolus sativus* (Ito and Kuribayashi) Drechsler ex. Dastur, 1942 [anamorph *Bipolaris sorokinana* (Sacc.), Shoemaker]. Finally, we investigated the ability of wireworms to discriminate between healthy and fungus infected roots. We discuss these results and previously reported ones, in relation to the scale at which we work and in terms of suitability of our olfactometers.

MATERIALS AND METHODS

Wireworms

Wireworms were collected in experimental plots in Montardon (Arvalis Institute, France), between 2010 and 2012. The collection area was chosen in control plots, free of pesticide treatments. Wireworms were transferred into rearings and separated from each other in order to prevent cannibalistic events. Each individual was kept in 80 cm³ vials filled with a mix of leaf mold and vermiculite (1/1, v/v, 16.5% water), and kept in the dark at room temperature (22±1 °C). Food consisted of meadow seeds from organic production (0.130-0.160 g, Prelac Bio, SCAR, Belgium). *Agriotes sordidus* wireworms were selected with reference to morphological criteria reported by Cocquempot et al. (1999) and Pic et al. (2008). Those having a length of at least 10 mm were selected for experimentation, as they represent critical instars for crops (Furlan, 2004). Seven days prior to testing, they were isolated from organic matter (either from food or their rearing substrate), by being transferred into separate vials filled with moist vermiculite (16.5%). On the experimentation day, individuals that were visibly inadequate for testing were excluded. These include larvae that just molted (not darkened mouth parts, light colored cuticle) or that are close to a subsequent molt (lowered activity and swollen, compared to their conspecifics). The remaining individuals (50-70% of isolated ones) were closer to the feeding phase, which lasts less than 25% of the whole development time in *A. sordidus* (Furlan, 2004). Such individuals are more prone to display a foraging behavior when exposed to plant originating cues than excluded ones.

Plant material

All barley plants used in these experiments were seven days old. However, they were produced with different methods. Chopped plants were simply grown in leaf mold, by groups of ten, in one-litre jars. They were watered daily. Other plants were produced with the same protocol as that detailed in Gfeller et al. (2013) for sterile plants grown on agar-agar medium. To infect plants with *C. sativus*, the caryopses were pre-germinated for two days and then sprayed with 1 mL of a solution containing 10^6 conidia/mL. Plants were stored in a growth chamber until experimentation, with 22 °C, 65 % RH, 20/4 L/D photoperiod and under LED light ($95 \mu\text{mol m}^{-2} \text{sec}^{-1}$).

Behavioral assays

Olfactometers

Individual behavioral experimentations on wireworms were performed in dual-choice glass pipes filled with moist vermiculite (grade 2, Sibli, Andenne, Belgium). Their suitability for assessing wireworm behavior towards plant-emitted VOC was already demonstrated in previous studies (Barsics et al., 2012, Gfeller et al., 2013). The cylinders (32 cm long, 3.6 cm internal diameter) are provided with one central tubular aperture (3 cm long, 1 cm diameter), allowing wireworm entry. Vermiculite is introduced through both extremities, which are closable with screwed caps. Two other tubular apertures are located 3 cm away from each extremity and are diametrically opposed to the one used for the introduction of wireworms. Pipes are therefore connectable to external odorant systems. These apertures also serve to introduce roots as baits, without cutting them from the rest of the plant, which is maintained out of the system.

Protocol for dual-choice bioassays

After filling pipes with moist vermiculite (30%), 4 cm of substrate is removed from each side, leaving room for baits. A stainless-steel boundary (3.6 cm diameter, 0.04 mm thickness and mesh-size) is inserted against the remaining substrate to avoid further contact between burrowing wireworms and roots, ensuring that the final choice is solely related to volatile cues rather than to physically encountered plant material. In the three different experiments, the control baits were introduced first. They consisted of 240 mg of leaf mold when chopped roots were tested, 240 mg of blank agar-agar medium when fungus infested roots were tested, and healthy roots in the experiments opposing healthy to fungus infected roots. The plants were systematically extracted from their substrate within 5 minutes before each test, and grouped by bundles of ten. When chopped, they were firstly cleaned with tap water to remove as much substrate as possible. There systematically were residues, explaining the use of leaf mold as a control. When entire plants were inserted, they were gently removed from the growth medium, and introduced by pulling the leaves through the cylinder and then through the lateral tubular aperture, until nothing else but the roots occupied the bait zone. As soon as baits were inserted, the pipes were closed with the screwed caps. Then, PTFE and aluminium tape were immediately wrapped around the plant necks and the lateral apertures to prevent any leak of volatiles out of the device. The left of

right position of the different baits inside olfactometers were assessed randomly before experimentation.

Experimentations were run by batches of ten. Time was noted down to the minute when each olfactometer was completely loaded. Exactly 40 min later, one of the selected wireworms was allowed to penetrate the glass-pipe through the middle entry. Each larvae was given 80 min to explore and orientate towards the olfactometer, at $21\pm 0.5^\circ\text{C}$ in a room without light. The latter was turned back on to retrieve wireworms and to record their final position. The zone comprised within 3 cm from the entry point was considered as the neutral zone, so that wireworms found at that level were ranked as non-choosing individuals. Tests were performed with pre-selected individuals until fifty effective choices were obtained. Results were treated with a Chi^2 -Goodness-of-fit test (1 df).

RESULTS

The results of the three experiments are reported in Table 1. Chopped roots highly attracted wireworms ($\text{Chi}^2=13.52$, $P<0.001$). Fungus infected roots were attractive as well, although the results were less significant ($\text{Chi}^2=3.91$, $P=0.048$). The larvae did not show any preference between healthy and infected roots in these experimental conditions. With chopped roots, only 18% of the tests resulted in neutral responses, whereas a third of the replicates were inconclusive when live roots were used. In each case, they were easily retrieved from the vermiculite since they form tunnels against the glass while burrowing. When healthy and fungus infected roots were used simultaneously, only 46 replicates were performed due to a reduced number of available wireworms.

Table 1. Amount of wireworms retrieved on in each side of the olfactometers for all behavioral tests and statistical values associated to the Chi^2 -Goodness-of-fit tests.

Bait on each side of the olfactometers		Sum of larvae retrieved on each side		Neutral responses (%)	Chi ²	P-value
		A	B			
Chopped roots	Leaf mold	38	12	18	13.52	<0.001***
<i>C. sativus</i> infected roots	Agar-agar	32	18	32.4	3.91	0.048*
	Healthy roots	21	25	34.3	0.35	0.555

DISCUSSION

The two first assays show that wireworms starved during seven days are attracted towards roots either decaying or in a critical physiological stage. The level of attraction is close to that observed when they are exposed to VOC emitted by healthy roots (Gfeller et al., 2013). When comparing healthy and fungus infected roots, our experimental set up failed to reveal a preferential target.

There are two hypotheses as to why wireworms did not discriminate between healthy and infected plants at this scale. Firstly, the experimental set up may have been saturated with volatile cues, annihilating guiding gradients that should have played a role in the orientation. The comparison of two live baits could be performed with adjusted experimental parameters. Reducing the diffusing time before wireworm release, or using fewer amounts of root material in the baits could avoid saturation of the system. Secondly, wireworms were unable to reach

the roots because of the steel boundaries between the baits and the substrate. Yet, after orientation during the foraging process, root contact is necessary to eventually confirm the suitability of the host (Johnson and Nielsen, 2012). Without the possibility to access the roots, the larvae may have orientated according to other cues, if available, such as when live baits were compared. In the experimentations with chopped or fungus infected roots, this step may have been necessary to reject or accept the unhealthy host. Complementary experiments in which boundaries are removed between the baits and the substrate would reveal whether wireworms remain in the roots. Since the position of wireworms was accurately recorded when they were retrieved from the pipes, it would be easy to compare these positions between cases with and without boundaries. Taking account of the visible tracks they leave while burrowing may reveal more of their foraging behavior as well.

The amounts of carbon dioxide released by roots in our experimentations were not measured. CO₂ is however known to be involved in wireworm foraging (Doane et al., 1975). To assess the semiochemical roles of other compounds of the cue than CO₂, behavioral tests could be performed in a CO₂-controlled environment. The olfactometers used here are suitable to assess the effect of selected volatile organic compounds, such as 2-pentylfuran (Barsics et al., 2012), a compound listed in blends sampled from barley roots (Fiers et al., 2013, Gfeller et al., 2013). But many compounds of one blend may be needed to induce a particular response. Exposing wireworms to these compounds would require using a synthetic blend as bait. Expressing results taking account of the relative amount of CO₂ is possible when its concentrations are measured. This could be done in experimentations with groups of individuals, for which results would be presented as ratios of wireworms retrieved on each side of the olfactometer, in relation to ratios of CO₂ produced by each type of bait (Robert et al., 2012). In this scenario, the impact of congeners on the foraging process would need to be investigated.

These olfactometers are useful tools to investigate the role of volatile organic compounds on the foraging behavior of wireworms. Further experimentations revealing interesting semiochemicals should provide new perspectives for wireworm management.

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