Attraction of wireworms to root-emitted volatile organic compounds of barley

Fanny Barsics, Eric Haubruge, François J. Verheggen
University of Liège, Gembloux Agro-Bio Tech, Unit of Functional and Evolutionary Entomology, 2 Passage des Déportés, B-5030 Gembloux, Belgium

Abstract: The ability of wireworms (*Agriotes sordidus* Illiger) to orientate towards a blend of volatiles emitted by chopped roots of barley was tested. During individual tests, the larvae chose between the two sides of a Y-shaped olfactometer. One side was connected to a chamber containing the chopped roots and the other was connected to an empty chamber. Wireworms chose significantly more often the side of the olfactometer providing the blend of root volatiles. This result underlines the importance of the identification of these compounds and their role assessment alone or combined, as for their effect on wireworms. Such compounds could be used in IPM strategies.

Key words: wireworms, barley roots, volatile organic compounds, integrated pest management.

Introduction

Wireworms, the soil dwelling larvae of click beetles (Coleoptera: Elateridae), remain unmanaged crop pests in many countries. Since the decrease of organochlorine pesticides use in the middle of the 1990s, considered alternatives in the struggle against wireworms are essentially parts of integrated pest management (IPM) strategies, such as risk assessment by site characteristics parameters (Parker and Seeney 1997), bait trapping and pheromone trapping both as monitoring and control methods (Parker 1996; Hicks and Blackshaw 2008), seed protection by treatment (Vernon *et al.* 2009), biopesticides (Cherry and Nuessly 2010), and biocontrol (Ansari *et al.* 2009). A gap persists concerning wireworms' chemical ecology while filling it would provide new angles of attack to combine to already existing ones and reinforce click beetles confinement.

If the general plant-insect relations are well documented for emerged parts of plants (Powell *et al.* 1998; Meiners and Hilker 2000; Verheggen *et al.* 2008; Webster *et al.* 2008; Mendesil *et al.* 2009), such information at root level is less frequent but tend to increase. Rasmann et al. (2011) underlined the suppression of root herbivores of milkweed (*Asclepias syriaca* L.) mediated directly by cardenolides and indirectly by the attraction of nematodes. The identification of root-emitted volatile organic compounds that impact on wireworms would lead to new trails in matter of click beetles control.

The work presented here is involved in a project aiming to the identification and role assessment of barley (*Hordeum vulgare* L.) root-emitted VOCs. Several actors of the rhizosphere are taken into account in the studied model and among them, wireworms (*Agriotes sordidus* Illiger) have been chosen to stand for belowground herbivory. We first tested the individual orientation of wireworms confronted to a blend of VOCs emitted by chopped roots of barley.

Material and methods

Experimental setups

Orientation of wireworms was observed thanks to the use of Y-shaped polypropylene olfactometers. Wireworms were introduced individually in the main branch of a Y while the two other branches were connected to two chambers: one filled with 3 g of chopped roots of barley, and the other one empty. We used the roots produced by 7 days old barley grown in 2 l pots sown with 50 caryopsis. Chopped roots were used for three hours in a row and then replaced for subsequent individual tests. A 0.2 l/min air flow was divided and pushed into the two chambers. We used 5 identical olfactometers in order to change and clean them between every test. The three branches of each Y measured 49 mm long and 12 mm diameter. They were filled in their inferior half with humidified and compressed vermiculite, which eased the progression of wireworms and allowed the formation of volatile trails by adsorption on the substrate. We observed the first choice made by each larva and considered it as done after they were no longer visible in the main branch. The overall available paths were covered with a translucent red envelope to darken the remaining space of the Y. Tests were performed over three days, with a room temperature varying between 18.3° and 20.3°C.

Wireworms

The larvae of *A. sordidus* were collected in early October 2010 in Montardon, South France. All individuals were taken from a plot previously cropped with wheat (from November 2009, after maize harvest, until July 2010). To trap wireworms, we used potato bait traps buried a few centimetres belowground and covered with opaque plastic pots to avoid humidity losses by evaporation. New shoots of wheat were removed from the ground in a 20 cm radius and their roots were used as complementary baits to potato slices.

Wireworms were kept individually in laboratory conditions, in 6 x 4 x 5 cm polystyrene crystal vials filled with humidified DCM leaf mould. We heated the mould for 3 hours under 95°C to kill any potential living enemies of the larvae such as entomopathogenic fungi. They were regularly fed with potato roots or thin tuber slices.

Before they were used in experimentations, larvae were selected according to their length. Furlan (2004) and Blot *et al.* (2008) mentioned that a 10 mm body length was a good inferior limit to consider for highly damaging instars. The time separating the extraction of the larvae out of its vial and its introduction in the experimental device is favourable to stress induction, which is not recommendable in orientation tests. Working on at least 10 mm long larvae allowed the reduction of stress induction risks, because they were easier to manipulate. Every larva was isolated from all organic matter 6 days before it was tested, in order to enhance their ability to detect attractive blends. They were put in similar vials than rearing ones, but filled with humidified and compacted vermiculite. A total of 60 larvae were used in these tests.

Results and discussion

Results

Out of the 60 larvae, 7 refused to make a choice. They preferred to dig in the vermiculite quickly after their introduction in the olfactometer. Thus, they weren't taken into account in the treatment of the results. Among the 53 remaining wireworms, 34 orientated towards barley blend. The application of a Chi-Squared Goodness of fit test on the results showed that wireworms had a significant preference for the side of the olfactometer containing barley ($\chi^2_{obs} = 4.25$, P-value = 0.039).

Discussion

Our experimentation only showed the ability of the larvae of *A. sordidus* to orientate towards a blend of VOCs coming from barley roots. It doesn't point out what compounds are responsible of this orientation. Carbon dioxide has been shown to attract wireworms (Doane *et al.* 1975). The amount of CO_2 produced by the chopped roots wasn't regulated in our experimental setups, but the attractive effect on wireworms of the released COVs cannot be associated to carbon dioxide alone until the assessment of the effects on *A. sordidus* larvae of the several volatiles merged in the blend.

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