Extended Abstract

The law of attraction: identification of Volatiles Organic Compound emitted by potatoes as wireworm attractants

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Context and Objectives

Wireworms (Coleoptera: Elateridae) are common polyphagous soil pests of various crops, including maize and potatoes, inflicting severe economic damage (Parker and Howard 2001). Their management with pesticides is often not successful or sustainable and more research on biological alternatives is required. One of these alternatives could be the use of traps baited with attractive volatile organic compounds (VOCs) in association with a microbial control agent. In this paper, we describe the methodology followed to identify volatile organic molecules with potential as wireworms attractants. We previously observed that cut potato tubers placed on the soil surface were highly attractive for wireworms. To identify the attractive cues released by potato tubers, we defined three specific objectives: 1) developing a behavioral assay suitable for the study of wireworm orientation behavior; 2) profiling VOCs released by potato tubers; 3) evaluating the role of the identified VOCs on wireworms foraging behavior.

Materials and Methods

Wireworms’ rearing

Wireworms’ larvae were collected in potato fields located in Pau (Southern France) during autumn 2016 and identified as Agriotes sordidus by dichotomous keys (Cocquempot et al. 1999; Pic et al. 2008). Each larva was isolated in single rearing boxes of 80 mL to avoid cannibalism. The rearing substrate consisted in a mixture of vermiculite and potting soil (1/1, humidified at 16.5% vol). Germinating meadow seeds were sown in each box (0.13–0.16g, Prelac Bio, SCAR, Belgium) as food source. The rearing was kept in the dark at room temperature (21–22 °C). The
larvae selected for behavioral assays were measuring at least 10 mm, corresponding to their most active instar in crop-threatening (Furlan 2004). Wireworms were starved prior to bioassays by being transferred to 80 mL vials filled with vermiculite (16.5 % vol) and soil two days prior to testing. We excluded larvae with reduced activity, in pre-molting phase and just out of the molting, easily spotted for displaying a light-colored cuticle and unhardened, non-darkened mouth-appendices.

Potatoes

All experiments were carried out on pesticide-free potato tubers (*Solanum tuberosum* var. *Grenaille*). The potatoes used for the analysis did not have any visible sprout and wound.

Olfactometer assays

The olfactometer system used for the behavioral assays was previously described in Barsics et al. 2012 and Gfeller et al. 2013. In Brief, the olfactometer is made of a glass tube (32 cm long, 3.6 cm internal diam.) closed at both extremities with GL45 caps (Duran, Belgium). The external openings allow the easy introduction of the substrate. The entrance of the larva is located in the center (sealable with a GL14 cap). During the bioassays, the olfactometers were filled with 29 g of vermiculites each. Wireworms were offered a piece of 15 g of potato on one side of the olfactometer and nothing on the other side. A control test was ran in parallel, consisting in a no choice situation where the wireworm was offered nothing on both sides of the olfactometer. The same protocol was used for the VOCs artificial blend made of hexanal, (E)-hex-2-enal, (E)-non-2-enal, (E,Z)-nona-2,6-dienal (Barsics et al. 2017) with the difference that a solution of pentane only was used as an alternative. In any cases, a single wireworm was inserted at the center of the olfactometer and was let free to choose for two hours at a room temperature of 18°C.

Odor collection

To collect tuber volatiles, 150 g of tubers were placed in a vial 75.5x22.5 mm (VWR, Belgium) placed in a bain-marie for one hour at 40°C. At the end of this period, a SPME fiber was introduced for two hours. The profile of collected VOCs was defined using gas chromatography (Agilent Technologies 7890A) coupled with mass spectrometry (Agilent Technologies 5975C) (GC–MS). The GC-MS method was carried out for thirty minutes in which the samples were maintained at 40°C for the three first minutes, then the temperature was progressively raised to 240°C at a rate of 10°C.min⁻¹ during the following 20 min. Once reached, the raise of
temperature was accelerated to 20°C.min⁻¹ for two minutes to finally reach 280°C. This temperature was maintained for the last five minutes. The identification was conducted by interpreting the mass spectra and confirmed with injection of standards.

**Statistical analysis**

The olfactometer was virtually divided in three zones. The six distal centimeters on each side of the olfactometer were considered as the zone of choice while the remaining central part was considered as a no-choice zone. The result were analyzed using a χ² test of independence to assess whether the wireworms actually made a choice and if they chose one arm or the other.

**Results**

**Olfactometer assays**

Wireworms did not express choice when only blanks were offered to them (p=0.368; χ²=2). On the contrary they we more present in the arm with the potato piece when offered it (p<0.001; χ²=42.25). In all tests, the wireworms were active: they did not stay in the no-choice zone and always moved to one arm or the other of the olfactometer.

**Identification of potato VOCs**

We identified ethylhexanol (RT: 11.089), limonene (RT: 11.8), 4-hydroxymandelic acid (RT: 12.92) and carbamic acid (RT: 19.64) as main components of potato tubers’ volatile profile.

**Discussions and Conclusions**

Wireworms are strongly attracted by potato tubers and olfactometer assays evidenced the role of VOCs in this attraction. The VOCs identified in this study are typical of plants but not necessarily specific of potato plants (Barsics et al. 2017; Petersen et al. 1998). One of them, limonene, may not be a compound of real interest as it has been found repellent for several insects (Johnson & Gregory 2006; Tapia et al. 2006). At this point, the role of the identified compounds in the attraction of wireworms cannot be evaluated.

Further experiments are required to identify the exact compounds responsible for the observed attraction. We also need to understand how they act, whether it is in an isolated way or in synergy. Finally, a quantification of the VOCs we identified in potato samples is a prior step in the elaboration of a new artificial blend. The attractiveness of the blend will have to be confirmed in olfactometer test and eventually in field conditions.
93 Bibliography


