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Wireworms (Coleoptera: Elateridae) Use Root Volatiles and CO2 to Discriminate Among Host Plants

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In the absence of visual signals, subterranean arthropods rely on olfactory and tactile cues to navigate toward resources. Here, in a series of pairwise dual-choice bioassays, we investigated the *Limonius californicus* (Col., Elateridae) larva response to wheat, pea, and bean seedlings in sand-filled olfactometers. We then quantified volatile organic compounds (VOC) emitted from roots. Wireworm preference for beans compared to wheat was attributed to the higher CO_2 emission. Wireworm preference for peas compared to wheat was attributed to the higher amounts (µg/hr) of hexanal emitted from pea roots. Wireworms preferred synthetic hexanal over clean air control and the higher amount of hexanal (200 µg) over the lower amount of 20 µg. In the presence of CO_2 at both ends of the olfactometer, wireworms did not respond preferentially toward hexanal. 2-Hexenal was also attractive to wireworms relative to the control, but wireworms did not discriminate between hexanal and 2-hexenal. While our results confirmed wireworms' positive response to the presence of CO_2 and some VOCs in isolation, their host choice appears to be driven by the combination and the concentrations of the present cues, allowing the insect to distinguish among host plants.

Keywords Subterranean pests, Cultural control, Trap crop, Olfactory cues, Click beetles

Insects use combinations of visual, olfactory, tactile, auditory, and gustatory cues to locate mates, food, and other resources[1]. For aboveground herbivorous insects, visual and olfactory cues can interact synergistically[2,3] or additively[1,4] to facilitate the host selection process. Some olfactory cues can elicit both negative and positive behavioral responses in these herbivores[5,6]. In addition, secondary metabolites released following herbivore attack can deter subsequent oviposition by herbivores while attracting carnivores[5,7]. Soil dwelling organisms cannot use visual cues, but other cues, including olfactory cues, can guide them through the soil profile toward resources[8,9].

Thorpe[10] suggested that root-feeding invertebrates encounter roots through random, unguided foraging[10]. However, it was later demonstrated that soil-dwelling insects do respond to CO_2 released by plant roots[11]. Although CO_2 can serve as a cue for host finding, it has its limitations as it is not host-specific, can be released from other sources in the soil, diffuses primarily vertically, and its attractiveness decreases in the presence of other root exudates[8]. Therefore, soil-dwelling organisms may rely on additional olfactory cues, including those released directly from plant roots, to detect and orient toward their hosts.

Root exudates can be attractive or repellant to soil-dwelling insects during host plant selection[12], and concentration-dependent repellency of hydrocarbons and attractiveness of alcohols, esters, and aldehydes have previously been reported[13]. The volatile compounds released from roots may be species- or even variety-specific, potentially providing soil-dwelling invertebrates with reliable cues to differentiate host plants[14]. Only a few studies, however, have demonstrated the attraction of root-feeding insects to volatiles of their host plants[15–19].

Wireworms are the larvae of click beetle (Coleoptera: Elateridae) species and generalist subterranean herbivores that feed on the belowground tissues of a wide range of cultivated and non-cultivated host plants[20,21]. Similar to other belowground insect pests, wireworms locate food sources in a three-step process[12,22]. First, they randomly move through the soil until they detect the presence of root signals[11]. Second, specific root-emitted volatile organic compounds (VOC) and CO_2 are used to orient and move toward the possible host

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plant. Finally, on contact, root surface chemosensory cues are used to accept or reject the potential host[12]. The relative importance of CO_2 and VOC in wireworm attraction is not well understood. In bioassays, Gfeller et al.[17] found that wireworms *Agriotes sordidus* (Illiger) were attracted to a blend of volatiles and CO_2 released from barley seedlings. Subsequently, Barsics et al.[18] demonstrated that in the absence of CO_2 a blend of hexanal, *(E)*-hex-2-enal, *(E)*-non-2-enal, and *(E, Z)*-non-2,6-dienal was attractive to *A. sordidus*. Wei et al.[19] documented a response from the wireworm *Melanotus cribriocollis* (Feldermann) to a combination of VOC and CO_2 released from bamboo plants, but suggested that for this species, VOC were more important attractants than CO_2. A better understanding of the relative contributions of CO_2 and VOC to wireworm attraction could help develop alternative management strategies for these pests.

For decades since the ban on organochlorines and until only very recently, there have been no insecticides effective for wireworm control in small grains[23–25]. This necessitated studies to evaluate alternative management tactics like trap cropping and intercropping to reduce damage by wireworms in these crops[26,27]. The sugar beet wireworm, *Limonius californicus* (Mannerheim) (Coleoptera: Elateridae), is a major pest of small grains in the Pacific Northwest region of the U.S[28]. Using peas and lentils as trap crops within wheat fields has been shown to significantly reduce damage caused by *L. californicus*[27]. Understanding the cues involved in this process could enhance the effectiveness of trap cropping and similar management strategies for this pest.

The present study quantified the preference of *L. californicus* for the roots of wheat versus bean and pea seedlings in laboratory olfactometer bioassays. It then sought to identify individual VOC released from these potential hosts that, in combination with CO_2, influence wireworm behavior, potentially accounting for the host preferences detected.

Results

Wireworm host preference

More wireworms moved toward pea (77%) than toward wheat (23%) (GLMM; $F_{1,50}$= 21.107; $P<0.001$). More wireworms also moved towards beans (91%) than toward wheat (9%) (GLMM; $F_{2,44}$= 93.90; $P<0.001$). More wireworms moved toward beans (65%) than peas (35%) (GLMM; $F_{2,44}$ = 4.098; $P=0.049$) (Fig. 1). Non-responding wireworms ranged from 7.2% in pea vs. wheat bioassays to 17.9% in bean vs. pea and bean vs. wheat bioassays.

CO_2 and VOCs detection and quantification in rhizosphere

CO_2 measurement

Belowground CO_2 concentration in the seedling rhizosphere differed among wheat, pea, and bean seedlings seven days after seeding (ANOVA; $F_{2,45}$= 29.552; $P<0.001$). Rhizosphere CO_2 concentrations were higher in bean seedlings than in wheat seedlings ($P<0.001$) and pea seedlings ($P<0.001$) but did not differ between pea and wheat seedlings (Fig. 2).

Belowground volatiles quantification

Overall, profiles of hexanal, 2-hexenal, and 1-hexanol differed among pea, bean, and wheat seedlings (MANOVA; *Pillai's Trace*: $F_{6.16}$= 5.30, $P=0.003$) (Fig. 3). Pea seedlings released hexanal at a higher rate (934 ± 195 ng/ root per hour) than wheat seedlings (70 ± 5 ng/root per hour) ($P=0.001$) and bean seedlings (220 ± 47 ng/root per hour) ($P=0.005$), but hexanal release rate did not differ between wheat and bean seedlings. Bean seedlings released 2-hexenal at a higher rate (108 ± 30 ng/root per hour) than wheat seedlings (29 ± 0.4 ng/root per hour) ($P=0.030$), but 2-hexanal release did not differ significantly between pea (61 ± 5 ng/root per hour) and bean seedlings roots.

Wireworm response to CO_2 and synthetic volatile compounds

Wireworm response to CO_2

More Wireworms moved towards CO_2 (80% of responses) than towards purified air (19%) (GLMM; $F_{1,50}$ = 16.62; $P<0.001$) (Fig. 4).

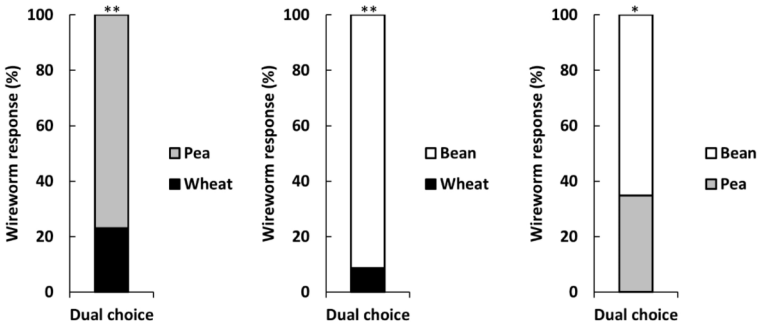

Fig. 1. Frequency of wireworm orientation toward wheat, pea and bean seedlings in dual choice experiments. **: $P<0.01$; *: $P<0.05$.

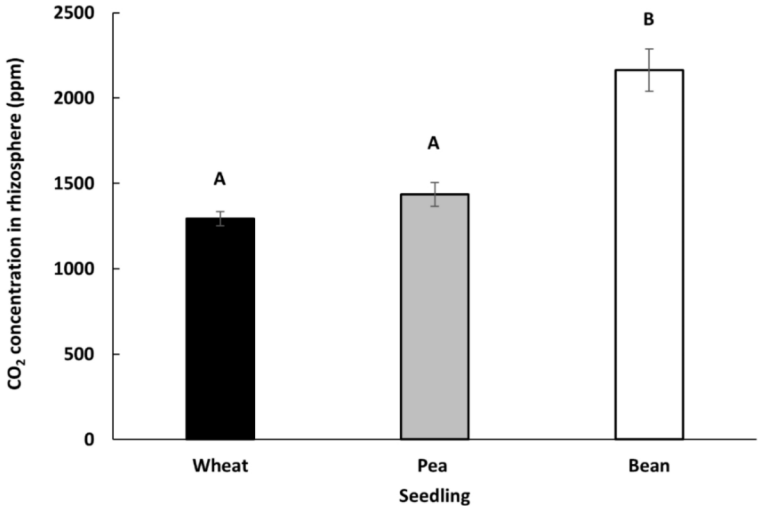

Fig. 2. CO_2 concentration in rhizosphere of wheat, pea and bean seedlings. Different letters show significant differences ($P < 0.05$).

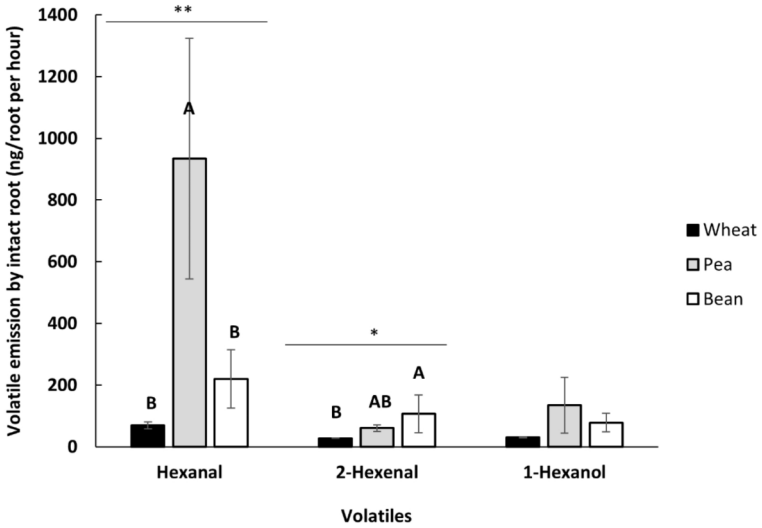

Fig. 3. Amounts of volatile compounds that released from each intact wheat, pea and bean seedling root over one-hour volatile collection. Different letters show significant differences ($P < 0.05$).

Wireworm response to synthetic VOC

More wireworms moved towards 20 µg of hexanal (GLMM; $F_{1,46}$= 38.40, $P < 0.001$) and 200 µg of hexanal (GLMM; $F_{1,44}$ = 13.53, $P = 0.001$) than towards the triacetin controls (respectively). More wireworms moved towards 200 µg hexanal than to 20 µg (GLMM; $F_{1,48}$ = 28.05, $P < 0.001$) (Fig. 5).

Similarly, the wireworms preferred 20 µg and 200 µg of 2-hexenal to the triacetin control (20 µg: GLMM; $F_{1,62}$ = 10.38, $P = 0.002$; 200 µg: GLMM: $F_{1,62}$ = 4.24, $P = 0.044$; Fig. 6). Since the amount of 2-hexenal released by seedlings did not reach 200 µg for any tested plant species, we removed the comparison between 20 and 200 µg of 2-hexenal in our preference bioassays.

Wireworms showed no preference for hexanal vs. 2-hexenal in olfactometer, whether tested with each compound at 200 µg (GLMM; $F_{1,52}$= 0.07, $P = 0.786$) or 20 µg (GLMM; $F_{1,60}$= 1.65, $P = 0.204$) (Fig. 7).

Wireworm response to CO_2 and synthetic volatiles

In dual-choice bioassays, wireworms preferred hexanal + air to CO_2 + solvent (GLMM; $F_{1,94}$= 7.88, $P = 0.006$) but showed no preference between hexanal + CO_2 or CO_2 + solvent (GLMM; $F_{1,56}$= 1.77, $P = 0.189$) (Fig. 8).

Discussion

Plant volatile compounds play an important role in the foraging and host selection processes of herbivorous insects, and this might be especially the case for belowground arthropods that need to navigate through the

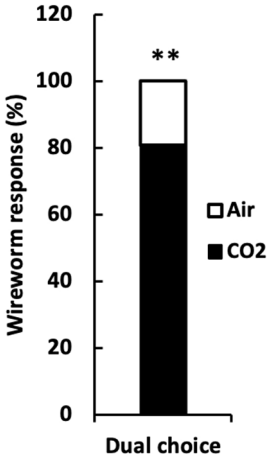

Fig. 4. Frequency of wireworm orientation toward CO_2 and air in a dual choice experiment. **: $P < 0.01$; *: $P < 0.05$.

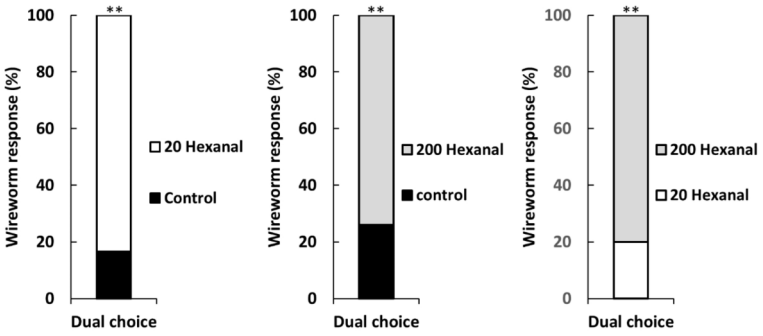

Fig. 5. Wireworm orientation toward two amounts of hexanal (i.e., 20 and 200 µg) against control (blank triacetin as a solvent) as well as orientation toward 20 and 200 µg hexanal in dual choice experiments. **: $P < 0.01$; *: $P < 0.05$.

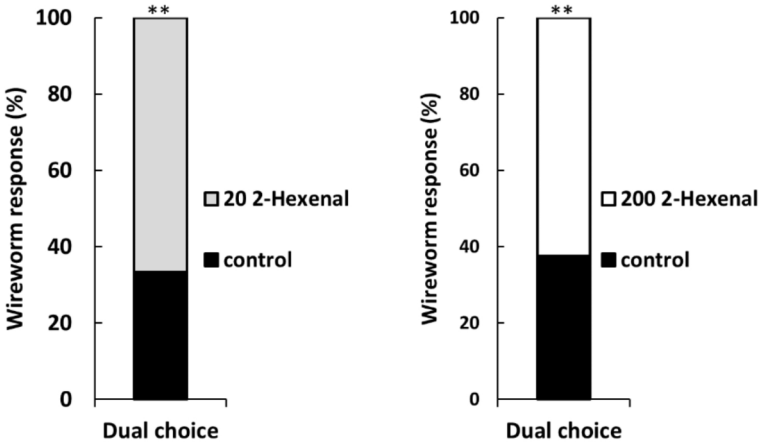

Fig. 6. Wireworm orientation toward two amounts of 2-hexenal (i.e., 20 µg [200 µL of 100 µg/mL solution] and 200 µg [200 µL of 1 mg/mL solution]) against control (blank triacetin as a solvent) in dual choice experiments. **: $P < 0.01$; *: $P < 0.05$.

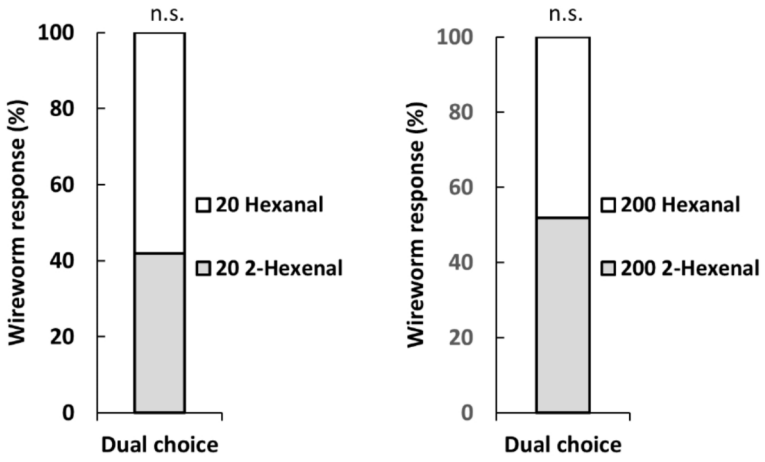

Fig. 7. Wireworm orientation toward two amounts of hexanal (i.e., 20 µg [200 µL of 100 µg/mL solution] and 200 µg [200 µL of 1 mg/mL solution]) against same amounts of 2-hexenal in dual choice experiments. n.s.: nonsignificant differences.

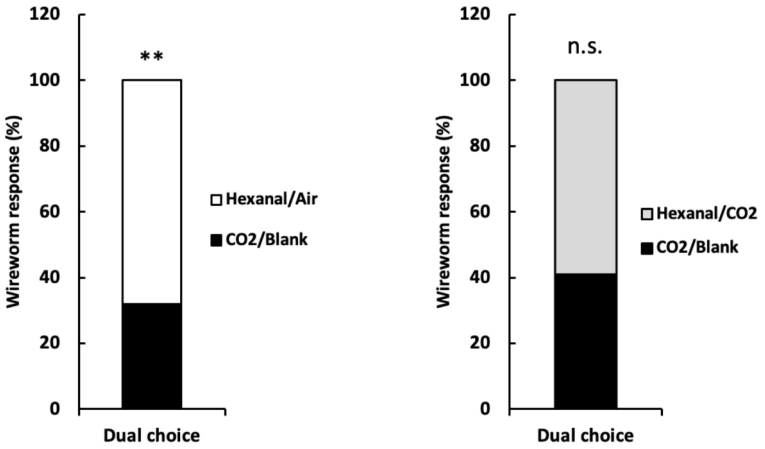

Fig. 8. Wireworm orientation toward hexanal + air against CO_2 (with triacetin as a solvent) and combination of hexanal + CO_2 against CO_2 in dual choice experiments. **: $P < 0.01$; *: $P < 0.05$; n.s.: nonsignificant differences.

complex soil environment in the absence of visual signals[11,16,29]. Several studies have shown that CO_2 is an important signal for belowground herbivores to locate their hosts[12,29]. Our results indicate that while CO_2 is a signal used in host selection by wireworms, root-emitted VOCs are also critical in the process. Wireworm responses were influenced by the amounts of two root VOCs and the combination of VOC and CO_2.

Wireworms showed a stronger preference for bean and pea seedlings over wheat seedlings, and a stronger preference for bean seedlings compared to pea seedlings. This preference can be explained by the observed higher CO_2 concentrations emitted from bean seedling roots than from the other two plant species (Fig. 2), an observation consistent with the previously-established role of CO_2 in host location by the belowground herbivores[8,11,12,16]. On the other hand, *L. californicus* preferred the roots of pea seedlings to roots of wheat seedlings despite their emitting similar concentrations of CO_2, indicating other cues are involved. Since there are numerous other sources of CO_2 in the soil including roots of most plant species[17], more host specific signals likely influence host selection by root herbivores[18]. Johnson et al.[12] and Barsics et al.[18] demonstrated that plant VOCs can play a secondary role in host selection by belowground herbivores. Our study shows this to be the case for *L. californicus.* Among-species differences in developmental stage (e.g., plant size) could have contributed to the observed wireworm preference and differences in the measured CO_2 and VOC concentrations[30]. It is possible that wireworms prefer the relatively faster-developing plants to plants with slower development, and this can be the topic of future studies.

Barsics et al.[18] demonstrated that the attraction of *Agriotes sordidus* to VOCs was concentration-dependent. High amount (1 mg) of a blend of four aldehydes; 9% hexanal, 8% (*E*)-hex-2-enal, 52% (*E*)-non-2-enal, and 31% (*E*, *Z*)-nona-2,6-dienal were more attractive than the lowest tested amount of this blend (0.01 mg). In our study, hexanal, the most abundant VOC detected (as high as 934 ng/hr from pea seedlings) (Fig. 3), was more attractive

to *L. californicus* at the higher amount of 200 µg (1 mg/ml) than the lower amount of 20 µg (100 µg/mL). This higher amount was equivalent to hexanal amounts released by the more preferred pea seedling roots, while the lower one was equivalent to less preferred wheat seedling roots, potentially accounting for the observed preference.

Higher amounts of root VOC can become repellent to wireworms. For example, *Agriotes sordidus* were repelled by the 100 mg of the four-aldehyde blend tested by Barsics et al.[18]. In preliminary bioassays, *L. californicus* was deterred by higher concentrations of hexanal [1000 µg (200 µL of 200 mg/mL pure hexanal solution); data not presented].

An important limitation of earlier studies[17,22] was that root VOCs were detected and quantified from ground root tissue, which can greatly alter the VOC profile qualitatively and quantitively compared to VOC from intact, living roots[31,32]. In our study, VOCs were quantified from intact roots of our test plants and those concentrations were used to guide our bioassay procedures, rendering our results more meaningful for understanding cues involved in host selection by *L. californicus*. The compounds we detected from intact roots, hexanal, (*E*)-2-hexenal, and 1- hexanol were previously reported from ground barley roots[17,18,31]. Other compounds we did not detect (*E*)-non-2-enal and (*E*, *Z*)-nona-2,6-dienal are induced in barley in response to mechanical damage[31,32] and may not be important in host selection of intact seedling root.

Because the concentration of *(E)*-2-hexenal released also differed among the three host plant species we tested, we evaluated its attractiveness to *L. californicus*. Compared to blank solvent control, *L. californicus* preferred *(E)*-2-hexenal at 20 µg and 200 µg. *(E)*-2-Hexenal and hexanal were equally attractive in a dual-choice bioassay when presented at the same concentration. In this bioassay, there was a high percentage of non-responsive wireworms, consistent with a lack of preference. 1-Hexanol was not tested in our bioassays because no significant differences were detected in the emission of this VOC among the three plant species.

Despite the importance of both CO_2 and VOC in locating the food source by wireworms, there is limited information on wireworm responses when both signals are present. We showed wireworms had stronger preference for synthetic VOC than for CO_2. However, when CO_2 was added to both sides of the olfactometer the preference for VOC disappeared. This finding suggests that although CO_2 and hexanal both attract *L. californicus*, their combination is no more attractive than CO_2 by itself. We only used one concentration of CO_2 (2000 ppm, equal to the CO_2 concentration in the bean rhizosphere) and our observations and evaluations were made over a fixed distance. This may explain the inconsistency of our findings with Wei et al.[19] who showed that wireworms preferred bamboo shoots buried in the soil over CO_2-releasing beads buried in the soil in a dual-choice assay. It is possible that the buried beads released less CO_2 compared to the approach we adopted. Future studies focusing on the interactions among different concentrations of volatiles and CO_2 at various distances (from the source) and soil types are warranted[18,33,34].

Trap cropping has been shown to have potential in managing belowground herbivores such as wireworms[27,35–37]. Realizing this potential may depend on knowledge of CO_2 and VOCs signals from trap crops and target crops to optimize deployment, develop synthetic lures, or even identify plant genotypes that are most effective as traps to divert pests from crops or bring them contact with biological[38,39] or synthetic[40] insecticides.

Methods

Plant material

Wheat (*Triticum aestivum* L.; var. SY-Ovation [Syngenta, Research Triangle Park, NC]), pea (*Pisum sativum* L.; var. Banner), and pinto bean (*Phaseolus vulgaris* L.; var. Quincy) were used in our volatile detection and host preference bioassays. SY-Ovation was provided by the South Central and Southeast Idaho Cereal Program, Aberdeen, ID. The pea seed was provided by Hamilton Triple C Farms, Ririe, ID, and the Quincy pinto beans were purchased from WinCo Foods, Pocatello, ID. Seeds were soaked in distilled water for 48 h to promote germination. Seeds were then transferred to Petri dishes lined with moist filter paper and kept at room temperature (23 ± 2 °C) until germination. For host preference bioassays, the sprouted seeds were planted directly into olfactometers, as described below. For VOC detection, the sprouted seeds were placed in 26.1 × 16 × 10.2 cm (L × W × H) stainless steel trays filled with moistened autoclaved sand and grown in the laboratory with an average daily temperature of 23 ± 2 °C and 16:8 h (Light: Dark) for 7 days.

Wireworms

Limonius californicus were collected from an organic vegetable plot located in Sandpoint, ID (48.369222, –116.401278), which had been planted in organic mustard, black beans, and broccoli in previous years, using multiple solar bait traps. Solar bait traps consisted of a mixture of water-soaked untreated wheat and barley seeds, buried 15 cm deep into the soil and covered with a black plastic bag[28]. After 2 weeks, the wheat and barley sprouts and the surrounding soil were transported to the laboratory with trapped wireworms[28]. Wireworms collected from these traps were placed individually in 5 × 5 × 10 cm (W×L ×H) round plexiglass containers filled with moistened sand and two barley seeds as their food source. Containers were kept at room temperature, and the sand was kept moist until wireworms were used in bioassays. Prior to bioassays, wireworms were transferred to containers filled with only moist sand to starve for ten days. To minimize variation, all the wireworms used in our study were between 1 and 1.5 cm in length.

Wireworm host preference

Olfactometer

Olfactometers were constructed of three pieces of glass tubing (7.5–9.0 cm long × 2.5–3 cm internal diam.) fitted together in line and sealed with parafilm (Supplementary Materials, Figs. S1, S2). The middle section had an opening on the top (1.25 cm internal diam.) through which wireworms could be introduced into the olfactometer. The two end pieces were closed at one end with open ends joined to the middle section of the

olfactometer. Each end piece had two holes (1.25 cm internal diam.), one on the upper side and one opposite to it on the lower side, spaced 1 cm from the end (see Supplementary Material, Fig. S1). The entire olfactometer was filled with dried, autoclaved sand. Depending on treatment, germinated seeds were planted in the sand through the upper openings of the olfactometer end pieces (Supplementary Materials, Fig. S2). After planting, the sand was moistened with 5 mL deionized water. The lower opening of each end section was sealed with a cork through which the needle of a 1.5 mL syringe was inserted to collect and measure CO_2 concentration surrounding the root rhizosphere.

Host preference bioassays

Germinated wheat, pea, or bean seeds were planted into the olfactometer seven days before running host preference experiments. The sand in each side of the olfactometer was moistened with 5 mL distilled water at planting to reach soil moisture of ~ 5.7% by volume. Seven days later, a single wireworm was placed in the middle section olfactometer. On that day 5 mL of distilled water was also added to each side of the olfactometer. Four hours after introducing the wireworm, its location was recorded by dissembling the olfactometer. If the wireworm was found in the middle section of the olfactometer, a 'non- responding' was recorded. If the wireworm was found in either end piece and/or feeding on the plant there, a choice was recorded. Three dual-choice bioassays were conducted: (1) pea vs. bean, (2) wheat vs. pea, and (3) wheat vs. bean. Experiments were conducted in three time-blocks with 8 (first time-block) or 10 (second and third time-blocks) replicates per time-block for a total of 28 replications for each bioassay. Within each time-block, all bioassays were run simultaneously in a completely randomized arrangement. The position of choices within olfactometers was also randomized.

CO_2 and VOCs detection and quantification

CO_2 measurement

Prior to placing wireworms in the tubes, the CO_2 concentration in the sand pore space in the vicinity of the root was measured by collecting 1 mL of air using a 1.5 mL syringe. The needle was inserted through the cork in the lower side of the olfactometer (Supplementary Materials, Fig. S3). The collected air was injected into a LI-COR LI-7000 CO_2/H_2O analyzer (LI-COR Inc., Lincoln, NE, USA). The CO_2 analyzer was calibrated using a one-point calibration standard of 2000 ppm CO_2 in pure nitrogen (zero air) gas. The area under the CO_2 curve was used to calculate the concentration of CO_2.

Belowground volatile collection

Germinated seeds were planted in sand in 26.1 × 16 × 10.2 cm (L × W × H) stainless steel trays and sand in trays kept moistened until seedlings were removed for the experiment. After seven days, each plant was gently removed, and roots were carefully washed with deionized (DI) water to minimize mechanical damage. To collect and quantify the organic volatiles from intact roots, the belowground tissues of each seedling were placed into a glass vial (25 mL) while the aboveground tissues remained outside of the vial, passing through a plastic cap and sealed with a polytetrafluoroethylene (PTFE) stopper (Supplementary material Fig. S4). Two other holes in the vial cap allowed the placement of Tenax (Tenax TA, Scientific Instrument Services Inc., Ringoes, NJ, USA) traps and carbon filters. The Tenax trap, used to collect plant organic volatiles, consisted of 120 mg Tenax in a GC liner packed with glass wool baked at 270 °C for 20 min before each collection. The trap was connected to a vacuum pump, and air (0.3 L/min) was drawn first through a carbon filter, then through the vial and onto the Tenax trap (Figure S4). An internal standard of 1 µL of 3,5,5- trimethylhexanal in triacetin solution (8.7 µg/µL) was added to each vial prior to the volatile collection. Collection from each intact root lasted for one hour at room temperature. The Tenax trap was then removed and placed into the inlet of a gas chromatograph (GC) for thermal desorption of the volatiles onto the GC column for detection and quantification of volatiles. We used four replicates of each plant species to collect and quantify volatile compounds.

Gas chromatography-mass spectrometry (GC-MS)

Volatile analyses were performed using a 7890 A GC System (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with a Hewlett Packard (HP) 5973 mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA) and an HP-5MS column (length = 30 m, internal diameter = 0.250 mm, film thickness = 0.25 µm; Agilent Technologies Inc., Santa Clara, CA, USA). The inlet of the GC system (set to 150 °C) served as a desorption oven[41] to thermally desorb the volatiles from the Tenax trap, which were then collected in a cold trap at the head of the GC column. The trap consisted of a jacket surrounding the first 3 cm at the column head which could be cooled to < − 30 °C using liquid CO_2. After the cold trap was turned off, the initial oven temperature was 30 °C for 3 min and then ramped up to 260 °C at 10 °C/min rate and held at 260 °C for 5 min. A single run required 31 min. Electron ionization (EI) mass spectra were collected by scanning between 50 and 550 m/z, and volatile compounds were identified by matching each peak with the NIST database using Mass Hunter Quantitative software (Version B.09.00/Build 9.0.647.0; Agilent Technologies Inc., Santa Clara, CA, USA) and the retention times of the authentic compounds (hexanal, 2-hexenal, and 1-hexanol) (Sigma-Aldrich, Inc., St. Louis, MO).

Wireworm response to CO_2 and synthetic volatile compounds

Wireworm response to synthetic VOC

The highest and lowest amounts (µg/hour/seedling) of VOC detected from seedling roots (see "Results"), were used for a wireworm preference bioassay. Purified hexanal and 2-hexenal (Sigma-Aldrich, Inc., St. Louis, MO) as the major volatiles detected in the rhizosphere of the selected seedlings were used for these bioassays. These two compounds were the two major VOC with significant differences among the assessed host plants. 1-hexanol was not included in bioassays because its released amount did not vary across plant species. The vial used for root volatile collection was filled with autoclaved sand moistened to 5.7% by volume as in host preference

bioassays. Different concentrations of purified hexanal (98%) in triacetin solvent were added to the vial and the released hexanal was collected and measured in the headspace for one hour. Two specific amounts, 20 µg (200 µL of 100 µg/mL solution) and 200 µg (200 µL of 1 mg/mL solution), released approximately 100 ng and 1000 ng hexanal per hour, respectively. These amounts (100 ng and 1000 ng) correspond to the amount of hexanal released from wheat and pea seedling roots (see "Results") and were used in preference bioassays.

To conduct bioassays, purified hexanal and 2-hexenal were dissolved in triacetin to make solutions of 1 mg/mL and 100 µg/mL, respectively. Then 200 µL of each solution were added to a 200 µL glass insert (5 × 31 mm) with flat bottom (Ibis Scientific Inc., Las Vegas, NV) filled with 100 mg glass wool (for slow releasing). Triacetin (200 µL) was used as a control.

Wireworm response to volatiles was assessed in the olfactometers described above in two separate sets of experiments. The first set included three bioassays: (1) control vs. 20 µg hexanal, (2) control vs. 200 µg hexanal, and (3) 20 µg hexanal vs. 200 µg hexanal in three time-blocks and 8 replicates per treatment in each time-block (a total of 24 replicates per bioassay). The second set included the four bioassays (1) control vs. 20 µg 2-hexenal, (2) control vs. 200 µg 2-hexenal, (3) 20 µg hexanal vs. 20 µg 2-hexenal, and (4) 200 µg hexanal vs. 200 µg 2-hexenal. This set was conducted in four time-blocks with 7, 7, 7, and 15 replicates per time-block (a total of 36 replicates per bioassay). Before starting each experiment, the volatile compounds were equilibrated in the olfactometer for 15 min at room temperature. Wireworm response to each treatment was assessed after 4 h based on the olfactometer section in which the wireworm was located. Wireworms found in the middle section were considered non-responding and excluded from statistical analysis.

Wireworm response to CO_2

A series of dual choice bioassays quantified wireworm preference for purified CO_2 (99.9% purified, dosed in purified air) versus purified air. A 99.9% purified commercial CO_2 tank (Norco Inc., Boise, ID) at the flow rate of 0.1 mL/min was used to simulate root CO_2 release into the olfactometer. The gas flow was adjusted with a regulator (Gasco, Cal Gas Direct Incorporated, Huntington Beach, CA) attached to the tank and monitored throughout the experiment using a flowmeter (VFA-21, DwyerOmega, Michigan City, IN). For the control treatment, a commercial purified air tank (Norco Inc., Boise, ID) was used at the same airflow used for the CO_2. Both CO_2 and air were distributed among 10 olfactometers using a glass manifold attached to the gas tank regulators by silicone tubing. Two flowmeters attached to silicon tubes between gas tanks and manifold on each side were used to adjust the gas flow into the olfactometers (Supplementary materials, Fig. S5). The gases entered the olfactometers through the 1.5 mL syringe needle inserted through corks in the lower olfactometer openings (Supplementary materials, Fig. S5). Olfactometers were filled with autoclaved sand moistened with 10 mL DI water (5 mL, each side; 5.7% moisture of soil volume) and all upper openings were sealed with cork stoppers. CO_2 and purified air flow were stabilized for 5 min before wireworms were introduced into the center section of the olfactometers, as in other bioassays in this study. Wireworm preference for each treatment (CO_2 vs. purified air) was assessed after 4 h by recording wireworm location among the olfactometer sections. Wireworms found in the middle section were considered non-responding and not included in the analysis. This set of experiments was conducted in three time-blocks with 10 replicates per time-block.

Wireworm response to VOC in the presence of CO_2

To evaluate wireworm response to VOC in the presence of CO_2, CO_2 (99.9% purified CO_2) and purified air were administered to both sides of the olfactometers (Supplementary Materials, Fig. S5) and responses of wireworms were assessed in response to 200 µL of either hexanal or triacetin solvent (control) in dual-choice experiments. Two treatment combinations were evaluated: (1) air + hexanal vs. CO_2 + solvent (six time-blocks with 10 replicates/time-block) and (2) CO_2 + hexanal vs. CO_2 + solvent (four time-blocks with 10 replicates/time-block). As in the VOC only preference bioassays, 200 µL of hexanal and 200 µL of solvent (triacetin) were loaded into the glass inserts filled with 100 mg of glass wool. Each bioassay lasted 4 h. Wireworms found in the middle section of the olfactometer were considered non-responding and excluded from the data set.

Statistical analysis

To define wireworm host preference in dual choice experiments, we used a generalized linear mixed model (GLMM) assuming a binomial distribution and a logit link function. Within each analysis, a randomized complete block design was assumed with the models, including the experiment-specific treatment (plant type, volatile compounds, or CO_2 treatments) as a fixed effect and time-block as a random effect. Analyses of CO_2 concentrations in the rhizosphere of the plant roots used a similar design and model assuming a normal distribution. Pairwise comparisons in models used Fisher's Protected LSD tests. The responses from volatile profiles emitted by the seedling roots were assessed through Multivariate Analysis of Variance (MANOVA) also employing Fisher's Protected LSD tests. All statistical analyses were conducted using IBM SPSS statistics software version 26.0 (IBM Corp., New York City, NY, USA).

Data availability

The data used to support the findings of this study are available from the corresponding authors upon request.

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Author contributions
AN participated in the study design, conducting experiments, data analysis and drafting the first version of the manuscript, and prepared all of the figures; RS participated in the study design, conducting experiments, data analysis and editing the manuscript; SE provided guidance for chemical analyses and edited the manuscript. WP provided guidance for statistical analysis and edited the manuscript. AR contributed to study design, conceptualization, writing and editing the manuscript. AR provided funding for the study.

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