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BEHAVIORAL RESPONSES OF WESTERN CORN ROOTWORM LARVAE TO VOLATILE SEMIOCHEMICALS FROM CORN SEEDLINGS

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Abstract—Corn seedling volatiles collected cryogenically are highly attractive to western corn rootworm larvae, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in a laboratory bioassay. Carbon dioxide is known as an attractant for western corn rootworm larvae, and the amount of carbon dioxide in the cryogenic collections was measured with an infrared gas analyzer. In a choice test between a source containing carbon dioxide alone and a source containing corn seedling volatiles with an equal amount of carbon dioxide (verified by infrared gas analysis), western corn rootworm larvae chose the corn volatile source significantly more often than the side with carbon dioxide alone. This indicates that carbon dioxide is only one of the volatiles from corn seedlings that is behaviorally important and that other compounds of behavioral importance are present as well.

Key Words—*Diabrotica virgifera virgifera*, Coleoptera, Chrysomelidae, western corn rootworm, rootworm, corn, *Zea mays*, kairomone, volatile substances, attractants, carbon dioxide, semiochemical.

INTRODUCTION

Diabrotica virgifera virgifera LeConte, the western corn rootworm, is an annual threat to corn throughout much of the Midwest. Larval damage to corn roots results in reduced plant height, reduced yield, and lodging of corn, which interferes with harvesting (Branson et al., 1980). Members of the genus *Diabrotica* have been estimated to cause more than $1 billion in damage per year in the United States, mostly to corn (Metcalf, 1986).

Larvae emerge from overwintering eggs in the spring and can feed only

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on certain species of Gramineae (Branson and Ortman, 1967, 1970). Because the eggs are laid in soil the previous year, the burden of host plant location lies entirely with the newly hatched larvae. The larvae have been reported to crawl through as much as a meter through the soil to find the roots of a suitable host (Short and Luedtke, 1970), although estimates to date have been complicated by a number of experimental difficulties (Branson, 1986). In surface olfactometer choice tests between cut roots of corn and broad-leaved plants, Branson (1982) found that significantly more western corn rootworm larvae were attracted to the roots of corn than to the roots of soybean, squash, or sunflower (no difference was observed between roots of corn and tomato). Behavioral bioassays in a soil olfactometer did not reveal differences in attraction, perhaps due to the difficulty of establishing equal amounts of root tissue for the choice test (Branson, 1982). Roots of a number of grass species were as attractive as corn roots or more so to western corn rootworm larvae in surface olfactometer choice tests, including several grass species that were nonhosts (Branson, 1982).

The only chemical cue known to be involved in the orientation of western corn rootworm larvae to corn roots is carbon dioxide (Strnad et al., 1986, Strnad and Bergman, 1987), which is known to attract a number of other soil insects (Jones and Coaker, 1977; Doane et al., 1975; Pline and Dusenbery, 1987; and references therein). Carbon dioxide is produced by the roots of most plant species, and it does not appear to provide a basis for the ability of the larvae to distinguish the roots of host plants from those of many other plant species. We wished to determine if carbon dioxide was the only attractive compound produced by corn root tissue, or if other semiochemicals may be involved in host selection by western corn rootworm larvae as well. Compounds that affect larval behavior might be expected to include two principal categories: compounds that are volatile and disperse mainly by diffusion in the soil atmosphere, and those that are water-soluble and disperse mainly by diffusion in aqueous solution. Our initial studies have focused on the possible contribution of volatile constituents of corn plants.

Cryogenic collection was used to recover volatile compounds from corn seedlings in the present study. This technique has been used successfully for the recovery of other semiochemicals, such as insect pheromones (Browne et al., 1974; Golub and Weatherston, 1984). Unlike conventional cold trapping, cryogenic collection involves condensing the air itself, along with any volatile organic compounds that the air might contain. Low-temperature distillation allows nitrogen (bp $-196°C$) and oxygen (bp $-183°C$) to be removed after the collection is complete, while volatiles with boiling points higher than $-183°C$ are retained. The principal advantage of cryogenic collection is that compounds with a wide range of volatilities can be recovered. This is important in the present study because the very volatile carbon dioxide is already known as a semiochemical important in host location by western corn rootworm larvae

(Strnad et al., 1986; Strnad and Bergman, 1987), and we wished to evaluate the possible behavioral importance of additional compounds that may have an array of large and small molecular weights. In many other studies of volatile semiochemicals, compounds have been extracted with solid adsorbants such as Tenax or Porapak Q, but these techniques fail to recover compounds with very small molecular weights, such as carbon dioxide (Byrne et al., 1975).

Two behavioral bioassays were developed to determine the role of volatile semiochemicals from corn seedlings in host location by western corn rootworm larvae. A single-choice bioassay was developed to determine if the full complement of corn volatiles collected cryogenically was attractive to the larvae and to verify the conclusion of Strnad et al. (1986) that carbon dioxide alone is attractive to western corn rootworm larvae. A choice test bioassay was also developed, to allow behavioral comparison of carbon dioxide alone with a cryogenic collection of corn volatiles that contained an equal amount of carbon dioxide. Instead of using a choice test bioassay with carbon dioxide levels equal on both sides, the behavioral role of carbon dioxide can alternatively be evaluated by using KOH to remove carbon dioxide from a volatile blend, and using single-choice bioassays to compare the attractiveness of KOH-treated host plant volatiles with untreated host plant volatiles (Doane et al., 1975; Jones and Coaker, 1979). However, there are two problems with this approach: First, semiochemicals that are behaviorally relevant only in the presence of carbon dioxide would be detected in a choice test, but would not be detected in single-choice bioassays of a KOH-treated volatile blend. Second, KOH reacts with many compounds other than carbon dioxide and may remove or chemically alter other semiochemicals in addition to carbon dioxide. A choice test bioassay was adopted in the present study to obviate both these possible problems.

METHODS AND MATERIALS

Insect Colony. Eggs of a nondiapausing strain of *Diabrotica virgifera virgifera* were obtained in June and July of 1986 from Dr. Jan Jackson at the Northern Grain Insects Research Laboratory. The colony was maintained according to methods described by Jackson (1986), with some modifications. Modifications (Jackson, personal communication) included the use of soil in covered, small plastic containers (17 cm diam. $\times$ 10 cm high) in which 75 larvae were reared on corn seedlings for their first six days of growth. Two of these smaller containers (along with the larvae) were then transferred into a larger covered plastic container (18 $\times$ 32 $\times$ 8 cm), which contained sufficient seedling corn in soil for completion of larval development and pupation.

Corn Seedlings. Dried whole kernels of field corn, not treated with insecticide or fungicide, were purchased from a local supplier (variety unspecified).

Dried kernels were washed with detergent solution (Ivory liquid) for 5 min to inhibit fungal contamination, rinsed thoroughly, and soaked in water for 24 hr. Soaked seeds were washed again with detergent solution, rinsed thoroughly, placed on moist germination paper (Anchor Paper Co., St. Paul, Minnesota), covered with moist paper towels, and kept in a closed plastic container. Corn seedlings were removed three to four days later for bioassays.

Cryogenic Volatile Collection. A cryogenic collection technique similar to that described by Browne et al. (1974) was used, based on a simple pump in which a glass sample tube closed at one end (12 mm × 35 cm) was immersed in a liquid nitrogen bath. A boiling chip was placed in a clean sample tube, and a glass seed-holding tube (30 cm × 30 mm, tapering to 12 mm) containing 70 g of 3- to 4-day-old corn seedlings (seeds not treated with insecticide or fungicide) was connected to the sample tube with Teflon tubing. The sample tube was then immersed 20 cm into the liquid nitrogen bath. As air condensed in the sample tube, a vacuum was created that pulled air through the corn seedlings at 300 ml/min (measured with a bubble flowmeter). After 10 min, the sample tube was removed from the liquid nitrogen bath and disconnected from the seed-holding tube. The sample tube was immediately placed into a snugly fitting Styrofoam sheath that had been precooled in liquid nitrogen. The condensed air boiled away within 10 min, allowing liquid nitrogen (bp −196°C) and liquid oxygen (bp −183°C) to escape slowly and leaving compounds that were tested in behavioral bioassays for their importance in western corn rootworm larval orientation.

Single-Tube Bioassay. Plastic Petri dishes (10 cm diam.) were used as arenas for the bioassay. The top of the sample tube was connected with Teflon tubing to a 12-mm hole cut in the bottom of the Petri dish (see Figure 1). Ten larvae (5–6 days old and 4–6 mm long) were placed equidistant in a ring near the wall of the Petri dish, and the cover was replaced. We chose to use second-instar larvae (5–6 days old and 4–6 mm long) for our bioassays because initial bioassays indicated that they behaved similarly to first-instar larvae (the instar that must locate a host), and second-instar larvae were easier to handle for the large numbers of bioassays required. All bioassays were performed in dim light. The number of larvae that entered the tube was recorded at 5-min intervals for 30 min. To prevent possible effects from previous testing, larvae were not reused in the bioassays.

Two treatments were tested in larval behavioral bioassays as controls. Clean sample tubes were tested to verify that the sample tubes themselves were not inherently attractive. Sample tubes containing cryogenic collections of ambient air were tested to determine if the circumstances of the cryogenic collection procedure might influence the bioassay results.

Water was observed to condense in the sample tubes in cryogenic collec-

tions from corn seedlings. Measurement of the weight change in the sample tube before and after the cryogenic collection indicated that 75 ± 5 (SE) mg of water collected in a sample tube during the 10-min collection interval. Sample tubes containing the same amount of water were tested to determine if water alone was attractive to western corn rootworm larvae.

Because carbon dioxide has been reported as an attractant for western corn rootworm larvae (Strnad et al., 1986, Strnad and Bergman, 1987), we wished to verify that carbon dioxide was attractive in our bioassay as well. Carbon dioxide from a gas cylinder was introduced into clean sample tubes for 15 sec, and the tubes were inverted for 30 sec to reduce the concentration of carbon dioxide and disperse it evenly in the tube. An infrared gas analyzer interfaced with a Porapak N gas chromatograph column (GC-IRGA, details below) was used to determine the carbon dioxide concentration in the tubes. The number of larvae attracted into the sample tubes was counted every 5 min for 30 min.

Double-Tube Bioassay. Because carbon dioxide was highly attractive in our single-tube bioassays, the additional influence of other behaviorally important corn volatiles would have been difficult to detect using the single-tube bioassay. In order to test if compounds other than carbon dioxide were involved, a choice test bioassay was designed to give larvae a choice between a sample tube containing carbon dioxide alone and a sample tube containing an equal amount of carbon dioxide in association with other corn volatiles. A bioassay apparatus similar to that described by Branson (1982) was designed, consisting of three plastic Petri dishes (5 cm diam.) connected in series by Teflon tubing (10 mm diam.). Holes (12 mm) were cut into the end dishes to allow connection of sample tubes (see Figure 3). One choice was a sample tube containing 4 mmol/mol of carbon dioxide at bioassay initiation (see Figure 3). The sample tube was prepared by flushing with carbon dioxide from a gas cylinder and inverting it for 30 sec to reduce the concentration of carbon dioxide and disperse it evenly in the tube. The carbon dioxide concentration was verified with the GC-IRGA (details below). For the other choice, a sample tube containing a cryogenic collection of corn seedling volatiles was prepared, the liquid nitrogen and liquid oxygen were removed by low-temperature distillation, the sample tube was raised to room temperature, and the carbon dioxide concentration therein was adjusted to 4 mmol/mol at bioassay initiation (see Figure 3) (by flushing the sample tube with carbon dioxide, inverting it, and verifying the final carbon dioxide concentration with the GC-IRGA). Each sample tube was connected to one of the end dishes of the bioassay apparatus, and a 5-min delay was allowed for volatiles to begin diffusing before larvae were added. Ten larvae were placed in the center of a small Petri dish lid (40 mm diam. with a lip 5 mm high); this was placed in the center chamber of the bioassay apparatus, and the cover to the chamber was replaced. The number of larvae in each of

the three Petri dishes was recorded every 5 min for 30 min. Bioassays were performed in dim light. To prevent possible effects from previous testing, larvae were not reused in the bioassays.

Carbon Dioxide Levels in Bioassays. An infrared gas analyzer (IRGA, Beckman model 865) was used to measure carbon dioxide concentrations. An interface of our own design was constructed to allow the IRGA to be used as the detector for a Porapak N gas chromatograph column (3 mm × 2 m) operated isothermally at 25°C, and the output from the GC-IRGA was analyzed with a HP 3390A integrator-recorder. Gas samples (1 ml) were injected with a Pressure-Lok gas sampling syringe (Precision Sampling Co.) for analysis. A mixture of carbon dioxide in nitrogen equal to the atmospheric concentration was purchased (340 ppm, Union Carbide Corp., Linde Division) and used as a standard for quantitation of carbon dioxide by GC-IRGA analysis. In addition, a 99.9% carbon dioxide source (local welding supplier) was used to make dilutions to generate a calibration curve of GC-IRGA response with respect to carbon dioxide concentration. For the single-tube bioassays, gas samples were taken from the mouth of the sample tube (see Figure 1). For the double-tube bioassays, gas samples were taken from the edges of the middle dish (see Figure 3), because this is the location at which larvae must make a choice to leave the dish. Samples were taken every 5 min for 30 min for analysis with the GC-IRGA.

Statistical Analysis. The statistical package BMDP (BMDP Program Librarian, Department of Biomathematics, University of California, Los Angeles, California 90024) was used for data analysis. Behavioral bioassay data for 30 min were analyzed with one-way analysis of variance for larval attraction in the single-tube and double-tube bioassays, because larvae that entered the sample tubes were usually unable to come back out of the tubes and the bioassay data were therefore essentially cumulative with time. Duncan's multiple-range test (Duncan, 1955) was used to discern differences among treatment means. Data for carbon dioxide levels at all times sampled were compared in a repeated measures design (Anonymous, 1981), because sequential samples for GC-IRGA analysis were taken from each sample tube.

RESULTS

Significantly more ($P \leq 0.01$) western corn rootworm larvae were attracted to sample tubes containing cryogenically collected corn volatiles than to control treatments, which included sample tubes containing ambient air, cryogenic collections of ambient air, or water (Figure 1A). Samples tubes containing cryogenic collections of ambient air attracted more larvae ($P \leq 0.05$) than tubes with ambient air not cryogenically collected.

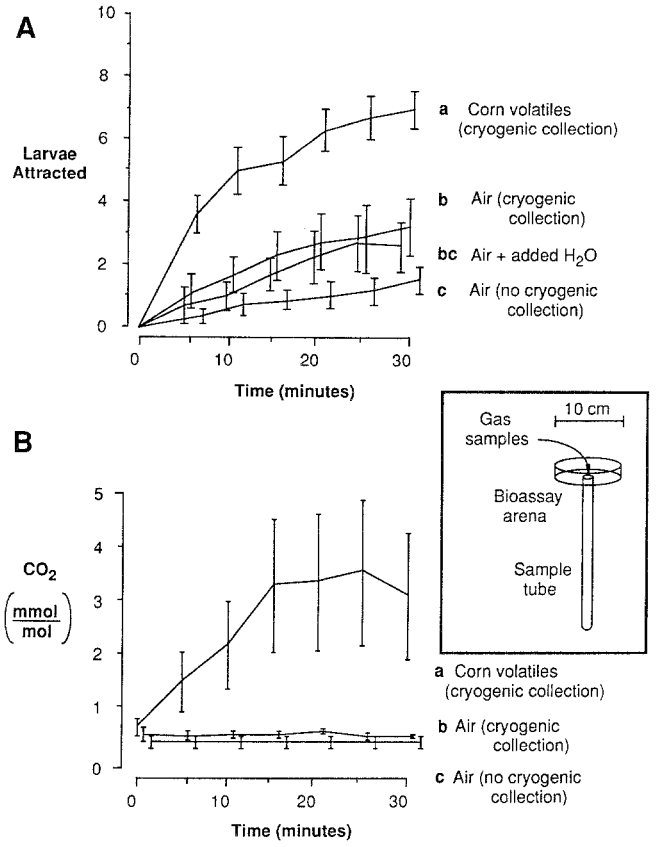

FIG. 1. (A) Single-tube bioassay to evaluate behavioral responses of western corn rootworm larvae to cryogenic collections of corn seedling volatiles and to control treatments. Treatment means at time 30 min were significantly different ($P \leq 0.01$) for curves followed by different letters according to Duncan's NMRT. Error bars represent 95% confidence intervals. (B) Carbon dioxide concentrations at the orifices of the sample tubes. Treatment means of overall carbon dioxide levels were significantly different ($P \leq 0.05$) for curves followed by different letters in a repeated measures analysis of all times according to Duncan's NMRT. Error bars represent 95% confidence intervals.

When a Petri dish with 10 larvae was added to sample tubes containing cryogenic collections of corn seedling volatiles, the first observable larval responses were head-waving, also observed by Strnad et al. (1986), and alignment of their body axes toward the center of the dish. Larvae crawled rapidly and more or less directly toward the source of corn volatiles, similar to the path that larvae took to carbon dioxide in Strnad et al. (1986). This behavioral sequence rarely occurred in response to control treatments (ambient air treat-

ment or water treatment). Larvae that lowered their heads into the sample tube usually crawled in and did not crawl back out.

The cryogenic apparatus for volatile collection provided an efficient vacuum. Air flow past the corn seedlings remained essentially constant throughout the 10-min collection period as indicated by measurement of the volume of liquid air that accumulated in the sample tube (0.35 ± 0.02 SE ml/min) and by measurement with a bubble flowmeter (300 ml/min). Collection rates remained relatively constant for collection periods as long as 30 min. Behavioral bioassay results indicated that it was effective in collecting compounds of interest to larvae, and after distillation of liquid oxygen and nitrogen, the odor of corn was apparent to the human nose as well.

Analysis of carbon dioxide standards with the GC-IRGA indicated a retention time for carbon dioxide of 1.8 min. No other peaks were observed in GC-IRGA analyses of air, cryogenic collections of air, or cryogenic collections of corn seedling volatiles. Calibration with known carbon dioxide concentrations showed the GC-IRGA to be linear in response ($r^2 = 0.995$) for the range of carbon dioxide found in the bioassays (0.34–30 mmol/mol). The use of the IRGA and GC in combination provided a sensitive, semiselective means of detecting carbon dioxide in conjunction with a characteristic retention time for this compound, allowing rigorous determination of carbon dioxide in the volatile samples even at low concentrations. The carbon dioxide concentration in ambient air was easily measurable (0.34 mmol/mol is atmospheric concentration).

The carbon dioxide concentration (Figure 1B) was significantly higher in sample tubes containing cryogenically collected corn seedling volatiles than in sample tubes containing cryogenically collected ambient air or sample tubes containing ambient air without cryogenic collection ($P \leq 0.01$). The concentration of carbon dioxide in sample tubes containing cryogenically collected ambient air (Figure 1B) was slightly higher than that found in ambient air (but significant, $P \leq 0.01$). The cryogenic collection evidently concentrated carbon dioxide from the surrounding air, and the greater carbon dioxide concentration may account for the significantly greater attraction of larvae to sample tubes containing cryogenically collected ambient air than to the sample tubes containing air alone.

Single-tube bioassays with carbon dioxide (Figure 2A) showed that this compound is highly attractive to western corn rootworm larvae ($P \leq 0.01$). The source of carbon dioxide in this experiment was a sample tube from which carbon dioxide emerged by diffusion. The dynamics of carbon dioxide release by the sample tube were determined by analysis with GC-IRGA (Figure 2B). For sample tubes prepared by filling them with carbon dioxide from a gas cylinder and inverting them for 30 sec to adjust the concentration to a low level,

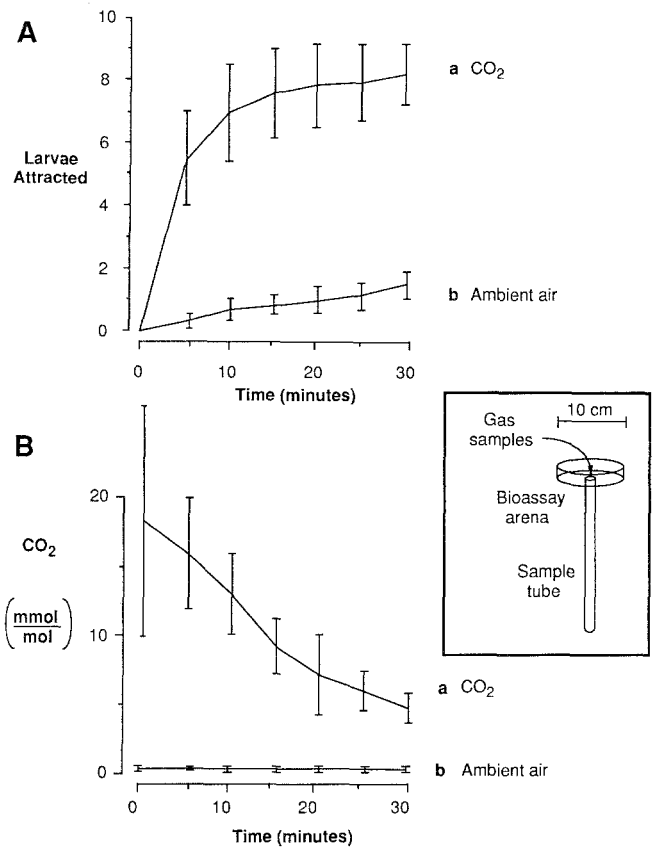

FIG. 2. (A) Single-tube bioassay to evaluate responses of western corn rootworm larvae to carbon dioxide and to ambient air. Treatment means at time 30 min were significantly different ($P \leq 0.01$) for curves followed by different letters according to Duncan's NMRT. Error bars represent 95% confidence intervals.(B) Carbon dioxide concentrations at the orifices of the sample tubes. Treatment means of overall carbon dioxide levels were significantly different ($P \leq 0.01$) for curves followed by different letters in a repeated measures analysis of all times according to Duncan's NMRT. Error bars represent 95% confidence intervals.

the average concentration of carbon dioxide at the orifice of the sample tube was initially 18 $\pm$ 8 (SE) mmol/mol and dropped to a third this value as the gas diffused from the sample tube during the 30 min bioassay (Figure 2B). This source of carbon dioxide was highly attractive to the larvae, in that 82% of the larvae tested entered the sample tube, and more than half of these entered the sample tube within the first 5 min of the bioassay (Figure 2A).

In a choice test between a sample tube containing carbon dioxide (4 mmol/mol at bioassay initiation; Figure 3B) in association with cryogenically collected corn seedling volatiles and a sample tube containing an equal amount of carbon dioxide alone (4 mmol/mol at bioassay initiation; Figure 3B), significantly more ($P \leq 0.01$) western corn rootworm larvae chose the sample tube

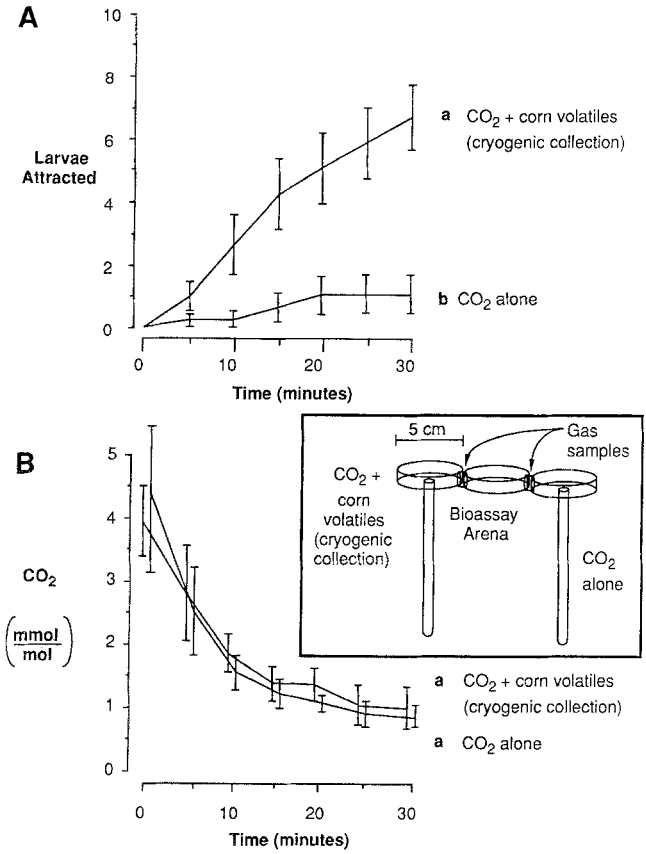

FIG. 3. (A) Double-tube bioassay to evaluate choice of western corn rootworm larvae between a sample tube containing carbon dioxide alone and a sample tube containing an equal concentration of carbon dioxide in conjunction with cryogenically collected corn seedling volatiles. Treatment means at time 30 min were significantly different ($P \leq 0.01$) for curves followed by different letters according to Duncan's NMRT. Error bars represent 95% confidence intervals. (B) Carbon dioxide concentrations at the orifices of the sample tubes. Treatment means of overall carbon dioxide levels were significantly different ($P \leq 0.05$) for curves followed by different letters in a repeated measures analysis of all times according to Duncan's NMRT. Error bars represent 95% confidence intervals.

with corn seedling volatiles (Figure 3A). It was essential that carbon dioxide concentrations be the same in both sample tubes, and GC-IRGA analysis verified that identical concentrations were effectively established by filling both types of sample tubes with carbon dioxide from a gas cylinder and inverting them for 30 sec (Figure 3B). The concentrations of carbon dioxide in each sample tube declined during the bioassay period as carbon dioxide diffused from the tubes, and the time course of carbon dioxide release from the two types of sample tubes was the same throughout the bioassay period (Figure 3B). The strong preference of the larvae for the sample tube containing corn seedling volatiles indicates that carbon dioxide is only one of the corn seedling volatiles attractive to western corn rootworm larvae and that other behaviorally important compounds are also produced by corn seedlings.

DISCUSSION

A central interest in studies in chemical ecology is to establish the complement of chemical compounds involved in a given ecological interaction and to determine how those compounds direct its course. We have shown that volatiles can be cryogenically collected from corn seedlings and that the isolated volatiles are attractive to western corn rootworm larvae. The only compound that has been implicated in host plant interactions with *Diabrotica* larvae is carbon dioxide (Strnad et al., 1986, Strnad and Bergman, 1987), a primary metabolite that is released by the roots of corn plants (Harris and van Bavel, 1957; Massimino et al., 1980). We found by GC-IRGA analysis that carbon dioxide is one of the compounds present in cryogenically collected corn seedling volatiles and verified that this compound was strongly attractive to larvae in our single-tube bioassay. The strong attraction of larvae to carbon dioxide in the single-tube bioassay (Figure 2A) would have made it difficult to determine any additional influence of other semiochemicals produced by corn seedlings. To make this distinction, we developed a choice test bioassay in which equal concentrations of carbon dioxide were present on both sides, and we found that the side containing corn root volatiles attracted significantly more western corn rootworm larvae. This indicates that a blend of volatile compounds from corn seedlings is involved in attraction of western corn rootworm larvae and that carbon dioxide is not the only compound in corn root volatiles of behavioral importance of *Diabrotica virgifera virgifera* larvae. Volatile secondary plant compounds have been shown to be important in location of host root systems by other species of insects that live in the soil but have not yet been implicated in host location by *Diabrotica* larvae (Jones and Coaker, 1978; Hsiao, 1985).

A number of volatiles from corn roots have been identified but have not been tested behaviorally with western corn rootworm larvae. Buttery and Ling

(1985) used the solid adsorbant Tenax to collect volatiles from the roots of young (60 cm high) and mature corn plants (2 m high). The principal volatiles recovered were sesquiterpene hydrocarbons that included beta-caryophyllene, logifolene, bazzanene (tentative), cyclosativene, alpha-ylangene, and a major hydrocarbon that was not identified. Volatile secondary compounds have also been identified from corn leaves (Buttery and Ling, 1984), buds (Thompson et al., 1974), silks (Flath et al., 1978; Cantelo and Jacobson, 1979), tassels (Buttery et al., 1980), and from husks and kernels (Buttery et al., 1978).

A review of the literature on volatile semiochemicals for soil insects indicates that two groups of compounds with very disparate vapor pressures have been considered. One group comprises gases such as carbon dioxide, nitrogen, oxygen, methane, and ethylene (see Jones and Coaker, 1977; Doane et al., 1975, and references therein). Carbon dioxide alone is attractive to a number of soil invertebrates, including insect larvae (Klingler, 1957, 1958, 1965, 1966; Doane et al., 1975; Städler, 1971, 1972; Paim and Beckel, 1963a, b; Meeking et al., 1974; Jones and Coaker, 1977), insect adults (Paim and Beckle, 1963a, b), mites (Moursi, 1962, 1970), chilopods (Moursi, 1970), nematodes (Pline and Dusenbery, 1987; Prot, 1980; Johnson and Viglierchio, 1961; Klingler, 1961, 1963, 1965), and bacteria (Scher et al., 1985). The other group of volatile semiochemicals for soil invertebrates comprises secondary compounds with considerably lower vapor pressures that can easily be collected by tissue extraction, steam distillation, or air extraction with solid adsorbants (Jones and Coaker, 1978; Hsiao, 1985).

Volatile secondary compounds important in underground attraction of larvae have been chemically identified for several insect species (all of them Diptera), including the carrot rust fly, *Psila rosae* (F)., the onion maggot, *Delia antiqua* (Meigen), the turnip maggot, *Delia floralis* (Fallen), and the related species *Delia brassicae* (Wiedemann). For the carrot rust fly *Psila rosae*, an insect that has a high degree of host specificity for certain species of Umbelliferae, carbon dioxide is one of several attractants that have been found for underground larvae (Jones and Coaker, 1977, 1979). Of 45 volatile secondary compounds that have been identified from fresh carrot root, five have been found to be most attractive to carrot rust fly larvae: bornyl acetate, 2,4-dimethyl styrene, alpha-ionone, beta-ionone, and biphenyl (Ryan and Guerin, 1982, Guerin and Ryan, 1984). Underground larvae of the onion maggot *Delia antiqua* are able to orient to onion volatiles (although host selection is primarily made by the ovipositing females). The larvae showed strong attraction to 27 synthetic sulfides, disulfides, and mercaptans in a laboratory bioassay (Matsumoto and Thorsteinson, 1968; Matsumoto, 1970, Soni and Finch, 1979), although only some of these compounds are known to be released from onions (Carson and Wong, 1961. Rotting onions are more attractive to larvae (and to ovipositing females) than are fresh onions, and it has been established that this is due to

the activity of *Klebsiella* bacteria, although it is not yet clear which volatiles of *Klebsiella*-infestated onions are responsible for the enhanced attraction (Ikeshoji et al., 1980, 1982, Yamada et al., 1981). Underground larvae of some *Delia* species feed only on certain species of Cruciferae. Larvae of the turnip maggot *Delia floralis* (Rygg and Somme, 1972) are attracted to allyl isothiocyanate, a characteristic volatile of many Cruciferae. A source of mustard oil glucosides is attractive (perhaps because of volatile degradation products) to the related species *Delia brassicae* (Finch and Skinner, 1974).

Volatiles have been shown to play a role in host location by underground larvae of several other insect species, but the compounds involved have not yet been chemically characterized. The false wireworm *Eleodes suturalis* (Say) was attracted to volatiles in an airstream passed over germinating wheat (Calkins et al., 1967). Sutherland (1972) found that subterranean larvae of the scarab beetle *Costelytra zealandica* (White) were attracted to fresh perennial ryegrass root in a laboratory bioassay. Neonate larvae of the clover root curculio *Sitona hispidulus* (F.) are attracted to roots of alfalfa *Medicago sativa* L. and red clover *Trifolium pratense* L. and are attracted in particular to *Rhizobium* nodules on the root systems (Wolfson, 1987).

Adults of some insect species have also been shown to orient underground to volatile secondary plant compounds, either for feeding or for oviposition. Underground adults of the scolytid *Hylastinus obscurus* Marsham, the clover root borer, feed on the diseased roots of red clover, *Trifolium pratense* (Leath and Byers, 1973), and behavioral bioassays for attraction have been performed with organic extracts of diseased roots and with organic extracts of a solid adsorbant (Tenax) used for air extraction of diseased roots (Kamm and Buttery, 1984). Of the compounds identified from diseased root tissue, the most attractive in a laboratory bioassay were ethyl laurate, ethyl benzoate, estragole, chavicol, pentadecanal, hexadecanal, and hexanoic acid. These compounds were not attractive in the field when presented alone or in combination (Kamm and Buttery, 1984). Adults of the pine weevil *Hylobius abietis* (L.) (Nordlander et al., 1986) and of the scolytid *Hylastes nigrinus* (Mann.) (Rudinsky and Zethner-Moller, 1967) locate conifer roots suitable for oviposition by using host volatiles diffusing through the soil.

The basis for host specificity in western corn rootworm larvae has not yet been determined. *Diabrotica virgifera virgifera* is an oligophagous species, feeding only on certain species of grasses. Branson and Ortman (1967, 1970) made a rigorous survey of the host plants suitable for larval development. Of 44 grass species tested, 18 supported rootworm larval growth for at least 10 days. No larvae survived 10 days on any broad-leaf species tested. In choice tests between cut roots of corn and of nonhost broad-leaved plants in a surface olfactometer, Branson (1982) found that significantly more western corn rootworm larvae were attracted to roots of corn than to roots of soybean, squash,

or sunflower (no difference was found for tomato roots vs. corn roots). In tests with a variety of grass species, many nonhost grass species were as attractive as corn or more so. Even the nonhost grass sorghum was found to be attractive (Branson, 1982) and to elicit feeding from western corn rootworm larvae (Branson et al., 1969), despite the fact that the roots contain cyanogenic glycosides that are lethal to the larvae. These results indicate that the olfactory cues used by western corn rootworm larvae in host location may be restricted to a range of grass species, limiting the amount of searching that must be done in the soil, but are not rigorously restricted to the grass species on which western corn rootworm larvae occur. We have shown that a blend of volatile compounds from corn seedlings is attractive to western corn rootworm larvae, and if these compounds are limited to some members of the Gramineae, this may provide a basis for olfactory discrimination by western corn rootworm larvae between grasses and broad-leaved plants.

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