Structure-Activity Study with Haloalkane Attractants of Western Corn Rootworm (Coleoptera: Chrysomelidae) Larvae Using a Behavioral Bioassay [1]

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ABSTRACT A two-choice laboratory behavioral bioassay was used to compare the dose-dependent responses of second-instar western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larvae to a series of structurally-related haloalkanes, including ones with different halogens, degree of halogen substitution, chain length, and degree of saturation. Disubstituted bromine and iodine analogs of dichloromethane attracted larvae at all doses tested, including 0.5, 1.0, 2.0, and 4.0 mg. Dibromomethane attracted significantly more larvae than dichloromethane at the lowest dose tested (0.5 mg). Analogs of dichloromethane with more chlorine substitutions attracted significantly fewer larvae than dichloromethane at most doses tested except for chloroform, which attracted significantly more larvae than dichloromethane at the lowest dose tested (0.5 mg). Although larvae were repelled by the two highest doses of 1,1-dichlorobutane tested (2.0 and 4.0 mg), orthogonal contrasts revealed no trend in responses of larvae to increasing doses of it or any of the other chain length analogs tested, 1,1-dichloroethene is an unsaturated analog of 1,1-dichloroethane, and orthogonal contrasts revealed a positive linear trend for responses of larvae to increasing doses of it.

KEY WORDS *Diabrotica virgifera virgifera,* Coleoptera, Chrysomelidae, dichloromethane, carbon dioxide, semiochemicals, attraction, synthetic analogs, host-finding, bioisosterism

Annual crop losses and treatment costs associated with activity of corn rootworms have been estimated at $1 billion by Metcalf (1986). The larvae are oligophagous, but corn is the most favorable host for their development (Branson and Ortman 1970). Consequently, larvae are regarded as the most immediate threat to fields because plants on which they feed are undersized and frequently lodge after heavy rains, rendering mechanical harvest difficult (Metcalf and

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Metcalf 1993). More land is treated with insecticide for corn rootworms than for any other agricultural pests in the United States (Suguiyama and Carlson 1985). Although their management has relied primarily upon soil insecticides, the need for alternative approaches has been expressed because of concerns for the environment.

In response to this need, attractants of diabroticite larvae have been sought as an objective in the development of improved methods for delivering insecticides to their target (Hibbard et al. 1995). Formulating these attractants with relatively smaller amounts of insecticide has been considered one approach to pest management (Hibbard et al. 1995), and many materials have already been reported to influence host-finding of diabroticite larvae. Carbon dioxide, which is released from corn roots (Harris and van Bavel 1957, Massimino et al. 1980), has previously been demonstrated to attract first- and second-instar (western corn rootworm larvae (Strnad et al. 1986, Hibbard and Bjostad 1988). A carbon dioxide source combined with an extract of germinating corn seedlings was reported to attract significantly more western corn rootworm larvae than carbon dioxide alone (Hibbard and Bjostad 1988, Hibbard and Bjostad 1990). Some components of these extracts have been identified, and they include 6-methoxy-2-benzoxazolinone (MBOA) (Bjostad and Hibbard 1992), stearic acid, oleic acid, and linoleic acid (Hibbard et al. 1994). Experiments concerning the activity of each component in an eluotropic series of materials revealed that western corn rootworm larvae were even attracted by dichloromethane (Jewett and Bjostad 1996).

That dichloromethane has many commercially available synthetic analogs, including ones with different halogens, degree of halogen substitution, chain length, and degree of saturation, presents an opportunity to study the effect of its specific structural parameters on the behavior of western rootworm larvae. We predicted that many of the analogs would still attract larvae, but that attraction would decline significantly with increasing structural changes. Understanding the various physical parameters inherent to dichloromethane and related compounds that are responsible for attraction of western corn rootworm larvae will facilitate identification or development of other materials that may be employed for their management.

Materials and Methods

Western corn rootworm larvae. Multiple generations of second-instar western corn rootworm larvae 5 to 7 days after hatching and 4 to 6 mm long were used for our experiments between 1992 and 1995. The results of initial bioassays suggested that their behavior was not significantly different from that of first-instar larvae, which is the host-seeking stage, and because second-instars are easier to manipulate and are less susceptible to desiccation in laboratory bioassays, we used them. Larvae were obtained from our colony, which was established and maintained using methods described by Jackson (1986) as modified by Hibbard and Bjostad (1988), and which has been supplemented occasionally with eggs of a non-diapausing strain from French Ag. Research Inc. (Lamberton, MN 56152).

Bioassay apparatus. Details regarding construction of the bioassay apparatus are provided in Jewett and Bjostad (1996). Briefly, it was comprised of an arena, a stand for the arena, and dispensers for the volatile compounds (Fig. 1). The arena was a plastic Petri dish with two holes in the bottom of it opposite from one another, and it was lined with moist filter paper to prevent larvae from desiccating. The arena rested on a stand of 110 lb paper, and a 4 ml glass shell vial was aligned beneath each hole. Each vial was sitting in a smaller Petri dish filled with water, which captured larvae that might escape.

Compounds tested (Fig. 2). Dichloromethane was purchased from Mallinckrodt Specialty Chemicals (Chesterfield, MO 63017-1077). Dibromomethane, diiodomethane, 1,1-dichloroethane, 1,1-dichloropropane, chloroform, bromoform, carbon tetrachloride, and 1,1-dichloroethene were purchased from Aldrich Chemical Company (Milwaukee, WI 53233). 1,1-dichlorobutane was purchased from Pfaltz and Bauer Chemical Company (Flushing, NY 11352).

Two-choice bioassay. Ten western corn rootworm larvae were used in each bioassay. They were selected randomly from our colony, and were transferred with a small, moist camel-hair paint brush to the center of a polystyrene cap from a 4 ml shell vial. The cap and larvae were kept on a moist paper towel to prevent desiccation until the apparatus was prepared. The cap was then placed over the larvae after they were inverted into the center of the arena, and remained there until commencement of the bioassay.

Dichloromethane had been tested in preliminary experiments, and because the four amounts, including 0.5, 1.0, 2.0, and 4.0 mg, were observed to elicit significant attraction (unpublished data), we used them to facilitate comparisons in the present study. An amount of any one compound was introduced in pure form to the bottom of a 4 ml glass shell vial dispenser on one side of the arena with a 10 µl glass, fixed-needle syringe (Alltech Associates, Inc., Deerfield, IL 60015). The dispenser on the other side of the arena remained empty and served as a control. The cap covering larvae was removed, and the lid was placed over the Petri dish arena.

A bioassay testing attraction to the single dose of a compound lasted 30 min and when it was finished, the number of larvae recovered from the bottoms of the two dispensers and from the moats around them was recorded. This procedure was conducted in the laboratory under fluorescent lamps at a temperature between 23 and 27 C. There were constraints upon the availability of larvae, so compounds were tested randomly until a generation was exhausted, and then larvae from subsequent generations were employed. Each western corn rootworm larva was used only once for testing. Arenas and dispensers also were used only once.

Statistical analyses. Data obtained from the bioassays were analyzed with SAS (SAS Institute Inc., Cary, NC 27512-8000). A t test was performed to determine if any differences existed between the average number of larvae attracted to the treatment dispenser and the control dispenser. For comparing the activity of compounds, including dichlormethane, in each group of analogs, an ANOVA was conducted using differences between the number of larvae recovered from the treatment and control dispensers. Analyses of the activity for compounds in each group of analogs that yielded significant P values (< 0.05) were compared dose-wise with Duncan's multiple range test. Orthogonal contrasts of average larval response to increasing doses of each compound were made, identifying any linear or quadratic trends that may exist.

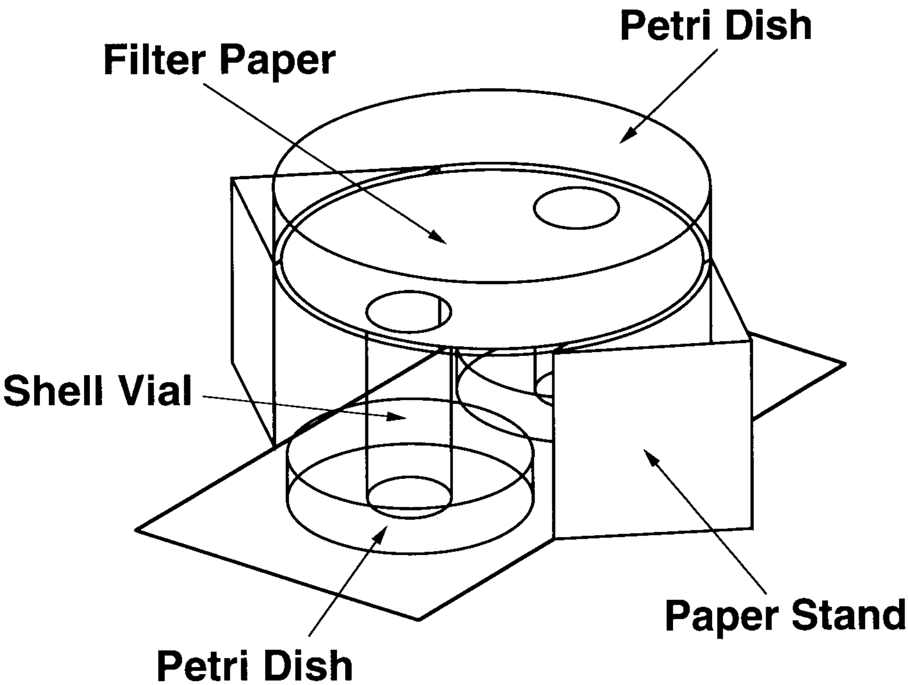


Fig. 1. Schematic of the two-choice bioassay apparatus and its five components, including three Petri dishes, two shell vials, filter paper lining, and a paper stand.

Results

Western corn rootworm larvae were attracted to all tested doses of compounds in the series of analogs with different halogens, including dichlormethane, dibromomethane and diiodomethane (Fig. 3). Orthogonal contrasts of average larval responses to increasing doses of dichloromethane and dibromomethane revealed a quadratic (convex) trend and a linear (negative) trend, respectively. Orthogonal contrasts of larval responses to increasing doses of diiodomethane revealed no trend. At the 0.5 mg dose, dibromomethane attracted significantly more larvae than dichloromethane and diiodomethane, which attracted an equal number of larvae. At the 1.0 mg dose, dichloromethane and diiodomethane, which attracted an equal number of larvae, but dichloromethane attracted significantly more larvae than diiodomethane. Dibromomethane and diiodomethane attracted an equal number of larvae. At the 2.0 mg dose, dichloromethane attracted significantly more larvae than dibromomethane and diiodomethane, which attracted an equal number of larvae. At the 4.0 mg dose, dichloromethane, dibromomethane, and diiodomethane attracted an equal number of larvae.

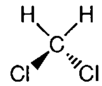
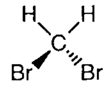
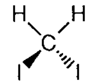
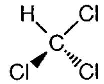
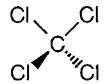
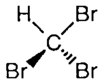
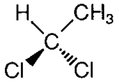
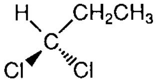
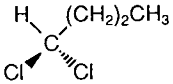
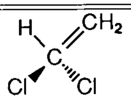
Analogs	Compound Name	Formula	Structure
Halogen Atoms	Dichloromethane	CH_2Cl_2	
	Dibromomethane	CH_2Br_2	
	Diiodomethane	CH_2I_2	
Number of Chlorine Atoms	Chloroform	$CHCl_3$	
	Carbon Tetrachloride	CCl_4	
Number of Bromine Atoms	Bromoform	$CHBr_3$	
Chain Length	1,1-Dichloroethane	$C_2H_4Cl_2$	
	1,1-Dichloropropane	$C_3H_6Cl_2$	
	1,1-Dichlorobutane	$C_4H_8Cl_2$	
Saturation	1,1-Dichloroethene	$C_2H_2Cl_2$	

Fig. 2. Names, formulas, and structures of the synthetic compounds tested in the two-choice bioassay.

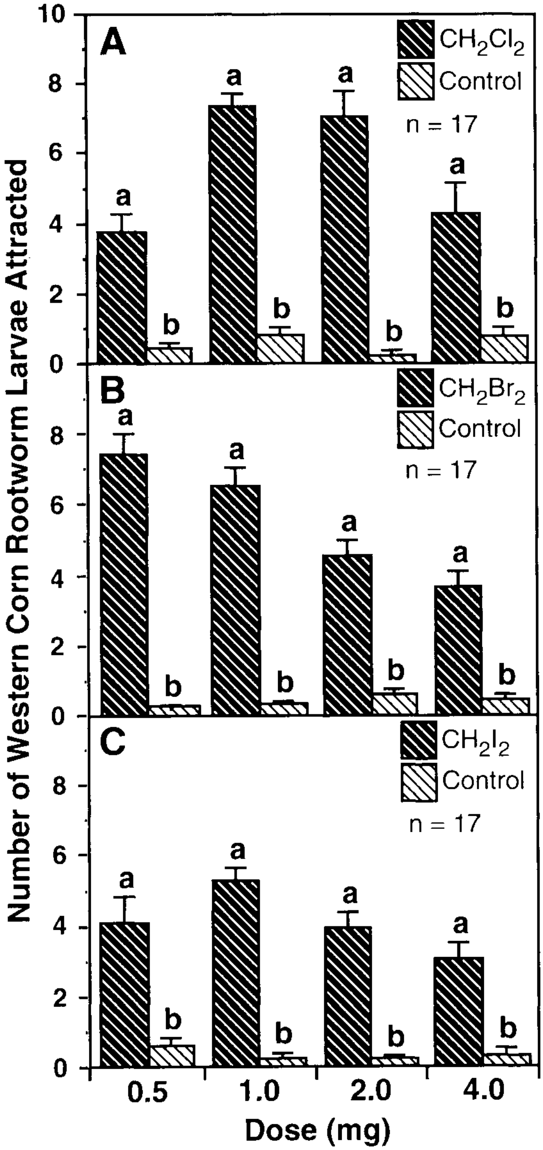


Fig. 3. Behavioral responses of western corn rootworm larvae *Diabrotica virgifera virgifera* to varying di-halogenated methanes, including A. dichloromethane (CH_2Cl_2), B. dibromomethane (CH_2Br_2), and C. diiodomethane (CH_2I_2). The n = number of times a dose of each compound was tested for attraction of ten larvae in a bioassay. Bars on columns represent standard error values. Average responses to control and treatment sides within a dose denoted by the same letter are not significantly different as determined with a t test ($P = 0.05$).

Significantly more western corn rootworm larvae were attracted to all tested doses of chloroform than to controls (Fig. 4). Orthogonal contrasts of average larval response to the four increasing doses of chloroform revealed a linear (negative) trend. Western corn rootworm larvae were attracted to only the 1.0 and 4.0 mg doses of carbon tetrachloride, and orthogonal contrasts revealed no trend in responses of larvae to the four increasing doses of it. Regarding the 0.5 mg dose of compounds chloroform attracted significantly more larvae than dichloromethane, which attracted significantly more larvae than carbon tetrachloride. At the 1.0 mg dose, dichloromethane attracted significantly more larvae than chloroform, which attracted significantly more larvae than carbon tetrachloride. The 2.0 mg dose of dichloromethane attracted significantly more larvae than the 2.0 mg dose of chloroform, which attracted significantly more larvae than the 2.0 mg dose of carbon tetrachloride. At the 4.0 mg dose, dichloromethane, chloroform, and carbon tetrachloride attracted an equal number of larvae.

Dibromomethane and bromoform attracted larvae at all doses tested (Fig. 5). At the 0.5 and 1.0 mg doses, dibromomethane attracted significantly more larvae than bromoform. At the 2.0 and 4.0 mg doses, dibromomethane and bromoform attracted an equal number of larvae. There was no trend in the average response of larvae to increasing doses of bromoform.

All tested doses of 1,1-dichloroethane attracted significantly more western corn rootworm larvae than controls (Fig. 6). Western corn rootworm larvae were attracted only to the 4.0 mg dose of 1,1-dichloropropane. None of the tested doses of 1,1-dichlorobutane were attractive, but the two highest doses were significantly repellent. Orthogonal contrasts revealed no trend in the average response of larvae to increasing doses of any compound in the series of chain length analogs except dichloromethane. At the 0.5 mg dose, dichloromethane attracted significantly more larvae than 1,1-dichloroethane. At the 1.0 mg dose, dichloromethane and 1,1-dichlorobutane attracted an equal number of larvae. 1,1-dichloropropane and 1,1-dichlorobutane elicited no response from larvae at either the 0.5 or 1.0 mg doses. The 2.0 mg dose of dichloromethane attracted significantly more larvae than 1,1-dichloroethane. 1,1-dichloropropane was unattractive at the 2.0 mg dose, and 1,1-dichlorbutane was significantly repellent. At the 4.0 mg dose, dichloromethane was not significantly more attractive than 1,1-dichloroethane, but it was significantly more attractive than 1,1-dichloropropane. 1,1-dichloroethane was not significantly more attractive than 1,1-dichloropropane at the 4.0 mg dose, and 1,1-dichlorobutane was significantly repellent.

Larvae were attracted to all but the lowest dose of 1,1-dichloroethene (Fig. 7). Orthogonal contrasts of average larval response to increasing doses of 1,1-dichloroethene revealed a linear trend (positive). The 0.5 and 1.0 mg doses of 1,1 dichloroethane attracted significantly more larvae than 1,1-dichloroethene. At the 2.0 mg dose, 1,1-dichloroethane and 1,1-dichloroethene attracted equal numbers of larvae. At the 4.0 mg dose, 1,1-dichloroethene attracted significantly more larvae than 1,1-dichloroethane.

Discussion

Ways of reducing dependency upon insecticides have been and continue to be sought. Consequently, formulations of attractants with insecticides are commercially

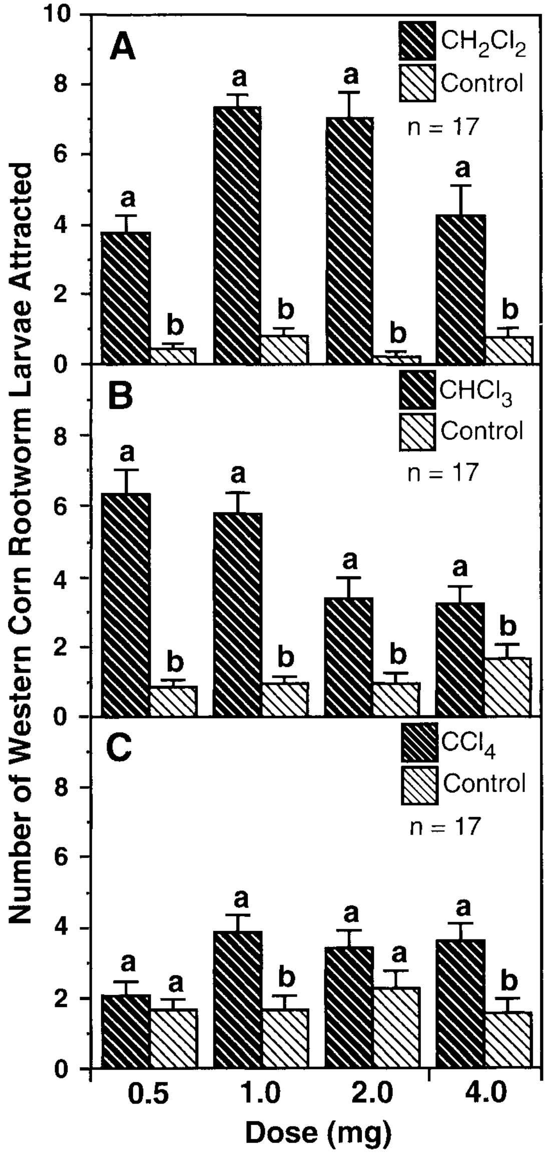


Fig. 4. Behavioral responses of western corn rootworm larvae *Diabrotica virgifera virgifera* to chlorinated alkanes with a single carbon and a varying number of chlorine atoms, including A. dichloromethane (CH_2Cl_2), B. chloroform ($CHCl_3$), and C. carbon tetrachloride (CCl_4). The n = number of times a dose of each compound was tested for attraction of ten larvae in a bioassay. Bars on columns represent standard error values. Average responses to control and treatment sides within a dose denoted by the same letter are not significantly different as determined with a t test ($P = 0.05$).

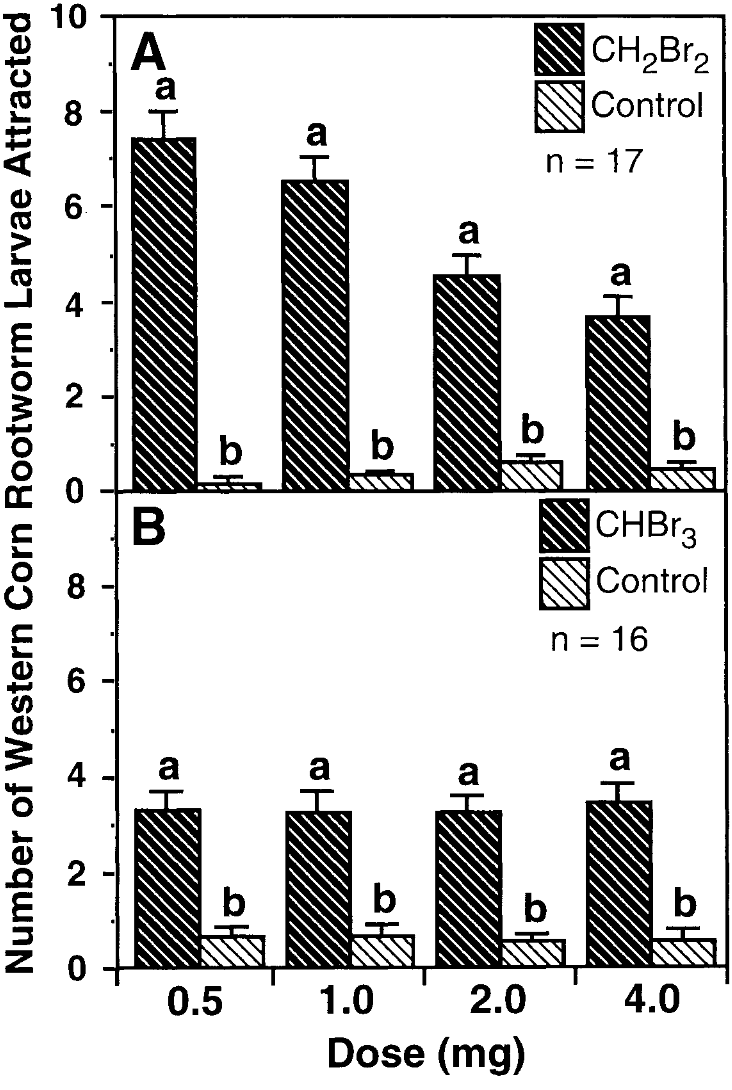


Fig. 5. Behavioral responses of western corn rootworm larvae *Diabrotica virgifera virgifera* to brominated alkanes with a single carbon and a varying number of bromine atoms, including A. dichloromethane (CH_2Br_2), B. bromoform ($CHBr_3$), The n = number of times a dose of each compound was tested for attraction of ten larvae in a bioassay. Bars on columns represent standard error values. Average responses to control and treatment sides within a dose denoted by the same letter are not significantly different as determined with a *t* test ($P = 0.05$).

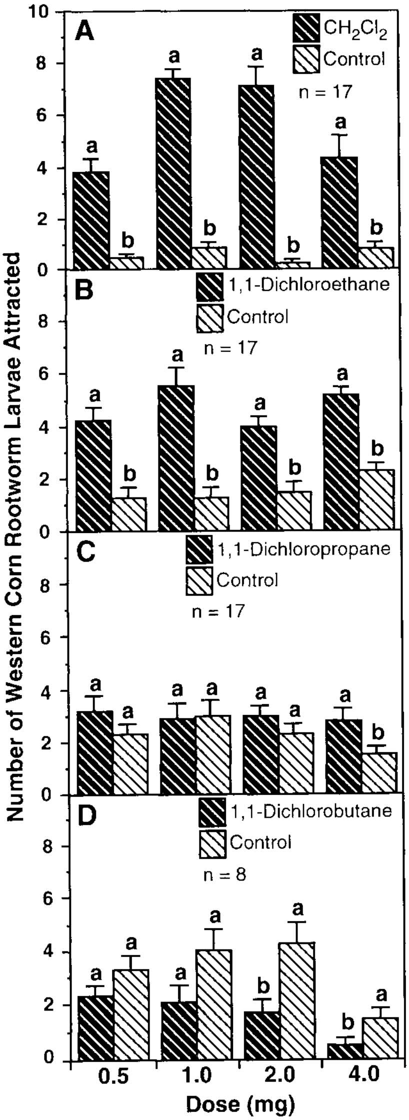


Fig. 6. Behavioral responses of western corn rootworm larvae *Diabrotica virgifera virgifera* to 1,1-chlorinated alkanes with a different chain-lengths, including A. dichloromethane (CH_2Cl_2), B. 1,1-dichloroethane, C. 1,1-dichloropropane, and D. 1,1-dichlorobutane. The n = number of times a dose of each compound was tested for attraction of ten larvae in a bioassay. Bars on columns represent standard error values. Average responses to control and treatment sides within a dose denoted by the same letter are not significantly different as determined with a *t* test (P = 0.05).

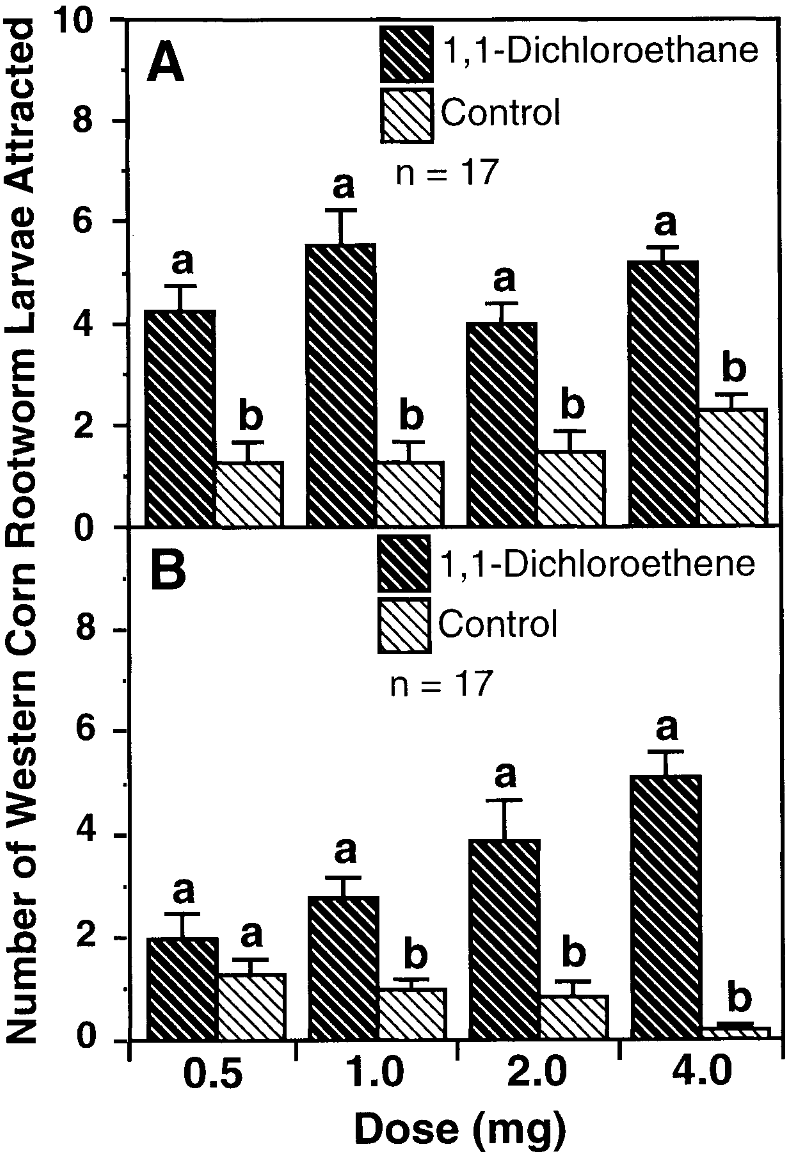


Fig. 7. Behavioral responses of western corn rootworm larvae *Diabrotica virgifera virgifera* to 1,1-chlorinated, two-carbon containing compounds including A. 1,1-dichloroethane and B. 1,1-dichloroethene. The n = number of times a dose of each compound was tested for attraction of ten larvae in a bioassay. Bars on columns represent standard error values. Average responses to control and treatment sides within a dose denoted by the same letter are not significantly different as determined with a *t* test ($P = 0.05$).

available for several insects and are being evaluated for others (Inscoe et al. 1990, Bjostad et al. 1993). Formulations of MBOA and the soil insecticide chlorethoxyphos have already been evaluated for their activity against western corn rootworm larvae, but the presence of this semiochemical did not consistently enhance the efficacy of chlorethoxyphos under variable field conditions (Hibbard et al. 1995).

Anticipating the identification of other attractants, a two-choice laboratory behavioral bioassay was used in the present study to compare dose-dependent responses of second-instar western corn rootworm larvae to a series of structurally-related haloalkanes, including ones with different halogens, degree of halogen substitution, chain lengths, and degree of saturation. All the structural parameters considered in this study had a measurable influence on activity of the analogs.

We predicted a decline in the attraction by analogs with graded dissimilarity in structure to dichloromethane. In general, our predictions were supported by the results of this study. We did not predict, however, that the lowest tested doses of dibromomethane and chloroform tested (0.5 mg) would attract significantly more larvae than the lowest dose of dichloromethane tested (0.5 mg). These results suggest that the optimal dose for attraction of larvae for these two compounds is lower than the ones we tested. Nor did we expect the two highest doses of 1,1-dichlorobutane tested (2.0 and 4.0 mg) to repel larvae. These results suggest that of all the structural parameters considered in our study, chain length had the greatest influence on activity. The degree of halogen substitution also influenced activity as illustrated by the general graded decline in attraction of chlorinated and brominated alkanes with increasing degree of halogen substitution. The type of halogen apparently did not influence activity much because all doses of all three analogs, including dichloromethane, dibromomethane, and diiodomethane were significantly attractive. Degree of saturation was also considered in this study, and the absence of a positive linear trend in larval response to increasing doses of 1,1-dichloroethane, but the presence of one to 1,1-dichloroethene suggests that 1,1-dichloroethene might demonstrate appreciable attraction at even higher doses.

Interaction of multiple chemical and physiological effects may have been interfering with the apparent attraction of larvae. For example, dichloromethane and dibromomethane vaporized immediately upon introduction to the dispensers, but diiodomethane did not, and an unmeasured amount remained at the conclusion of each bioassay. Therefore, behavioral activity of the halogen analogs may have been confounded by differences in their volatility. In addition to differences in volatility that may have interfered with our results, some compounds were acutely toxic to larvae at higher doses and visibly affected their performance. Some of the compounds were still attractive despite their toxicity. Multiple generations of larvae were employed for this study, and we assumed that there were no physiological differences among them which might have affected any differential response to semiochemicals.

In addition to some of the haloalkanes, carbon dioxide has been reported to attract western corn rootworm larvae, and many other agriculturally or medically important invertebrates (Kellogg 1970, Meeking et al. 1974, Prot 1980, Warnes and Finlayson 1986, Bogner 1992, Steullet and Guerin 1992, Perritt et al. 1993, Rasch and Rembold 1994, Jewett and Bjostad 1996). Dichloromethane, which has also been demonstrated to attract other corn rootworms in laboratory behavioral bioassays (Jewett and Bjostad 1996), and carbon dioxide may behave analogously when they interact with chemoreceptors on larvae as a consequence of their structural similarity (Jewett and Bjostad 1996). Alternatively, carbon dioxide may become oxidized in the antennal hemolymph, and bicarbonate may actually be received by chemoreceptors in the dendrites (Jewett and Bjostad 1996). Halogenated analogs of semiochemicals have been investigated previously for their influence on the behavior of several adult Lepidoptera (Lucas et al. 1994, Svatos et al. 1990, Camps et al. 1984, McLean et al. 1989, Prestwich et al. 1986, Bengtsson et al. 1990, Linn et al. 1992). None of these semiochemicals are as ubiquitous as carbon dioxide, though.

Confirming a physiological relationship between behaviorally active haloalkanes and carbon dioxide could facilitate the development or identification of new chemical attractants not only for western corn rootworm larvae, but for other agriculturally or medically important invertebrates that rely on carbon dioxide for host-finding. Presently, many of the materials considered in this study are moderately toxic to humans (Sax 1968), and might not enjoy commercial application. A proposed order of increasing toxicity to humans of some materials considered in this study is dichloromethane, chloroform, carbon tetrachloride, dichloropropane, and dichloroethane (Sax 1968). Furthermore, because of the effect they may have on the behavior of invertebrates that use carbon dioxide for host-finding, results obtained in behavioral studies after using materials like dichloromethane or chloroform to extract, isolate, identify or purify semiochemicals should be regarded critically.

Acknowledgments

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