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RESPONSE OF *Diabrotica virgifera virgifera*,[1] *D. v. zeae*,[1] AND *D. porracea*[1] TO STEREOISOMERS OF 8-METHYL-2-DECYL PROPANOATE[2]

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Abstract—The four stereoisomers of 8-methyl-2-decyl propanoate were tested in the United States and Mexico for attractiveness to *Diabrotica virgifera virgifera* LeConte, the western corn rootworm, *D. v. zeae* Krysan and Smith, the Mexican corn rootworm, and *D. porracea* Harold. Males of *D. v. virgifera* and *D. v. zeae* responded strongly to the (2R,8R)-isomer and secondarily to (2S,8R), while *D. porracea* responded exclusively to the (2S,8R)-isomer. The (2S,8S)- and (2R,8S)-isomers were inactive in all tests. Synergism or inhibition was not detected when various mixtures of the isomers were tested with *D. v. virgifera*. These phenomena were not tested with *D. v. zeae* and *D. porracea*.

Key Words—Coleoptera, Chrysomelidae, *Diabrotica*, western corn rootworm, Mexican corn rootworm, sex pheromone, stereospecificity.

INTRODUCTION

The sex pheromone of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (WCR), was recently shown to be the propionate ester of 8-methyl-2-decanol (Guss et al., 1982). This compound was the first pheromone to be identified from the family Chrysomelidae, and, like most known coleopteran pheromones, it is chiral (two

[1]Coleoptera: Chrysomelidae.
[2]Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

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asymmetric centers). Lack of sufficient quantities of pure pheromone precluded elucidation of the specific configuration of the natural pheromone, but at low doses (about 350 ng), racemic synthetic 8-methyl-2-decyl propanoate dispensed from cotton wicks was equal to a similar amount of pure natural pheromone in its ability to attract feral WCR males to baited traps (Guss et al., 1982).

Racemic 8-methyl-2-decyl propanoate has been shown to be attractive to a number of *Diabrotica* spp. (Guss et al., 1982). The Mexican corn rootworm, *D. virgifera zeae* Krysan and Smith (MCR), is attracted to racemic 8-methyl-2-decyl propanoate in a manner identical to that of the WCR with respect to time-of-day arrival at baited traps. These two taxa are subspecies and have been shown to intergrade in those areas where their ranges contact (Krysan et al., 1980). Another congener, *D. porracea* Harold, which cohabits the same areas as the MCR in this study, also responds to the racemic synthetic pheromone (Guss et al., 1982), but no information is available concerning reproductive interaction between the MCR and *D. porracea*.

Synthesis of the four stereoisomers of 8-methyl-2-decyl propanoate at high isomeric purity has been accomplished (Sonnet and Heath, 1982; Carney et al., unpublished), and here we report the response of the WCR, MCR, and *D. porracea* to these individual isomers. The data yield an insight into the stereochemical composition of the natural pheromone produced by WCR females.

METHODS AND MATERIALS

Syntheses of the two stereoisomers used in this study involved a convergent approach in which two fragments, each containing one asymmetric center, were joined to complete the required sequence. The configurational purity of each fragment, which assured the configurational purity of the final product, was determined absolutely by GLC and/or HPLC using diastereomeric derivatives (Sonnet and Heath, 1982; Carney et al., unpublished). The isomeric purity of the target isomers in these preparations ranged from 97.4 to 98.8% (Table 1).

Usually, the individual isomers or specific mixtures were diluted to appropriate concentrations in hexane and dispensed into the "cup" portion of rubber septa (A.H. Thomas No. 8753-D22) in 50-μl quantities to produce the pheromone sources. In Mexico cotton wicks (30 $\times$ 10-mm diameter) were used as dispensers to compare the activities of unfractionated WCR volatiles with synthetic pheromone because the volume of the unfractionated preparation (1 ml) was too large to be accommodated by rubber septa. When cotton wicks were used, synthetic pheromone was diluted with hexane containing 10% trioctanoin to regulate coarsely volatilization of the phero-

TABLE 1. ISOMERIC COMPOSITION OF 8-METHYL-2-DECYL PROPANOATE PREPARATIONS

Excess isomer	% isomeric composition			
	2*S*,8*S*	2*R*,8*S*	2*S*,8*R*	2*R*,8*R*
2*S*,8*S*	98.8	0.4	0.8	—
2*R*,8*S*	0.9	98.3	—	0.8
2*S*,8*R*	1.9	—	97.4	0.7
2*R*,8*R*	—	1.9	0.3	97.8

mone (Guss, 1976). Collection of unfractionated volatiles from WCR females is described elsewhere (Guss et al., 1982).

Field traps for studies with the WCR consisted of plastic (PVC) cylinders (45 × 5-cm diameter) wrapped in waxed paper and coated with Stickem Special. The traps were placed between corn rows on wooden stakes (2.5 cm^2 × 1.3 m) equipped with pegs to control trap height. The trap height at the top was normally first ear height or about 1 m, and the distance between traps was about 20 m. For studies with the MCR and *D. porracea* in Mexico, traps consisted of wax-coated cylindrical drink cups (11.2 × 9.0-cm diameter) coated inside and out with Stickem Special. Pheromone sources were anchored to the tops of the traps, and the traps were monitored daily.

Experiments involving the WCR were done near Brookings, S.D., during August 1981, and those involving the MCR and *D. porracea* were done near Ameca in the state of Jalisco, Mexico, during September and October 1981.

RESULTS AND DISCUSSION

Western Corn Rootworm. Under field trapping conditions, the response threshold of racemic 8-methyl-2-decyl propanoate for the WCR is about 10 ng when dispensed from rubber septa (Guss et al., 1982). When assessing the activity of the individual isomers, we at first chose a loading of 250 ng in order to obviate as much as possible influences that might be attributable to the small amounts of nontarget isomers in these preparations (see Table 1). Thus, in most cases with the 250-ng sources, the amount of any given nontarget isomer was less than that found in 10 ng of racemic pheromone. In the case of (2S,8S) in (2S,8R) and 2R,8S) in (2R,8R), the amount of nontarget isomers was slightly more than that found in 10 ng of the racemate.

With the 250-ng sources, data in Table 2 show that (2R,8R) was the preferred configuration, being about four times as active as (2S,8R). Both (2S,8S) and (2R,8S) were inactive. With 1-μg sources, (2R,8R) was about twice as active as (2S,8R), while (2S,8S) and (2R,8S) were again inactive.

TABLE 2. RESPONSE BY *D. v. virgifera* MALES TO STEREOISOMERS OF 8-METHYL-2-DECYL PROPANOATE

Isomer	Mean No. of males/trap ± SD[a]	
	250 ng	1 μg
Racemate	104.3 ± 19.7 b	—
2*R*,8*R*	148.3 ± 44.7 a	607.3 ± 66.1 a
2*S*,8*R*	39.0 ± 5.3 c	306.7 ± 93.3 b
2*R*,8*S*	2.0 ± 1.0 d	8.0 ± 2.0 c
2*S*,8*S*	4.0 ± 3.5 d	10.7 ± 3.2 c
Solvent blank	6.3 ± 1.2 d	5.3 ± 2.9 c

[a]Means followed by different letters are significantly different at the 0.05 level of confidence (Duncan's multiple range).

The difference in the ratio of trapped insects between (2R,8R) and (2S,8R) at 250-ng and 1-μg loadings may not be meaningful because the experiments were carried out at different locations with different population densities. Also, 250 ng of (2S,8R) may be approaching the response threshold for this isomer under these conditions. Thus, at 250 ng the mean for (2S,8R) was significantly different at the 0.05 level but was not significantly different from (2R,8S), (2S,8S), and the solvent blank at the 0.01 level.

The R configuration at carbon 8 appears to be critical, while either configuration at carbon 2 (site of the ester bond) at least partially satisfies the criteria for positive response for the WCR. Most chiral pheromones thus far identified contain asymmetric centers at or near functional groups (Silverstein, 1979); in several recent cases, however, specific configurations at centers relatively remote from functional groups of pheromones have been shown to be essential (Kraemer et al., 1981) or extremely important (Silverstein et al., 1980); Guss et al., 1983a) for attraction of target insects to baited traps.

The most active isomer, (2R,8R), was combined with the other three isomers, individually, at 1:1 ratios to determine if synergistic or inhibitory phenomena existed. The results in Table 3 indicate that the trap catches obtained with these mixtures were no different from those from traps baited with (2R,8R) only and that the other three isomers, including (2S,8R), neither synergize nor inhibit the response. In further studies involving the two independently active isomers, mixtures containing a constant amount of (2R,8R) plus up to 67% (2S,8R) [1 μg (2R,8R) + 2 μg (2S,8R)] produced captures (35.44 ± 11.22) insignificantly different from those of traps baited with (2R,8R) alone (27.75 ± 5.85).

A dose–response effect was demonstrated with (2R,8R) from 0.25 through 1000 μg, with the data in Table 4 being best characterized by the

TABLE 3. RESPONSE BY *D. v. virgifera* MALES TO MIXTURES OF 8-METHYL-2-DECYL PROPANOATE

Isomer mixture	Mean No. of males/trap ± SD[a]
1 μg 2*R*,8*R*	347.0 ± 123.5 a
1 μg 2*R*,8*R* + 1 μg 2*R*,8*S*	312.5 ± 70.4 a
1 μg 2*R*,8*R* + 1 μg 2*S*,8*R*	277.3 ± 76.2 a
1 μg 2*R*,8*R* + 1 μg 2*S*,8*S*	321.0 ± 92.0 a
Solvent blank	8.3 ± 1.5 b

[a]Means followed by different letters are significantly different at the 0.01 level of confidence (Duncan's multiple range).

equation $y = 136.49 + 90.32 \ln x$ ($R^2 = 0.97$). Notwithstanding the high R^2, the number of WCR captured in traps baited with 1 mg of (2R,8R) may not accurately reflect the number of beetles actually influenced by the source. Several meters downwind from those traps baited with 1 mg of (2R,8R), dramatic increases in beetle density and activity were observed, and it appeared that many of the beetles attracted to the area had ceased searching and were attempting to copulate with other males.

Mexican Corn Rootworm and D. porracea. We showed in an earlier report that both the MCR and *D. porracea* are attracted to traps baited with racemic 8-methyl-2-decyl propanoate (Guss et al., 1982). The response of these two species to the resolved isomers is shown in Table 5, Expt. I. The response of the MCR to (2R,8R) and (2S,8R) is virtually identical to that of the WCR. This was not surprising considering the close relationship of these two taxa (Krysan et al., 1980). The response of *D. porracea*, which cohabited this trap area in very low numbers, was limited exclusively to (2S,8R).

TABLE 4. DOSE-RESPONSE EFFECTS OF 8*R*-METHYL-2*R*-DECYL PROPANOATE ON TRAP CATCHES OF *D. v. virgifera*

Dose (μg)	Mean No. of males/trap ± SD
0.25	66.0 ± 18.5
1	123.3 ± 53.3
10	279.7 ± 41.7
100	524.3 ± 176.6
1000	811.7 ± 405.2
Solvent blank	1.7 ± 2.9

TABLE 5. RESPONSE BY *D. v. zeae* AND *D. porracea* TO STEREOISOMERS OF 8-METHYL-2-DECYL PROPANOATE

Isomer	Experiment I[a] (Mean No. of males/trap ± SD)		Experiment II[b] (Mean No. of males/trap ± SD)	
	D. v. zeae	*D. porracea*	*D. v. zeae*	*D. porracea*
2*R*,8*R*	33.3 ± 3.8	0	285 ± 102	1 ± 1.4
2*S*,8*R*	17.3 ± 4.6	17.0 ± 1.7	119 ± 5.7	35 ± 2.8
2*R*,8*S*	2 ± 1	0	—	—
2*S*,8*S*	0	0	—	—
D. v. virgifera volatiles	—	—	370 ± 98	0
Solvent blank	0	0	0	0

[a]Sept. 15–18, 1981, 10 μg/source dispensed from rubber septa.
[b]Oct. 20–23, 1981, 1 μg of (2R,8R) or (2R,8S) and an estimated 1 μg of active material in *D. v. virgifera* volatiles dispensed from cotton wicks.

At a later date, unfractionated volatiles from WCR females were compared with (2R,8R) and (2S,8R) in the same area (Table 5, Expt. II). As expected, the MCR responded to all three sources, but *D. porracea* again responded overwhelmingly to the synthetic (2S,8R)-isomer and not at all to the unfractionated WCR volatiles. Circumstances associated with this experiment suggest that the two *D. porracea* males captured on one of the (2R,8R) traps may not be accidental. The (2R,8R) preparation is known to contain 0.3% (2S,8R) (Table 1), and the inherently rapid release rate of pheromone from cotton wicks may have resulted in the release of (2S,8R) in quantities sufficient to attract *D. porracea* in the immediate trap area. The very low numbers of *D. porracea* in the trap area would tend to support this hypothesis; intense visual searches failed to produce a single, free-moving *D. porracea* adult except in areas immediately adjacent to traps baited with the (2S,8R) preparation.

The result that *D. porracea* responds specifically to (2S,8R) but also to racemic 8-methyl-2-decyl propanoate indicates that none of the other three isomers inhibits to extinction the response of *D. porracea* to (2S,8R)-8-methyl-2-decyl propanoate. Thus, the failure of *D. porracea* to respond to unfractionated volatiles (known to contain pheromone) from WCR females would indicate that (2S,8R) is not a component of the natural WCR pheromone, despite the fact that WCR males respond to the (2S,8R)-isomer. It would seem remote that either (2S,8S) or (2R,8S) would be a component of the natural WCR pheromone since neither displayed any discernable biological activity, either alone or in conjunction with (2R,8R). Therefore, the

data collected in this study would suggest that the sex pheromone produced by WCR females is exclusively (2R,8R)-8-methyl-2-decyl propanoate.

The untested possibility remains that the unfractionated WCR volatiles could contain a compound(s), other than any of the four pheromone isomers, that inhibits the response of *D. porracea* to the (2S,8R)-isomer, in which case (2S,8R) could be present in the WCR volatiles and be undetectable by the behavior of *D. porracea*. A partial negation of this possibility is available in the response to various pheromone preparations by another congener, *D. barberi* Smith and Lawrence. Thus, *D. barberi* responds strongly to WCR virgin females and unfractionated volatiles therefrom (Guss, 1976; Bartelt and Chiang, 1977), responds only to (2R,8R) among the individual isomers, and is strongly inhibited in its response to (2R,8R) by the presence of very small amounts of the (2S,8R)-isomer (Guss et al., unpublished).

In his review, Silverstein (1979) has listed the response of insects to chiral pheromones in nine possible categories. Assuming from our data that (2S,8R) is not a component of the natural pheromone of the WCR, the response of male WCR to (2S,8R) would place it in category 1, i.e., "The insect produces only a single enantiomer (stereoisomer) and it is more active than the other enantiomer (stereoisomer), which is an artifact." This category of response appears to be the most common among those insects using chiral pheromones.

In the case of the WCR, the relatively high response to (2S,8R) is of no conceivable consequence since there are no other known cohabiting *Diabrotica* that respond secondarily or exclusively to (2S,8R), and therefore, (2S,8R) is probably not encountered by WCR males in their natural environment.

A somewhat different situation exists with the MCR. Since *D. porracea* responds exclusively to (2S,8R), it seems likely that the sex pheromone of this insect is largely, if not exclusively, composed of the (2S,8R)-isomer.

Cohabiting some areas with *D. porracea*, the MCR male, which responds strongly but secondarily to (2S,8R), would probably be exposed to (2S,8R) as an environmental constituent. The consequences, if any, are difficult to predict since relatively little is known about the reproductive behavior of the MCR, and virtually no such information is available for *D. porracea*. It is possible, of course, that the response of *D. porracea* to the (2S,8R)-isomer is fortuitous or that *D. porracea* females, in addition to (2S,8R), produce another compound which inhibits the response of the MCR to (2S,8R), in which cases the cohabiting situation might well be inconsequential.

In addition to those mentioned in this report, three other *Diabrotica* are now known to be attracted to 8-methyl-2-decyl propanoate. They include *D. barberi* (the northern corn rootworm), *D. longicornis* (Say), and an undescribed species found in Peru (Guss et al., 1982; J.L. Krysan, personal communication). These three *Diabrotica* and the three mentioned earlier are

all in the *virgifera* species group (Smith and Lawrence, 1967). This group was erected on morphological grounds, and subsequently, members of this group were found to share similar life histories with respect to host relationships and egg diapause (Branson and Krysan, 1981; Krysan, 1982).

With one exception, all *virgifera* group *Diabrotica*, that we have knowingly exposed to 8-methyl-2-decyl propanoate (racemic and/or certain of the resolved stereoisomers), have responded to baited traps. The exception of which we are aware is *D. cristata* (Harris), a nonpest species found primarily in relict prairies throughout the eastern United States (Wiesenborn and Krysan, 1980). In the summer of 1982, however, we found that males of *D. cristata* strongly respond to both racemic and (2S,8R)-8-methyl-2-decyl acetate (Guss et al., 1983b). It would thus appear that 8-methyl-2-decanol may be a common biosynthetic precursor among *Diabrotica* in the *virgifera* species group.

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