Inhibition of *Carpophilus freemani* Dobson (Coleoptera: Nitidulidae) Aggregation Pheromone Response by a *Z*-Double-Bond Pheromone Analog

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Males of *Carpophilus freemani* Dobson (Coleoptera: Nitidulidae) produce an aggregation pheromone that attracts beetles of both sexes. The major component of the pheromone was previously identified as (2*E*,4*E*,6*E*)-5-ethyl-3-methyl-2,4,6-nonatriene. The response of *C. freemani* to this pheromone in a wind tunnel bioassay was inhibited 26−47% ($P < 0.01$) by a 10-fold excess of (2*E*,4*E*,6*Z*)-5-ethyl-3-methyl-2,4,6-nonatriene, a double-bond configurational isomer. This artifact of synthesis is not produced by *C. freemani* and does not attract *C. freemani*. In the presence of a synergist, significant inhibition was not observed with equivalent amounts of pheromone and pheromone analog. Although technical-grade pheromone used to monitor *C. freemani* in the field contains 8−30% of the pheromone analog, this amount of the analog is insufficient to cause significant inhibition in laboratory wind-tunnel bioassays.

Keywords: *Triene; hydrocarbon; Coleoptera; Nitidulidae; Carpophilus freemani; Freeman sap beetle*

INTRODUCTION

The Freeman sap beetle, *Carpophilus freemani* Dobson (Coleoptera: Nitidulidae), is an important pest of sweet corn in the field (Sanford and Luckman, 1963) and after harvest (Connell, 1975). The Freeman sap beetle and related nitidulids can vector aflatoxin-producing fungi into corn (Lussenhop and Wicklow, 1990). The insect is also a significant pest of figs (Smilanick and Ehler, 1976). Males of *C. freemani* produce an aggregation pheromone that attracts beetles of both sexes, and the major component of the pheromone was identified as (2*E*,4*E*,6*E*)-5-ethyl-3-methyl-2,4,6-nonatriene (Bartelt *et al.*, 1990a). The major component, by itself, is sufficient to elicit attraction. Previous studies have shown that the bioassay count is enhanced by the presence of a synergist (Bartelt *et al.*, 1990a,b).

The final step of the synthesis of the pheromone results in a mixture of 6*E*- and 6*Z*-isomers (Bartelt *et al.*, 1990a). This technical-grade pheromone, containing 8−30% of the unnatural 6*Z*-isomer, is used to monitor fields for *C. freemani*. The attractiveness of the undesired isomer and the potential inhibitory properties of this compound were unexplored in previous work (Bartelt *et al.*, 1990a).

In this paper, we report inhibition of the response of *C. freemani* to its major pheromone component in a flight-tunnel bioassay by (2*E*,4*E*,6*Z*)-5-ethyl-3-methyl-2,4,6-nonatriene, an unnatural synthetic analog. The significance of the inhibition is discussed in terms of whether this pheromone needs to be supplied at higher purity for field monitoring.

MATERIALS AND METHODS

Beetle Culture. The *C. freemani* culture was the same as for earlier pheromone identification (Bartelt *et al.*, 1990a). This culture was originally collected in an area of oak woods and corn fields near Bath, IL, in 1989 and has been maintained using the artificial diet described previously (Dowd, 1987), except that the pinto beans were replaced by an equal weight of brewer's yeast.

Preparation of Pheromone *Z*-Isomer Analog. A synthetic mixture of (2*E*,4*E*,6*E*,*Z*)-5-ethyl-3-methyl-2,4,6-nonatriene was available from previous work (Bartelt *et al.*, 1990a). A silica HPLC column coated with $AgNO_3$ (Heath and Sonnet, 1980) was used to separate and purify the 6*E*- and 6*Z*-pheromone isomers. The solvent was 0.5% 1-hexene in hexane using a flow rate of 0.6 mL/min. The HPLC equipment was described previously (Bartelt *et al.*, 1990b). Purification of the 6*Z*-isomer was achieved with size exclusion chromatography using a PL GEL 5μ 100 Å column (Polymer Laboratories) with hexane as the solvent at a flow rate of 0.6 mL/min.

HPLC fractions were analyzed by gas chromatography (GC) using a Hewlett-Packard (HP) 5890 Series II instrument equipped with flame ionization detector, splitless injector, and an HP 7673 autosampler and interfaced to an HP 3396 integrator. The oven temperature was programmed from 50 to 250 °C at 10 °C/min; the injector temperature was 220 °C, and the detector temperature was 270 °C. A DB-1 capillary column (15 m × 0.25 mm, 1.0-μm film thickness, J&W Scientific, Folsom, CA) was used with 2-μL sample injections. An internal standard (*n*-nonadecane) was used for isomer quantitation.

Electron impact mass spectra (70 eV) were obtained with an HP 5970 MSD instrument, with a DB-1 capillary GC column as described above was used. Proton NMR spectra (C_6D_6) were obtained on a Bruker (Billerica, MA) ARX 400 MHz spectrometer.

Bioassay. The wind-tunnel bioassays were conducted as described by Bartelt *et al.* (1990a). The wind tunnel contained ca. 500−1000 beetles, and ca. 100 at a time were in flight during bioassays. Two different treatment preparations to be compared were applied to pieces of filter paper, and those were hung side by side in the upwind end of the wind tunnel. Each bait was tested at each side to minimize bias resulting from position effects. Behavioral responses by the beetles to an active preparation included an upwind, casting flight, followed by alighting on the filter paper. Each test lasted 3 min, and the numbers of landings were recorded. Propyl acetate (20 μL, 1% solution in mineral oil) was added as a synergist in some bioassay treatments; previous studies have shown that

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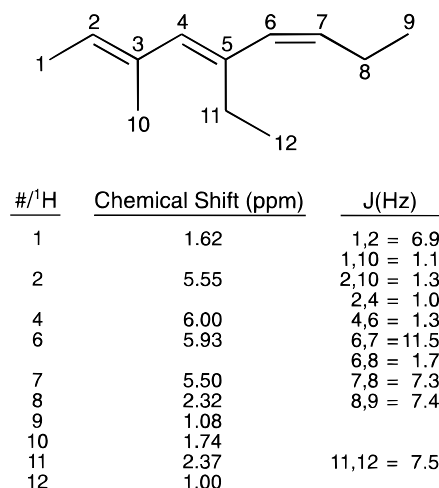

#/¹H	Chemical Shift (ppm)	J(Hz)
1	1.62	1,2 = 6.9 1,10 = 1.1
2	5.55	2,10 = 1.3 2,4 = 1.0
4	6.00	4,6 = 1.3
6	5.93	6,7 = 11.5 6,8 = 1.7
7	5.50	7,8 = 7.3
8	2.32	8,9 = 7.4
9	1.08	
10	1.74	
11	2.37	11,12 = 7.5
12	1.00	

Figure 1. Structure of (2*E*,4*E*,6*Z*)-5-ethyl-3-methyl-2,4,6-nonatriene and proton NMR data.

bioassay count is enhanced by the presence of a synergist (Bartelt *et al.*, 1990a,b). Controls consisted of filter paper alone (studies conducted in the absence of synergist) or filter paper containing only propyl acetate (studies conducted in the presence of synergist). Tests were replicated 16 times using the same beetles on the same day except in the case of checking for increased attractiveness of the 6*E*-isomer in the presence of a synergist (eight replicates). Attractiveness of the pheromone at doses of 0.13 or 1.3 ng was compared side by side with the same dose of the pheromone and either an equivalent amount or a 10-fold excess of the pheromone 6*Z*-isomer analog. When the pheromone was used at a dose of 1.3 ng, inhibition studies were conducted both in the presence and in the absence of the synergist.

Statistical Analysis. Wind-tunnel data were transformed to the $\log(X + 1)$ scale before analysis to stabilize variance. Analysis of covariance (ANCOVA) was used to analyze bioassay data with bait location as the covariate.

RESULTS AND DISCUSSION

Pheromone Analog Structure Proof. The coupling constant for the olefinic protons on the disubstituted double bond is 11.5 Hz (Figure 1), and the structure has been assigned as (2*E*,4*E*,6*Z*)-5-ethyl-3-methyl-2,4,6-nonatriene on the basis of comparison with the natural 6*E*-isomer. The NMR spectrum of the natural 6*E*-isomer was reported previously (Bartelt *et al.*, 1990a), and a coupling constant of 15.7 Hz was observed for the olefinic protons on the disubstituted double bond.

Effect of Synergist. At a dose of 0.13 ng of the natural pheromone, the synergist was necessary to obtain sufficient bioassay counts; without the synergist only two landings were observed in each of two tests. Bioassay counts higher than this are needed to show the effect of an inhibitor. In side by side comparisons, bioassay counts increased more than 3-fold when propyl acetate was used as a synergist. A mean count of 41 landings was observed at a pheromone dose of 1.3 ng without the synergist, and a mean count of 137 landings was observed at the same pheromone dose in the presence of the synergist [eight tests; $F = 195$; df, 1, 6; $P < 0.01$; MSE (treatment × test) = 0.007]. This result is consistent with previous results (Bartelt *et al.*, 1990a).

Inhibition by Pheromone *Z*-Isomer. The 6*Z*-isomer by itself did not attract *C. freemani* at a dose of 13 ng (six tests, no landings). At a dose of 0.13 ng of pheromone (Table 1), 47% inhibition was observed when the 6*Z*-isomer was used at a dose of 1.3 ng (i.e., ten times the dose of the natural pheromone). In all cases, when the analog was tested at a level of ten times the dose of pheromone, 26−47% inhibition ($P < 0.01$) was observed after accounting for bait location effect. Without the synergist, an equivalent dose (i.e., 1.3 ng) of pheromone and pheromone analog showed 18% inhibition ($P < 0.05$). In the presence of the synergist, significant inhibition was not observed with an equivalent dose (i.e., 1.3 ng) of pheromone and pheromone analog. Bioassay count varied considerably from day to day; for this reason, comparisons between tests (within columns in Table 1) cannot be made.

Inhibition of pheromonal response by double-bond configurational isomers of insect pheromones has been reported in the literature. A 5% addition of *E*,*Z*, *Z*,*E*, or *Z*,*Z* geometric isomers of the pheromone (*E*,*E*)-8,10-dodecadien-1-yl acetate to lures containing the pheromone significantly reduced male pea moth (*Cydia nigricana* F.) attraction (Witzgall *et al.*, 1993). A 10% addition of the *Z*-isomer of (*E*)-11 tetradecadecen-1-yl acetate inhibited the behavioral responses of the male light-brown apple moth (*Epiphyas postvittana* Walker) (Rumbo *et al.*, 1993).

Our results strongly suggest that technical grade pheromone is sufficient for field use because the technical grade pheromone, used to monitor the presence of *C. freemani* in the field (Bartelt *et al.*, 1990a, 1994, 1995a), contains only 8−30% of the 6*Z*-isomer. If the double-bond isomer had been inhibitory at the 5% concentration as reported for the pea moth (Witzgall *et al.*, 1993) or the 10% concentration as reported for the light-brown apple moth (Rumbo *et al.*, 1993) then higher purity pheromone would be needed for field monitoring of *C. freemani*. Fortunately, there is no justification to supply more highly purified pheromone since laboratory bioassay data extrapolate to what is observed in the field (Bartelt *et al.*, 1995b).

In contrast to the results reported in this paper, all four mono-*Z*-isomers of (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene showed significant activity as attractants to the driedfruit beetle *Carpophilus hemipterus* (L.) (Bartelt *et al.*, 1992). The 6*Z*-isomer, which is inhibitory to *C. freemani*, is a triene whereas all the mono-*Z*-isomers of the major pheromone component for *C. hemipterus* are tetraenes. Still, we cannot explain the differences in biological activity without extensive structure−activity relationship studies using many synthetic analogs and computational chemistry.

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