Identification of the Sex Pheromone of the Webbing Coneworm Moth, *Dioryctria disclusa* (Lepidoptera: Pyralidae)[1]

WENDY L. MEYER, GARY L. DEBARR,[2] C. WAYNE BERISFORD,[3] LARRY R. BARBER,[4] AND WENDELL L. ROELOFS

Department of Entomology, New York State Agricultural Experiment Station, Geneva, New York 14456

ABSTRACT

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A sex pheromone component for the webbing coneworm, *Dioryctria disclusa*, has been identified as (Z)-9-tetradecenyl acetate. Traps baited with rubber septa impregnated with this compound at loadings of 30 to 300 μg caught the same as or significantly better than traps baited with two virgin female moths. Addition of 3 to 30% of the *E* isomer did not increase trap catch, and only (Z)-9-tetradecenyl acetate was found in the female extract.

The webbing coneworm, *Dioryctria disclusa* Heinrich is found in the southern United States on loblolly pine, *Pinus taeda* L., and Virginia pine, *P. viginiana*, Mill., where it attacks and destroys second-year cones (Neunzig et al. 1964, Ebel et al. 1975). It usually causes minor economic losses (Yates and Ebel 1978), but outbreaks in loblolly pine seed orchards in 1979 and 1980 had severe economic impact on production of genetically improved seed (Barber and DeBarr, unpublished data). The presence of a female-produced sex pheromone was demonstrated (DeBarr and Berisford 1981), and a reliable monitoring system was needed for this species, so we initiated a cooperative project to isolate and identify the chemical component(s). This is the first report identifying a sex pheromone for a species in the genus *Dioryctria*.

Materials and Methods

Cones infested with *D. disclusa* larvae were collected from loblolly pine orchards in Georgia or North Carolina from 24 April to 16 May 1980. The cones were dissected, and 3rd to 5th instar larvae were removed and reared individually at 28°C in 12-ml plastic cups containing artificial diet (Fedde 1974). Upon pupation, the sexes were separated, and the insects were allowed to emerge on a photocycle of LD 16:8 at 25°C and were collected daily.

The pheromone gland of this species appeared to be a ring of modified intersegmental membrane between abdominal segments 8 and 9, similar to the gland described for *D. abietella* (Fatzinger 1972). Crude female extract was prepared by taking 3- to 4-day-old females during their calling period, 4 h into their scotophase (DeBarr and Berisford 1981), and excising the ovipositor just anterior to the 9th abdominal segment. Removing just this portion of the ovipositor minimized the amount of oils extracted. The abdominal tips were transferred to a 4-ml vial containing dichloromethane and extracted overnight. After 24 h, the extract was pipetted into another vial and stored at −10°C until use.

Electroantennograms (EAG) were conducted as described by Roelofs (1977). Responses were recorded from a digital voltmeter display (Bjostad and Roelofs 1980).

The following gas-liquid chromatography (GLC) phases were used in glass columns, OV-101 (methyl silicone, 3% on 100- to 120-mesh Gas-Chrom Q, 4 mm by 1.8 m) and XF-1150 (GE XF-1150, 50% cyanoethyl, methyl silicone, 10% on 100- to 120-mesh Chromosorb W AW-DMCS, 2 mm by 1.8 m). Mass spectra were obtained with a HP-5985 GC-MS interfaced with a 10-m OV-101 glass capillary column.

Chemicals used for field testing were obtained from the following sources: (Z)-9-tetradecenyl acetate (Z9-14:Ac) and (Z)-9-dodecenyl acetate (Z9-12:Ac) were from Farchan Company (Willoughby, Ohio); E9-14:Ac was synthesized in our laboratory; dodecyl acetate (12:Ac) was from Eastman Co. (Rochester, N.Y.). The unsaturated acetates contained ca. 1% of the opposite geometrical isomer as indicated by GLC analysis on XF-1150.

Field tests were conducted from 24 May to 30 June 1980, in Putnam County, Ga. (test 1), Beaufort County, N.C. (test 2), Halifax County, N.C. (test 3), and New Kent County, Va. (test 4). The pine seed orchards used were loblolly pine 12 to 18 m high, in rows spaced 4.5 or 6 by 9 m. Blocks of trees equal to the number of treatments were selected, and treatments were randomly assigned to trees within each block. Pherocon 1C traps (Zoecon Co., Palo Alto, Calif.) were used and baited with a rubber septum dispenser (A. H. Thomas Co., Philadelphia, Pa.) or two live virgin female *D. disclusa* held in a divided cylindrical screen wire cage, 5 cm in length and 2.5 cm in diameter. The traps were hung in the upper 3 m of the tree (DeBarr and Berisford 1981). Trap catches were counted at 1- to 3-day intervals, and male moths were removed. Females were replaced when dead or at 5- to 7-day intervals. Every 3 to 5 days the traps were rerandomized. Test 1 was replicated four times, whereas tests 2, 3, and 4 were replicated five times each.

Results

Crude female extract was collected from OV-101 (190°C) in 1-min fractions and assayed for EAG activity. The only activity occurred between 5 and 7 min,

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[2]USDA Forest Service, Southeastern Forest Experiment Station, Athens, GA 30602.

[3]Dept. of Entomology, University of Georgia, Athens, GA 30602.

[4]USDA Forest Service, Southeastern Area, State and Private Forestry, Asheville, NC 28803.

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with tetradecyl acetate (14:Ac) having a retention time of 6.4 min. These active fractions were combined and collected from XF-1150 (170°C) in 1-min fractions. EAG activity occurred between 5 and 8 min, with 14:Ac and Z9-14:Ac having retention times of 5.0 and 5.9 min, respectively. When the collected material was injected on OV-101, a peak was produced at a retention time of 16.6 min with an impurity at 15.3 min. Z9-14:Ac under the same conditions had a retention time of 16.6 min, whereas 14:Ac had a retention time of 17.7 min. On XF-1150, the retention times of Z9-14:Ac and E9-14:Ac were 6.8 and 6.3 min, respectively. Injection of the active material produced a peak with a retention time of 6.7 min as well as other later impurity peaks. No peak with a retention time corresponding to E9-14:Ac was observed, but an amount smaller than 10% of Z9-14:Ac would not have been detected, due to the small amount of collected active material.

A mass spectrum (EI, 70eV) of OV-101-collected material exhibited an M-60 ion at 194 m/e, which is characteristic of a monounsaturated 14-carbon acetate. The spectrum was identical to authentic Z9-14:Ac. Further chemical tests were not possible, due to the small amounts of material present. We estimate that each female contained ca. 0.5 ng of Z9-14:Ac.

EAGs to 10 μg of synthetic 12-carbon, 14-carbon, and 16-carbon monounsaturated acetates, alcohols, and aldehydes on filter paper showed the highest millivolt response to Z9-14:Ac (3.53 ± 0.45 SEM) relative to that of a standard (E5-14:Ac, 1.30 ± 0.36 SEM). These data support (Roelofs 1977) the GLC and mass spectral analyses in an assignment of Z9-14:Ac for the main pheromone component.

Field test results (Table 1) show that 30 μg of Z9-14:Ac alone captured male *D. disclusa* in traps as well as or better than virgin females. Catches with dosages of 100, 300, and 3,000 μg (tests 1 and 2) were not significantly different from the 30-μg dosage, but when the dosage was lower than 30 μg (test 2), trap catch was significantly decreased. To test the possibility that small amounts of the opposite geometrical isomer would enhance trap catch we added 3, 10, and 30% E9-14:Ac and found there was no significant increase in trap catch (test 3). Indeed, when 30% E9-14:Ac was added a significant decrease in male capture occurred.

At this time we were also conducting field tests, involving Z9-12:Ac and 12:Ac, for another *Dioryctria* sp., *D. clarioralis* (Walker). When included in a test for *D. disclusa* (test 4), no increase in trap catch was observed when either Z9-12:Ac (1:20) or 12:Ac (1:1) was added to Z9-14:Ac.

Table 1.—Male *D. disclusa* trap catches by Z9-14:Ac on rubber septa in loblolly pine seed orchards, 24 May to 30 June 1980[a]

Test no.	Treatment	Total catch
1; Putnam County, Ga.[b]	30 μg Z9-14:Ac	140a
	300 μg Z9-14:Ac	135a
	3000 μg Z9-14:Ac	81ab
	Blank	11c
	Females[c]	89b
2; Beaufort County, N.C.[c]	3 μg Z9-14:Ac	217b
	10 μg Z9-14:Ac	218b
	30 μg Z9-14:Ac	316a
	100 μg Z9-14:Ac	293ab
	Blank	103c
	Females	251ab
3; Halifax County, N.C.[c]	30 μg Z9-14:Ac + 3% E9-14:Ac	290a
	30 μg Z9-14:Ac + 10% E9-14:Ac	267ab
	30 μg Z9-14:Ac + 30% E9-14:Ac	133c
	30 μg Z9-14:Ac	248ab
	Blank	4d
	Females	227bc
4; Kent County, Va.[c]	30 μg Z9-14:Ac	204a
	30 μg Z9-14:Ac + 1.5 μg Z9-12:Ac	173a
	30 μg Z9-14:Ac + 30 μg 12:Ac	206a
	Blank	33b
	Females	38b

[a]Catches within each test followed by the same letter are not significantly different, by Waller and Duncan's BSD test (Waller and Duncan 1969) data transformed $\sqrt{x + 0.5}$.

[b]Replicated four times.

[c]Replicated five times.

On 22 and 23 May 1980, 17 males of a sympatric species, *D. clarioralis* were caught in traps baited with female *D. disclusa*. This occurred at a time when *D. disclusa* females from the natural population were not yet present, since they are univoltine with an adult emergence peak in June, whereas *D. clarioralis* is multivoltine with emergence peaks in May, July, and September (Yates and Ebel 1975). No other *D. clarioralis* males were caught after this date. This cross attraction was not observed in a previous study (DeBarr and Berisford 1981), although blacklight trap catches indicated a small overlap in adult activity periods. These seasonal differences in emergence are likely a major factor in reproductive isolation of these two species, because Z9-14:Ac was also found to be a pheromone component for *D. clarioralis* (W. L. Meyer, W. L. Roelofs, and R. S. Cameron 1981, unpublished data).

Discussion

The chemical and biological data show that Z9-14:Ac is the main sex pheromone component produced and perceived by *D. disclusa*. Z9-14:Ac is also a pheromone component of another pyralid species, *Ephestia cautella* (Walker) (Brady et al. 1971, Brady 1973). It later was found to increase trap catch when added to the primary pheromone component, (Z,E)-9,12-tetradecadienyl acetate (Read and Haines 1979).

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