NOTE

(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-Pentacosapentaene and (9*Z*,11*E*)-Tetradecadienyl Acetate: Attractant Lure Blend for *Dioryctria ebeli* (Lepidoptera: Pyralidae)[1]

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Feeding damage by larval coneworms (Lepidoptera: Pyralidae) on flowers and cones of pines can cause significant economic losses in pine seed orchards in the southern USA (Ebel et al. 1980, USDA Forest Service GTR SE-8, Asheville, NC). The south coastal coneworm, *Dioryctria ebeli* Mutuura & Monroe, is a common pest in seed orchards of slash pine (*Pinus elliottii* L.) in the coastal region of southeastern USA, particularly Florida (Ebel et al. 1980). With multiple generations per year, infestations of *D. ebeli* can be severe, especially in seed orchards infected by the southern pine cone rust, *Cronartium strobilinum* Hedgc. & Hahn (Merkel 1958, J. Forestry 56:651).

Monitoring coneworm moths with pheromone-baited traps to time aerial insecticide applications can increase the efficacy of control programs for coneworms in southern pine seed orchards (Hanula et al. 2002, Can. Entomol. 134: 255 - 268). Sex pheromone-based lures are known for 4 economically important *Dioryctria* species in the southern states: *D. amatella* (Hulst), *D. clarioralis* (Walker), *D. disclusa* Heinrich, and *D. merkeli* Mutuura & Munroe (Hanula et al. 1984, Environ. Entomol. 13: 1298 - 1301). A sex pheromone for *D. ebeli* has not been identified.

Dioryctria ebeli was classified as either *D. abietella* (Denis & Schiffermüller) or *D. abietivorella* (Grote) until 1979, when it was taxonomically segregated from these species based on differences in morphology, ecology and geographic ranges (Mutuura and Munroe 1979, J. Georgia Entomol. Soc. 14: 290 - 304). *Dioryctria ebeli* is found in the southeastern USA, whereas, *D. abietella* and *D. abietivorella* are found in Europe, and western and northern North America, respectively (Neunzig 2003 *in* Dominick et al., The Moths of America North of Mexico, fasc. 15.5).

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Given the close taxonomic relationship among these 3 allopatric species, we expected that the pheromone for *D. ebeli* would be similar to that of *D. abietivorella* and *D. abietella*. (9*Z*,11*E*)-Tetradecadienyl acetate (9*Z*,11*E*-14:Ac) is a component of the pheromone blends of both *D. abietella* (Löfstedt et al. 1983, Ent. Exp. & Appl. 34: 20 - 26) and *D. abietivorella* (Millar et al. 2005, J. Chem. Ecol. 31: 1229 - 1234; Strong et al. 2008, Ent. Exp. & Appl. 126: 67 - 77; Grant et al. 2009, Can. Entomol. 141: 129 - 135). Löfstedt et al. (1983) cite unpublished work by Roelofs, Meyer, and DeBarr indicating that *D. ebeli* also produces *Z*9,*E*11 - 14:Ac.

Our initial objective was to verify the production of *Z*9,*E*11 - 14:Ac by female *D. ebeli*. In 1996, female pupae of *D. ebeli* were collected from infested cones of slash pine (*Pinus elliottii* Engelm.) at the Withlacoochee Seed Orchard near Inverness, FL. Pheromone glands from calling virgin females held in a 16:8 L:D light regimen were excised at the beginning of the photophase while females were still calling, and extracted for 4 h in small volumes of pentane. Aliquots from 2 composite extracts of 59 and 39 females, respectively, were analyzed separately by GC-MS, using splitless injections on DB-1 and DB-23 columns, respectively. We also conducted selected ion monitoring (SIM) analyses on the extracts, searching specifically for 12, 14, and 16 carbon acetates and alcohols at the retention times of likely pheromone components. Authentic standards of *Z*9,*E*11 - 14:Ac and (9*Z*,11*E*)-tetradecadienyl alcohol (*Z*9,*E*11 - 14:OH) were obtained from Sigma-Aldrich (Oakville, ON, Canada).

Using full scan GC-MS analyses on DB-1 and DB-23 columns, we obtained good matches between the mass spectrum of a component of the extract and a database spectrum of *Z*9,*E*11 - 14:Ac. The insect-produced compound and the authentic standard had identical retention times on both columns. No other likely pheromone compounds were detected by full scan mass spectrometry. However, SIM analysis of the extracts with the DB-23 column detected traces of a compound whose SIM mass spectrum showed ions at *m/z* 192 (M-18) and *m/z* 210 (M^+), consistent with an unsaturated 14-carbon alcohol, at the retention time of authentic *Z*9,*E*11 - 14:OH. Based on area counts of the GC peaks corresponding to these compounds in several extracts, the ratio of acetate to alcohol was about 1:0.2, with a range of 0.51 - 1.58 ng of the *Z*9,*E*11 - 14:Ac detected per female. The diene alcohol was reported previously in pheromone gland extracts from female *D. abietella* (Löfstedt et al. 1983).

In an initial field trial, we found that *Z*9,*E*11 - 14:Ac was not attractive to male *D. ebeli*. On 10 May 1996, wing traps were baited with 0 - 1000 μg/septum dosages of *Z*9,*E*11 - 14:Ac and deployed in the upper crowns of mature slash pine at the Withlacoochee Seed Orchard. Catches of *D. ebeli* were low with a total of 48 male *D. ebeli* caught among 30 baited traps over a 14-wk period; no males were captured in blank control traps (unpubl. data).

Our results suggest that additional pheromone components such as *Z*9,*E*11 - 14:OH might be needed to improve attraction. Therefore, a second field trial was conducted in 1999 at the same seed orchard. Traps were baited with 100 μg of *Z*9,*E*11 - 14:Ac alone and in combination with 2 and 10% *Z*9,*E*11 - 14:OH, with 10% *Z*9 - 14:OH, and with 1% 9*Z*,12*E*-tetradecadienyl acetate (*Z*9,*E*12 - 14:Ac; Bedoukian Research Inc, Danbury, CT). *Z*9,*E*12 - 14:Ac was included because it is a common pheromone component of several related phycitine species (El-Sayed 2008, www.pherobase .com). Populations of *D. ebeli* were low and no more than 1 moth was caught per lure treatment except for traps baited with the 100:1 blend of *Z*9,*E*11 - 14:Ac and *Z*9,*E*12 - 14:Ac, which caught a total of 27 moths (unpubl. data).

In 2004, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene (pentaene) was identified in pheromone gland extracts of *D. abietivorella* and, when combined with *Z*9,*E*11 - 14:Ac in various ratios, significantly enhanced attraction of male *D. abietivorella* (Millar et al. 2005). Subsequent field tests established that a lure ratio of 200:2000 µg of *Z*9,*E*11 - 14:Ac to pentaene was optimally attractive to *D. abietivorella* (Strong et al. 2008; Grant et al. 2009).

In 2005, we conducted a third field trial to determine if attraction of *D. ebeli* to traps baited with *Z*9,*E*11 - 14:Ac could be enhanced similarly by the addition of pentaene. Pentaene and *Z*9,*E*11 - 14:Ac were synthesized as described by Millar et al. (2005). *Z*9,*E*12 - 14:Ac was obtained from Bedoukian Research. Compounds were loaded onto gray rubber septa (11 mm; The West Co., Lionville, PA) in 100 µL hexane, with butylated hydroxytoluene and Sumisorb 300 (5 mg/ml for each) added as stabilizers to the solutions. The experiment was conducted 14 June - 8 August 2005 in a slash pine stand at the Withlacoochee Seed Orchard. Five replicate blocks of 6 wing traps per block were set in the crowns of mature slash pine (one trap per tree). One of 6 treatments (specified in Fig. 1) was randomly assigned to each trap within a replicate. Lures were replaced after 4 wks. Total catches of *D. ebeli* over the 8-wk period were transformed by ln(y+1) to remove heteroscedasticity (Pepper et al. 1997, USDA Forest Service RP SRS-5, Asheville, NC). We compared treatment means with the Holm-Sidak multiple comparison test (SigmaStat for Windows, version 3.11, SYSTAT Software Inc., Point Richmond, CA). Data for control traps were not included in the analyses due to the lack of variance associated with zero trap catches.

Catches of *D. ebeli* were highest in traps baited with lures that contained both the pentaene and *Z*9,*E*11 - 14:Ac (Fig. 1). The presence of *Z*9,*E*12 - 14:Ac in this binary blend had no effect on catches. No moths were caught in unbaited control traps. Our results are consistent with those obtained with *D. abietivorella* with similar blends of these compounds, indicating that the pentaene is a key pheromone component for *D. ebeli* as well. The magnitude of catches in our trial suggests that the blend of

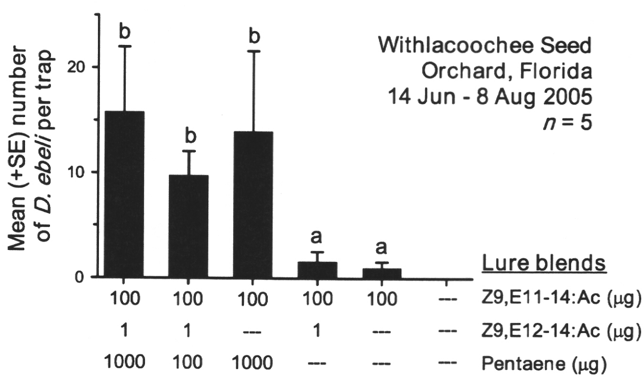

Fig. 1. Mean catches of male *Dioryctria ebeli* in wing traps baited with (9*Z*,11*E*)-tetradecadienyl acetate (*Z*9,*E*11 - 14:Ac), (9*Z*,12*E*)-tetradecadienyl acetate (*Z*9,*E*12 - 14:Ac), and (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene (pentaene). Means followed by a different letter are significantly different at *P* = 0.05 (Holm-Sidak multiple comparison test).

pentaene and *Z*9,*E*11 - 14:Ac could be used in monitoring programs to time aerial applications of insecticides for control of *D. ebeli* in slash pine seed orchards.

Additional analyses of pheromone gland extracts are required to identify other minor components produced by female *D. ebeli* and establish an optimal blend for monitoring *D. ebeli.* Furthermore, there are currently 40 recognized species of *Dioryctria* in North America, with 8 in southern pines alone (Neunzig 2003). *Dioryctria* species are often difficult to identify because of their similar wing patterns, color and hosts. As a result, the taxonomic status of some species is questionable. Further detailed work on the pheromone chemistry and responses of *Dioryctria* moths from various populations and geographic locations may provide useful information to help resolve taxonomic problems while concurrently providing the information needed for development of species-specific monitoring and management programs.

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