

## (3Z,6Z,9Z,12Z,15Z)-Pentacosapentaene and (Z)-11-Hexadecenyl Acetate: Sex Attractant Blend for *Dioryctria amatella* (Lepidoptera: Pyralidae)

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J. Econ. Entomol. 103(4): 1216–1221 (2010); DOI: 10.1603/EC09412

**ABSTRACT** In 2006–2008, we tested (3Z,6Z,9Z,12Z,15Z)-pentacosapentaene (pentaene) with the pheromone components (Z)-11-hexadecenyl acetate (Z11-16:Ac) and (Z)-9-tetradecenyl acetate (Z9-14:Ac), as sex attractants for four sympatric species of coneworms, *Dioryctria* Zeller (Lepidoptera: Pyralidae) in slash (*Pinus elliotii* Engelm.) and loblolly pine (*Pinus taeda* L.) seed orchards in Georgia and Louisiana, respectively. The addition of pentaene increased catches of male southern pine coneworm, *Dioryctria amatella* (Hulst), in wing traps baited with Z11-16:Ac, whereas catches of *Dioryctria disclusa* Heinrich in traps baited with Z9-14:Ac were unaffected by the addition of pentaene. The effect of pentaene on male *Dioryctria merkei* Mutuura & Munroe was inconsistent. In 2006, pentaene seemed to inhibit attraction of *D. merkei* to traps baited with Z9-14:Ac, whereas in a subsequent trial in 2008, moths were equally attracted to Z9-14:Ac with or without the pentaene. We caught too few *Dioryctria clarioralis* (Walker) in any experiment for meaningful analyses. Our field results with pentaene and the unresolved complexity of the taxonomy, ecology, and management of southern coneworms support the need for a comprehensive examination of the chemical ecology of *Dioryctria* spp.

**KEY WORDS** (3Z,6Z,9Z,12Z,15Z)-pentacosapentaene, *Dioryctria amatella*, *Dioryctria merkei*, *Dioryctria disclusa*, *Dioryctria clarioralis*

Tree improvement programs for pines in the southern United States have led to substantial increases in seed production within pine (*Pinus* spp.) seed orchards as well as superior growth characteristics and disease resistance of progeny in pine plantations (Byram et al. 2000). Most pine plantations in the southern states are established with genetically improved seed (Hodge et al. 1997). However, southern pine seed orchards are at significant risk from attack by cone and seed insects that can reduce seed production by 50–90% if left uncontrolled (Powell and White 1994).

Coneworms, *Dioryctria* Zeller (Lepidoptera: Pyralidae), are the most important pests of pine cones and seed in the southern United States (Ebel et al. 1980).

Feeding by larval coneworms damages flowers, conelets, and cones of all southern pine species. In 1967, DeBarr (1974) found that at the time of harvest, 27% of mature cones in a slash pine (*Pinus elliotii* Engelm.) stand in northern Florida were damaged by *Dioryctria* spp. In Louisiana, coneworms damaged 48% of conelets and 35% of cones in a loblolly pine (*Pinus taeda* L.) seed orchard during 1973–1974 (Goyer and Nachod 1976). Sartor and Neel (1971) found that infestation by coneworms in Mississippi seed orchards reduced the numbers of seeds extracted from slash and loblolly pine cones by 87 and 78%, respectively.

Insecticide applications to control coneworms can double the production of high-value seed in southern pine seed orchards, especially during low-cone crop years (Powell and White 1994). Control of cone and seed pests in southern pine seed orchards is complicated. The four most economically important species of coneworms in loblolly and slash pine seed orchards are southern pine coneworm, *Dioryctria amatella* (Hulst), *Dioryctria clarioralis* (Walker), *Dioryctria disclusa* Heinrich, and *Dioryctria merkei* Mutuura & Munroe (Ebel et al. 1980, Neunzig 2003). Two species, *D. disclusa* and *D. merkei*, are univoltine, with adult flight in spring and late fall, respectively, whereas *D. amatella* and *D. clarioralis* are multivoltine, with considerable overlap in generations from spring to fall

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(Yates and Ebel 1975, Hanula et al. 1984). In addition, three or more coneworm species can be present in the same orchard at the same time. Consequently, managers use a generic strategy against coneworms in southern pine seed orchards, with three to six applications of insecticides at roughly monthly intervals each season (Nord et al. 1985, Mangini et al. 1998).

Pheromone-based monitoring can minimize the need for insecticide applications when population levels of coneworms are low as well as improve efficiency by targeting sprays at optimal times for species that are present in the orchard at critical levels (Hanula et al. 2002). Pheromone lures are known for all four species of southern coneworms (Hanula et al. 1984). Traps baited with (Z)-11-hexadecenyl acetate (Z11-16:Ac) are attractive to *D. amatella*, whereas the remaining three species are attracted to traps baited with (Z)-9-tetradecenyl acetate (Z9-14:Ac). A degree-day spray-timing model using traps baited with Z11-16:Ac was developed for *D. amatella* (Hanula et al. 2002). These authors reported that two spray applications, timed using the model, were as effective as four sprays applied monthly. Spray-timing models have not been developed for the other coneworm species in the southeastern states.

The use of lures that ensure capture of a single species of *Dioryctria* would be invaluable to orchard managers because a critical component of any pest management program is simple and unequivocal identification of pest species to time management actions appropriately. The use of traps with species-specific lures can minimize confusion in species identification, particularly when moths quickly become covered in glue within sticky traps. Although *D. amatella* uses a pheromone component (Z11-16:Ac) distinct from that used by *D. disclusa*, *D. merkei*, and *D. clarioralis*, the latter three species use the same pheromone component (Z9-14:Ac) (Hanula et al. 1984). Peak flight periods clearly separate *D. disclusa* (spring emergence of adults) from *D. merkei* (fall emergence) but do not separate *D. disclusa* and *D. merkei* from *D. clarioralis* which, like *D. amatella* has multiple generations per year (Yates and Ebel 1975, Hanula et al. 1984).

Historically, pheromone researchers have assumed that moths produce either type I lepidopteran pheromones consisting of 10–18 carbon esters, alcohols, or aldehydes, or type II pheromones, consisting of longer chain hydrocarbons and epoxides (Ando et al. 2004). Recently, the first examples of moths in the families Pyralidae and Crambidae that use combinations of type I and type II pheromones have been found, including *Dioryctria abietivorella* Grote, whose pheromone consists of a blend of (9Z,11E)-tetradecadienyl acetate (Z9,E11-14:Ac) and (3Z,6Z,9Z,12Z,15Z)-pentacosapentaene (pentaene) (Millar et al. 2005). Pentaene significantly enhanced attraction of male *D. abietivorella* to traps baited with Z9,E11-14:Ac in Douglas-fir (*Pseudotsuga* Carrière) seed orchards in British Columbia, Canada, where a blend of pentaene and Z9,E11-14:Ac is now used routinely for monitoring *D. abietivorella* (Strong et al. 2008). In Quebec, Canada, pentaene is also a critical pheromone component

for *D. abietivorella* in white spruce, *Picea glauca* (Moench) Voss seed orchards (Grant et al. 2009).

The biological activity of pentaene as a possible pheromone component is not restricted to *D. abietivorella*. Miller et al. (2010) found that the addition of pentaene significantly increased attraction of male *Dioryctria ebeli* Mutuura & Monroe to traps baited with Z9,E11-14:Ac in a slash pine seed orchard in northern Florida. Phylogenetically, *D. abietivorella* and *D. ebeli* are closely related species, forming the *abietella* group of *Dioryctria* species, along with the European species *Dioryctria abietella* (Denis & Schiffermüller) (Neunzig 2003, Roux-Morabito et al. 2008).

The objective of our study was to determine whether the addition of pentaene enhances or inhibits attraction of the four common species of coneworms that attack southern pines to their respective sex pheromones. Our goal is to develop species-specific lures to improve our ability to monitor the various *Dioryctria* species effectively so that pesticide applications can be used most efficiently for each species.

### Materials and Methods

The compounds Z9-14:Ac and Z11-16:Ac were purchased from Bedoukian Research Inc. (Danbury, CT). Pentaene was synthesized as described by Millar et al. (2005) (all chemical purities >98%). Lures were prepared by loading compounds onto gray rubber septa (11 mm; The West Co., Lionville PA) in 100  $\mu$ l of hexane, with butylated hydroxytoluene and Sumisorb 300 (each 5 mg/ml) added as stabilizers to the solutions. These adjuvants had no effect on trap catches of *D. abietivorella* (Strong et al. 2008). Commercial lures for *D. amatella* (SPC) were obtained from APTIV Inc. (Portland OR). As reported by APTIV Inc., the major component of the southern pine coneworm lure was Z11-16:Ac (100- $\mu$ g dose; chemical purity unknown).

We conducted four experiments over a 3-yr period (2006–2008). Experiment 1 was designed to test the effect of pentaene on the attraction of *D. amatella*, *D. clarioralis*, *D. disclusa*, and *D. merkei* to their respective pheromones. The experiment was conducted at two locations: the Baldwin Seed Orchard near Eatonton, GA (12 April–1 November 2006) and the Stuart Seed Orchard, Kisatchie National Forest near Bentley, LA (15 March–13 October 2006). At each location, five replicate blocks of six wing traps per block were set in the crowns (one trap per tree) of mature slash pine at the Baldwin Seed Orchard and loblolly pine at the Stuart Seed Orchard. One of the following six treatments was randomly assigned to each trap within a replicate: 1) blank control, 2) Z9-14:Ac (100  $\mu$ g), 3) Z11-16:Ac (100  $\mu$ g), 4) pentaene (1,000  $\mu$ g), 5) Z9-14:Ac + pentaene (100:1,000  $\mu$ g), and 6) Z11-16:Ac + pentaene (100:1,000  $\mu$ g). Lures were replaced every 4 wk. Traps were replaced as needed.

Experiment 2 was designed to verify the effect of pentaene on the attraction of *D. amatella* to Z11-16:Ac, and for comparison with the commercial lure (southern pine coneworm) for *D. amatella*. The experiment was conducted at the Baldwin Seed Or-

chard, GA (22 February – 6 November 2007) and the Stuart Seed Orchard, LA (20 March – 11 June 2008). In Georgia, six replicate blocks of three wing traps per block were set in the crowns of mature slash pine (one trap per tree). One of the following three treatments was randomly assigned to each trap within a replicate: 1) SPC lure, 2) Z11-16:Ac (100  $\mu\text{g}$ ), and 3) Z11-16:Ac + pentaene (100:1,000  $\mu\text{g}$ ). In Louisiana, eight replicate blocks of four wing traps per block were set in the crowns of mature loblolly pine (one trap per tree). One of the following four treatments was randomly assigned to each trap within a replicate: 1) SPC lure, 2) Z11-16:Ac (100  $\mu\text{g}$ ), 3) Z11-16:Ac + pentaene (100:1,000  $\mu\text{g}$ ); and 4) blank control. Lures were replaced every 4 wk at both locations.

Experiment 3 was designed to verify the effect of pentaene on the attraction of *D. amatella* to Z11-16:Ac, providing a third consecutive test over the 3-yr period. The experiment was conducted at the Baldwin Seed Orchard, GA (22 April–20 May 2008). Eight replicate blocks of two wing traps per block were set in the crowns of mature slash pine (one trap per tree). One of the following two treatments was randomly assigned to each trap within a replicate: 1) Z11-16:Ac (100  $\mu\text{g}$ ); and 2) Z11-16:Ac + pentaene (100:1,000  $\mu\text{g}$ ). Lure replacement was unnecessary because the experiment ran for only 4 wk.

Experiment 4 was designed to verify the effect of pentaene on the attraction of *D. merkei* to Z9-14:Ac. The experiment was conducted at the Baldwin Seed Orchard, GA (4 September–22 October 2008). Fall is the typical flight period of *D. merkei* (Ebel et al. 1980). Ten replicate blocks of two wing traps per block were set in the crowns of mature slash pine (one trap per tree). One of the following two treatments was randomly assigned to each trap within a replicate: 1) Z9-14:Ac (100  $\mu\text{g}$ ) and 2) Z9-14:Ac + pentaene (100:1,000  $\mu\text{g}$ ). Lures were replaced once after 4 wk.

Statistical analyses were conducted with SigmaPlot for Windows, version 11.0 (Systat Software, Inc., Point Richmond CA). Total catches of moths in experiments 1, 3, and 4 were transformed by  $\ln(y + 1)$  to remove heteroscedasticity (Pepper et al. 1997). Transformation of data in experiment 2 was unnecessary due to homogeneity of variances. Trap catch data where variances were homoscedastic were subjected to analysis of variance (ANOVA) using the following model components: replicate and treatment. We compared treatment means with the Holm-Sidak multiple comparison test when  $P \leq 0.05$  (Glantz 2005). In some experiments, total catches of some species to some treatments were zero, resulting in zero variance and a clear violation of the assumption of homoscedasticity (Cobb 1998). In such cases, treatments with total catches of zero were excluded from the analyses (Reeve and Strom 2004). Voucher specimens were deposited in the Entomology Collection, Museum of Natural History, University of Georgia (Athens, GA). In addition, vouchers of each species were frozen for future genetic analysis.

## Results

We captured a total of 723 *D. amatella* in experiment 1 (638 in Georgia and 85 in Louisiana). There were significant treatment effects on catches of *D. amatella* in both Louisiana and Georgia ( $F_{3,12} = 37.15$ ;  $P < 0.001$  and  $F_{5,20} = 43.01$ ;  $P < 0.001$ , respectively). No moths were captured in traps baited with two treatments in Louisiana, thereby precluding analysis for interactions between the two locations. At both locations, the addition of pentaene to lures loaded with Z11-16:Ac significantly increased trap catches of *D. amatella* compared with traps baited with Z11-16:Ac alone (Fig. 1). In Georgia, catches of moths in traps baited solely with Z11-16:Ac were higher than those in traps baited with solvent alone, pentaene alone, Z9-14:Ac alone, or Z9-14:Ac + pentaene. Traps baited with pentaene alone were not attractive to *D. amatella* at either location.

In total, 815 *D. amatella* were captured in experiment 2 in Georgia with catches significantly affected by treatments ( $F_{2,10} = 5.68$ ;  $P = 0.023$ ). As in experiment 1, catches of *D. amatella* in traps baited with Z11-16:Ac + pentaene were significantly greater than those in traps baited solely with Z11-16:Ac (Fig. 2A). Catches of moths in traps baited with the commercial lure (SPC) were intermediate between the two other treatments. The exact pheromone blend used in the SPC lure was unknown although the major component was reported to be Z11-16:Ac with a load of 100  $\mu\text{g}$ /septum (APTIV Inc.). We captured too few moths in Louisiana for analysis (11 *D. amatella*) in 2008. In experiment 3 (2008), we captured 310 *D. amatella* in total in Georgia, with a significant difference in catches between the two treatments ( $F_{1,8} = 5.32$ ;  $P = 0.050$ ). As in previous years, traps baited with the binary blend of Z11-16:Ac + pentaene were more attractive than those baited solely with Z11-16:Ac (Fig. 2B).

In 2006, we caught 348 *D. disclusa* in experiment 1 in Georgia. There was a significant treatment effect on responses of *D. disclusa* ( $F_{3,12} = 37.15$ ;  $P < 0.001$ ). The highest catches were in traps baited with Z9-14:Ac and Z9-14:Ac + pentaene, with no difference between the two treatments (Fig. 3A). No *D. disclusa* was captured in blank control traps or those baited with pentaene alone. We did not capture any *D. disclusa* in the remaining experiments.

We caught 232 *D. merkei* in experiment 1 in Georgia in 2006 with a significant treatment effect on responses ( $F_{1,4} = 152.12$ ;  $P < 0.001$ ). Catches of *D. merkei* in traps baited with the binary blend of Z9-14:Ac and pentaene were significantly lower than those in traps baited with Z9-14:Ac alone (Fig. 3B). No *D. merkei* was caught in traps baited with the remaining treatments. We caught too few *D. merkei* in Louisiana for statistical analyses (31 moths) in 2006. In 2008, we caught 226 *D. merkei* in experiment 4 in Georgia. In contrast to results in experiment 1, we found no significant treatment effect on catches of moths between traps baited with Z9-14:Ac alone or Z9-14:Ac + pentaene in experiment 4 ( $F_{1,4} < 0.01$ ;  $P < 0.961$ ). The

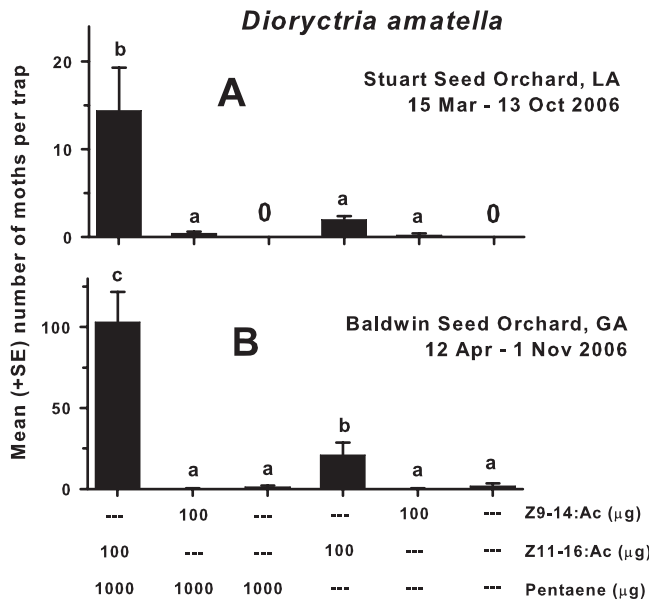


Fig. 1. Effects of Z9-14:Ac, Z11-16:Ac and pentaene on trap catches of male *D. amatella* in experiment 1 in Louisiana (A) and Georgia (B) in 2006 ( $n = 5$ ). Means with the same letter within a figure are not significantly different at  $P = 0.05$  (Holm-Sidak multiple comparison test). Means with zero catches of moths are denoted with a zero.

numbers (mean  $\pm$  SE) of *D. merkei* in traps baited with the two treatments were  $15.1 \pm 4.0$  and  $13.1 \pm 1.1$ , respectively. No *D. merkei* were captured in the remaining experiments.

We caught too few *D. clarioralis* in any experiment for meaningful analyses. In experiment 1, we captured 36 moths in Georgia and 46 moths in Louisiana. In

experiment 2, 31 *D. clarioralis* were captured in Georgia in 2007 and 21 in Louisiana in 2008. In Georgia, 11 moths were caught in experiment 4 and none in experiment 3.

Discussion

It is likely that pentaene is a sex pheromone component for *D. amatella*. Although not attractive by itself, pentaene synergized attraction of male *D. amatella* to Z11-16:Ac (Fig. 1), the major sex pheromone component produced by female *D. amatella* (Hanula et al. 1984). Our results for *D. amatella* in Georgia were consistent in each of three consecutive years of field trials (2006-2008) (Figs. 1 and 2). Verification of pheromone status will require demonstration of pentaene production by female *D. amatella*. The increase in attraction of *D. amatella* to baited traps by pentaene may provide seed orchard managers with precise and possibly earlier detection of moths in spray-timing programs, thereby adding greater flexibility in the planning of spray applications. Therefore, the spray-timing model developed by Hanula et al. (2002) for *D. amatella* should be revised for operational use in southern pine seed orchards to account for the increased effectiveness of the binary blend.

Intraspecific or geographic variation may complicate the chemical ecology of *Dioryctria* species. For example, in British Columbia, male *D. abietivorella* were attracted to a 1:10 ratio of Z9,E11-14:Ac and pentaene, with no effect from the addition of Z9-14:Ac (Strong et al. 2008). In contrast, Z9-14:Ac was essential for eliciting attraction of *D. abietivorella* to the same combination of Z9,E11-14:Ac and pentaene in Quebec (Grant et al. 2009). Such geographic vari-

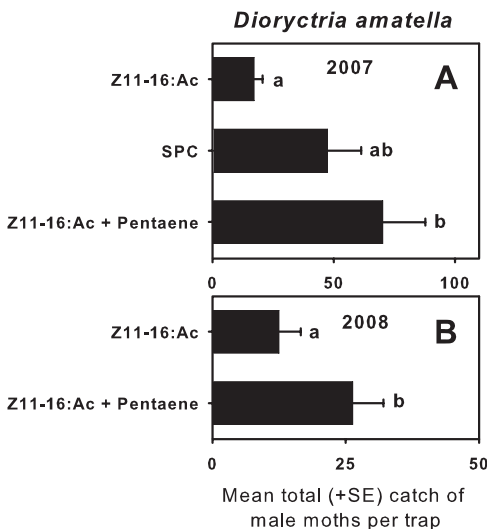


Fig. 2. Effects of pentaene on catches of male *D. amatella* in traps baited with Z11-16:Ac at the Baldwin Seed Orchard, GA, in 2007 (A;  $n = 6$ ) and 2008 (B;  $n = 8$ ) in experiments 2 and 3, respectively. Means with the same letter within a figure are not significantly different at  $P = 0.05$  (Holm-Sidak multiple comparison test). SPC, commercial lure loaded primarily with Z11-16:Ac (see text).

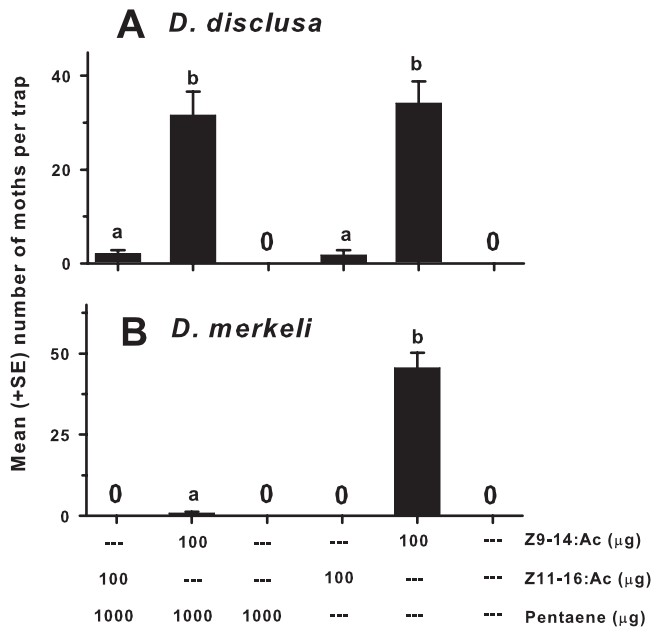


Fig. 3. Effects of Z9-14:Ac, Z11-16:Ac, and pentaene on trap catches of *D. disclusa* and *D. merkei* in experiment 1 at the Baldwin Seed Orchard GA ( $n = 5$ ). Means with the same letter within a figure are not significantly different at  $P = 0.05$  (Holm-Sidak multiple comparison test). Means with zero catches of moths are denoted with a zero.

ation can have significant implications in developing region-specific pheromone-based tactics for managing this species. Whereas we did not discover any regional variations in responses by *D. amatella*, there was a discrepancy in responses by *D. merkei* between years at a single location.

It is possible that our catches of *D. merkei* in a slash pine seed orchard in northern Georgia may have included *Dioryctria taedivorella* Neunzig & Leidy. Forest managers have traditionally considered *D. taedivorella* and *D. merkei* as one species (Ebel et al. 1980) due to the morphological similarities between the two species (Hedlin et al. 1981, Neunzig 2003). It is possible that some of the variation in responses we attributed to *D. merkei* alone may have arisen from changes in relative abundance of the two species between years. The range of *D. taedivorella* extends from eastern Virginia and North Carolina to northern Georgia, Alabama, and Mississippi, whereas the range of *D. merkei* is limited to Florida and southern regions of South Carolina, Georgia, Alabama, Mississippi, and Louisiana (Neunzig 2003). However, loblolly pine is the host species for *D. taedivorella*, whereas slash and longleaf pine are the host species for *D. merkei*. Our studies were conducted in north central Georgia in stands of slash pine.

Seed orchard managers in the southern and western United States have identified the need for improved timing of spray programs and new pheromone-based tactics as two high-priority research items (Hodge et al. 1997). To date, a pheromone-based spray-timing model has been developed for only one coneworm species, *D. amatella* in North America (Hanula et al. 2002). The only work on pheromone-based control of

southern coneworms was conducted by DeBarr et al. (2000). They found that the release of synthetic coneworm pheromones in a loblolly pine seed orchard greatly reduced catches of three species of *Dioryctria* in traps baited with synthetic pheromones or live conspecific females. The development of more effective lures from a comprehensive study on the chemical ecology of southern coneworms, particularly within the *zimmermani* group, could help in the development of spray-timing models for numerous other species of coneworms as well as the development of alternative management tactics such as pheromone-based mating disruption in southern pine seed orchards. Furthermore, studies on the chemical ecology of coneworms may be useful in resolving species relationships and identities, in concert with morphological and genetic analyses (Roe et al. 2006).

#### Acknowledgments

We thank M. G. Cody for field assistance; the Kisatchie National Forest and the Georgia Forestry Commission for permission to conduct the studies in the Stuart and Baldwin Seed Orchards, respectively. Personnel from the Forest Health Protection, Alexandria Field Office installed and assisted in monitoring the traps at the Stuart Seed Orchard. We thank A. D. Roe, W. B. Strong, and two anonymous referees for reviews of the manuscript.

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Received 4 December 2009; accepted 27 March 2010.