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# BINARY FLORAL LURE ATTRACTIVE TO VELVETBEAN CATERPILLAR ADULTS (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Evaluation of combinations of flower odor compounds in northern Florida revealed that linalool was synergistic in attractiveness with phenylacetaldehyde (PAA) to the migratory moth velvetbean caterpillar (*Anticarsia gemmatalis* Hübner). This noctuid was the most common species collected from traps with a binary lure composed of PAA and linalool, with over 900 males and females collected in Sep 2005 and almost 13,000 collected in a 4-week period in Aug and Sep 2006. Different lid openings of the vials containing the chemical blend in an attempt to vary the release rate of the binary lure did not affect the number of velvetbean caterpillar moths captured. Traps baited with the binary lure and placed in May, Jun and early Jul of 2005 and 2009 failed to capture adults demonstrating the absence of adult moths early in the season. Management application of floral attractants in an attract-and-kill strategy is discussed.

Key Words: floral lure, attractant, trapping, moths, *Anticarsia gemmatalis*

RESUMEN

Una evaluación de las combinaciones de olores de compuestos florales en el norte de Florida revelo que linalol era sinergista con el fenilaldehido (PAA) en la atracción a la polilla migratoria gusano del frijol (*Anticarsia gemmatalis* Hübner). Este noctuido fue la especie más común en trampas colectadas con cebos del compuesto binario PAA y el linalol, con más de 900 machos y hembras colectados en septiembre de 2005 y casi 13.000 recogidos en un período de 4 semanas en agosto y septiembre de 2006. Con el fin de variar la velocidad de liberación del atractivo binario se usaron tapas de diferente abertura las cuales no afectaron el número de adultos del gusano del frijol capturados. Trampas cebadas con un señuelo binario colocadas en mayo, junio, y principios de julio del 2005 y 2009 no capturaron adultos demostrando la ausencia de polillas adultas a principios de la temporada. Se discute el manejo para la aplicación de atrayentes florales en una estrategia para atraer-y matar.

Translation provided by the authors.

Many species of moths are known to be attracted to volatile compounds emitted by flowers (Knudsen et al. 2006). Some of the flower species studied include *Abelia grandiflora* (Haynes et al. 1991), *Cestrum nocturnum* L. (Heath et al. 1992), 3 species of *Gaura* (Teranishi et al. 1991; Kint et al. 1993; Shaver et al. 1997), *Lonicera japonica* (Schlotzhauer et al. 1996), *Platanthera bifolia* L. (Rich.) (Plepys 2001), and *Mahonia* = *Berberis aquifolium* (Pursh) (Landolt & Smithhisler 2003). The volatiles released by these flowers contain multiple compounds. One of the compounds that has been shown to be attractive to moths is phenylacetaldehyde (PAA). It has been isolated from many flowering plants and shrubs that are attractive to moths (Haynes et al. 1991; Heath et al. 1992; Kint et al. 1993; Pair & Horvat 1997; Landolt & Smithhisler 2003). Phenylacetaldehyde as a single attractant or in mixtures has been used to attract various species of moths (Smith et al. 1943; Creighton et al. 1973; Cantelo & Jacobson 1979; Lopez et al. 2000; Landolt et al. 2001, 2006). In Florida, PAA is a strong attractant for several noctuid moths from different subfamilies, but particularly for soybean looper moths *Chrysodeixis* = *Pseudoplusia includens* (Walker) (Meagher 2001a, 2002). A recent study showed that the floral odorants *cis*-jasmone, benzyl acetate, limonene, linalool, β-myrcene, methyl salicylate, and methyl-2-methoxy benzoate all increased captures of some moths when added to traps with PAA, but responses varied among the moth species that were trapped (Meagher & Landolt 2008). One interesting result was that velvetbean caterpillar moths (*Anticarsia gemmatalis* Hübner) responded very strongly to PAA plus linalool (Meagher & Landolt 2008). Linalool has been found in several species of moth-visited flowers (Bruce & Cork 2001; Honda et al. 1998; Schlotzhauer et al., 1996).

Velvetbean caterpillar is a migratory noctuid that infests cultivated legumes including soybean

*Glycine max* (L.) Merrill, peanut *Arachis hypogaea* L., alfalfa *Medicago sativa* L., cowpeas *Vigna unguiculata* (L.) Walpers, and velvetbean *Mucuna pruriens* (L.) DC in the southeastern U.S. (Buschman et al. 1977; Herzog & Todd 1980; Buschman et al. 1981; Slansky 1989). It apparently overwinters in central and southern Florida and southern Texas where it feeds on wild legumes such as clovers *Melilotus* spp. and *Trifolium* spp., medics *Medicago* spp., kudzu *Pueraria lobata* (Willd.) Ohwi, and vetches *Vicia* spp. (Watson 1916; Buschman et al. 1981; Slansky 1989). Mating behavior studies (Johnson et al. 1981) led to the identification of the sex pheromone (Heath et al. 1983) and to the design of trapping systems (McLaughlin & Heath 1989; Mitchell & Heath 1986). However, the pheromone components are costly to synthesize and are not commercially available.

Meagher & Landolt (2008) found large numbers of velvetbean caterpillar moths in traps baited with a 2-component blend of PAA and linalool. Two experiments following this research were designed to test whether both floral volatiles (PAA and linalool) were needed for attraction and if capture number was influenced by the release rates of the chemicals.

## Materials and Methods

Standard Universal Moth Traps, "Unitraps" (Great Lakes IPM, Vestaburg, MI, USA) were used to capture moths that were attracted to chemical compounds. These traps were constructed of a white bucket, a yellow cone on top of the bucket, and a dark green cover above the cone. A 2.5 × 2.5-cm piece of Vaportape (Hercon Environmental, Emigsville, PA, USA), releasing the pesticide dichlorvos, was stapled to a string and hung inside the bucket to kill captured moths. Lures were suspended by a wire inside the bucket of the trap. Both chemicals tested were dispensed from 8-ml polypropylene vials (Nalg Nunc, Rochester, NY, USA). Five milliliters of active ingredient were added to cotton balls in the bottom of the vial. When 2 chemicals were tested in a single trap, each chemical was dispensed from a separate vial. Phenylacetaldehyde (W287407) and linalool (W263508, racemic mixture) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

Traps were placed on 1.5-m metal poles along roads and edges of 400-ha fields of commercial peanuts near Williston, FL (Levy County). Previous research suggested that velvetbean caterpillar moths are not found in north-central Florida until late Jul or Aug (Watson 1916, Watson 1932). Early season samples were collected in 2005 and 2009 with traps baited with a binary lure of PAA + linalool in vials with 3.2-mm lid opening diameters. One trap was placed at Williston on 6 May 2005 and removed 2 Jun 2005; in 2009 one trap was placed on 28 May and removed 18 Aug. Lures and vaportape were replaced every 2 weeks.

The 2 main experiments were conducted in 2005 and 2006 at Williston. A randomized complete block experimental design was used, with 5 replicate blocks for each experiment. Lures were replaced every 2 weeks and Vaportape was replaced every 4 weeks. The first experiment was designed to test linalool as a single attractant compared to it as a co-attractant. The 4 treatments were no lure as a control, PAA alone, linalool alone, and PAA + linalool. Traps were placed at least 30 m apart, and treatment order was randomized at each sample date. Release of the chemicals was allowed through lid opening diameters of the vials of 3.2 mm. Traps were set up at Williston on 13 Sep and moths were removed 15, 20, 23, 26, 28, and 30 Sep 2005. The second experiment in 2006 was designed to determine if different release rates from traps with the binary blend (PAA + linalool) affected moth capture. Different release rates were achieved by changing the diameter of the hole in the lid opening. The treatments were hole diameters of 1.0, 1.6, 3.2, 6.4, and 12 mm. This trapping test was set up 22 Aug 2006 and moths were removed 25 Aug and 7, 18, and 28 Sep 2006.

Moths captured were identified to species (Kimball 1965; Covell 2005), and then sorted by sex. Voucher specimens are deposited in the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. Trap catch data were subjected to an ANOVA (PROC MIXED, SAS 9.2, SAS Institute 2008), where block, date (block), and block*treatment were random variables (Littell et al. 1996). If the treatment variable produced a significant $F$ value, treatment means were separated by simple effect differences of the least square means. Sex ratios (number of males/number of females) were analyzed to determine if they were significantly different from 1.0 by a chi-square test (GraphPad Software, Inc., San Diego, CA).

A gravimetric method was used to estimate the loss of PAA and linalool from vials with lid opening diameters of 1.0, 1.6, 3.2, 6.4, and 12 mm that were placed outdoors and indoors. For both chemicals, four 8-mL vials were loaded with 5 mL chemical and placed in traps on 2 Jul 2009 in Gainesville, FL. The vials were reweighed weekly until 30 Jul. Because of high humidity outdoors, the vials were brought inside and placed in a laboratory fume hood and weighed periodically until 5 Nov 2009. Vial weight change over time was analyzed with regression analysis (PROC REG, SAS 9.2, SAS Institute 2008) and slopes were compared by Student's $t$ test statistic (Wuensch 2007).

## Results

### Early Season Samples

Traps baited with the binary lure of PAA + linalool and placed in Williston from 6 May to 2 Jun 2005 and from 28 May to 18 Aug 2009 captured several species of moths but not velvetbean caterpillar adults. Peanuts were planted in Williston by the time of sampling, but there was no evidence of larval infestation by velvetbean caterpillar.

### Importance of Blend Components

This experiment tested whether both compounds, PAA and linalool, were needed to attract moths. During a 17-d-period, a total of 934 male and female velvetbean caterpillar adults were collected in the Unitraps baited with floral lures. Captures varied between sampling dates but were generally highest in traps with the binary lure and lowest in traps with linalool (Fig. 1a). The binary lure of PAA + linalool attracted 81% of all captured velvetbean caterpillar moths, whereas the low captures by the single lures of PAA or linalool were not statistically different from captures by the unbaited traps ($F = 13.4$; $df = 3, 12$; $P = 0.0004$) (Fig. 1b). Although several other pest moth species were collected in high numbers in traps baited with PAA and PAA + linalool, very few moths of any species were collected in traps baited with linalool singly (data not shown).

### Testing of Different Release Rates

This experiment tested the attraction of moths to traps releasing different amounts of the binary lure compounds. Release rates were adjusted by changing the size of the opening in the lid of the vials containing the chemicals. Large numbers of velvetbean caterpillar moths (12,924) were collected in traps during the 4-week test (Fig. 2a). Average total captures varied between 1470 moths on 18 Sep in the 6.4 mm treatment to 2 moths on 25 Aug in the 12 mm treatment. Across all dates there was a non-significant trend for an inverse relationship between lid opening and moths captured as fewer moths were found in traps with the higher release rates ($F = 0.62$; $df = 4, 16$; $P = 0.6556$) (Fig. 2b).

An additional experiment attempted to estimate the release rates of each chemical from vials with different lid openings by measuring the weight loss of vials over time under outdoor and indoor conditions. Lid openings affected the release of the chemicals as shown by different regression slopes (mg change in weight per day) among the openings. For the outdoor experiment, vials containing PAA gained weight with the 1.0, 1.6 and 3.2 mm openings, but lost weight with the 12 mm openings (weight loss = 4.1 mg per day) (Fig. 3a). Vials containing linalool all gained weight except for the 12 mm opening (weight loss = 1.34 mg per day) (Fig. 3b). For both chemicals, slopes among openings were significantly different ($P < 0.05$) except for the 1.0 mm versus 1.6 mm comparisons ($P > 0.05$). Weight loss rates of vials placed in a dry environment inside the laboratory hood were higher, although PAA vials with 1.0 mm and 1.6 mm openings, and linalool vials with all openings except 12 mm, still gained weight (Fig. 4). Weight loss of vials with PAA increased from 0.2 to 1.4 to 4.9 mg per day for openings of 3.2, 6.4 and 12 mm, respectively. Vials with linalool had a loss of 5.3 mg per day when the opening was 12 mm. Loss rates among openings for both chemicals were significantly different ($P < 0.05$) except for the 1.0 mm versus 1.6 mm comparisons ($P > 0.05$).

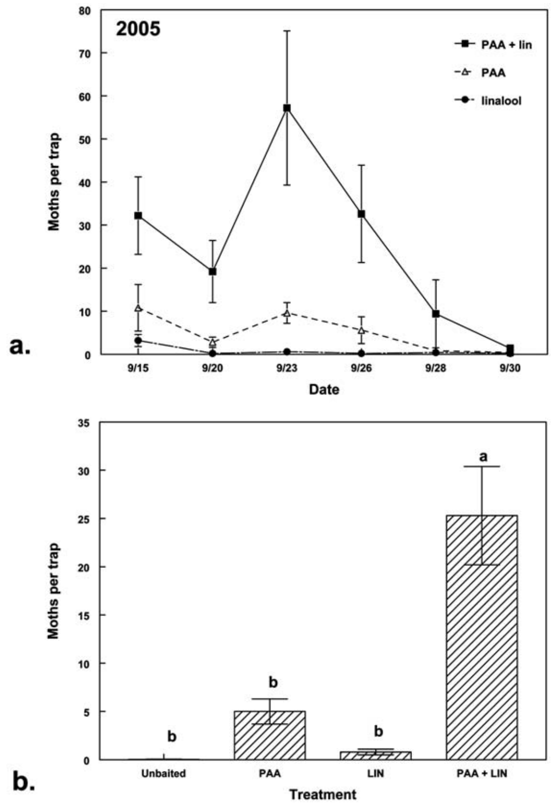


Fig. 1. Mean (±) SE numbers of velvetbean caterpillar moths captured in Universal moth traps ($n = 5$ per date per treatment) baited with phenylacetaldehyde (PAA) and linalool (LIN) dispensed from polypropylene vials, 2005, Williston, FL, USA. A. Captures over time. B. Summarized and compared per treatment. Bars followed by the same letter are not significantly different (P > 0.05, Contrasts, PROC Mixed).

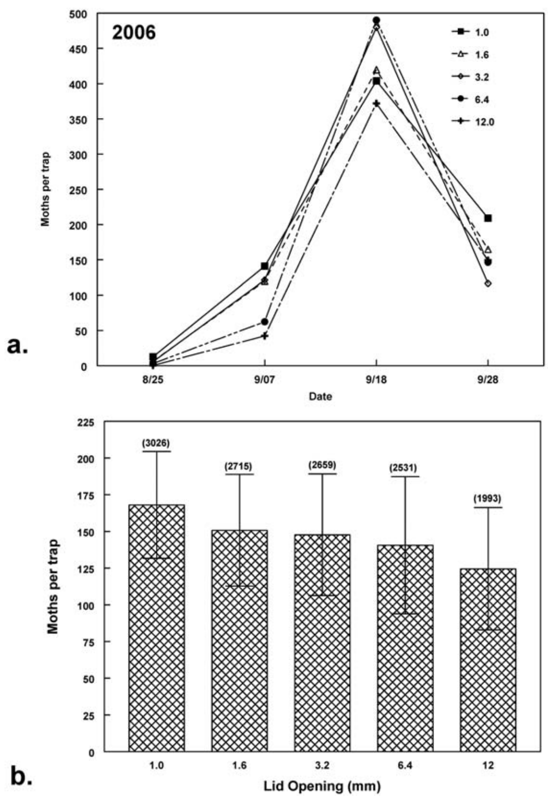


Fig. 2. Mean (±) SE numbers of velvetbean caterpillar moths captured in Universal moth traps ($n$ = 5 per date per treatment) baited with different release rates (lid opening diameter = mm) of phenylacetaldehyde + linalool dispensed from polypropylene vials, 2006, Williston, FL, USA. A. Captures over time. B. Summarized and compared per treatment with capture numbers in parenthesis. Means among openings were not significantly different (P > 0.05, Contrasts, PROC Mixed).

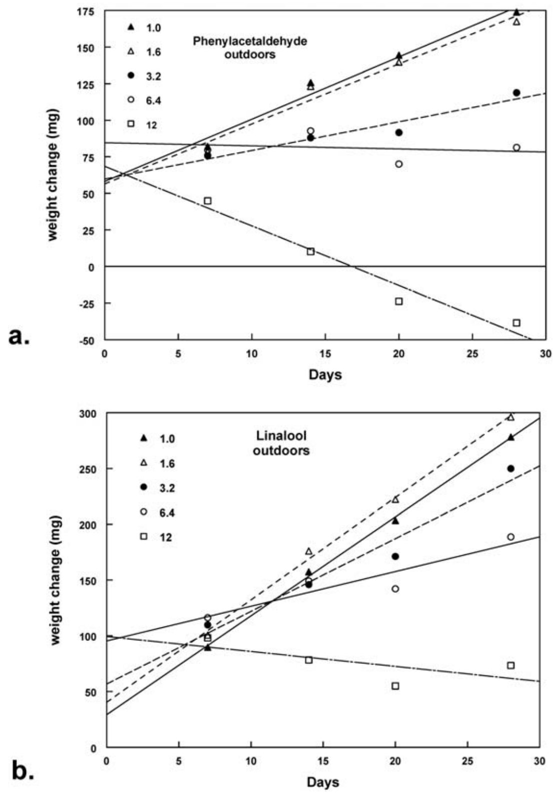


Fig. 3. Mean weight changes of 8-mL polypropylene vials placed outdoors with 5 mL of phenylacetaldehyde (A) or linalool (B) and with varying opening diameters of the vial lids. Regression slopes were all significantly different than 0 except for the 6.4 mm opening of phenylacetaldehyde vials. Regression equations for phenylacetaldehyde: 1.0, y = 58.1 + 4.3x; 1.6, y = 56.5 + 4.1x; 3.2, y = 59.8 + 2.0x; 12, y = 68.5 - 4.1x. Regression equations for linalool: 1.0, y = 29.2 + 8.9x; 1.6, y = 40.4 + 9.2x; 3.2, y = 56.7 + 6.5x; 6.4, y = 95.3 + 3.1x; 12, y = 99.3 - 1.34x.

### Sex Ratio

The sex ratio of captured velvetbean caterpillar moths in traps baited with PAA and PAA + linalool was male-biased. In 2005, over 5 times as many male velvetbean caterpillar moths were captured as female moths (sex ratio for PAA, 5.818, $\chi^2$ = 74.9, $P$ < 0.0001; PAA + linalool, 5.333, $\chi^2$ = 355.8, $P$ < 0.0001). In the 2006 release rate experiment, male moths were again more commonly collected than female moths. However, as the release rate increased the sex ratio decreased (lid opening 1.0 mm, 4.688, $\chi^2$ = 1272.1, $P$ < 0.0001; 1.6 mm, 4.507, $\chi^2$ = 1101.1, $P$ < 0.0001; 3.2 mm, 4.427, $\chi^2$ = 1060.2, $P$ < 0.0001; 6.4 mm, 3.982, $\chi^2$ = 906.8, $P$ < 0.0001; 12 mm, 3.205, $\chi^2$ = 547.9, $P$ < 0.0001; correlation coefficient = 0.997).

## Discussion

This study follows up on research completed with several lure combinations in Florida (Meagher & Landolt 2008). In that study velvetbean caterpillar moths and other moth species such as *C. includens*, *Argyrogramma verruca* (F.), *Mocis disseverans* (Walker), *M. latipes* (Guenée), *Diaphania hyalinata* (L.), *Heliothis virescens* (F.), and *Spodoptera eridania* (Stoll) all were captured in higher numbers in PAA + linalool-baited traps than in unbaited traps. Our experiments following that research showed the synergistic effect of the addition of linalool to PAA-baited traps because velvetbean caterpillar moths were captured in higher numbers in traps baited with the binary blend than in traps baited with either chemical alone.

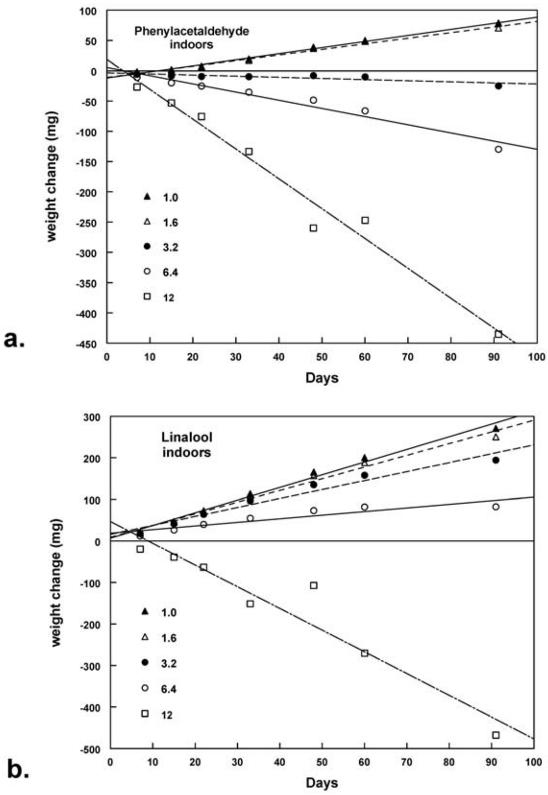


Fig. 4. Mean weight changes of 8-mL polypropylene vials placed indoors with 5 mL of phenylacetaldehyde (A) or linalool (B) and with varying opening diameters of the vial lids. Regression slopes were all significantly different than 0 ($P < 0.05$). Regression equations for phenylacetaldehyde: 1.0, y = -11.9 + 1.0x; 1.6, y = -10.9 + 0.9x; 3.2, y = -3.6 - 0.2x; 6.4, y = 5.4 - 1.4x; 12, y = 18.7 - 4.9x. Regression equations for linalool: 1.0, y = 6.5 + 3.1x; 1.6, y = 8.8 + 2.8x; 3.2, y = 16.0 + 2.2x; 6.4, y = 18.5 + 0.9x; 12, y = 46.9 - 5.3x.

Because the peanut fields were being managed commercially, it was initially surprising that large numbers of velvetbean caterpillar moths were captured starting in Aug. However, it was soon discovered that large areas of hairy indigo (*Indigofera hirsuta* L.) bordered the fields and were heavily infested by velvetbean caterpillars. This exotic plant is known to be a good host and in Brazil velvetbean caterpillar can maintain populations on it for most of the year (Panizzi et al. 2004).

Velvetbean caterpillar moths were captured in similar numbers in baited traps that had different vial lid openings, a result that was unlike captures of 6 other moth species which were captured in higher numbers with larger openings (data not shown). Attempts to determine the release rate of the 2 chemicals by vial weights over time provided interesting results. It was apparent, because of the large numbers of moths captured, that vials with small lid openings (1.0 and 1.6 mm) released undetermined amounts of chemical, but we hypothesize that the cotton balls consistently gained water weight, especially under high humidity weather conditions in Florida during the summer. However, vials with the 12-mm opening readily lost weight under the humid conditions. Vials with the 3.2- and 6.4-mm openings gained or lost weight in between those extremes. Bringing the experiment indoors increased the rate of weight loss of the vials, especially of vials containing PAA. The weight loss of vials with PAA in our study was 2.5 times less than what was found with a different release vessel (our study, 12 mm = 4.9 mg/d; Meagher 2001b, 0.493 mg/h × 24 h = 11.8 mg/d). Landolt et al. (2004) reported an estimated 4 mg per h loss of linalool with *p*-cresol and *m*-cresol from 15 mL vials with a 25 mm-diameter hole in the vial lid. The hygroscopic (water absorption) properties of linalool have been determined (Varutbangkul et al. 2006), although it is not known how these properties affect moth attraction. The gravimetric method used in this study proved inapplicable to determine release rates of PAA and linalool from the vials utilized in traps.

The large numbers of velvetbean caterpillar moths captured in traps suggest the possibility of using these attractants in an attract-and-kill strategy to manage moth populations (Landolt et al. 1991). Previous research showed the utility of the attract-and-kill strategy when alfalfa looper *Autographa californica* (Speyer) populations were controlled and oviposition on host plants was reduced in a screenhouse with pesticide-coated stations baited with a floral odor-based lure (Camelo et al. 2007). The PAA + linalool lures were attractive to both male and female moths, although there was a strong male bias of velvetbean caterpillar captured in traps. The sex ratio decreased with increasing release rates of the blend, but traps still contained high numbers of male moths relative to female moths. The success of the attract-and-kill strategy will depend on several other factors, such as the age of the population responding to the lures and the percentage of the actual population trapped. However, results shown in this study provide an incentive to future attractants research.

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