Research Article

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Interaction of acetic acid and phenylacetaldehyde as attractants for trapping pest species of moths (Lepidoptera: Noctuidae)

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Abstract

BACKGROUND: Phenylacetaldehyde is a flower volatile and attractant for many nectar-seeking moths. Acetic acid is a microbial fermentation product that is present in insect sweet baits. It is weakly attractive to some moths and other insects, but can be additive or synergistic with other compounds to make more powerful insect lures.

RESULTS: Acetic acid and phenylacetaldehyde presented together in traps made a stronger lure than either chemical alone for moths of the alfalfa looper *Autographa californica* (Speyer) and the armyworm *Spodoptera albula* (Walker). However, this combination of chemicals reduced captures of the cabbage looper moth *Trichoplusia ni* (Hübner), the silver Y moth *Autographa gamma* (L.), *MacDunnoughia confusa* (Stephens) and the soybean looper moth *Chrysodeixis includens* (Walker) by comparison with phenylacetaldehyde alone.

CONCLUSION: These results indicate both positive and negative interactions of acetic acid, a sugar fermentation odor cue, and phenylacetaldehyde, a floral scent cue, in eliciting orientation responses of moths. This research provides a new two-component lure for the alfalfa looper *A. californica* and for the armyworm *S. albula* for potential use in pest management.

Keywords: acetic acid; attractant; lure; moth; phenylacetaldehyde; insect trap

1 INTRODUCTION

Phenylacetaldehyde (PAA) has been identified as an odorant of several moth-visited flowers and an attractant for a number of species of Lepidoptera, including pest loopers (Noctuidae, Plusiinae). Flowers that are visited by moths and produce PAA include *Araujia sericofera*,[1] *Gaura drummondi*,[2] *Abelia grandiflora*,[3] *Cestrum nocturnum*,[4] *Lonicera japonica*,[5] *Berberis aquifolium*,[6] *Buddleia davidii*[7] and *Cirsium arvense*.[8] Much of the research on moth orientation to floral scent chemistry has involved pest species of Plusiinae (Noctuidae), with the goal of discovering and developing lures for females or for both sexes. Plusiinae moths attracted to PAA include the cabbage looper *Trichoplusia ni* (Hübner), the soybean looper *Chrysodeixis includens* (Walker),[9] the alfalfa looper *Autographa californica* (Speyer),[10] the silver Y moth *Autographa gamma* (L.), *MacDunnoughia confusa* (Stephens),[11] the golden looper *Argyrogramma verruca* (F.)[12] and *Thysanoplusia orichalsea* (F.).[13] It is assumed but not well documented that these moths respond to PAA and other floral scent compounds as a means of seeking sugar-rich floral nectars.

Acetic acid (AA) is produced by microbial fermentation of sugars and is a volatile chemical produced by sweet baits that attract insects.[14,15] It is by itself a weak attractant for insects[15] but is a coattractant or synergist with other compounds to form more powerful attractants for a variety of different insect taxa. Examples include the combination of AA and ethanol as an attractant for *Calliphora* sp. blowflies,[16] AA and isobutanol as a lure for temperate vespid wasps,[17,18] AA and 3-methyl-1-butanol as an attractant for moths[11,19–23] and AA and pear ester as a lure for codling moth *Cydia pomonella* (L.).[24]

Acetic acid may be a general indicator or cue to insects of sugar-rich materials that are colonized by microbes. Natural and man-made sugar sources or sweet materials, including floral nectar, have potential for colonization by microbes such as yeasts[25] that can produce AA and other fermentation byproducts. Although microbial colonization of a floral nectar may degrade nectar as an insect food source,[25] it is hypothesized that AA as a microbial-produced volatile may be an important food cue, along with flower-produced scents, for some nectar-seeking insects.

This paper reports the results of experiments that tested the hypothesis that acetic acid enhances attraction of pest Plusiinae moths to floral scent compounds. Experiments were conducted in Washington State to study *A. californica* and *T. ni*, in Florida to

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study *C. includens* and in Hungary to study *A. gamma*. Information was incidentally obtained for several other species of pest moths.

2 MATERIALS AND METHODS

Experiments conducted in Washington State and Florida used the universal moth trap, or Unitrap® (Agrisense BCS, Pontypridd, UK), which is a white bucket topped by a yellow cone and a green lid about 3 cm above the cone. Vaportape® (Hercon Environmental Inc., Emigsville, PA) was placed in each trap bucket to kill captured moths. Chemicals tested (AA, PAA, β-myrcene and methyl salicylate) were dispensed from 8 mL polypropylene vials (Nalge Nunc, Rochester, NY), each with a 3 mm diameter hole in the lid to provide chemical release. Chemicals were loaded at 4 mL per vial, in cotton balls pushed into the bottom of the vial. The cotton holds the chemicals (all liquids at room temperature) and reduced the risk of spillage. Each chemical was dispensed from a separate vial. Acetic acid, PAA, methyl salicylate and β-myrcene were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Experiments in Hungary used the CSALOMON VARL+ trap produced by the Plant Protection Institute (Hungarian Academy of Sciences, Budapest, Hungary). In one of the two tests conducted in Hungary, AA, PAA and β-myrcene were formulated in polyethylene bag dispensers. For making these baits, 400 mg of each compound was loaded onto a 1 cm piece of dental roll (Celluron; Paul Hartmann AG, Heidenheim, Germany) which was put into a polyethylene bag (1.0 × 1.5 cm) made of 0.02 mm linear polyethylene foil. The bait dispensers were heat sealed and attached to 8 × 1 cm plastic strips for easy handling when assembling and baiting traps. Lures were wrapped singly in aluminum foil and stored at −18 °C until use. In a second test in Hungary, vials were used as chemical dispensers as described for the experiments in Washington and Florida. In the field, lures were changed at 2–3 week intervals, as previous experience with similar baits had shown that they may start to lose activity after this period.[26]

2.1 Washington field experiments

Two experiments were conducted in Washington to evaluate the effects of acetic acid on moth response to floral lures. The first experiment was planned for the trapping of *T. ni* and used PAA plus methyl salicylate as a positive control for that moth. The second experiment targeted *A. californica* moths and included PAA plus β-myrcene as a positive control because it is particularly attractive to this species.[27]

The first Washington experiment, with eight treatments, compared (1) unbaited traps with (2) AA, (3) PAA, (4) AA and PAA, (5) methyl salicylate, (6) PAA and methyl salicylate, (7) AA and methyl salicylate and (8) AA, PAA and methyl salicylate. For multicomponent treatments, each chemical was dispensed from a separate vial. A randomized complete block experimental design was used, with ten replicate blocks. Traps were located along the edges of apple orchards near Zillah, Yakima County, Washington. Traps were set up in the field on 2 September 2008 and taken down on 29 September 2008. Each week during that time interval, insects were removed from traps and placed in prelabeled Ziplock® plastic bags for transport to the laboratory, and traps were rerandomized within blocks. Lures were replaced on 15 September.

The second experiment, with eight treatments, compared (1) unbaited traps with (2) AA, (3) PAA, (4) AA and PAA, (5) β-myrcene, (6) PAA and β-myrcene, (7) AA and β-myrcene and (8) AA, PAA and β-myrcene. Chemicals were dispensed from vials as described above. A randomized complete block experimental design was used, with ten replicate blocks. Traps were located along the edges of apple orchards near Zillah, Yakima County, Washington. Traps were set up and maintained as described for the preceding experiment from 29 September to 20 October. Lures were replaced on 14 October.

2.2 Florida experiment

Two experiments conducted in Florida compared the following four treatments: (1) unbaited trap, (2) AA, (3) PAA and (4) AA and PAA. Chemicals were loaded at a rate of 5 mL in an 8 mL vial with a 3 mm diameter hole in the lid. For multicomponent treatments, each chemical was dispensed from a separate vial. A randomized complete block experimental design was used, with ten replicate blocks. Traps were located along the edges of fields of peanut (*Arachis hypogaea*) in Levy County, Florida. For the first experiment, traps were maintained from 4 to 20 August 2009. For the second experiment, traps were maintained from 5 August to 14 September 2010. Lures were replaced after 2 weeks in the field, and traps were checked each week. When traps were checked, captured insects were removed, and treatments were rerandomized within each block.

2.3 Hungary experiment

Two experiments compared AA, PAA and the combination of AA with PAA, using two types of dispenser (vials and bags). The treatments were the same for both experiments, and were (1) an untreated control, (2) AA in a vial, (3) PAA in a vial, (4) AA in a vial and PAA in a second vial, (5) AA in a bag, (6) PAA in a bag and (7) AA in a bag and PAA in a second bag. A randomized complete block design was used, with five replicate blocks. The first experiment was set up near Debrecen, Hungary, and was maintained from 3 June to 28 September 2009. Traps were checked each week, and lures were replaced every 3 weeks. The second experiment was set up near Halásztelek, Hungary, on 27 July and was maintained until 5 October 2009. Traps were checked twice per week, and lures were replaced every 3 weeks.

For each trap, catch data were summed over the duration of the experiment. For each experiment, data were analyzed using a one-way analysis of variance (ANOVA). Treatment means were separated using Tukey's test (Washington and Florida) or the Tukey–Kramer test (Hungary).

3 RESULTS

3.1 Washington field experiments

In the first trapping experiment, male and female *A. californica* were trapped with PAA and combinations of chemicals that included PAA (for males: ANOVA $F = 15.9$, df $= 79$, $P < 0.001$; for females: $F = 14.1$, df $= 79$, $P < 0.001$) (Table 1). Greater numbers of male and female moths were captured in traps baited with the combination of PAA and AA as opposed to PAA alone, and greater numbers of both sexes of moths were captured with the combination of PAA, methyl salicylate and AA as opposed to PAA plus methyl salicylate (Table 1). *Autographa californica* moths were not captured in traps baited with methyl salicylate, and this chemical did not increase *A. californica* response when added to PAA or when added to PAA with AA. *Autographa californica* moths were not captured in traps baited with acetic acid, and acetic acid did not increase *A. californica* response to methyl

Table 1. Mean (± SE) numbers of male and female *A. californica* and *T. ni* moths captured in traps baited with combinations of acetic acid (AA) and the floral odorants phenylacetaldehyde (PAA) and methyl salicylate (MS). Washington[a]

	Control	AA	PAA	AA + PAA	MS	PAA + MS	AA + MS	AA + PAA + MS
A. californica ♂	0.0 ± 0.0 c	0.1 ± 0.1 c	10.8 ± 2.2 b	19.1 ± 4.3 a	0.0 ± 0.0 c	10.9 ± 1.8 b	0.6 ± 0.2 c	19.4 ± 3.2 a
A. californica ♀	0.0 ± 0.0 c	0.0 ± 0.0 c	7.2 ± 1.8 b	12.8 ± 3.4 a	0.0 ± 0.0 a	8.6 ± 1.2 ab	0.0 ± 0.0 c	13.6 ± 2.0 a
T. ni ♂	0.0 ± 0.0 c	0.0 ± 0.0 c	1.0 ± 0.4 b	0.0 ± 0.0 c	0.0 ± 0.0 c	1.5 ± 0.8 a	0.0 ± 0.0 c	0.1 ± 0.1 c
T. ni ♀	0.0 ± 0.0 d	0.0 ± 0.0 d	1.5 ± 0.3 b	0.2 ± 0.1 d	0.0 ± 0.0 d	2.0 ± 0.3 a	0.0 ± 0.0 d	0.7 ± 0.3 c

[a] Means in a row followed by the same letter are not significantly different at $P \leq 0.05$ by Tukey's test.

Table 2. Mean (± SE) numbers of male and female *A. californica* and *T. ni* moths captured in traps baited with combinations of acetic acid (AA) and the floral odorants phenylacetaldehyde (PAA) and β-myrcene (BM). Washington[a]

	Control	AA	PAA	AA + PAA	BM	PAA + BM	AA + BM	AA + PAA + BM
A. californica ♂	0.0 ± 0.0 d	0.0 ± 0.0 d	21.0 ± 2.4 c	36.6 ± 8.1 b	0.0 ± 0.0 d	73.2 ± 8.4 a	0.1 ± 0.1 d	72.9 ± 15.5 a
A. californica ♀	0.0 ± 0.0 d	0.0 ± 0.0 d	5.5 ± 0.8 c	8.2 ± 2.3 c	0.0 ± 0.0 d	22.5 ± 3.6 a	0.0 ± 0.0 d	18.0 ± 4.5 b
T. ni ♂	0.0 ± 0.0 a	0.0 ± 0.0 a	0.2 ± 0.1 a	0.1 ± 0.1 a	0.0 ± 0.0 a	0.2 ± 0.1 a	0.1 ± 0.1 a	0.1 ± 0.1 a
T. ni ♀	0.0 ± 0.0 b	0.0 ± 0.0 b	0.7 ± 0.3 a	0.1 ± 0.1 b	0.0 ± 0.0 b	0.8 ± 0.3 a	0.0 ± 0.0 b	0.1 ± 0.1 b

[a] Means in a row followed by the same letter are not significantly different at $P \leq 0.05$ by Tukey's test.

salicylate. *Trichoplusia ni* numbers captured were small, and were significantly greater than unbaited traps only in traps baited with PAA and PAA plus methyl salicylate (for males: $F = 3.4$, df = 79, $P = 0.004$; for females: $F = 10.2$, df = 79, $P < 0.001$) (Table 1). There was no evidence of attractiveness or coattractiveness of AA for *T. ni* moths in this experiment, but AA added to traps baited with PAA resulted in fewer moths captured by comparison with PAA alone.

In the second experiment (for males: $F = 21.8$, df = 79, $P < 0.001$; for females: $F = 16.6$, df = 79, $P < 0.001$) (Table 2), results were similar to those of the preceding experiment; male and female *A. californica* moths were attracted to PAA, and not to acetic acid. Again, AA enhanced the attractiveness of PAA to both sexes. *Autographa californica* moths were not trapped with β-myrcene, but this compound enhanced the attractiveness of PAA to both males and females. The three-component blend of AA, PAA and β-myrcene was not more attractive than PAA plus β-myrcene, but was more attractive than PAA plus AA. As in the prior experiment, small numbers of male and female *T. ni* were captured in this test (for males: $F = 0.8$, df = 79, $P < 0.55$; for females: $F = 4.6$, df = 79, $P < 0.001$) (Table 2). PAA and PAA + β-myrcene were attractive to *T. ni* females. As in the previous experiment, AA significantly reduced numbers of female *T. ni* moths when added to traps with PAA and when added to traps with PAA and β-myrcene.

3.2 Florida field experiments

In 2009, *C. includens* moths, as well as *Spodoptera albula* (Walker), were trapped in Florida (Table 3). Numbers of female and male *C. includens* in traps baited with PAA were significantly greater than in unbaited traps, while numbers of *C. includens* in traps baited with AA were not significantly greater than in unbaited traps (for males: $F = 25.4$, df = 136, $P < 0.0001$; for females: $F = 22.7$, df = 136, $P < 0.0001$) (Table 3). Numbers of *C. includens* moths of either sex were significantly reduced in traps baited with the two chemicals together as opposed to PAA alone. Both sexes of *S. albula* were attracted to PAA in this test. Numbers of *S. albula* female moths, but not males, were increased in traps baited with PAA plus AA compared with PAA alone or AA alone (for males: $F = 9.2$, df = 136, $P < 0.0001$; for females: $F = 10.6$, df = 136, $P < 0.0001$) (Table 3).

In 2010, *C. includens* moths were trapped again in Florida (Table 3). As in 2009, numbers of female and male *C. includens* in traps baited with PAA were significantly greater than in unbaited traps, while numbers of *C. includens* in traps baited with AA were not significantly greater than in unbaited traps (for males, $F = 24.6$, df = 102, $P < 0.0001$; for females: $F = 24.0$, df = 102, $P < 0.0001$) (Table 3). Numbers of *C. includens* moths of either sex were significantly reduced in traps baited with the two chemicals together compared with PAA alone. The numbers of *S. albula* and other pest species of moths were too few for statistical analyses.

3.3 Hungary field experiments

Autographa gamma moths were captured in traps baited with PAA in vials, both at Debrecen and Halásztelek (for Debrecen: $F = 7.6$, df = 6, $P < 0.0001$; for Halásztelek: $F = 15.1$, df = 6, $P < 0.0001$) (Table 4). Moth capture was not affected by AA, and numbers of moths in traps baited with PAA plus AA were greatly reduced compared with traps with PAA in vials. At Debrecen the numbers of moths in traps baited with PAA in bags were considerably fewer than in traps baited with PAA in vials, but at Halásztelek the responses by moths to PAA from bags and vials were similar. At Debrecen, numbers of *A. gamma* moths captured with AA plus PAA were numerically lower but not statistically different from numbers captured with PAA alone.

MacDunnoughia confusa moths were also captured in traps baited with PAA in vials and in bags (for Debrecen: $F = 10.4$, df = 6, $P < 0.0001$; for Halásztelek: $F = 10.7$, df = 6, $P < 0.0001$) (Table 4), and fewer moths were captured when acetic acid and PAA were presented together in traps as opposed to PAA alone.

Table 3. Mean (± SE) numbers of *C. includens* and *S. albula* moths captured in traps baited with acetic acid (AA) and the floral odorant phenylacetaldehyde (PAA). Florida, 2009 and 2010[a]

	Control	AA	PAA	AA + PAA
2009				
C. includens ♂	0.04 ± 0.03 c	0.02 ± 0.02 c	9.9 ± 2.1 a	3.8 ± 0.8 b
C. includens ♀	0.08 ± 0.04 c	0.02 ± 0.02 c	14.3 ± 3.0 a	5.1 ± 1.4 b
S. albula ♂	0.04 ± 0.03 b	0.06 ± 0.03 b	0.33 ± 0.10 a	0.41 ± 0.09 a
S. albula ♀	0.00 ± 0.00 c	0.28 ± 0.10 bc	0.53 ± 0.17 b	1.20 ± 0.32 a
2010				
C. includens ♂	0.00 ± 0.00 b	0.00 ± 0.00 b	3.11 ± 0.69 a	0.46 ± 0.13 b
C. includens ♀	0.09 ± 0.05 b	0.03 ± 0.03 b	4.20 ± 0.85 a	0.57 ± 0.25 b

[a] Means in a row followed by the same letter are not significantly different at $P \leq 0.05$ by an ls means test using Proc Mixed SAS v.9.2.

Table 4. Mean (± SE) numbers of *A. gamma* and *M. confusa* moths captured in traps baited with acetic acid (AA) and the floral odorant phenylacetaldehyde (PAA). Hungary 2009[a]

	Control	AA in vial	PAA in vial	AA + PAA in vial	AA in bag	PAA in bag	AA + PAA in bag
Debrecen							
A. gamma	0.0 ± 0.0 b	0.2 ± 0.2 b	3.6 ± 1.3 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.6 ± 0.2 b	0.2 ± 0.2 b
M. confusa	0.0 ± 0.0 b	0.0 ± 0.0 b	3.4 ± 1.1 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.4 ± 0.2 b	0.6 ± 0.4 b
Halasztelek							
A. gamma	0.0 ± 0.0 b	0.0 ± 0.0 b	9.6 ± 2.9 a	0.6 ± 0.4 b	0.0 ± 0.0 b	7.2 ± 1.1 a	3.8 ± 1.2 a
M. confusa	0.0 ± 0.0 b	0.0 ± 0.0 b	6.6 ± 1.7 a	0.4 ± 0.2 ab	0.0 ± 0.0 b	7.6 ± 1.3 a	5.4 ± 3.3 a

[a] Means in a row followed by the same letter are not significantly different at $P \leq 0.05$ by the Tukey–Kramer test.

4 DISCUSSION

Acetic acid enhanced *A. californica* moth attraction to PAA when dispensed from vials in traps, but did not have the same effect for the related moths *T. ni, C. includens, A. gamma* and *M. confusa*. For the latter pest looper species, AA had a negative or deterrent effect on moth attraction to PAA, as evidenced by their reduced capture in traps. All of these species are Plusiinae and are attracted to PAA.

These results add to an increasing body of information indicating roles of AA in the food-finding behavior of insects. Acetic acid enhances the response of many moths to 3-methyl-1-butanol,[19,23,28,29] calypterate flies and spotted wing drosophila, *Drosophila suzukii* (Matsumura), to ethanol,[16,30] social Vespidae to isobutanol[17,18] and codling moth to pear ester.[24] Microbial fermentation of sweet baits that are attractive to insects produces AA, among other volatile odorants,[14,15] suggesting that AA may indicate a source of sugar. This compound may serve as a feeding coattractant in conjunction with an attractive floral scent where nectar is colonized by microbes that ferment the nectar sugar. Perhaps this is why *A. californica* and *S. albula* moth attraction to the floral scent compound PAA is enhanced by the presence of AA. A similar explanation can be suggested for the case of certain green lacewings (Neuroptera: Chrysopidae). In that group, species with adults that feed at flowers (*Chrysoperla carnea* sensu lato) respond better to a blend of AA and PAA than to either compound on its own.[31]

It is puzzling that some moths (*A. californica, S. albula*) respond positively to AA released with PAA, while others (*T. ni, C. includens, A. gamma*) respond negatively. Perhaps there are fundamental differences in where the different species of moths seek and obtain nectar in the natural environment that would lead some species to respond to AA as a coattractant with a floral scent compound, and lead other species to be deterred by the presence of AA with floral scent chemicals. Yeast colonization of floral nectars degrades nectar quality for bees,[25] suggesting that it may reduce the quality of nectar as food for moths. However, information on this question is unavailable for Lepidoptera. Perhaps, then, moth species differ in their tolerance or use of clean versus fermented nectars as foods.

The dispenser and release rate (by vial hole size) of AA used in this study were drawn from results of prior work to optimize responses of other moths such as *Lacanobia subjuncta* (Barnes and MacDunnough) to acetic acid plus 3-methyl-1-butanol.[32] Similar information on the effects of varied release rates of AA on attraction responses of other moth species such as the looper species studied here is unavailable. So, it is possible that different results may be obtained with changes in the amounts of AA released from vials. In Hungary, the release rates of chemicals dispensed from polyethylene bags probably differed from the release rates of chemicals from vials, providing a possible explanation for the general differences in catches of moths in traps baited with floral chemicals in bags versus vials. Future evaluations of varying lure component release rates and ratios should be pursued to determine optimum lure parameters for these pests.

This work confirms attraction of these species to PAA, confirms coattractiveness of PAA and β-myrcene for *A. californica* and *T. ni* and demonstrates coattractiveness of methyl salicylate and PAA for *T. ni*. These combinations of floral compounds have been found to be preferentially attractive to different species of Plusiinae.[10,12,27]

It has been suggested that chemical lures that are attractive to female moths, or to both sexes, may be more useful for some pest management purposes than lures that attract only males. For example, the trapping of females, compared with the trapping of males, may provide better information on the timing of adult moth activity[33] or as a predictor of damaging pest populations.[34] The trapping or baiting of females versus males may have a more direct negative impact on pest populations. Preliminary demonstrations have been made of attract-and-kill approaches using similar floral-based lures against the cabbage looper[35] and the alfalfa looper.[36] The development of attract-and-kill technology using these floral-based feeding attractants could provide an alternative to pesticidal cover sprays on numerous vegetable crops that are damaged by pest species of Plusiinae loopers, reducing both pesticide amounts used and pesticide contact with the crop.

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