

# An Attracticide for Control of *Amyelois transitella* (Lepidoptera: Pyralidae) in Almonds

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**ABSTRACT** A wind-tunnel bioassay was used in conjunction with field trapping to determine long-range behavioral response of navel orangeworm, *Amyelois transitella* (Walker), females to almond by-products and to assess potential for developing an attracticide by combining these materials with pyrethroids. Crude almond oil (CAO) was 10-fold more effective in attracting navel orangeworm females, on a per-weight basis, than almond presscake (PC); the combination of PC with CAO elicited no greater response than did CAO alone. Mummy almonds, which are those that remain on the tree after harvest, provide the primary sites for oviposition by *A. transitella* females in the spring, but CAO attracted twice as many females as did larvae-infested mummy almonds and 6.5-fold more than did uninfested mummies. Upwind-flight response to almond materials seemed to be restricted to gravid females, as these materials evoked no response in either virgin males or virgin females. When CAO was combined with pyrethroids, upwind attraction was not significantly affected by either 0.1 or 0.5% formulations of either permethrin or cypermethrin; however, response was reduced when either of these compounds was present at a 1% level. Fenvalerate did not interfere with response to CAO, even at the 1% level. Mortality studies of females attracted to a formulation of nonracemic fenvalerate with CAO showed greatest efficacy (90% mortality) when the toxicant was present at a 2.5% concentration.

**KEY WORDS** *Amyelois transitella*, attracticide, host odors, almonds, pyrethroids

DESPITE CONSIDERABLE ADVANCES that have been made in cultural control (Barnes & Curtis 1979), the navel orangeworm, *Amyelois transitella* (Walker), remains the key pest of almonds and continues to inflict millions of dollars in damage each year. Past studies have suggested that almond odors may play a role in host selection by females (Curtis & Clark 1979, Andrews & Barnes 1982, Rice et al. 1984); however, the extent of this role is not clear. Since these studies have focused almost exclusively on egg laying, it is impossible to know if these odors elicit long-distance attraction, or if their effect is only short range, by evoking either oviposition or in-flight arrestment.

The objectives of the present study were to measure the long-range response of navel orangeworm females to host odors, and to explore the potential for a novel means of navel orangeworm control by incorporating a toxicant into an attractive bait formulation.

## Materials and Methods

**Wind-tunnel Bioassay.** Navel orangeworm larvae were reared at  $23 \pm 1^\circ\text{C}$  and a photoperiod of 16:8 (L:D) on a honey/bran/glycerol diet (Strong et al. 1968). Adults eclosed from the pupal stage as mixed sexes. Two to 2.5 h before lights were

turned off, 2- to 4-d-old females (>99% mated) were placed in groups of 10 into screen cylinders (55 mm diameter by 80 mm long), the open end of which was closed by a petri-dish cover. The females were allowed to acclimate to simulated twilight conditions ( $1.5 \text{ lx}$  and  $21 \pm 3^\circ\text{C}$ ) for 0.5 h in the wind-tunnel room. Bioassays were then conducted for 2 h.

In the processing of almonds for oil, navel orangeworm-damaged almonds are combined with rice hulls and compressed, producing "crude" almond oil (CAO) and a residual solid, "presscake" (PC). Subsequently, the crude oil is passed through a series of purification steps, resulting in "refined" almond oil (RAO). These three almond by-products were tested as potential lures and were provided by Liberty Vegetable Oil (Santa Fe Springs, Calif.). In addition, the long-range response of navel orangeworm females to larvae-infested and uninfested "mummy" almonds was examined. Mummy or "stick-tight" almonds are those nuts that remain on the tree after harvesting. Overwintering by the navel orangeworm occurs primarily in these nuts, which also represent the primary sites for oviposition by females in the spring (Wade 1961).

The search for a candidate toxicant for the attracticide formulation focused on three pyrethroids: permethrin, cypermethrin, and fenvalerate. Preliminary studies of topical toxicity (M. M. Barnes, University of California, Riverside, personal communication) indicated that these com-

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pounds possessed similar toxicities, which were more than an order of magnitude greater than those of most organophosphate insecticides. These compounds were tested for any tendency either to be repellent or at least to diminish the response of mated females to host odors by combining them with CAO at the rates of 0, 0.1, 0.5, and 1.0% (wt/wt).

All experimental materials were bioassayed in a previously described wind tunnel (0.6 by 0.6 by 1.8 m) (Willis & Baker 1984) using a wind speed of 0.5 m/s. Oil treatments were applied to a filter-paper circle (Whatman no. 1, 5.5-cm diameter), which was held by an alligator clamp connected to a ring stand 20 cm from the upwind end of the tunnel and 30 cm from the floor. PC was ground to a coarse powder, placed in a petri-dish cover (5.5-cm diameter), and positioned in the wind tunnel like the oil treatments. To evaluate female response to almond mummies, almond by-products, and CAO combined with insecticides, a screen cylinder containing 10 females was placed on a release platform 2 m downwind from and at a height parallel with the filter-paper odor source. The cover was gently removed from the cylinder and the numbers of females exhibiting the following behaviors were recorded: taking flight, flying upwind in the chemical plume, and landing on the source. For each bioassay, a randomized complete-block experimental design was employed with nine replicates of 10 females per replicate, except for the permethrin experiment, in which 12 replicates were used. Percentages of responses to treatments were compared using two-way analysis of variance (ANOVA) and Duncan's (1955) multiple range test after arcsine  $\sqrt{x}$  transformation.

The potential of a CAO/toxicant formulation to attract and kill females also was assessed, using resolved fenvalerate (Shell MO70616) with a toxicity to lepidopteran pests 3- to 4-fold greater than that of racemic fenvalerate. Using the same wind-tunnel procedure as before, females were allowed to fly up to and contact the attracticide formulation; however, upon leaving, females were captured with a small fish-tank net and held 24 h to assess mortality. Treatments were presented randomly and percentages of mortality were compared using Ryan's (1960) multiple comparison test for proportions ( $n = 40$  females).

**Field Trapping.** Results from the wind tunnel were corroborated by field trapping. During the period 17 April–29 May 1985, materials were assessed for their ability to lure female navel orangeworms. Pherocon 1C traps (Zoecon, Palo Alto, Calif.) were placed in three widely separated mature almond orchards located in the Central Valley of California. Liquid treatments were applied to a cotton dental wick (3 by 1 cm) and hung in the center of the trap. Treatments containing PC were held in Zoecon egg traps, which also were hung in the Pherocon traps. Since Zoecon egg traps containing PC with CAO are currently used to monitor

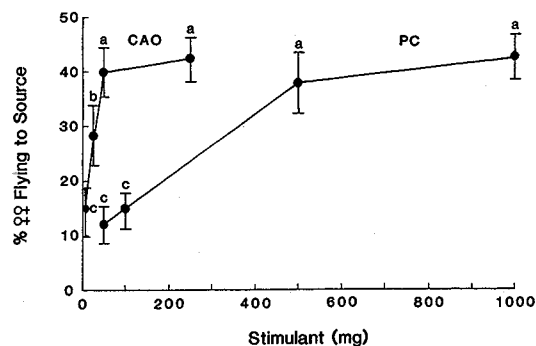


Fig. 1. Mean percentage of response ( $\pm$ SEM) of mated navel orangeworm females to varying quantities of CAO and PC in a wind tunnel. Means marked by the same letter are not significantly different by Duncan's (1955) multiple range test after arcsine  $\sqrt{x}$  transformation ( $n =$  nine groups of 10 females each).

the navel orangeworm (Van Steenwyk et al 1986), their inclusion in the present study was meant to provide comparable almond-volatile release rates. Finally, a trap containing three larvae-infested mummies was included to provide a comparison with natural oviposition sites. In a second field study, the effect on trap catch of various concentrations of fenvalerate (0, 0.5, and 1.0%) in 500 mg of CAO was assessed. For each experiment, traps within a block were hung along a single row, separated by four trees, and blocks ( $n = 9$ ) were separated by at least four rows. Trap capture was recorded weekly, at which time treatments were replaced and rerandomized. Results were analyzed using two-way ANOVA followed by Duncan's (1955) multiple range test after  $\sqrt{x + 0.5}$  transformation.

## Results

In the wind-tunnel bioassay, we found CAO to be ca. 10-fold more effective than PC in attracting mated navel orangeworm females (Fig 1). Maximum response was elicited by 50 mg of CAO, compared with 500 mg of PC necessary for a similar level of response. Moreover, combining PC and CAO in a 10:1 ratio did not increase the level of response over that to CAO alone (Fig 2). RAO evoked only a minimal response, suggesting that the behaviorally active components of CAO are removed by the refining process.

The specificity of response to almonds with regard to mating status and sex is striking. CAO elicited no response from either virgin males or virgin females, whereas the response of concurrently tested mated females was quite high (68%), indicating that a significant switchover in the behavioral program is brought about by mating. Thus, if almond odors are used in an attracticide, it is clear that only gravid females will be targeted.

Because mummy nuts represent the primary sites for oviposition by females during spring and early

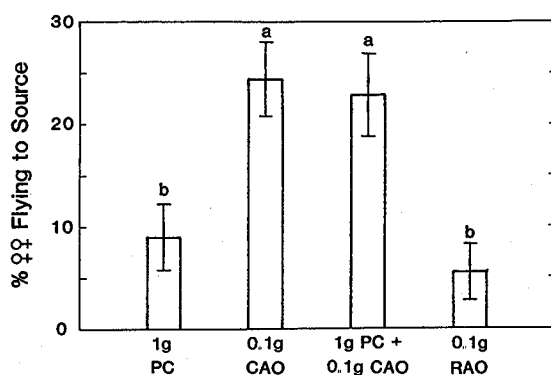


Fig. 2. Mean percentage of response ( $\pm$ SEM) of mated navel orangeworm females to PC, CAO, combined PC and CAO, and RAO in a wind tunnel. Means marked by the same letter are not significantly different by Duncan's (1955) multiple range test after arcsine  $\sqrt{x}$  transformation ( $n =$  nine groups of 10 females each).

summer, they become the standard by which other potential attractants are measured. Wind-tunnel results suggest that 100 mg of CAO was indeed competitive with mummies (Fig. 3), evoking a response 2.5-fold greater than that to a cluster of three mummies infested with navel orangeworm larvae and 6.5-fold greater than that to three uninfested mummies. The higher response of females to infested versus uninfested nuts is consistent with previous field observations (Andrews & Barnes 1982).

To confirm that wind-tunnel results are an accurate predictor of navel orangeworm behavior in the orchard, almond by-products were also tested by field trapping. Although only low numbers of females were captured, the relative pattern was consistent with the results from the wind tunnel (Fig. 4). The 50- and 500-mg doses of CAO performed better than PC, which did not attract significantly more moths than the blank. These doses of CAO were just as attractive as the combined PC + CAO. As in the wind tunnel, the response to 50 mg of CAO was about twice that to the infested nuts, although this relationship was not significant in the field results, probably because of the low number of females captured in this experiment.

Assessment of the effect of the pyrethroids on attraction to CAO showed that none of the compounds altered the response of females when present as either 0.1 or 0.5% of the CAO formulation (Table 1); however, when present as 1% of the CAO formulation, both permethrin and cypermethrin caused females to abort their orientation before reaching the odor source. Fenvalerate, on the other hand, did not interfere with CAO attraction, even at the 1% level. This lack of behavioral disruption also was confirmed by field studies in which mean trap captures for 500 mg of CAO containing 0, 0.5, and 1.0% fenvalerate were 1.9, 1.6, and 1.8 females per trap per week, respectively, differences that were not significant.

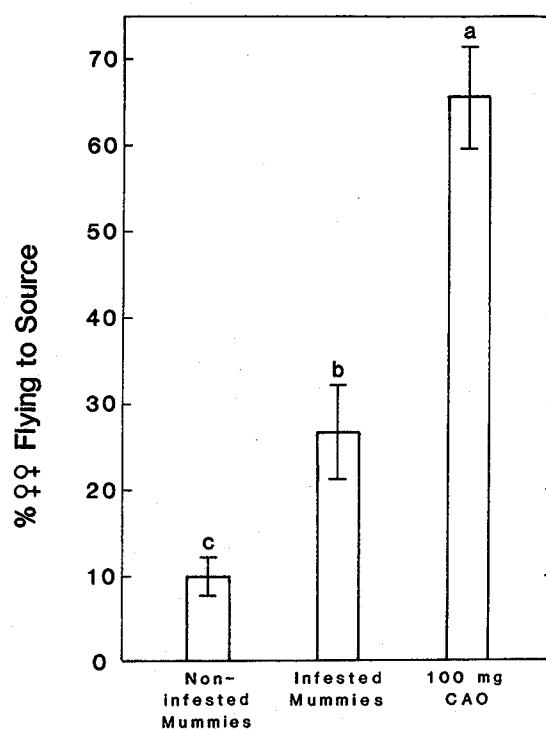


Fig. 3. Mean percentage of response of mated navel orangeworm females to a cluster of three larvae-infested or three uninfested almond mummy nuts or CAO in a wind tunnel. Means marked by the same letter are not significantly different by Duncan's (1955) multiple range test after arcsine  $\sqrt{x}$  transformation ( $n =$  nine groups of 10 females each).

In assessing the susceptibility of females flying to and contacting the attracticide formulations, significant mortality was brought about by any of the formulations containing the resolved fenvalerate (Fig. 5); however, the 2.5% formulation proved to be the most effective, with almost 90% mortality seen in females landing on this material. In addition, significant sublethal effects would be expected in those surviving females, rendering many of them incapable of finding suitable oviposition sites (Haynes & Baker 1985). Mortality to navel orangeworm females decreased slightly at the highest (5%) toxicant formulation (Fig. 5). Although no data were collected on the duration of time before leaving the attracticide, females appeared to remain on this formulation for a shorter period.

### Discussion

The wind tunnel has proven to be an extremely powerful tool for the investigation of lepidopteran sex pheromones (Baker & Linn 1984); however, its use in the study of host finding in free-flying insects has been limited (Kennedy 1977) and, to our knowledge, unprecedented in the Lepidoptera. Nevertheless, the wind tunnel provided us with a

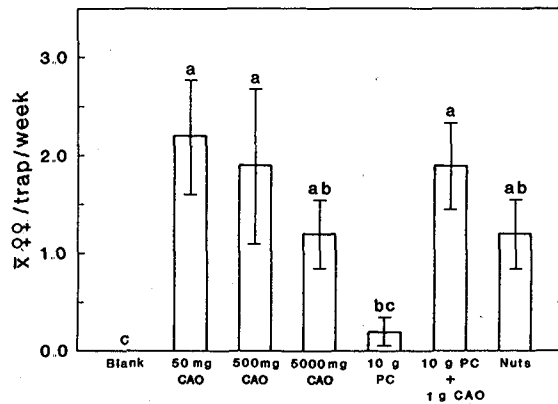


Fig. 4. Mean number of female navel orangeworms captured in traps baited with varying quantities of CAO, PC, a combination of PC and CAO, or three larvae-infested almond mummies. Means marked by the same letter are not significantly different by Duncan's (1955) multiple range test after  $\sqrt{x + 0.5}$  transformation ( $n = 9$ ).

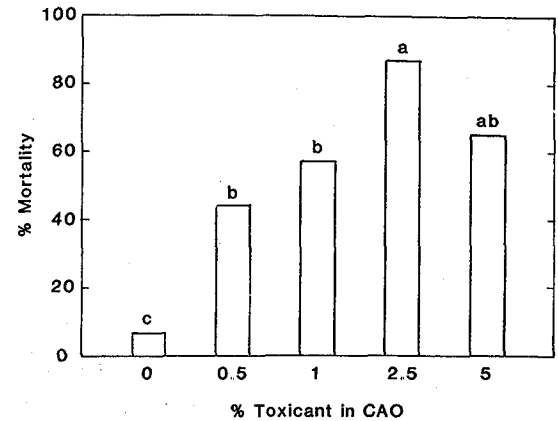


Fig. 5. Percentage of mortality of mated navel orangeworm females after flying up-tunnel to and landing on formulations of resolved fenvalerate in CAO. Values marked by the same letter are not significantly different by Ryan's (1960) multiple comparison test for proportions ( $n = 40$ ).

discriminating long-range bioassay for host finding by navel orangeworm females because of their in-flight behavioral response to almond odors. In fact, flight patterns of responding navel orangeworm females were very similar to those of their male counterparts responding to female pheromone. The behavior pattern began with wing-fanning in the release cage, followed by an upwind flight with narrow cross-tunnel zigzagging.

The importance of volatiles in navel orangeworm host finding was suggested by the early observation that damaged host material elicited greater egg deposition (Caltagirone et al. 1968, Curtis & Barnes 1977). Curtis & Clark (1979) later demonstrated that the stimulus was indeed chemical by showing that egg laying could be evoked with solvent extracts of frass from larvae feeding on almonds. Andrews & Barnes (1982) partially clarified the behavioral role of the active constituents by showing that the greater number of eggs found on infested mummies was due to a greater number of visits by females, not increased oviposition by similar numbers of females; however,

whether the role of the chemicals was one of attraction, arrestment, or the stimulation of oviposition after arriving randomly to the nut remained unresolved. The results of our wind-tunnel studies allowed us to distinguish these mechanisms by demonstrating that volatiles in almonds act to elicit upwind attraction from  $>2$  m.

The propensity of almond PC to elicit oviposition by the navel orangeworm was first demonstrated by Rice et al. (1984), and it proved an effective lure in egg traps for monitoring this pest. Recent improvements in this trap design have included the addition of 10% CAO to the PC (Van Steenwyk et al. 1986). Our results concerning the efficacy of CAO and PC suggest that CAO alone is more attractive than PC and just as attractive as the combined PC and CAO, although it requires considerably less material for optimal response. The use of a liquid of substantially lower volume may allow the development of improved trap designs that are more convenient to handle.

In the population dynamics of the navel orangeworm, a bimodal flight pattern occurs in the spring, followed by low-level adult activity throughout the early summer and then a very large increase in activity in late summer and early fall, coinciding with almond hull-split and subsequent harvest (Rice [1976] and references therein). In addition to acting as a significant source of navel orangeworm inoculum, mummy nuts provide the primary oviposition sites during the spring and early summer, allowing populations to increase until the new almond crop becomes accessible to this pest after the hulls split. Winter sanitation practices, involving the manual removal of mummies from the trees, significantly reduce navel orangeworm populations and infestations of the new almond crop (Engle & Barnes 1983, Zalom et al. 1984). It is during the

Table 1. Upwind flight response of gravid navel orangeworm females to 100 mg of CAO containing varying concentrations of toxicant

Toxicant	$n^a$	$\bar{x}\%$ upwind response by ♀♀			
		% toxicant in CAO <sup>b</sup>			
		0	0.1	0.5	1.0
Permethrin	12	55.8a	60.8a	44.2ab	35.8b
Cypermethrin	9	60.0a	60.0a	73.3a	26.7b
Fenvalerate	9	56.3a	44.4a	44.4a	57.8a

<sup>a</sup>  $n$  = groups of 10 females each.

<sup>b</sup> Values within a row followed by the same letter are not significantly different ( $P > 0.05$ ; Duncan's [1955] multiple range test after arcsine  $\sqrt{x}$  transformation).

spring period, when oviposition sites are limited, that the attracticide strategy would be expected to be most effective. We have demonstrated the CAO lures to be more attractive than mummies, with which they would compete in the spring. By incorporating a toxicant into the lure, we hope to target the pest insect without the use of broadcast sprays, which are disruptive to beneficial arthropods and commonly allow secondary outbreaks of phytophagous mites because of the incidental elimination of predaceous mites (McMurtry et al. 1970). Resurgence of phytophagous mites in almond orchards is particularly pronounced after the use of pyrethroid insecticides (Bentley et al. 1987); however, directing a toxicant to the navel orangeworm through the attracticide should preserve beneficial insects and mites, while still allowing the use of these more effective pyrethroids.

In conclusion, we have shown that almond odors elicit long-distance attraction in gravid navel orangeworm females. On a per-weight basis, CAO seems to be the most concentrated source of the active constituents of all the almond materials tested. Although the addition of higher levels of either permethrin or cypermethrin reduced female response to CAO lures, fenvalerate did not affect female response at any of the levels tested. Moreover, the addition of 2.5% resolved fenvalerate proved effective in killing 90% of females visiting the attracticide. When used in conjunction with good winter sanitation, a field-durable formulation of the attracticide could prove to be an effective strategy for controlling the navel orangeworm while preserving beneficial arthropods.

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