

# Effects of Exposure to Pheromone and Insecticide Constituents of an Attracticide Formulation on Reproductive Behavior of Oriental Fruit Moth (Lepidoptera: Tortricidae)

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**ABSTRACT** The effect of exposure to both the pheromone and insecticide constituents of an attracticide formulation on subsequent pheromonal response of male oriental fruit moth, *Grapholitha molesta* (Busck) (Lepidoptera: Tortricidae), was tested in several wind tunnel bioassays. Male response to the attracticide formulation was significantly reduced in all behavioral categories, including source contact 1 h after sublethal exposure (both by voluntary contact in the wind tunnel and forced application in the laboratory) to the attracticide formulation containing inert ingredients, pheromone, and insecticide. Sublethal exposure to the attracticide formulation in the laboratory (forced application) 24 h before the bioassay resulted in a significantly lower proportion of males subsequently responding to attracticide droplets in the wind tunnel. However, voluntary contact of male moths with the toxic formulation in the wind tunnel had no effect on subsequent response 24 h later. Exposure of males to different constituents of the attracticide formulation demonstrated that both pheromone and insecticide exerted effects on subsequent male pheromonal response. Exposure to the formulation containing the inert ingredients plus the pheromone (no insecticide) significantly reduced male behavioral responses to an attracticide droplet in the wind tunnel 1 h but not 24 h after exposure, compared with males treated with inert ingredients alone. Response to attracticide droplets was further reduced by exposure to the entire attracticide formulation containing inert ingredients, pheromone and insecticide at both 1 and 24 h postexposure. Similarly, males exposed to inert ingredients plus pheromone were less likely to orient to female-produced plumes 1 h but not 24 h after exposure than males treated with inert ingredients alone. Response to female-produced plumes was further reduced at 1 h but not at 24 h after exposure to the entire attracticide formulation. Mating success of males was significantly reduced by exposure to the entire attracticide formulation but not to the formulation without insecticide when placed with females 1 and 24 h postexposure. These findings suggest that sublethal poisoning of males exposed to the attracticide formulation will enhance the effectiveness of this formulation under field conditions.

**KEY WORDS** pheromone, attract and kill, lure and kill, orchard pest management, mating disruption

COMMERCIAL ATTRACTICIDE FORMULATIONS combine two active ingredients: an attractant to lure the target insect pest and an affector agent to remove the pest from the population after contact (Jones 1998). In this way, only insects attracted to the formulation are susceptible to the treatment. The attractant component of the formulation generally consists of visual cues such as an attractive color or shape or odor cues such as synthetic pheromones or host volatiles. The affector agent can be physical such as a sticky surface or chemical such as an insecticide-treated surface; it can be synthetic or natural such as a pathogen. Attracticides developed to control lepidopteran pests contain synthetic sex pheromones to attract male moths and pyrethroid insecticides that achieve rapid knockdown of

attracted moths (Butler and Las 1983; Haynes et al. 1986; Miller et al. 1990; Downham et al. 1995; Charmillot et al. 1996, 2000; Charmillot and Hofer 1997; Brockerhoff and Suckling 1999; Suckling and Brockerhoff 1999; Krupke et al. 2002).

Optimal effectiveness of a lepidopteran attracticide depends on male exposure to insecticide through contact with the formulation. Some studies have shown that 50% of the activity of the attracticide was a mating disruption effect of pheromone alone and the additional 50% was due to removal of males from the population as a result of insecticide poisoning (Charmillot et al. 1996, Suckling and Brockerhoff 1999). However, other studies indicated that the insecticide component of the attracticide formulation has little added effect to pheromone alone on disruption of male orientation and mating with sentinel females (Downham et al. 1995, Evenden and McLaughlin

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2004a). The effectiveness of an attracticide also will depend on the impact of sublethal exposure to the insecticide (Haynes et al. 1986, Krupke et al. 2002) and previous exposure to pheromone in the formulation. Sublethal exposure of moths to pyrethroid insecticides can alter male moth response to sex pheromone (Haynes and Baker 1985, Haynes et al. 1986, Floyd and Crowder 1981, Linn and Roelofs 1984, Moore 1988) as well as to close range mating behaviors (De Souza et al. 1992, Krupke et al. 2002).

A recent attracticide formulation consists of a viscous paste that incorporates insecticide and sex pheromone in a UV-protective carrier material (Hofer and Brassel 1992). This formulation has been registered in the United States for use against a key pest of tree fruit production, the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). The commercial attracticide formulation with the trade name LastCalloFM (IPM Tech, Inc., Portland, OR) contains 0.16% of the oriental fruit moth sex pheromone and 6.0% of the pyrethroid insecticide permethrin. Topical treatment of oriental fruit moth males with permethrin at sublethal doses resulted in fewer males orienting to a sex pheromone source (Linn and Roelofs 1984). In addition, exposure of male oriental fruit moths to pheromone has a significant impact on subsequent pheromone response that has been attributed to an inhibition of both peripheral (Sanders and Lucuik 1996, Rumbo and Vickers 1997) and central nervous system processing of the signal (Figueredo and Baker 1992). Therefore, exposure to both active ingredients in this attracticide formulation may contribute to the effectiveness of the formulation by impeding subsequent mate finding and mating behaviors.

Our initial studies have shown that male oriental fruit moths successfully orient to individual droplets of this attracticide formulation and are exposed to insecticide by source contact. Mortality rates post-exposure vary depending on exposure technique and elapsed time since exposure (Evenden and McLaughlin 2004b). Here, we examine the impact of previous exposure to the formulation with and without the insecticide constituent on subsequent male response to synthetic and natural sources of pheromone and mating success.

### Materials and Methods

**Attracticide Formulations.** LastCalloFM formulations were formulated at IPM Tech, Inc. Formulations consisted of a clear viscous paste with a base of a proprietary product plus other inert ingredients (93.8% of the formulation). Oriental fruit moth pheromone used in the LastCalloFM formulation was purchased as a premixed three-component blend that consisted of 90–94.5% (*Z*)-8-dodecenyl acetate (*Z*8-12:Ac), 5.5–7.2% (*E*)-8-dodecenyl acetate (*E*8-12:Ac), and 0.75–1.5% (*Z*)-8-dodecenol (*Z*8-12:OH) with each component 97% pure (Bedoukian Research, Inc., Danbury, CT). In each formulation, the pheromone was incorporated into the LastCall formulation

at 0.16%. In formulations containing insecticide permethrin was added at 6.0%. Individual attracticide sources consisted of a 50- $\mu$ l droplet dispensed from a calibrated hand pump on to the center of a 1 by 2-cm piece of aluminum foil and suspended vertically by a short length of wire in the wind tunnel. Droplets were weighed to between 42.5 and 52.5 mg and used in bioassays within 1 h of preparation.

**Insects.** Moths used in bioassays came from a laboratory colony maintained on a lima bean-based diet at a photoperiod of 16:8 (L:D) h and 24°C. Pupae were separated by sex, and males and females were held separately until moth eclosion and subsequent use in the bioassay. Adult moths were held in individual 30-ml cups and provided with a water source.

**Wind Tunnel.** The flight section of the wind tunnel was 1.7 m in length and 0.85 m in height. Air was pushed through the tunnel at speeds between 0.4 and 0.5 m/s and the pheromone plume was exhausted out of the room by a centrally located fan at the downwind end. Uniform dim white light was provided by six, 25-W incandescent bulbs, diffused through white paper. Temperature was maintained between 24 and 25°C in all experiments.

**Wind Tunnel Experiments.** Wind tunnel bioassays were conducted during the last 2 h of the photophase and the first hour of the scotophase. Two- to 5-d-old males were collected 30–40 min before testing and placed under bioassay conditions in the wind tunnel room. Individual males in conical screen release cages (5 cm in diameter by 5 cm in height) were introduced into the wind tunnel and placed on a platform 40 cm off the ground and 20 cm from the downwind end of the tunnel. Formulations were presented to male moths on foil tabs suspended from a bar extended from a ring stand, 40 cm off the floor and 10 cm from the upwind end of the tunnel. Males were evenly distributed among treatments by age in all wind tunnel experiments. Male response to the various formulations was graded as + or – for wing fanning, take-off, locking-on to the plume, oriented upwind flight, and source contact. Males had 2 min to respond to the pheromone source.

Experiments 1 and 2 tested the hypotheses that voluntary contact with the attracticide formulation containing 0.16% pheromone and 6% permethrin in the wind tunnel would influence subsequent response to the formulation one and 24 h later, respectively. Males were released and observed following the standard protocol, but upon completion of the 2-min observation period they were categorized as either exposed or unexposed and captured using a small net and placed in a 30-ml cup until the subsequent flight. All exposed males were captured after their first encounter with the attracticide droplet. All unexposed males had successfully conducted upwind oriented flight toward the attracticide source but had not contacted the droplet and were captured and handled in the same manner as the exposed moths. Surviving males were flown one (experiment 1) or 24 (experiment 2) h later to a new attracticide droplet. Exposed and unexposed males were presented individually in

an alternating order to the new attracticide source and observed for characteristic behaviors following the standard protocol. In experiment 1, bioassays were conducted on day 6, and a total of 55 exposed and 46 unexposed males were tested. In experiment 2, bioassays were conducted on day 8, and a total of 34 exposed and 35 unexposed males were tested. In each case, a similar number of males in each treatment group were flown on each day of experimentation. The proportion of males responding in each behavioral category in wind tunnel bioassays was compared using logistic regression of a randomized block model in which day of bioassay was treated as an explanatory variable (PROC LOGISTIC, SAS Institute 1996).

Experiments 3 and 4 tested the hypotheses that forced contact with various components of the attracticide formulation would influence subsequent response to the attracticide in a wind tunnel 1 and 24 h later, respectively. Virgin males, 2–5 d old, were chilled at 5°C for 15 min. Chilled males were removed from individual cups by grasping their forewings with forceps. Males were evenly distributed among treatments by age. Males were positioned so that their tarsi momentarily touched the formulation. Individual males were exposed to one of three formulations (treatments) containing 1) inert ingredients plus both insecticide (6% permethrin) and pheromone (0.16%) constituents; 2) inert ingredients plus pheromone (0.16%) but no insecticide; and 3) only the inert ingredients and neither pheromone nor insecticide constituents. Exposure to formulations was conducted between 2 and 3 h before the onset of scotophase. After exposure, males were returned to containers and held until transport to the wind tunnel room, 15 min before the bioassay. Test males were flown to a fresh droplet of the complete attracticide formulation (0.16% pheromone and 6% permethrin) 1 (experiment 3) and 24 h (experiment 4) after contact. In experiment 3, 11–15 males were flown in each treatment group on each of 6 d, for a total of >65 moths flown per treatment. In experiment 4, 6–16 males were flown in each treatment group on each of 6 d, for a total of >60 moths flown per treatment. In each case, a similar number of males in each treatment group was flown on each day of experimentation. The proportion of males in each treatment group displaying each of the behaviors to the complete attracticide formulation were compared using logistic regression of a randomized block design model in which day of bioassay was treated as an explanatory variable (PROC LOGISTIC, SAS Institute 1996). The contrast statement in PROC LOGISTIC was used to compare any significant treatment effects.

Experiments 5 and 6 tested the hypotheses that exposure to various components of the attracticide formulation would influence subsequent response to calling virgin female moths as the pheromone source in a wind tunnel 1 and 24 h later, respectively. Cohorts of 2- to 3-d-old females were placed individually in mesh screen bags (9 by 6.5 cm) 30 min before the bioassay and held at the upwind end of the wind tunnel. At the start of the bioassay, one female that

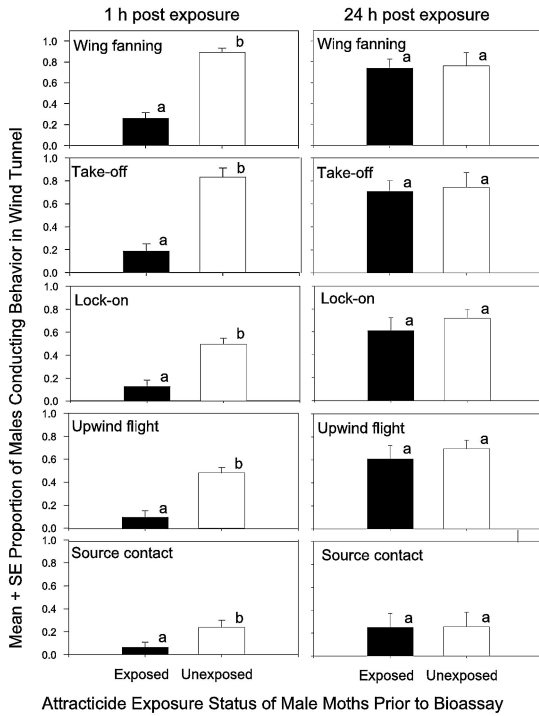
exhibited calling behavior was selected, and the mesh bag was positioned at the upwind end of the tunnel, and all other females were removed from the wind tunnel room. Only one calling female was used as a pheromone source on each day of the experiment.

In both experiments 5 and 6, cups containing 1–4-d-old males were placed on ice and subdued males were grasped with forceps and positioned so that their tarsi momentarily contacted the formulation. Individual males were exposed to one of the same three treatments tested in experiments 3 and 4, 3 h before the onset of scotophase. Males were evenly distributed by age among the three treatment groups. In experiment 5, 5–12 males were flown in each treatment group on each of 8 d for a total of >50 moths per treatment. In experiment 6, 7–18 males were flown in each treatment group on each of 5 d for a total of >55 moths per treatment. In each case, a similar number of males in each treatment group was flown on each day of experimentation. The proportion of males in each treatment group displaying each of the behaviors to the calling virgin female were compared using logistic regression of a randomized block design model in which day of bioassay was treated as an explanatory variable (PROC LOGISTIC, SAS Institute 1996). The contrast statement in PROC LOGISTIC was used to compare any significant treatment effects.

**Mating Experiments.** Experiments 7 and 8 tested the hypotheses that exposure to various components of the attracticide formulation would influence the subsequent mating success of males placed with females 1 and 24 h postexposure, respectively. Female and male pupae were held separately under a photoperiod of 8:16 (D:L) h, and adult moths were held in 30-ml cups and provided with a water source until use. Two- to 4-d-old (experiment 7) and 0–4-d-old (experiment 8) individual males were exposed as in experiments 5 and 6 to one of the same three treatments tested in experiments 3 and 4, 1–3 h before the onset of scotophase. Males were paired with 1–3-d-old female moths in a fresh cup 1 h (experiment 7) or 24 h (experiment 8) after exposure. Pairs remained together for the following scotophase, and the condition of male moths was noted as healthy, incapacitated, or dead at 24 h (experiment 7) and at 24 and 48 h postexposure (experiment 8). The mating status of females from pairs in which their partners were rated as healthy was determined by dissection to reveal the presence (mated) or absence (virgin) of a spermatophore in the bursa copulatrix. Between 30 and 42 pairs of moths were established for each treatment in each of two trials, and the data from the two trials were pooled. The proportions of females that mated with males exposed to the various treatments were compared using logistic regression of a two-factor (pheromone, insecticide) randomized block model (PROC LOGISTIC, SAS Institute 1996).

## Results

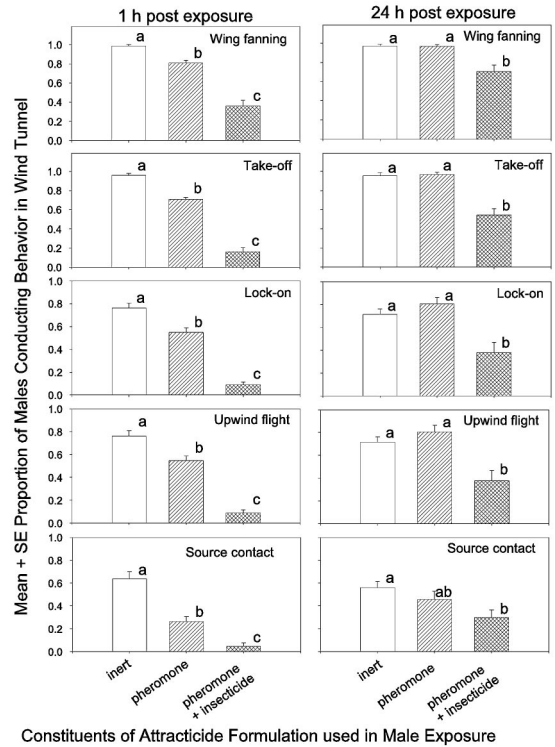
**Wind Tunnel Experiments.** The sublethal effects of exposure to the attracticide formulation in the wind



**Fig. 1.** Mean + SE proportion of male oriental fruit moths exhibiting behavioral responses to a 50- $\mu$ l droplet of the attracticicide formulation 1 h (experiment 1) and 24 h (experiment 2) postexposure by voluntary moth contact with the formulation in the wind tunnel. Exposed males came into contact with the droplet and unexposed males flew upwind but did not contact the droplet during the exposure flight. Within each experiment and behavioral category bars with different letters are significantly different, logistic regression ( $P < 0.05$ ).

tunnel varied with time since exposure in experiments 1 and 2. Male response to the attracticicide formulation was significantly reduced in all behavioral categories 1 h after sublethal exposure to the formulation containing inert ingredients, pheromone, and insecticide. However, there was no reduction in male response 24 h after sublethal exposure (Fig. 1). Fifty-six male oriental fruit moths were exposed to the attracticicide formulation in the wind tunnel in experiment 1 and there was no mortality observed before the test flight 1 h later. However, of the 67 moths that contacted the attracticicide droplet in the initial exposure flight in the wind tunnel in experiment 2, 28 were dead at 24 h postexposure (48%). Exposure of males to this same formulation in a wind tunnel bioassay in a previous study resulted in 58% mortality at 24 h postexposure (Evenden and McLaughlin 2004b).

Exposure of males to different constituents of the attracticicide formulation in experiments 3 and 4 demonstrated that both pheromone and insecticide exerted effects on subsequent male response to the attracticicide formulation. Male response in all behavioral categories was significantly reduced at 1 h postexposure but not at 24 h postexposure (Fig. 2) to the



**Fig. 2.** Mean + SE proportion of male oriental fruit moths exhibiting behavioral responses to a 50- $\mu$ l droplet of the attracticicide formulation in the wind tunnel 1 h (experiment 3) and 24 h (experiment 4) after forced exposure to various constituents of the attracticicide formulation in the laboratory. Within each experiment and behavioral category bars with different letters are significantly different, logistic regression ( $P < 0.05$ ).

formulation containing inert ingredients and pheromone compared with exposure to inert ingredients alone. Response in all categories was further reduced at 1 h postexposure and at 24 h postexposure to the full attracticicide formulation containing inert ingredients, pheromone, and insecticide (Fig. 2).

Exposure to the attracticicide formulation impacted subsequent male response to calling virgin females in the wind tunnel to a lesser extent than subsequent response to the attracticicide formulation. Exposure to various constituents of the attracticicide formulation significantly influenced male flight response to calling females at 1 h postexposure but not at 24 h postexposure (Fig. 3). An equal proportion of males wing fanned in response to calling females in the wind tunnel 1 h after exposure to inert ingredients alone or inert ingredients plus pheromone. However, the proportion of males that took-off, locked-on to the pheromone plume, conducted upwind oriented flight, and contacted the bag containing the calling female was significantly reduced 1 h postexposure to the formulation containing inert ingredients plus pheromone (Fig. 3). The proportion of males that displayed all behaviors in the wind tunnel to calling virgin females

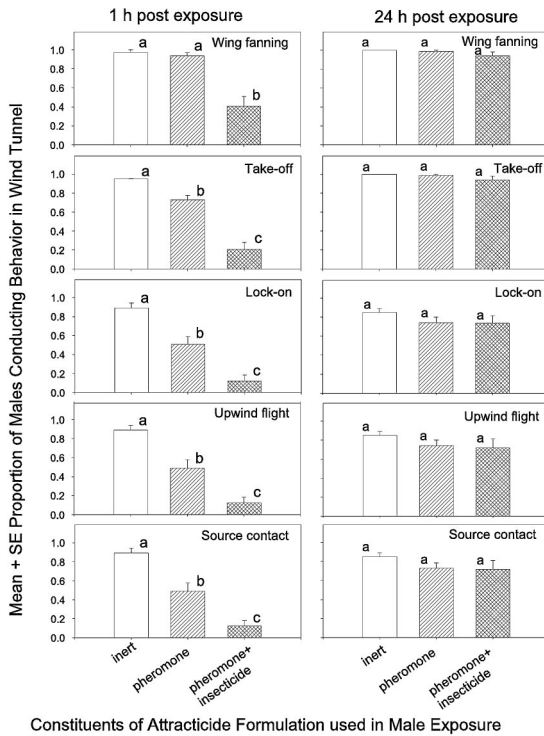


Fig. 3. Mean + SE proportion of male oriental fruit moths exhibiting behavioral responses to a calling virgin female in the wind tunnel 1 h (experiment 5) and 24 h (experiment 6) after forced exposure to various constituents of the attracticide formulation in the laboratory. Within each experiment and behavioral category bars with different letters are significantly different, logistic regression ( $P < 0.05$ ).

was further reduced as the result of sublethal exposure 1 h before testing to the attracticide formulation containing both pheromone and insecticide (Fig. 3). The proportion of males displaying all behaviors in the wind tunnel in response to calling virgin females was not influenced at 24 h postexposure to the formulation with inert ingredients plus pheromone or the formulation with inert ingredients, pheromone, and insecticide, compared with that of inert ingredients alone (Fig. 3).

Although in experiments 3–6 direct mortality was not assessed, 73% of males that were exposed to the full attracticide formulation in a similar manner in a previous study were dead at 24 h postexposure, whereas no mortality occurred as a result of exposure to the attracticide formulation containing the inert ingredients alone or the inert ingredients plus pheromone (Evenden and McLaughlin 2004b).

**Mating Experiments.** There was no impact of prior exposure to the pheromone constituent of the attracticide formulation on the males' ability to mate with females in experiments 7 and 8. A similar proportion of females were mated after one night together with males exposed to the inert ingredients of the formulation and the inert ingredients with the pheromone constituent 1 h postexposure ( $P = 0.5800$ ) and 24 h

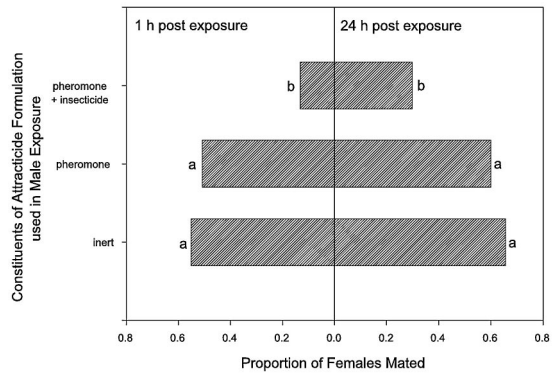


Fig. 4. Proportion of females mated after one night with males exposed to various constituents of the attracticide formulation 1 h (experiment 7) and 24 h (experiment 8) before pairing. Within each experiment, bars with different letters are significantly different, logistic regression ( $P < 0.05$ ) (experiments 7 and 8).

postexposure ( $P = 0.5262$ ) (Fig. 4). However, exposure of males to the formulation containing inert ingredients plus both pheromone and insecticide constituents resulted in a significant reduction in mating. The sublethal effect of male exposure to the entire attracticide formulation was significant when males were exposed 1 h ( $P = 0.0001$ ) and 24 h ( $P = 0.0005$ ) before pairing with the female (Fig. 4).

## Discussion

Our data demonstrate that exposure to both of the active ingredients in the attracticide formulation impact subsequent response to synthetic and natural sources of pheromone. In addition, male mating success is impaired by exposure to the insecticide but not to the pheromone component of the formulation. This may enhance the effectiveness of this formulation under field conditions. In the field, males would most likely contact attracticide droplets by flying upwind in response to the pheromone and alight on the insecticide-laden droplet. Exposure of male oriental fruit moths to the attracticide formulation by source contact with this formulation in a wind tunnel (Evenden and McLaughlin 2004b) resulted in  $\approx 58\%$  mortality at 24 h postexposure. Therefore, a potentially large proportion of the males that contact the formulation under field conditions could still be alive during the first and second scotophase after exposure. Sublethal effects of exposure to the attracticide on pheromone response could impact subsequent contact with sources of the attracticide formulation and may limit the possibility of multiple exposures to the formulation. However, sublethal exposure to the attracticide also may decrease the males' ability to locate and court mates (Haynes et al. 1986, Krupke et al. 2002) and therefore increase the overall effectiveness of the formulation.

Males exposed to the attracticide formulation through flight in the wind tunnel in experiment 1 were

less likely to locate subsequent sources of the attracticide when tested within the same scotophase (Fig. 1). A smaller proportion of exposed males conducted all of the behavioral responses monitored in the wind tunnel compared with unexposed males in experiment 1 (Fig. 1). Similarly, Linn and Roelofs (1984) found that male oriental fruit moths topically treated with sublethal levels of permethrin 5 h before wind tunnel bioassays were less likely to orient and initiate upwind flight to a synthetic pheromone source. However, only the initial behaviors conducted by male pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), including wing fanning and initiation of flight were impacted by topical treatment with sublethal concentrations of permethrin (Haynes and Baker 1985) and exposure to attracticide formulations containing 10% permethrin (Haynes et al. 1986).

The sublethal effect of exposure to the attracticide formulation in the wind tunnel was depleted by 24 h postexposure, as an equal proportion of exposed and unexposed males conducted all behaviors upon subsequent testing to the formulation in experiment 2 (Fig. 1). However, after experimental exposure to the attracticide formulation in the laboratory in experiment 4, a lower proportion of males displayed all behaviors 24 h postexposure in the wind tunnel to the attracticide formulation (Fig. 2). This suggests that males forced to touch the formulation in the laboratory received more insecticide or an increased exposure period than males exposed in the wind tunnel. Similar incongruous results have occurred in other species. Recovery of male pink bollworm moths from the sublethal effects of exposure to an attracticide formulation containing 0.3% cypermethrin in a wind tunnel assay occurred at 48 h postexposure (Haynes et al. 1986). In contrast, only 5–55% of pink bollworm males responded to pheromone sources by wing fanning 96 h after topical application with permethrin (Floyd and Crowder 1981). Alternatively, males exposed to the attracticide formulation in the laboratory in our experiments may be more sensitive to permethrin poisoning because they were chilled before exposure. Permethrin has been shown to be more toxic at lower temperatures in some Lepidoptera (Sparks et al. 1982, Toth and Sparks 1988).

Experiments 3–6 were the first attempts to test the independent effects of the inert, pheromone, and insecticide constituents of any lepidopteran attracticide. The LastCall formulation is very viscous and can easily adhere to male moths that contact the formulation either by alighting on droplets or by artificial exposure in the laboratory. This feature of the formulation may not only promote enhanced toxicity of the contact insecticide but also result in constant exposure of males to low levels of pheromone that may impact male behavior (Krupke et al. 2002). Exposure to the inert ingredients and pheromone constituent of the formulation resulted in a reduction in the proportion of males that were able to respond, orient to, and contact subsequent sources of the attracticide (Fig. 2) or calling virgin females (Fig. 3) 1 h postexposure.

Male oriental fruit moth orientation to calling virgin females in a wind tunnel was significantly reduced when females were surrounded by rubber septa loaded with 100 or 1000  $\mu\text{g}$  of synthetic pheromone but not by septa releasing pheromone levels lower than females (Sanders and Lucuik 1996). This suggests that the amount of pheromone remaining on males exposed to the attracticide formulation is greater than that released by females. The constant exposure to and proximity of the pheromone to males probably resulted in adaptation of sensory receptors on the male antennae, because large amounts of pheromone (3,200 female equivalents) are required to reduce subsequent male pheromonal response after a recovery period of 20 min (Rumbo and Vickers 1997). The adaptation effect of residual pheromone had completely dissipated by 24 h postexposure, because there was no reduction in the proportion of males that displayed all behaviors in the wind tunnel to a subsequent source of attracticide (Fig. 2) or to calling females (Fig. 3). The lack of effect of the residual pheromone on males flown 24 h postexposure further suggests that adaptation of sensory receptors is impacting male oriental fruit moths exposed to the formulation and not long-term habituation of the central nervous system that has been demonstrated for male oriental fruit moths repeatedly exposed to pheromone (Figueredo and Baker 1992).

Contact with the complete attracticide formulation containing inert ingredients, pheromone, and insecticide further reduced subsequent male response 1 h postexposure to the attracticide formulation (Fig. 2) and calling females (Fig. 3) compared with that of the formulation without insecticide. This suggests that contact with permethrin significantly impacts male response to pheromone. Pyrethroids such as permethrin induce changes in nerve axon membrane permeability that result in repetitive nerve discharges (Pedigo 2002). Sublethal permethrin poisoning of male oriental fruit moths seems to impact the males' ability to conduct all precopulatory flight behaviors monitored in the wind tunnel to both natural and synthetic sources of pheromone 1 h postexposure to LastCallOFM (Figs. 2 and 3). Oriental fruit moths dosed with a sublethal topical application of permethrin were less likely to orient to the pheromone plume and initiate upwind flight than control males in a wind tunnel 5 h postexposure (Linn and Roelofs 1984). It was suggested that permethrin disrupted the basic motor units involved in normal flight behavior and not the initial activation response, because wing fanning was not impaired by sublethal poisoning (Linn and Roelofs 1984). However, our results suggest that the activation response is influenced by exposure to permethrin because wing fanning was significantly reduced in males treated with the entire attracticide formulation (Figs. 2 and 3). Similarly, pink bollworm males treated with sublethal levels of permethrin were less likely to wing fan in response to pheromone at 24, 48 (Haynes and Baker 1985), and 96 h (Floyd and Crowder 1981) postexposure.

Male response to the attracticide formulation 24 h after initial exposure was still significantly reduced in all categories compared with males exposed to the pheromone and inert components of the formulation (Fig. 2). However, male response to calling females 24 h after exposure to the attracticide formulation was not significantly different from males exposed to the inert ingredients alone or inert ingredients plus pheromone (Fig. 3). This difference may be because the female-produced signal is more attractive to untreated male oriental fruit moths than this attracticide (Evenden and McLaughlin 2004b). Permethrin might be acting at both the peripheral and central nervous system levels to alter the quality or the quantity of the signal perceived by the male (Haynes and Baker 1985), which may further degrade the suboptimal signal of the attracticide formulation but not alter the female-produced signal enough to result in reduced response. Therefore, it seems that males surviving exposure to the attracticide formulation for 24 h will be less likely to contact subsequent sources of the attracticide but just as likely to find females as unexposed males.

Although the males' ability to orient to calling females was reduced 1 h after exposure to the attracticide formulation and not at 24 h postexposure, males were less likely to mate when placed with females both at 1 and 24 h postexposure (Fig. 4). Male oriental fruit moths conduct a sequence of courtship behaviors that culminate in hairpencil display (Baker and Cardé 1979) and release of a courtship pheromone (Baker et al. 1981) that attracts the female at short distances. After the courtship display, the female approaches the male and touches his abdomen, this is quickly followed by the male turning and attempting to copulate (Baker and Cardé 1979). It seems that sublethal exposure to permethrin interferes with some of these close range behaviors as male mating success was significantly reduced after exposure to the attracticide in experiments 7 and 8. It is most likely that the characteristic movement of males in the courtship behavior (Baker and Cardé 1979) and copulation attempts are impaired by attracticide treatment because males treated with attracticide are just as likely to display their hairpencils as control males after contacting a calling virgin female (M.L.E., unpublished data). Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), males exposed to similar attracticide formulation (LastCallCM) and placed with calling females one and 24 h after treatment were unable to sustain directed orientation to the female that resulted in few copulation attempts and no mating success in treated males (Krupke et al. 2002). Mating success of attracticide-treated codling moth males increased slightly by 48 and 72 h postexposure but was still low (3.3%) (Krupke et al. 2002). Mating success of male *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was completely suppressed for 48 h postexposure to residues of lambda-cyhalothrin sprayed on cotton leaves (De Souza et al. 1992). However, male pink bollworm moths topically treated with sublethal levels

of permethrin showed no reduction in mating success 96 h posttreatment (Floyd and Crowder 1981).

Here, we demonstrated that treatment with both the pheromone and insecticide constituents of the LastCalloFM attracticide formulation impair the ability of male oriental fruit moths to locate synthetic and natural sources of pheromone within the same scotophase. This may result in delayed mating of female moths in the population, particularly at low population densities. A delay in mating of as little as 2 d after female eclosion can significantly reduce female oriental fruit moth fecundity and egg fertility (Fraser and Trimble 2001) and may play a role in the population dynamics of this pest at low densities. Although treated males were able to locate calling females 24 h postexposure, their mating success was significantly reduced at both 1 and 24 h postexposure, which may further reduce successful reproduction in orchards treated with LastCalloFM.

These findings suggest that sublethal poisoning of oriental fruit moth males exposed to the attracticide formulation will enhance the effectiveness of this formulation. However, survival of males exposed to the attracticide under field conditions could induce selection for resistance to the insecticide in the formulation, in a similar fashion as conventional pyrethroid insecticides. The toxicity of an attracticide formulation containing 6% permethrin was significantly lower for a pyrethroid-resistant strain of codling moth compared with a susceptible strain (Poullot et al. 2001). It remains to be seen whether resistance would develop under field conditions to an attracticide formulation, but it is likely that this formulation would be less effective against populations of pyrethroid-resistant oriental fruit moth.

Because mortality rates of male oriental fruit moths exposed to LastCalloFM are negligible between 1 and 3 h postexposure and range from 58 to 73% 24 h postexposure (Evenden and McLaughlin 2004b), the sublethal effects on pheromone response and mating behavior demonstrated here are likely to contribute to the effectiveness of this formulation under field conditions.

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