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## ATTRACTION OF MATED FEMALE CODLING MOTHS (LEPIDOPTERA: TORTRICIDAE) TO APPLES AND APPLE ODOR IN A FLIGHT TUNNEL

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## ABSTRACT

In a flight tunnel, mated female codling moths, *Cydia pomonella* L., were attracted (upwind flight with zigzagging flight patterns) to cold-stored thinning apples. Greater numbers of codling moths were attracted to apples infested with codling moth larvae than to uninfested apples. However, codling moth response to piped odor from cold-stored thinning apples infested with larvae was not significantly greater than that of moths to piped odor from uninfested apples. In a flight tunnel, significant numbers of mated female codling moths were captured in traps baited with fresh-picked immature apples or in traps through which odor from such apples was piped. Also, more codling moths were captured in traps baited with infested versus uninfested apples, and more were captured in traps with odor from infested apples compared to odor from uninfested apples. These studies demonstrate upwind attraction by flying female codling moths to apple fruit and odors from apple fruit and show increased response by moths to odors of fruit that are infested with codling moth larvae. It is suggested that this heightened response to infested apples may be due to increased apparency of infested fruit that may release greater amounts of volatile odorants.

Key Words: codling moth, host-finding, attraction, apple, kairomone

## RESUMEN

En un túnel de vuelo, hembras apareadas de *Cydia pomonella* L. fueron atraídas (vuelo contra el viento en un patrón zigzag) a manzanas inmaduras y refrigeradas. Un mayor número de palomillas fueron atraídas a manzanas infestadas con larvas que a manzanas no infestadas. Sin embargo, la respuesta de las palomillas al olor de manzanas inmaduras refrigeradas e infestadas con larvas introducido por un tubo no fué significativamente mayor que al de manzanas no infestadas. En el túnel de vuelo, un número significativo de hembras apareadas fueron capturadas en trampas cebadas con manzanas inmaduras recién cosechadas o en trampas con el olor introducido de tales manzanas. También se capturaron más palomillas en trampas cebadas con manzanas infestadas que no infestadas, y se capturaron más palomillas en trampas con el olor de manzanas infestadas que con el olor de manzanas no infestadas. Estos estudios demuestran la atracción de las palomillas hembra de *Cydia pomonella* en vuelo contra el viento hacia manzanas y hacia el olor de manzanas y demuestra una respuesta mayor de las palomillas hacia los olores de frutos infestados con larvas de *C. pomonella*. Esta respuesta mayor a manzanas infestadas puede ser debido a una mayor apariencia de las frutas infestadas por que tal vez emiten mayores cantidades de olores volátiles.

Adult codling moths are attracted to host fruit and codling moth attraction responses to apple odorants are thought to be important host-finding behavior (Wearing et al. 1973, Wearing and Hutchins 1973, Sutherland et al. 1974, Hern and Dorn 1999, Yan et al. 1999). Although Wearing et al. (1973) could not show upwind movement by moths in an olfactometer in response to apple odor, they and others (Wearing and Hutchins 1973, Sutherland et al. 1974) speculated that apple odor, as well as the apple odorant  $\alpha$ -farnesene, may be an attractant for the adult female codling moth. An ambulatory upwind response to apple odor by adult codling moths was recently demonstrated by Yan et al. (1999). In that study, both virgin and mated female moths moved farther up-

wind in tubes with apple odor compared to tubes without apple odor. Using a Y-tube olfactometer design, Hern and Dorn (1999) demonstrated an ambulatory orientation response by codling moth females to the apple odorant (E,E)- $\alpha$ -farnesene. Attraction of flying codling moths to apple odor has yet to be shown, although Light et al. (2001) trapped male and female codling moth with the pear chemical ethyl (E,Z)-2,4-decadienoate. Understanding such behavior is critical to the development of appropriate assays to explore codling moth host-finding and to isolate and identify host plant kairomones that codling moths use to locate and select oviposition sites.

Adult moth host-finding behavior can include chemoanemotactic flight in response to host odor

(Phelan and Baker 1987, Landolt 1989, Tingle et al. 1989, Rojas and Wyatt 1999). Such responses appear to be similar to those documented for male moth responses to female pheromone, a combination of upwind chemoanemotaxis with self-steered countering (Baker 1989). We investigated whether codling moths, like other moths, respond to host (apple) odor with zigzagging upwind flights that lead them to the odor source.

Attraction or orientation responses of phytophagous insects to host plant odor may be enhanced or increased with injury to the plant. This has been noted in flight tunnel studies of cabbage looper moth, *Trichoplusia ni* Hübner, attraction to cotton plants, but not to cabbage or potato plants (Landolt 1993, 2001), and in both wind tunnel and olfactometer ambulatory assays of Colorado potato beetle, *Leptinotarsa decemlineata* Say, responses to potato plants (Bolter et al. 1997, Landolt et al. 1999), and larval codling moth attraction to apple fruit (Landolt et al. 1998, 2000). Particularly because of the increased response of codling moth larvae to apple fruit infested with other codling moth larvae (Landolt et al. 2000), adult codling moths may also respond more strongly to the odors of infested apple fruit, compared to un-infested fruit. Additional experiments were conducted to test this hypothesis, using a flight tunnel assay.

## MATERIALS AND METHODS

### General

Moths used in assays were obtained as pupae from a laboratory colony maintained on an artificial diet (Toba and Howell 1991) at the Yakima Agricultural Research Laboratory, Wapato, Washington. Pupae were sorted by sex and were placed in plastic-screened cages in a controlled environment room. The room was at 23°C, 50-70% RH, with both a red incandescent light on 24 h per day and white fluorescent lamps on a reversed 14 h light:10 h dark photoperiod. As adults emerged, pupae were moved to new cages daily to provide males and females of known ages. Cages of moths were provided water on cotton.

To obtain mated female codling moths for bioassays, 15-20 females (2-3 day old) were placed in one cage with 20-25 males (2-5 day old) for one scotophase (dark period of the light cycle). Females were removed during the following photophase (light period) and were then used in flight tunnel tests during the next 2 scotophases. Females used in bioassays were dissected to confirm the presence of spermatophores in the bursa copulatrix. Data sets for groups of females that were <90% mated were rejected (one group).

For flight tunnel experiments involving observation of moth responses to cold-stored thinning apples, Red Delicious apples were obtained from a

commercial apple orchard in June of 1999 and were placed in cold storage at 2°C until used in experiments from December 1999 through February 2000. Apples were infested by placing 2 neonate codling moth larvae with individual apples in paper cups with lids for 5 to 12 days. Infestation was evident by physical damage to the fruit and the presence of frass. Apples were 30 to 45 mm in diameter.

Fresh-picked Granny Smith apples were obtained from commercial apple orchards in June and July of 2000 and in July of 2001. Apples were picked at 800 to 900 h the morning of the day they were used in flight tunnel experiments. Apples were infested by confining 3rd instar codling moth larvae with sleeved fruit on trees for 5 days prior to picking of fruit and their use in assays. Again, successful infestation of fruit was confirmed by the presence of physical damage and frass. Fresh-picked apples were 30 to 50 mm in diameter, depending on the date.

### Observational Experiments

A set of experiments was conducted as observational assays of moth responses in a flight tunnel. The flight tunnel was a 1 m wide × 1 m tall × 2 m long plexiglas box with a blower motor pushing air into the upwind end and a second blower motor pulling air through the downwind end. Airflow was balanced to produce a slight positive pressure in the tunnel, with an in-tunnel air speed of 22 cm/sec. Charcoal-coated filters were installed at both ends of the flight tunnel. Moths were tested one at a time, released from a horizontal polystyrene vial near the center of the downwind end of the tunnel, during the 2nd and 3rd h of the scotophase. Moths were observed for 3 min and were scored for upwind oriented flight and contact with the odor source (apples or airflow vent) at the center of the upwind end of the tunnel. Upwind oriented flights were zigzagging upwind flights within the likely odor plume downwind of the fruit or pipe vent. These flights are here referred to as attraction, or upwind oriented flights.

The first experiment compared moth responses to 3 cold-stored thinning apples that were not infested with a codling moth larva, 3 cold-stored thinning apples infested with a codling moth larva, and a control (no apples). Apples were placed on a petri plate on a ring stand at the center of the upwind end of the flight tunnel. On each of 11 days, five moths were tested per treatment, with the 3 treatments rotated in the treatment sequence each day. Totals of 55 moths were tested per treatment in this experiment. Treatment means were separated by Tukey's test following a significant ANOVA F value (DataMost 1995).

The second experiment compared mated female codling moth responses to 1) airflow that was passed over 6 un-infested cold-stored thin-

ning apples in a glass jar, 2) airflow passed over 6 such apples each infested by a codling moth larva, or 3) airflow through a "system blank" consisting of the jar and plumbing but with no apples. On each of 10 days, 5 females were tested per treatment, with the 3 treatments rotated in the treatment sequence each day. Totals of 50 moths were tested per treatment. Treatment means were separated by Tukey's test following a significant ANOVA F value (DataMost 1995).

#### Flight Tunnel Trapping Assays

Two sticky traps containing a bait or odor source were placed near the center of the upwind end of the flight tunnel at the beginning of the scotophase. Traps were triangular cardboard tent traps (10 cm wide, 15 cm tall and 10 cm deep) painted yellow and coated on all 3 inside panels with adhesive. Two traps were set up for each assay, with one serving as a control and the other containing an apple treatment. Groups of 20-25 female codling moths were released from five 20-ml clear plastic vials hung horizontally at the center of the downwind end of the flight tunnel at the end of the second hour of the scotophase. Vials were open on the upwind end and screened on the downwind end to permit moth escape upwind into the tunnel. Traps were 20 cm apart and 30 cm from the tunnel walls. Four hours after the release of codling moths into the flight tunnel, traps were checked to count codling moth captured in the traps. Three experiments were conducted to evaluate codling moth responses to two or three fresh-picked, immature apples, using this experimental design. Data were analyzed as mean percentages of released moths recaptured in traps. Treatment and control means were compared by a paired t-test (DataMost 1995) to determine if they were significantly different.

The first trapping experiment evaluated moth response to two un-infested apples in a trap compared to a control trap (no apples). The assay was conducted six times, with releases of moths in the flight tunnel made on each of six days. The second trapping experiment evaluated moth response to two infested apples in a trap compared to a control trap (no apples). This assay was conducted five times, with releases of moths in the flight tunnel made on each of five days. The third trapping experiment compared moth response to two un-infested apples and to two infested apples, in a trap. This assay was conducted six times, with releases of moths in the flight tunnel made on each of six days.

Three additional trapping tests evaluated moth response to airflow passed over fresh picked immature apples. For each test replicate, three apples were placed in a glass jar and air was pumped through the jar and into a trap in the flight tunnel. Airflow was also pumped through

an empty jar and into a second trap that served as an experimental control. Twenty to 25 female codling moths were released at the downwind end of the tunnel at the end of the second hour of the scotophase and traps were checked 4 h later to count moths captured on the sticky insides of the traps. The fourth trapping experiment compared moth response to airflow with and without uninfested apples in the jar. This test was replicated five times over five days. The fifth trapping experiment compared moth response to airflow with and without apples infested with codling moth in the jar. This test was also replicated five times over five days. The sixth trapping experiment compared moths trapped in response to infested apples compared to un-infested apples. This test was replicated 10 times over 10 days. Apples were infested in the field by placing 3rd instar codling moth larvae on fruit in sleeves on the tree, five days prior to assays.

Data for moths captured in traps were analyzed as percentages of released moths recaptured in traps. Differences between treatments or between treatments and controls were determined using a paired t-test (DataMost 1995) at  $p \leq 0.05$ .

## RESULTS

#### Flight Tunnel Observational Assays

Mated female codling moths were attracted to and contacted cold-stored thinning apples presented in the flight tunnel (Table 1). Percentages of moths exhibiting upwind oriented flights towards either un-infested or infested apples were significantly greater than percentages of moths responding to the control. Also, percentages of moths contacting un-infested or infested apples were significantly greater than percentages of moths contacting the control. Percentages of moths attracted to infested apples were significantly greater than percentages of moths attracted to un-infested apples (Table 1), but source contact was similar on infested and un-infested apples in this test.

Mated female codling moths were also attracted to odors of cold-stored thinning apples. Percentages of moths exhibiting upwind oriented flights towards the pipe that vented odor of un-infested or infested apples were significantly greater than the percentages of moths responding to the control airflow (Table 1). Also, percentages of moths contacting the odor source (pipe vent) were greater in response to odor from over un-infested or infested apples compared to the system control (Table 1). Percentages of moths responding (attraction or contact) to odor of infested apples were not statistically different than percentages of moths responding to odor of un-infested apples.

TABLE 1. MEAN ( $\pm$ SEM) PERCENTAGES OF MATED FEMALE CODLING MOTHS RESPONDING TO COLD-STORED THINNING APPLES IN A FLIGHT TUNNEL.

Treatment	n	% Upwind oriented flight	% Source contact
Experiment 1			
Blank	55	0.0 + 0.0 a	0.0 + 0.0 a
Un-infested apples	55	18.2 + 3.3 b	12.7 + 3.0 b
Infested apples	55	38.2 + 8.3 c	18.2 + 6.3 b
Experiment 2			
Blank airflow	50	0.0 + 0.0 a	0.0 + 0.0 a
Un-infested apple airflow	50	10.0 + 3.3 b	8.0 + 0.0 b
Infested apple airflow	50	8.0 + 4.4 b	6.0 + 4.3 b

Within a column and within an experiment, means followed by a the same letter are not significantly different by Tukey's test, at  $p \leq 0.05$ .

### Flight Tunnel Trapping Assays

Significantly more moths were captured in flight tunnel traps baited with immature apples, compared to moths captured in un-baited traps, when those apples were infested with a codling moth larva (Table 2). In a direct comparison of un-infested and infested apples, significantly more moths were captured in traps baited with infested apples (Table 2).

Female codling moths were captured in traps into which odor (as airflow) from over fresh-picked apples was introduced, indicating attraction to the apples (Table 2). This response was significant in comparison to traps without odor from apples, both using apples infested with codling moth and using apples that were not infested. In a direct comparison of odor from infested apples and odor from un-infested apples, significantly more moths were captured in traps baited with odor of infested apples (Table 2).

### DISCUSSION

These results demonstrate attraction responses of flying mated female codling moths to apple fruit and to apple fruit odor. Previous studies had shown attraction by walking codling moth adult to apple fruit, apple odor, and the apple odorant E,E- $\alpha$ -farnesene (Hern and Dorn 1999, Yan et al 1999), but not flight responses. Flight responses by moths to host plants have been shown for the noctuids *T. ni* (Landolt 1989), *Heliothis subflexa* Tingle et al. 1989), and *Mamestra brassica* L. (Rojas and Wyatt 1999), and the plutellid *Plutella xylostella* (L.) (Palaniswamy et al. 1986, and Pivnick et al. 1990). Additionally, the codling moth has been captured in traps baited with the pear volatile ethyl-(Z, E)-2,4-decadienoate (Light et al. 2001). Such flight responses indicate a potential for long distance host-finding by the codling moth, with a significant role of host odor in host finding.

TABLE 2. MEAN ( $\pm$ SEM) PERCENTAGES OF MATED FEMALE CODLING MOTHS CAPTURED IN FLIGHT TUNNEL TRAPS BAITED WITH FRESH PICKED IMMATURE GRANNY SMITH APPLES.

Treatment	N	% Upwind oriented flight	
		Apples in trap	Apple airflow into trap
Experiment 1			
Blank	6	1.3 $\pm$ 0.8 a	2.0 $\pm$ 1.2 a
Un-infested apples		3.3 $\pm$ 1.0 a	7.0 $\pm$ 1.2 b
Experiment 2			
Blank	5	1.6 $\pm$ 1.6 a	1.4 $\pm$ 1.4 a
Infested apples		18.0 $\pm$ 2.7 b	9.0 $\pm$ 2.1 b
Experiment 3			
Un-infested apples	5	0.9 $\pm$ 0.9 a	2.0 $\pm$ 0.8 a
Infested apples		7.5 $\pm$ 1.6 b	5.5 $\pm$ 1.6 b

Means within an experimental comparison that are followed by the same letter are not different by a paired t-test at  $p \leq 0.05$ .

These results also indicate that host-attraction responses of adult codling moth, like that of neonate larvae, are enhanced by prior infestation of fruit by the codling moth. Both anemotactic and klinotactic responses by neonate larvae of the codling moth are enhanced when larvae are in the airstream with odor from apple fruit infested with other codling moth larvae, compared to uninfested fruit (Landolt et al. 2000). Similar enhanced orientation responses by herbivores to damaged or induced plant odors have been shown for the cabbage looper moth and for the Colorado potato beetle (Landolt 1993, Landolt et al. 1999). Although it might appear disadvantageous for a phytophagous insect to respond to an infested plant as an oviposition site, because the moth's offspring would likely face immediate competition for food, there are other explanations for the observed behavior. If host plants occur in patches, locating an infested plant or fruit may provide proximity to other, uninfested, plants or fruits. In flight tunnel studies of cabbage looper attraction to cotton plants, moths were likely to fly to plants damaged by conspecific larvae but were also likely to then oviposit on undamaged adjacent plants, when given that choice (Landolt 1993). Plants, including apple and pear, that are injured or induced, often produce, or produce increased amounts of, particular odorants (Boeve et al. 1996, Landolt et al. 2000, Scutareanu et al. 1997) and may be more chemically apparent to a plant-seeking insect compared to an un-injured plant. Perhaps the codling moth cannot easily detect apple fruit from a distance unless it is injured or infested.

The attraction response rates of codling moths in these flight tunnel assays were not high (up to 35%), compared to male moth responses to pheromones, but are not out of line with studies of other moths and their responses to host plants. Rates of attraction to cabbage plants by cabbage looper moths in a flight tunnel ranged from 22% for males up to 41% for females (Landolt 1989). Attraction responses of the same moth to potato plants were 20-26% (Landolt 2001). Rojas (1999) reported attraction (upwind flight) response rates of ca 20-55% for female *Mamestra brassicae* (L.) moths to cabbage plants in a flight tunnel.

This information should assist efforts to isolate and identify additional host attractants for codling moth and to understand the role of volatile chemicals in host finding and host selection behavior of this insect. If moths use flight responses to apple odor as a means of locating oviposition sites, then flight attraction assays might be useful in studying codling moth host finding behavior and also in isolating those chemicals emitted from apple fruit that attract codling moth. Additionally, if infested fruit are more strongly attractive to codling moth adults and larvae, then the odors of these fruits might best be used in studies to isolate and identify improved

host attractants for the codling moth. Qualitative and quantitative comparisons of the odor of un-infested and infested apple fruit may provide clues as to which volatile chemicals are important to codling moth host finding and host selection.

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