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FINAL REPORT

PROJECT NO. US-807-84

Development of Synthetic Fruit Odor/Visual Traps for Apple Maggot and Mediterranean Fruit Flies

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FINAL REPORT OF US-807-84: DEVELOPMENT OF SYNTHETIC
ODOR/VISUAL TRAPS FOR APPLE MAGGOT AND MEDITERRANEAN FRUIT FLIES

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ABSTRACT

A potential new form of non-pesticidal control of apple maggot flies and Mediterranean fruit flies in commercial fruit orchards is the removal of females through attraction to odor/visual traps. Understanding the dynamic nature of female response to real or synthetic fruit odor and visual stimuli is essential to creation of an effective trapping system. Under this grant, we found that apple maggot flies are stimulated to move upwind, through a series of tree to tree flights, toward the source of synthetic apple odor from distances as great as 12 meters. We showed that after a female arrives on a host tree, it detects abundant and conspicuous host fruit solely by vision and uses fruit odor as a guide to locating individual fruit within a tree only when fruit are relatively scarce and inconspicuous. In commercial orchards, we used synthetic fruit odor to attract apple maggot flies to perimeter host trees and used conspicuously-positioned sticky red sphere fruit visual mimic traps to capture flies after arrival on such trees. By intercepting immigrating flies on perimeter trees before they could penetrate the orchard interior, we were able to achieve a very high level of apple maggot fly control and a clean crop of fruit. Under this grant, we obtained strong evidence that Mediterranean fruit flies are attracted to the odor of guava fruit. We developed a sensitive olfactometer assay for future measurement of fly response to fractions of guava fruit odor components. Hopefully, this will soon lead to development of a synthetic fruit odor attractant that can be used in conjunction with fruit visual mimics to trap out medfly females.

OBJECTIVES

The apple maggot fly in North America and the medfly in Israel are among the most damaging pests of fruit. Much environmentally-damaging insecticide is applied annually to control these pests. It is in the strong interest of pest managers, citizens of the general public, and those concerned with the health of the environment that alternative non-pesticidal methods be developed for controlling these pests. One potential major form of non-pesticidal control lies in employing host fruit odor and visual stimuli in traps to capture out female flies before they can lay eggs in orchard fruit. Because little quantitative information was available on the responses of females of either species to host fruit odor and visual stimuli, we undertook to investigate the dynamic nature of these responses as the major thrust of this grant. From our findings, we hoped to design and test out behavioral methods of fly control in commercial orchards using traps.

Our specific objectives for the apple maggot fly were five-fold (all work on apple maggot flies was conducted in Massachusetts);

- (1) To quantify within-tree search dynamics of individual females foraging for host fruit odor and visual stimuli or their synthetic equivalents,

- (2) To quantify among- tree search dynamics of individual females foraging for host odor and visual stimuli or their synthetic equivalents,

- (3) To assess patterns of female movement in response to fruit odor stimuli under laboratory wind tunnel conditions,

- (4) To determine distances at which females respond to host odor and visual stimuli;

- (5) To use the knowledge gained from research on Objectives 1-4 and apply it to development of a behavioral control method for apple maggot flies in commercial orchards.

Our studies on medflies were much less ambitious, because no previous information was available indicating whether or not medflies do in fact respond to host fruit odor. Thus, the major focus under this grant was to collect volatiles of medfly host fruit and conduct preliminary electrophysiological assays of medfly responses to such volatiles (carried out by personnel at Geneva, New York) and to design and carry out various assays for evaluating behavioral responses of medfly females to host odor and visual stimuli (carried out by personnel in Israel).

Our specific objectives for the Mediterranean fruit fly were fourfold:

- (1) To study inter-tree movement of medfly females,
- (2) To characterize olfactory responses of medfly females to fruit and fruit odor in traps,
- (3) To examine medfly behavior in a wind tunnel,
- (4) To evaluate medfly attraction to colored spheres

BODY OF THE REPORT

The body of our report consists of a detailed description of our accomplishments under each objective. Because some of these descriptions have been published, others have been submitted for publication, and still others are simply in scientific report form, we have chosen to place all of these descriptions in an Appendix at the end of this report. Here, we present a summary of each description, according to the objective to which it applies.

For Apple Maggot Flies:

Objective 1: within-tree search dynamics of apple maggot flies.

Description #1 entitled "Novel Approach for Tracking and Quantifying the Movement Patterns of Insects in Three Dimensions under Seminatural Conditions". Summary: We describe a method that permits tracking and quantifying the movement patterns of insects in three dimensions under seminatural conditions. We released individual Rhagoletis pomonella flies onto an apple tree and recorded all movement and behavior of the foraging insect. The type of information obtained included: measurement of relative distance flown (net and gross displacement, average distance between stops), relative directness of flight from the fly release point to any point within the tree, relative speed of flight, relative angle and vector information for individual and total displacements, and time spent performing individual behaviors. All these parameters were calculated over the total time the insect spent on the tree and for specific time and spatial windows of particular interest. We also describe a computer program that processes and partially analyzes the gathered data. The usefulness of 3-D analysis is illustrated by providing results that show a trend for reduction in the relative distance flown and for an increase in the directness of flight by flies that searched for and landed on fruit models that released synthetic apple odor compared with models that did not.

Description #2 entitled "Host Odor and Visual Stimulus Interaction During Intra-Tree Host Finding Behavior of Rhagoletis pomonella". Summary: Responses of apple maggot females to host visual stimuli (apples or models of apples) and fruit chemical stimuli (synthetic apple volatile blend) were studied in semi-dwarf field-caged apple trees. We tested fly response to 3 different fruit or model densities (1, 4 or 16 fruit or models/tree) and 2 odor release rates (ca. 0.7 ug/h and ca. 500 ug/h). Individually released flies were followed as they moved within a tree for a maximum of 20 min. By using a method developed under "Novel Approaches to Tracking...", we were able to record three-dimensional search paths followed by foraging flies and compute such variables as total relative distance traveled before alighting on a fruit or model, track length between individual alightment sites, and directness of flight to fruit or models. Effect of odor on propensity to alight on fruit or models depended on fruit or model color and density. If the visual stimulus were strong (e.g. red color) odor did not increase the probability of finding fruit or fruit models. As the visual stimulus became progressively weaker (red to green to clear), odor appeared to aid flies during the fruit finding process. For example, we recorded a significant reduction in the mean number of fly visits to leaves before alighting on a green model with odor compared with a green model without odor. The greater the density of fruit or models, the greater the propensity of flies to find a fruit or model (e.g. only 50% of

flies found a red fruit model at a model density 1/tree while 90% found a red model at a model density of 16/tree; only 4% of flies found a clear model with odor at a model density of 1/tree while 35% found a clear model with odor at a model density of 16/tree). Continuous exposure to high odor concentration appeared to arrest fly activity, while intermittent exposure to odor appeared to stimulate fly activity. Findings reported under Objective 2 indicate that R. pomonella flies are able to discover a point source of odor by flying upwind in response to intermittent exposure to odor. After arrival on a host plant (point source), findings here indicate that flies discover individual apparent and abundant host fruit on the basis of vision. If fruit are less apparent or scarce, odor interacts with vision during the host finding process.

Objective 2: among-tree search dynamics of apple maggot flies.

Description entitled "Host search behavior by Rhagoletis pomonella flies: inter-tree movement patterns in response to wind-born fruit volatiles under field conditions". Summary: Responses of apple maggot females to synthetic host fruit volatiles were studied in the field. Individually marked females were released in the center of a 25 m² patch containing 25 host trees and followed as they moved within and between trees. Fly response to three experimental conditions was studied: 1) clean air; 2) synthetic host fruit volatiles (apple) permeating the patch; and 3) a single point source of odor placed 1 m away from the edge of the patch. Flies in clean air exhibited area concentrated search and moved at a slower rate when compared to flies exposed to host-odor permeated air. Flies exposed in a patch permeated with host odor exhibited more straightened-out moves and reached the edges of the patch more quickly than those exposed to clean air. Flies exposed to a point source of odor exhibited clear orientation responses, landing consistently on the tree harboring the point source of odor. Here, odor exposure was intermittent and brief (ca. 20 s/exposure) with intervals between exposure periods averaging 103 s. Wind speed and direction were highly variable. Flies moved during wind ranging in speed from 0-4.4 m/s, with fly activity greatest at winds below 2 m/s. Strong winds (> 3.5 m/s) either completely arrested movement or enhanced downwind displacement.

Objective 3: patterns of movement of apple maggot flies under wind tunnel conditions.

Description entitled "Wind tunnel assays of olfactory responses of Rhagoletis pomonella flies to apple volatiles". Summary: Responses of apple maggot fly females to synthetic host apple volatiles were studied using a novel wind tunnel bioassay. Three apple tree models were placed in the wind tunnel (upwind, central and downwind sections) after which flies were released individually on the central tree and allowed to forage for 15 minutes. The experiment was designed as a two-way factorial with three odor release rates (0, ca. 18 and ca. 500 ug/hour) and three wind speeds (0, 0.8 and 1.6 m/s) as treatments. Both wind speed and presence of odor had significant effects on the total time flies spent foraging and on rate of movement (landings/minute). Fly movement decreased as wind speed increased from 0 m/s to 0.8 and 1.6 m/s, but this decrease was attenuated when air carried apple volatiles. Flies exposed to the highest wind speed (1.6 m/s) showed a tendency to move downwind when the air was odor-free. Host odor appeared to induce arrestment near the

source under calm air conditions and to induce a weak positive anemotactic response at a wind speed of 0.8 m/s.

Objective 4: distance of host stimulus detection by apple maggot flies.

Descriptions entitled: "Distances at which Female Apple Maggot Flies Respond to Host Odor and Visual Stimuli" and "Dynamics of Host Odor and Visual Stimulus Interaction in Host Finding Behavior of Apple Maggot Flies". Summary: Responses of mature female apple maggot flies to artificial host plant visual and olfactory stimuli were examined to determine the maximum distance of detection of host plant cues. Flies regulated time spent foraging on a fruitless host tree according to the distance to neighboring host tree models, foraging for less time if models were nearby, and for more time when models were absent or further away. Flies foraged as if no models were present when 1m² or 2m² models were further than 2.5 m away from the real tree. The presence of synthetic apple odor (500 ug/h) decreased foraging time, but not when placed further than 2.5 m away from the real tree. In another assay, individually caged flies spent a greater proportion of time in the upwind portion of the cage when synthetic apple odor (500 ug/h) was placed 6 or 12 m upwind of the cage than when odor was not present or was placed at 20 m upwind, or when released at 6000 ug/h at distances of 6, 12 or 20 m upwind of the cage.

Objective 5: application of knowledge gained to apple maggot fly control.

Description entitled "Second-Stage Integrated Management of Apple Arthropod Pests". Summary: To date, integrated pest management (IPM) of arthropods in commercial apple orchards has been comprised almost exclusively of a suite of what we term "first-stage" IPM practices focused upon pest monitoring. These practices predict first appearance, rate of development, and abundance of pest and beneficial arthropods, which leads to recommendation of selective properly-timed pesticides as the sole tactic of controlling pests not effectively suppressed by beneficials. Here, we evaluated in Massachusetts the effectiveness of what we term "second-stage" IPM practices. These involved integration of pesticidal control of early season apple arthropod pests and behavioral, cultural and biological control of mid and late season apple arthropod pests in the absence of insecticide and acaricide application after mid June. The behavioral approach consisted of ringing the perimeter of several apple orchard blocks (each ca. 1 ha) with sticky-coated red spheres baited with synthetic apple odor to intercept immigrating Rhagoletis pomonella (Walsh) flies. The cultural approach focused on removing unmanaged host trees in a ring around the orchard block perimeter to prevent entry of Cydia pomonella (L.) and Argyrotaenia velutinana (Walker) females. The biological approach emphasized buildup of natural enemies of foliar pests in an orchard environment free of insecticide and acaricide use after mid June.

During the second year (1988) of second-stage IPM implementation, test blocks in which baited red spheres were placed 5 m apart on perimeter apple trees and in which all unmanaged host trees within 100 m of the block perimeter had been removed received only 0.6% combined fruit injury by the above and all other pests active after mid June. Nearby blocks treated under first-stage IPM practices received 0.4% combined injury from such pests. As

revealed by unbaited within-block monitoring traps, comparatively few R. pomonella flies penetrated the ring of interception traps, despite the large numbers caught on the latter. Beneficial predators of pest mites and aphids were nearly twice as common in second-stage as first-stage IPM blocks. Total cost was essentially the same for second-stage as first-stage IPM practices. But we propose a potentially much less costly, labor-saving method as a substitute for using a sticky substance to control R. pomonella flies alighting on perimeter-tree spheres.

For Mediterranean Fruit Flies

Objective 1: Collection of host fruit volatiles at Geneva, New York and conduct of preliminary electrophysiological assays by Geneva personnel. Description entitled "Report of Wendell Roelofs and Anne Averill ..."

Summary: Odor of whole guava, apricot and peach fruit was collected, fractionated, and sent to Israel for behavioral assay. At Hilo, Hawaii, preliminary electrophysiological assays were conducted of medfly response to a range of host and non-host volatiles, with no positive indication of significant antennal response of medfly females.

Objective 2: inter-tree movement of medfly females (descriptions for Objectives 2-5 are given in "FINAL REPORT - ISRAEL.

Summary: we assessed how host fruit odor affected medfly female movement away from a central fruitless host tree on which mature females were released individually. The source of odor was fresh-picked host fruit placed in cloth bags (ca. 3 kg/bag) attached at an amount of 2 bags on each of 4 green tree models surrounding the central tree at a distance of 2 meters. A high proportion (65%) of released females alighted on models harboring guava fruit. Fewer alighted on models harboring peaches (45%), apricots (19%) or no fruit (9 %). Furthermore, females spent a shorter time on the release tree when the surrounding models harbored guava than the other fruits or no fruit. This study suggests that the odor of guava fruit is particularly positively stimulating to medfly females.

Objective 3: Olfactory responses of medflies to fruit odor in traps.

Summary: Medfly response to the odor of intact host fruit or chemical fractions of host fruit was studied using both an outdoor and an indoor olfactometer. The olfactometer in each case consisted of MacPhail traps (inverted hollow glass) loaded with intact test fruit, chemical fractions of test fruit, or traps that remained empty as controls. Fruit in the traps was concealed by the yellow color of the lower half of each trap. Females were highly attracted to the odor of guava fruit, substantially attracted to the odor of peaches and apricots, and slightly attracted to the odor of tangerines. However, neither females nor males were attracted to fractions of volatiles collected from any of these types of host fruit.

Objective 4. Olfactory responses of medflies to fruit odor in a wind tunnel.

Summary: A wind tunnel 70 cm diam x 210 cm long was constructed to evaluate medfly responses to host fruit odor in a laboratory arena larger than was used in laboratory McPhail trap assays in Objective 2. Flies were released at the downwind end of the tunnel and their movement toward the upwind end emitting the odor of host fruit or isolated volatiles (at several concentrations) of host fruit was observed. Unfortunately, there was no greater response of wild-origin females to the odor of whole guava, peach, apricot, orange or grapefruit fruit than to clean air. However, lab-origin females did exhibit a slight tendency to move toward an isolated blend of guava fruit volatiles.

Objective 5. Visual responses of medflies to fruit-mimicking spheres.

Summary: attraction of medflies to colored plastic spheres was studied in the outdoor olfactometer setup used in Objective 3. Males proved more responsive to colored vs. clear spheres than females, but males did not discriminate well among colors. Females alighted more often on black, green, orange, and red spheres than on yellow, white or clear spheres.

DESCRIPTION OF COOPERATION

In our opinion, cooperation on the project among personnel in Israel, Geneva, and Amherst was very good. The Geneva lab under Drs. Roelofs and Averill collected host fruit of medflies, isolated and fractionated volatiles from these fruit, and sent the volatiles to Israel for assays of medfly response. The Israel and Geneva labs were fully informed of up-to-date results of experiments on apple maggot flies as soon as results could be interpreted with confidence. Likewise, the Geneva and Israel labs informed one-another (and Amherst) on progress in medfly experiments as soon as reliable results were obtained and special needs occurred. The USDA lab in Hilo, Hawaii provided special cooperation by allowing Drs. Averill and Roelofs access to equipment and medflies for conduct of electrophysiological assays. There existed much good will among all participants. Ms. Tova Rivnay (the principal experimenter on medflies at Rehovot) made a 2-week visit to Amherst and Geneva during the first year to learn about the more advanced methods of assaying apple maggot fly responses to fruit odor and visual stimuli. The information gained from her trip was then used to modify or create appropriate behavioral assays for medfly in Israel. Ms. Rivnay also saw advanced methods used for collecting fruit volatiles at Geneva and the process for fractionating these volatiles. This information likewise had an impact on subsequent fruit volatile collection procedures in Israel. Overall, we feel that progress on research with medflies benefited considerably from prior or coincident work on apple maggot flies, of which our knowledge was much more advanced even before work was initiated under this grant.

MAIN ACHIEVEMENTS OF THE RESEARCH

We feel we have achieved a significant level of understanding of the dynamics of apple maggot fly foraging behavior for real and synthetic host odor and visual stimuli and have made a substantial step forward along these lines with the medfly. With both fly species, we are now in a better position

to design effective methods for behavioral fly management using odor/visual traps. In fact, work under this grant has already led to development and implementation of a pilot-stage program to control apple maggot flies in commercial apple orchards behaviorally.

With apple maggot flies, research carried out under this grant has revealed that when a mature female in a non-fruiting tree gets a whiff of host fruit odor, she is stimulated to move often and to proceed in an upwind direction during tree-to-tree flights, eventually bringing her to the source of the odor. We found a female capable of detecting about 700 apples worth of synthetic apple odor (500 ug/h) from distances up to 12 meters but not (under our assay conditions) from a distance of 20 meters. Female movement toward a source of synthetic odor occurred largely during slight to moderate winds of 0.4 to 2.0 meters per second. Under calm conditions or during winds greater than 3.4 meters per second, females exhibited rather little upwind movement toward a fruit odor source. Females were unable to detect a visual model of a medium-size host tree beyond 2-3 meters, suggesting females may be less likely to find host trees surrounded by open space than host trees in close proximity to non-hosts.

After fly arrival on a host tree, we have found (using a novel approach never used heretofore in studying the movements of insects in 3 dimensions under natural conditions) that apple maggot females find strongly visually stimulating individual fruit (eg. dark color, large size) only by vision. As the visual stimulus of a fruit becomes progressively weaker (red to green color, large to small size), or fruit becomes scarce, fruit odor increasingly aids females in discovering individual fruit. Continuous exposure of females to a high concentration of fruit odor appears to arrest fly movement, suggesting that synthetic odor released in high amount and placed too close to a fruit visual mimic (eg. a red sphere trap) may inhibit alightment of females on the mimic.

The combined knowledge gained from these basic studies on apple maggot flies led us to initiate a new behavioral management scheme for this pest in 6 commercial apple orchard blocks. In place of 3-4 insecticide sprays during July and August against the flies, we ringed each orchard block with synthetic-apple-odor-baited sticky red spheres to intercept immigrating flies at the orchard perimeter (apple maggot flies originate not within commercial orchards but from abandoned hosts neighboring orchards). When the spheres were placed 5 meters apart on perimeter apple trees, they proved highly effective in capturing very large numbers of maggot flies and preventing maggot injury to the fruit. When placed 10 meters apart on perimeter apple trees or on neighboring non-host trees, the traps were less effective. This demonstration will serve as a springboard to large-scale future application of this new apple pest control practice.

With Mediterranean fruit flies, research carried out under this grant has demonstrated rather conclusively that medflies indeed respond positively to the odor of preferred host fruit, such as guava. This suggests that synthetic attractive odor of guava may one day be very useful for attracting medfly females to visual fruit-mimicking sphere traps. Our attempts to isolate the attractive components of guava fruit odor did not fully succeed. But the way has been paved for future success along these lines, as research

conducted under this grant led to development of a sensitive olfactometer for assaying medfly responses to fruit odor components.

PUBLICATIONS

Prokopy, R.J., M. Aluja, and T.A. Green. 1987. Dynamics of host odor and visual stimulus interaction in host finding behavior of apple maggot flies. p. 161-166. In Proceedings 6th International Symposium on Insect-Plant Relationships V. Labeyri, G. Fabres, and D. Lachaise (eds.). Junk, Netherlands.

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Prokopy, R.J., S.A. Johnson, and M.T. O'Brien. 1989. Second-stage integrated management of apple arthropod pests. Entomologia Experimentalis Applicata (in press).

Aluja, M., R.J. Prokopy, J.P. Buonaccorsi and R.T. Cardé. Wind tunnel assays of olfactory responses of Rhagoletis pomonella flies to apple volatiles. Submitted to Physiological Entomology.

Aluja, M. and R.J. Prokopy. Host odor and visual stimulus interaction during intra-tree host finding behavior of Rhagoletis pomonella. Submitted to Journal of Chemical Ecology.

Aluja, M. and R.J. Prokopy. Host search behavior by Rhagoletis pomonella flies: inter-tree movement patterns in response to wind-borne fruit volatiles under field conditions. Submitted to Ecological Entomology.