AN ABSTRACT OF THE THESIS OF

Mohammad Moizuddin Mumtaz for the degree of Master of Science in Entomology presented on December 30, 1976

Title: PHEROMONAL COMMUNICATION IN THE WESTERN CHERRY FRUIT FLY, WITH PARTICULAR REFERENCE TO OVIPOSITION

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Studies conducted with the adult western cherry fruit fly, Rhagoletis indifferens Curran, revealed that successful oviposition occurred in three distinct phases, the prepenetration phase, the penetration phase and the postpenetration phase. During the postpenetration phase which averaged 51 seconds (range 18 to 86), the females dragged their ovipositor over the fruit surface following the deposition of an egg. During this process, they apparently released and deposited a highly specialized pheromone which affected the behavior of incoming flies. The pheromone was soluble in methanol and water which indicated that it could be a polar compound. Methanolic and water extracts were stable and retained biological activity under laboratory and field conditions. The pheromone(s) was stable for at least two weeks at 3°C and did not lose its biological activity even upon heating to 80°C. The pheromonal compound(s) prevented repeated oviposition in a fruit already containing an egg, thus contributing to a uniform egg distribution throughout the available fruits.

Identification and synthesis of the pheromone could result in its application in an integrated pest management program against the western cherry fruit fly.

Pheromonal Communication in the Western Cherry Fruit Fly, With Particular Reference to Oviposition

by

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INTRODUCTION

Based on their physiological and ecological characteristics, fruit flies of the family Tephritidae (= Trypetidae) are divided into two major groups; the univoltine and multivoltine flies (Bateman, 1972). The univoltine species usually have a winter diapause and are found in the temperate regions. The multivoltine species are nondiapausing and are commonly found in the tropical regions.

The Genus Rhagoletis Loew, of the univoltine group includes at least 50 described species, several of which are important economic pests (Wasbauer, 1972). The members of this genus are widely distributed over the Holarctic and Neotropical regions on various host plants (Bush, 1966; Smyth, 1960; Foote and Blanc, 1963; Rohdendorf, 1961). The range of environments to which these flies are exposed is extremely broad and there is no single environmental factor responsible for limiting their distribution. The larvae of many Rhagoletis species cause serious damage on a wide range of fruit and vegetable crops throughout the world.

The western cherry fruit fly, Rhagoletis indifferens Curran, is a major pest of cultivated cherries, Prunus avium L. and Prunus cerasus L., in the Pacific Northwest, including British Columbia, Washington and Oregon (Frick et al. 1954, Blanc and Keifer, 1955). In Oregon, it was first reported near Stayton in 1908 (unpublished data) and within half a century it was established throughout the cherry growing areas of the state.

Most Rhagoletis adults emerge in the spring from infested soil (Boyce, 1934; Lathrop and Nickels, 1932; Frick et al., 1954) and the flies invade nearby host trees soon after emergence. Adults feed on honeydew and other available food such as plant liquids exuding from glandular structures, wounds and oviposition scars (Boyce, 1934). Each female lays several hundred eggs (Boller and Prokopy, 1976) within the fruit of host plants. The larvae feed on the pulp of developing fruit and complete their development in 6 to 8 weeks. Pupation occurs in the soil at a depth of 5 to 10 cm after mature larvae drop to the ground surface (AliNiazee, 1974a). There is one generation per year.

Laboratory rearing of tephritid flies has been only partially successful. Many simple, efficient and relatively cheap diets are now available for most of the tropical fruit flies such as Dacus dorsalis Hendel, Dacus cucurbitae Coquillett and Ceratitis capitata (Wiedmann), (Mitchell et al., 1965), but rearing of the temperate flies such as Rhagoletis pomonella (Walsh), R. cerasi (L) and R. indifferens has been difficult. Several artificial diets have been tested for R. pomonella and R. cerasi (Prokopy, 1967; Haisch, 1968). A satisfactory larval diet consisting of wood pulp, agar, wheat germ, Brewer's yeast, sugar, salt, choline chloride, ascorbic, citric and propionic acids, cholesterol, methyl para-hydroxybenzoate (MPH) and water has been developed for rearing larvae of R. indifferens (AliNiazee and Brown, 1977).

In addition to larval rearing, oviposition on artificial substrates has been a major problem in the laboratory rearing of Rhagoletis flies. Various objects resembling the host fruit in size and shape have been tested for oviposition. These include wax domes, gelatin balls, paraffin foam balls and agar balls (Hagen et al., 1963; Prokopy, 1966; Boller, 1968; Haisch and Boller, 1971). Prokopy and Boller (1970) and AliNiazee and Brown (1977) obtained good oviposition of the European and the western cherry fruit flies on artificial soft ceresin wax substrates.

Little is known about the process of host-plant selection in Rhagoletis flies. In R. pomonella the odor of susceptible fruits is known to attract flies (Prokopy et al., 1973). A contact arrestant on host leaves has also been suggested (Bush, 1974). Response to color stimuli are being explored to possibly develop new methods for surveying and monitoring fruit fly populations (Prokopy, 1972b; Bateman, 1976; AliNiazee, 1977). The flies are strongly attracted to surfaces of spherical or rectangular objects painted yellow, green or orange (Prokopy, 1968a, 1968b, AliNiazee, 1977).

Under natural conditions, female tephritid flies have been observed dragging their extended ovipositor over the surfaces where they have laid their eggs. The dragging, which occurs immediately after oviposition, is apparently responsible for the deposition of pheromone(s) which affects the oviposition behavior of incoming flies. In the black cherry fly, Rhagoletis fausta (Osten Sackon), the walnut husk fly, Rhagoletis completa Cressan, the apple maggot and the

European cherry fruit fly the pheromone prevents repeated oviposition in the same fruit and/or in the same general area (Prokopy, 1972a, 1975b; Cirio, 1973; Katsoyannos, 1975). This factor may be responsible for the uniform distribution of eggs in available fruit within an orchard (Bateman, 1976). Several other factors such as the spreading of exuding juice from an oviposition puncture as well as the deposition of an oviposition deterrent pheromone may be responsible for aggregation of male flies reported in apple maggots (Prokopy and Bush, 1972). Aggressive interaction between females may also be involved in uniform distribution of eggs (Pritchard, 1969; Cirio, 1971; AliNiazee, 1974b).

The deposition of marking pheromones preventing repeated oviposition in the host and causing a uniform egg distribution is common in parasitic hymenoptera (Greany and Oatman, 1972; Vinson, 1972; Rabb and Bradley, 1970). For example, the females of Telenomus sphingis (Ashmead), mark the eggs of Manducta sexta (Johannson) after ovipositing inside the egg, which deters further oviposition in the same egg. Orgilus lepidus Muesebeck, was found to discriminate between parasitized and nonparasitized larvae based on perception of a pheromone left near the host by previous females and by detection of an oviposition deterrent within parasitized hosts (Greany and Oatman, 1972). The pheromone was extractable from O. lepidus females and was localized within the abdomen of the females.

An oviposition deterrent pheromone in Rhagoletis indifferens was first suggested by AliNiazee (1974b). However, no detailed studies have been conducted to demonstrate the existence and properties of

such a pheromone. The purpose of my study was to investigate the pheromonal communication in R. indifferens with particular reference to the role of fruit marking pheromone(s) in deterring repeated oviposition. Experiments were designed and conducted to obtain information about the presence of the marking pheromone, and then to determine different properties of the pheromone, such as solubility, biological activity and stability in the laboratory and field conditions.

MATERIALS AND METHODS

LABORATORY STUDIES AND BIOASSAY TECHNIQUES

Virgin male and female flies were put together in plastic culture cages (15 x 29 x 10 cm) approximately seven days before they were used in the experiments. Fresh artificial cherries were provided each day to serve as oviposition sites. Cherry fruit fly diet (AliNiazee and Brown, 1977) and a 10% sucrose solution were provided on absorbent cotton for adult food. Cherries which had received a large number of eggs were collected from the cages every other day, eggs were counted and the cherries were stored at $3^{o}C$ until needed.

Preliminary studies conducted by releasing different numbers of flies for 24 to 48 hours in the cages with three to five treated and untreated cherries indicated that a single gravid female deposited an average of five to seven eggs in 24 hours if an adequate number of cherries were available. If enough cherries were not available, the female flies oviposited more than once on a single visit to a cherry. A satisfactory egg count was obtained when 25 cherries were exposed to two females and two males for 24 hours in a large cage.

Based on preliminary results, an experimental arrangement was designed which included placing treated and untreated cherries (12 or 13) alternately in a row as shown in Figure 1. This arrangement gave the female flies an equal chance of ovipositing on treated or untreated cherries.

All laboratory studies were conducted in a constant temperature chamber maintained at 26.7 ± 1°C with a 19 hour photoperiod at a light intensity of 300 to 400 foot candles. The humidity inside the cage was maintained between 45 and 55% by adding water on cotton puffs in paper cups. To avoid mold and yeast growth, new food cups were provided every other day. Special precautions were taken to avoid contact of any non-experimental object with the treated or untreated cherries before the experiment. Unless otherwise stated two female and male flies were released in each cage and eggs were counted 24 hours later. The location of the artificial cherries and the number of eggs were recorded on a cage diagram.

Experiment 1: General observations on oviposition behavior

Observations were conducted by releasing six or seven mature mated flies in an experimental cage (23 x 31 x 11 cm) with twenty-five fresh artificial cherries. As soon as a female alighted on a cherry, all other flies were aspirated out of the cage and the oviposition activities were carefully observed and recorded. After a female fly oviposited four or five eggs, it was removed from the cage and another fly introduced into the cage. Each cherry on which any ovipositional behavior was recorded was removed and the number of egg(s) recorded as soon as the female fly flew to another cherry. The cherry was returned to its position after the eggs were counted.

Similar observations were made with natural cherries except that once the female oviposited in the cherry, the cherry was dissected

under a binocular microscope to count the eggs and could not be replaced at its position in the cage.

Experiment 2: Distribution of eggs in fresh artificial cherries

Since under field conditions, the western cherry fruit fly adults distribute their eggs evenly with about 97.5% of the cherries receiving only one egg (AliNiazee, 1974b), tests were conducted to determine whether or not similar trends were exhibited under laboratory conditions. Twenty-five artificial wax cherries were placed in each experimental cage and flies were released as described earlier. Eggs were counted and the location of each cherry containing eggs was determined 24 hours later. The percent oviposition was calculated (Figure 5).

Experiment 3: Fly discrimination between fresh and oviposited cherries

Artificial cherries which had been oviposited in by females were obtained in the laboratory by exposing five to ten artificial cherries to several mature mated females for extended periods. Eggs were counted and removed from the cherries. These test cherries with various levels of oviposition were compared with an equal number of unused freshly made cherries. The experimental procedures described earlier were followed and the number of eggs laid on each cherry was counted 24 hours later. The experiment was replicated 23 times.

Experiment 4: Water solubility of the oviposition deterrent compound(s)

The water solubility of the suspected compound(s) was determined by comparing artificial cherries in which flies had oviposited with

fresh artificial cherries, both washed for three minutes with water and held at room temperature for an hour. Eggs were removed from cherries prior to extracting. The same experimental procedures as described earlier were followed. The experiments were replicated five times.

Experiment 5: Biological activity of the deterrent compound(s) in solution

1) Water washings: Concentrated water washings of the deterrent compound(s) were obtained by washing heavily oviposited cherries (with about 350 ovipositions) in 25 ml distilled water. These washings were obtained according to the method described earlier. The experiments were conducted by spraying 120 fresh artificial cherries twice with the above water washings, using a 20 ml hand atomizer. All sides of the cherries were sprayed uniformly with approximately 0.2 ml of the solution. Equal numbers of fresh artificial cherries were sprayed with about 25 ml distilled water and treated similarly as test cherries.

Test and check cherries were dried for 60 to 90 minutes and placed in experimental cages. The same bioassay techniques as described earlier were followed and eggs were counted 24 to 36 hours later. The experiments were replicated six times.

2) Methanol washings: The concentrated 95% methanol washings of the suspected oviposition deterrent compound(s) were obtained in the same manner as the water washings, although the volume of methanol was a problem. In the process of extraction some of the methanol evaporated.

To keep this factor constant so that a comparison could be made between methanol and water washings, the total volume of washings was brought to 25 ml by adding enough methanol at the end of the washing process. A set of 120 fresh artificial cherries was sprayed with methanol washings in a similar manner as the water washings. The test cherries were placed in the experimental cages 90 minutes after the treatment along with an equal number of fresh cherries sprayed with methanol only. The experimental cages with test and check cherries were held at 26.7 $\pm$ 1^{o}C for an additional 30 to 60 minutes before releasing the flies. This additional precaution was necessary to avoid fly mortality due to methanol. The tests were conducted in a similar manner as reported for water washings and replicated eight times.

Experiment 6: Stability of deterrent compound(s)

Heavily oviposited artificial cherries were obtained from culture cages and checked for number of ovipositions. These cherries were then stored at three different temperatures; 3^{o}C, room temperature (20^{o}C to 23^{o}C) and 26.7 $\pm$ 1^{o}C for varying periods and compared with the freshly made unoviposited check cherries by employing bioassay techniques described earlier.

In addition, another set of experiments was conducted in which the stability of the pheromone was checked at a high temperature. A large number of heavily oviposited cherries with a considerable amount of suspected oviposition deterrent compound(s) were heated to 80-85^{o}C, and new cherries were made from this melted wax. Similarly,

check cherries were made using fresh, unused wax. These two types of cherries were compared employing the bioassay techniques discussed earlier and ovipositional behavior was observed on each of these two types of cherries. Experiments were replicated six times.

FIELD STUDIES

The cherry fruit fly infestation levels were monitored in an abandoned sweet cherry orchard located near Albany, Oregon. As soon as a 100% infestation was reached, over 300 cherries were picked with stems and brought to the laboratory for further studies. On the same day over 300 check cherries were picked from a cherry fruit fly free orchard and brought to the laboratory for comparative tests. All the cherries were held at room temperature for 48 hours before initiation of the experiment. Precautions were taken to prevent contact of the cherries with other objects in the laboratory though it could not be completely avoided.

About 75 infested and uninfested cherries of same quality, size, color and ripeness were selected and tied individually by their stems with different colored threads to identify treatments and used in the following field studies.

Experiment 1: Oviposition of flies on cherries with and without deterring compound(s)

From the selected cherries, 12 infested (test) and 12 uninfested (check) cherries were tied alternately in rows on small branches of sweet cherry trees (variety Royal Anne and Bings) located at the

Entomology Farm, Oregon State University, Corvallis, Oregon. A sleeve cage was put over these branches and the open ends were fastened around the branch (Figure 2). Three female and two male flies were released in each cage, and the fly activity checked every 12 hours. Based on fly activity in each cage the experiments were terminated after 48 or 60 hours. The flies were removed and released in other cages and the cherries picked (with their threads) and placed in two premarked bags. These bags were taken to the laboratory. Each cherry was observed and individually dissected under a binocular microscope. The total number of scars, the number of hatched and unhatched eggs and the number of larvae were recorded. The experiments were conducted during the month of July, 1976. No unusual weather conditions occurred during the experiments.

Experiment 2: Biological activity of oviposition deterring compound(s) in solution

Concentrated water and methanol washings of the oviposition deterring compound(s) were obtained in the laboratory by washing heavily oviposited artificial cherries (with approximately 700 ovipositions), with 50 ml of solvent. The final volume of both solutions was brought to 50 ml at the end of extraction to compensate for evaporation.

About 150 fresh uninfested cherries were picked with stems, from stock cages in the field at the Entomology Farm. Maximum efforts were made to standardize the test conditions including cherry size,

ripeness, color, etc. Efforts were also made to avoid contacting any object with the test or check cherries after the treatments. Two sets of 75 cherries each were selected, and individual cherries were tied by the stem to a coded colored thread. The cherries in each set were suspended by threads and treated as follows:

Seventy-five cherries were sprayed three times with 50 ml water washings using a 20 ml hand atomizer. After each spray the cherries were set aside for 60 minutes and retreated. After the final spray, they were allowed to dry completely. The check cherries were sprayed three times with distilled water using a similar procedure. The remaining 75 cherries were treated with methanol washings. The check cherries were sprayed with 95% methanol alone. The experiments were conducted by alternatively placing 12 cherries of each treatment on enclosed branches of sweet cherry trees at the Entomology Farm. A sleeve cage was put over the branch and the ends fastened to the branch. Three female and two male flies were released in each cage, and observations on fly activity were made every 12 hours. Both the water washing and the methanol washing experiments were terminated after 48 to 60 hours.

The flies were removed and the cherries were taken to the laboratory and checked under a binocular microscope for the number of scars and eggs.

All experiments were replicated at least five times, and data were analyzed using a two-sample paired t-test. The check and test values were compared for significant difference at $P < 0.05$ level.

INSECTS

Adult insects used in the study were obtained by collecting soil samples 5 to 10 cm deep from infested orchards and sifting to obtain overwintering pupae. Soil underneath trees where high infestation had been recorded in past years was sifted using the wet sifting system (AliNiazee, 1974a). About 1000 to 1500 pupae thus obtained were held in petri dishes (9 cm diameter) at $3^{o}C \pm 0.5^{o}C$ for 3 to 9 months. The pupae were removed from the cold room as needed and moved to a rearing chamber maintained at $26.7 \pm 1^{o}C$ for adult emergence which occurred in 26 to 40 days. The newly emerged male and female flies were collected daily and held separately in small, one pint cardboard cartons and provided with 10% sucrose solution and drops of honey as food.

Artificial cherries were made as described by AliNiazee and Brown (1977). Soft ceresin wax (Texaco Wax MP 121, Deutsche Texaco, Hamburg, Germany) was melted with 10% wt/wt orange candle dye (Craf-Trims, Tacoma, Washington) in a beaker at $80^{o}C$. The artificial cherries, which were actually hollow domes, were made by dipping the ends of test tubes (1.2 cm dia.) in warm soapy water, then in molten wax. To ensure a uniform solidification and texture, hot air was blown on the end of the test tube after it was dipped in the wax. The artificial cherries were cut to a uniform height (1.1 cm) and then removed from the test tube.

CAGES

a) Experimental cages: Large clear plastic cages (23 x 31 x 11 cm) were used in all bioassay studies. The experimental cages had removable floors. Twenty-five small pieces of sponge (0.5 x 0.5 cm) were glued to the bottom surface and one artificial cherry was placed over each piece (Fig. 1). For air circulation, two windows (2.5 x 3.5 cm) covered with fine cheesecloth were made on two sides of each cage. A hole 3 cm in diameter was made in the roof of each cage through which flies were released or collected. When not in use, the hole was closed with a cotton plug.

b) Sleeve cages: All cages used in field studies were cylindrical 46 x 38 cm diameter, made of chicken wire (2.54 cm mesh) covered with fiber glass (6 meshes per linear cm) and cheesecloth (11 meshes per linear cm). These sleeve cages were placed over medium sized branches and held in position by tying them with strings and wires (Fig. 2).

Twenty sleeve cages were placed on selected branches prior to maturity of the fruit and/or emergence of the natural population of flies. These cages protected the cherries from fly infestation, but allowed the cherries to be exposed to natural environmental and weather conditions.

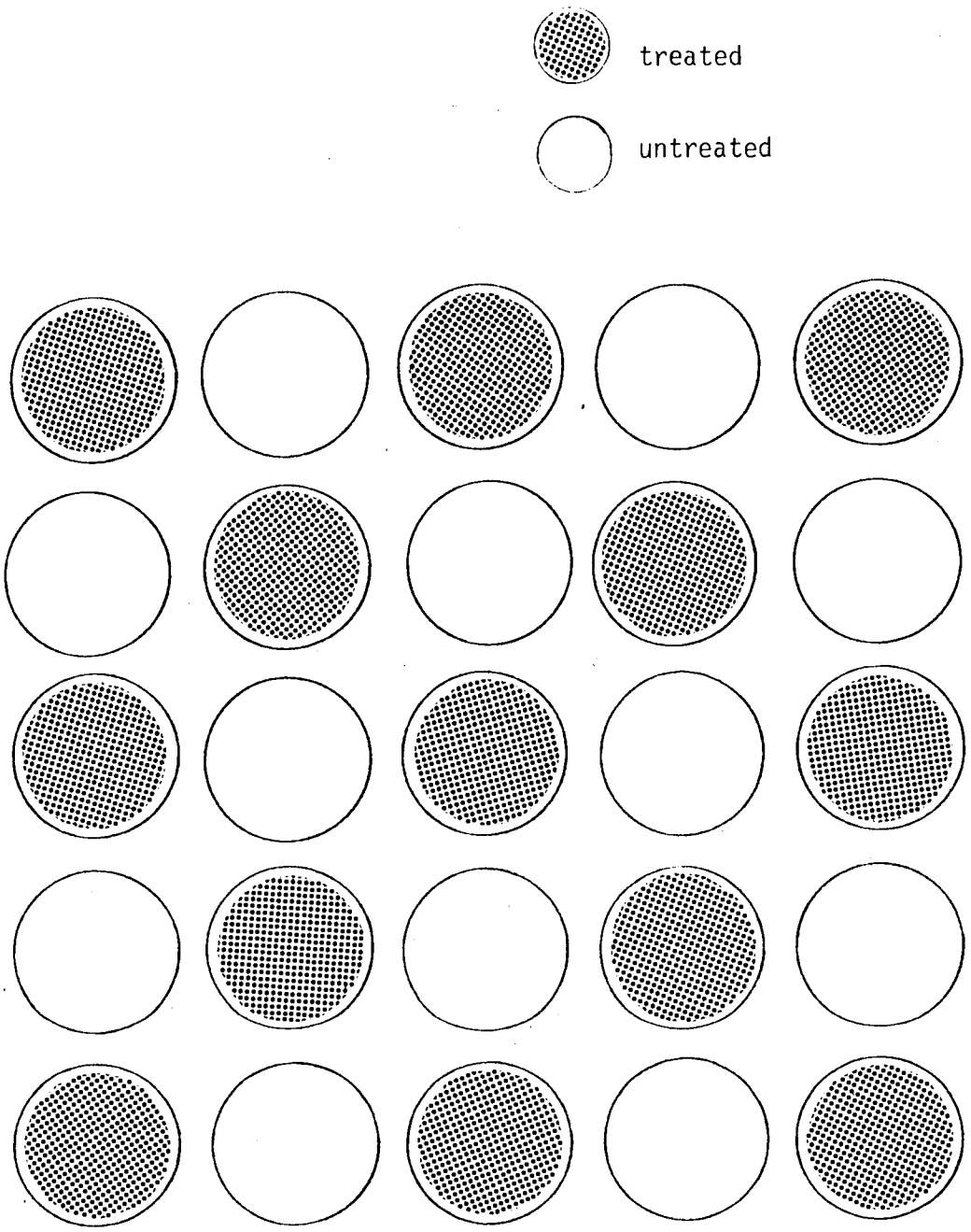

Figure 1. Arrangement of treated and untreated artificial cherries on the bottom of an experimental cage

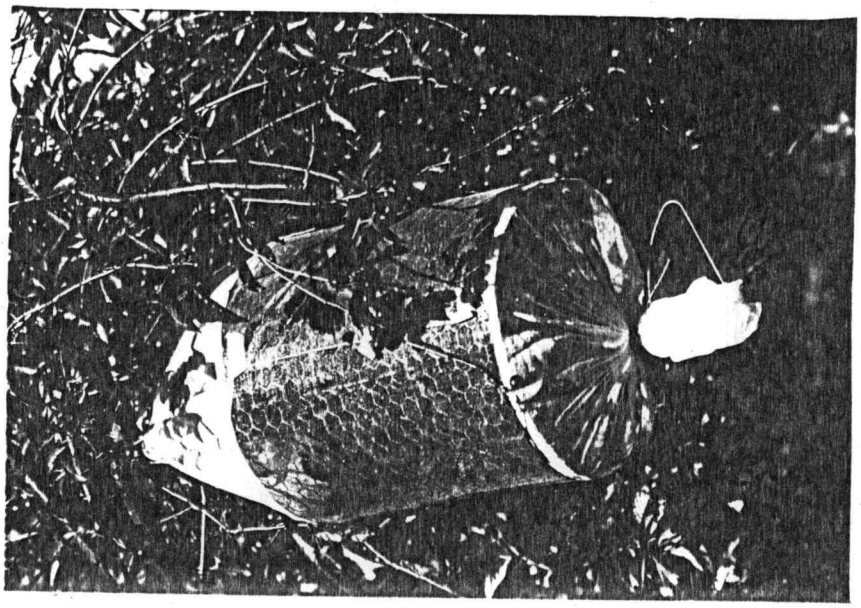

Figure 2. A field cage

EXTRACTION AND REAPPLICATION OF THE OVIPOSITION DETERRING COMPOUND(S)

Artificial cherries in which flies had oviposited were washed by dipping in water or solvent for three minutes. The volume of the solvent was kept at a minimum to get a relatively concentrated solution. The washings were collected in a Teflon^{R} capped large vial and stored at 3^{o}C.

Commonly used solvents such as cyclohexane, ether, methanol (95%) and water were used in preliminary studies. Cyclohexane and ether dissolved most of the wax components of the artificial cherries including the candle dye used for coloring. These solutions were difficult to handle at room temperatures and their high solubilities could create unanticipated problems in chemical analysis of the suspected pheromone at a later date. Methanol and water on the other hand, were suitable solvents and were used in all subsequent studies.

In the preliminary studies, a small chromatographic syringe was used to apply the suspected pheromonal washings in fine droplets of approximately one microliter all over the surface of the cherries. In later studies a 20 ml hand atomizer was used to spray the washings uniformly on the cherries. The treated cherries were set aside for about 30 minutes at room temperature and then the coating process was repeated. Treated cherries were set aside for 60 minutes before testing in bioassays. Similar methods were used to treat cherries with only solvents such as methanol or water.

RESULTS AND DISCUSSION

OVIPOSITION BEHAVIOR OF *RHAGOLETIS INDIFFERENS* CURRAN

Males usually arrive on cherries before females. The female flies arriving on a cherry for oviposition exhibit the following:

General activities like landing and sitting on the cherries, quick movements on the surface of the cherry, cleaning legs, wings, and antennae are quite common. Under laboratory conditions females and males excrete small droplets of liquid on the surfaces of the cage. It is not known whether or not the excretion process has any significance to the overall communication in these insects.

The characteristic oviposition behavior is composed of three distinct phases:

Pre-penetration phase: Soon after arriving on a cherry, female flies explore the cherry surface, typically touching the surface with their proboscis while moving in an irregular fashion. During this phase the body and wing movements were quite pronounced. This initial search time averaged 12 seconds in duration (range 5 to 30 seconds), at the end of which penetration with the ovipositor began.

Penetration phase: After finding a suitable spot, the female pushed the ovipositor into the cherry skin by a pumping action of the abdomen (Figure 3). The average penetration time was 61 seconds (range 15 to 240 seconds). The duration of penetration was independent of the deposition of eggs. For example, it was found that even during

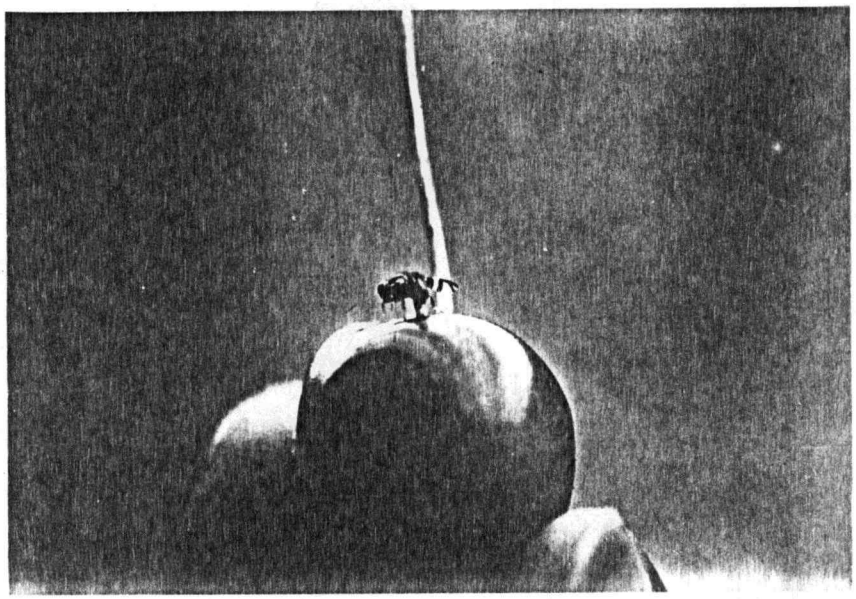

Figure 3. Oviposition

a short penetration time an egg might be deposited while many long penetrations may not result in any oviposition. Only one egg was deposited at each of the oviposition sites.

Post-penetration phase: After depositing an egg, the female rapidly circled around the cherry surface dragging her extended ovipositor for about 51 seconds (range 18 to 86 seconds). In some cases, the dragging was interrupted by a two to three second pause. The number of eggs a cherry contained had no influence on the post-oviposition dragging process. In nature female flies feed on droplets of juices exuding from the puncture caused by penetration of the ovipositor either at the beginning or during the dragging process. The flies cleaned the ovipositor with their legs before flying to another cherry.

If the egg was not deposited, only the first two phases viz. pre-penetration and penetration were observed while the third phase post-penetration (dragging) was observed only when an egg was deposited. In one of the 60 depositions observed, only one egg was deposited without dragging while in another case dragging was observed but no egg was found. Thus it is a "no oviposition, no dragging process".

EGG DISTRIBUTION IN AVAILABLE FRUIT

Studies on the distribution of eggs in available fruits in the laboratory indicated that cherries with one egg were maximum in number

(Figures 4, 5). Under laboratory conditions about 80% of the artificial cherries had only one egg per cherry while 16% had two eggs per cherry and 4% had three eggs per cherry. Earlier studies (AliNiazee, 1974b) indicated about 97.5% of the cherries in the field contained only one egg.

Field studies revealed a significant negative correlation ($R = -0.89$) between the number of scars and the number of egg(s) per scar. There was an inverse relationship between the number of egg(s) per scar and the number of scars, indicating that the probability of finding one egg per scar decreased as the number of scars increased (Figure 6).

PHEROMONE DETERRING REPEATED OVIPOSITION

Studies in the laboratory indicated that R. indifferens was capable of discriminating between the treated (previously oviposited) and untreated (unoviposited) artificial cherries and preferred laying eggs on untreated rather than treated cherries (Figure 7). Females oviposited in an average of 77% of the untreated cherries compared to an average of 24% in the treated cherries. This respresents a 69% reduction in oviposition on treated cherries. There was a marked difference in the number of eggs deposited in treated cherries as compared to untreated cherries with the untreated cherries receiving two to four times more eggs (Appendix, Table 1).

Similar results were obtained in field studies (Figure 8 and Appendix, Table II). The female flies oviposited in 78% of the

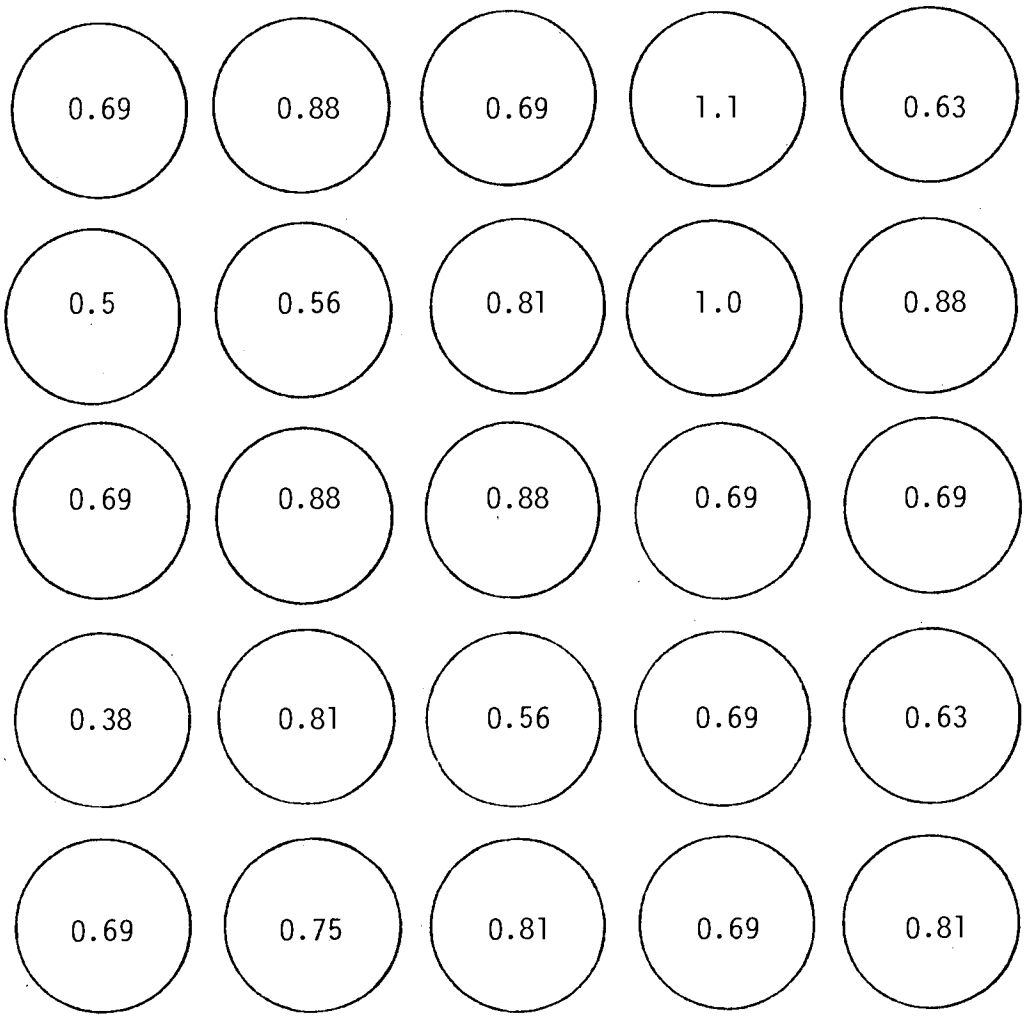

Figure 4. Distribution of Eggs in Artificial Cherries in a Cage (Average of 16 replicates)

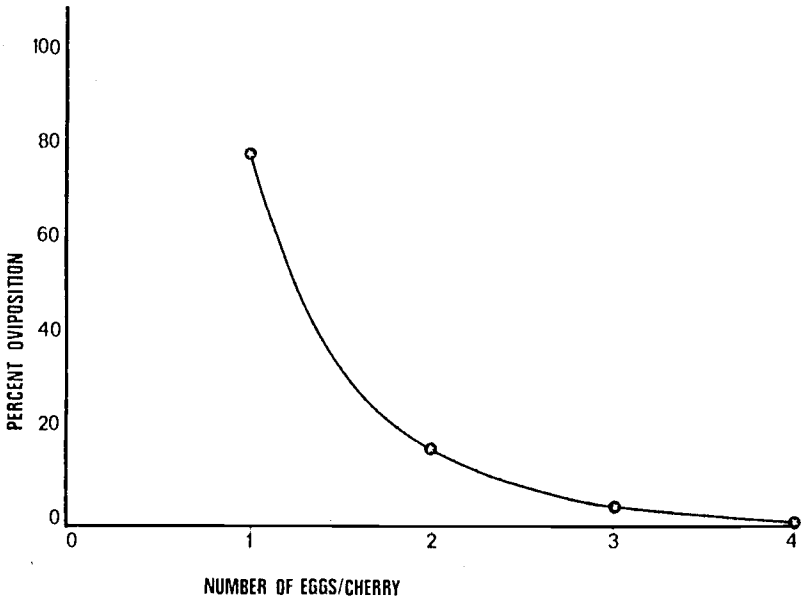

Figure 5. Percentage of cherries with varying number of eggs showing distribution of eggs

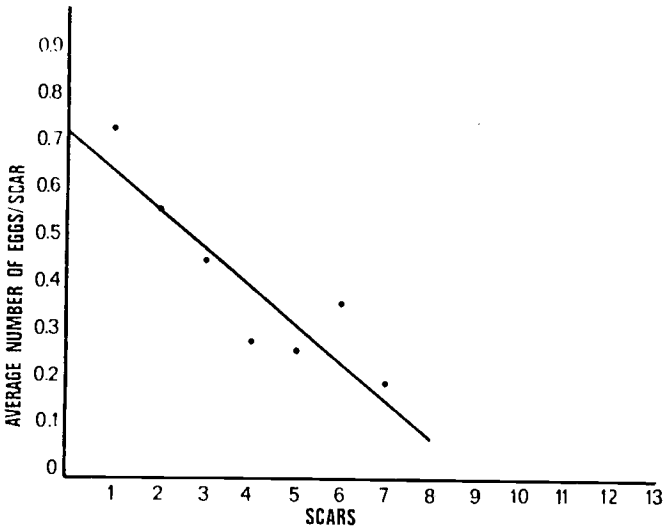

Figure 6. Relationship between number of eggs and scars

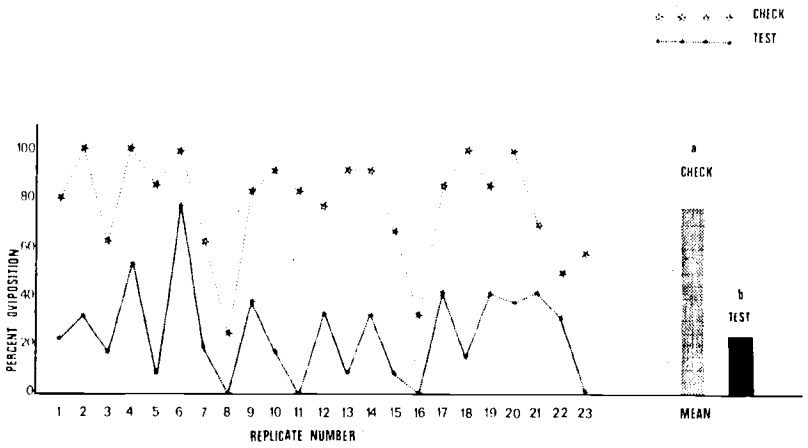

Figure 7. Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (previously oviposited) artificial cherries under laboratory conditions at 26.7^{o}C, 45 to 55% RH and 300 to 400 FC light intensity

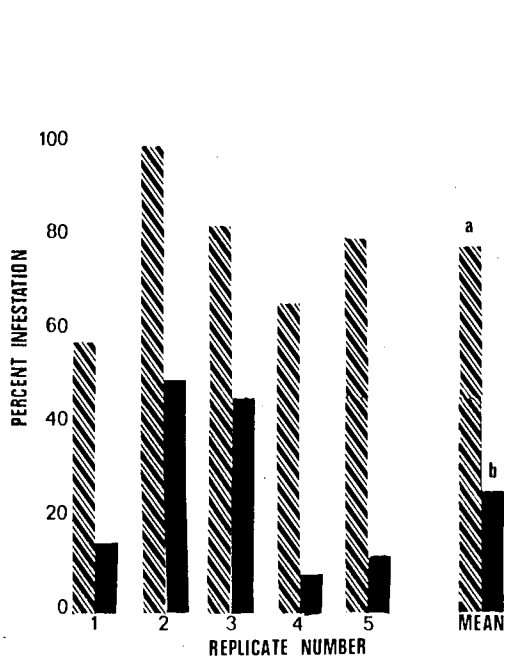

Figure 8. Field studies comparing oviposition of the western cherry fruit fly adults on check (uninfested) vs test (infested) natural cherries conducted at Entomology Farm, OSU, Corvallis

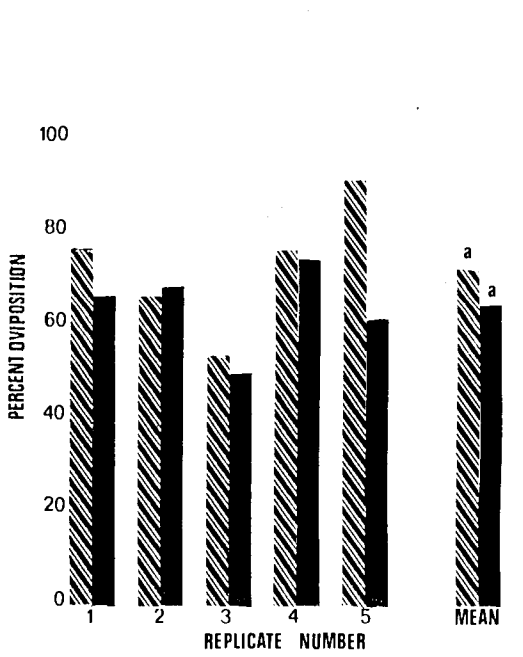

Figure 9. Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (water washed oviposited) artificial cherries under laboratory conditions at 26.7°, 45 to 55% RH and 300 to 400 FC light intensity

untreated cherries and in 26% of the treated cherries. Also a larger number of eggs was deposited in untreated as compared to treated cherries.

A comparison of the number of eggs laid in cherries in the laboratory and field indicated a significant difference between the untreated and treated cherries ($P<0.05$). This indicates that the female flies deposited some marking compound(s) on the cherry surface which was responsible for deterring further oviposition in that fruit. The compound(s) was deposited during the period when the female was dragging the ovipositor over the cherry surface. Similar results were found with apple maggots (Prokopy, 1972a), black cherry fly (Prokopy, 1975b) and the European cherry fly (Katsoyannos, 1975).

SOLUBILITY OF THE PHEROMONE

Washing of treated cherries with water for three minutes dissolved and removed the marking pheromone. The female flies were unable to discriminate between the check cherries (fresh artificial cherries) and the treated cherries (washed in water) (Figure 9, Appendix, Table III). The mean oviposition in untreated cherries was 73% compared to 65% in treated cherries, which was not significant ($P>0.05$). The possibility that marking resulted in a physical rather than a chemical clue seems unlikely, since washing marked cherries removed the factor(s) and allowed repeated oviposition.

Laboratory studies on biological activity of the compound(s) consisted of two experiments. One experiment with water washings reapplied to fresh artificial cherries (treated cherries) compared with water sprayed on artificial cherries (untreated cherries) showed that the flies perceived the compound(s) in the water washings and discriminated between treated and untreated cherries. Figure 10 (Appendix, Table IV) shows that flies oviposited in an average of 25% of the treated cherries compared to an average of 73% in the untreated cherries ($P < 0.05$). This represents a 65% reduction of oviposition on treated cherries.

The second experiment with cherries treated with methanol washings and untreated cherries (sprayed only with 95% methanol), showed that the methanol washings retained biological activity (Figure 11, Appendix, Table V). Flies oviposited in 69% of the untreated cherries and in 17% of the treated cherries which was significant at the 5% level. The mean reduction in oviposition in treated cherries was 75%.

Results of field studies support the evidence obtained in the laboratory. The oviposition deterrent pheromone(s) retained their biological activity in water and in methanolic extracts. The flies perceived the pheromone when water extracts were sprayed on fresh cherries (Figure 12, Appendix, Table VI). A mean of 45% of the untreated cherries was infested compared to 15% of the treated

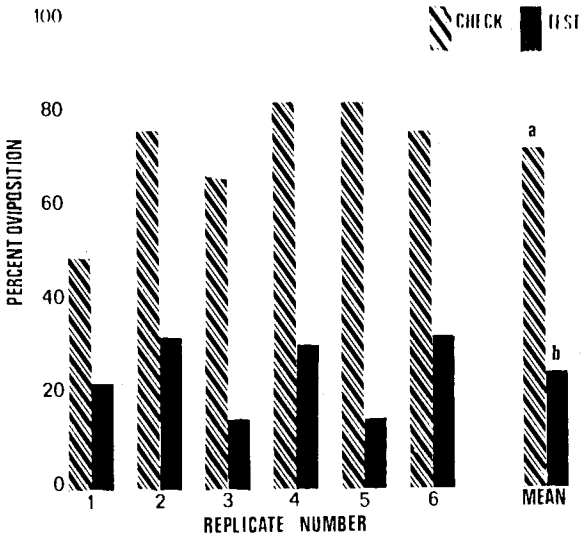

Figure 10. Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (water washings coated) artificial cherries under laboratory conditions at 26.7°C, 45 to 55% RH and 300 to 400 FC light intensity

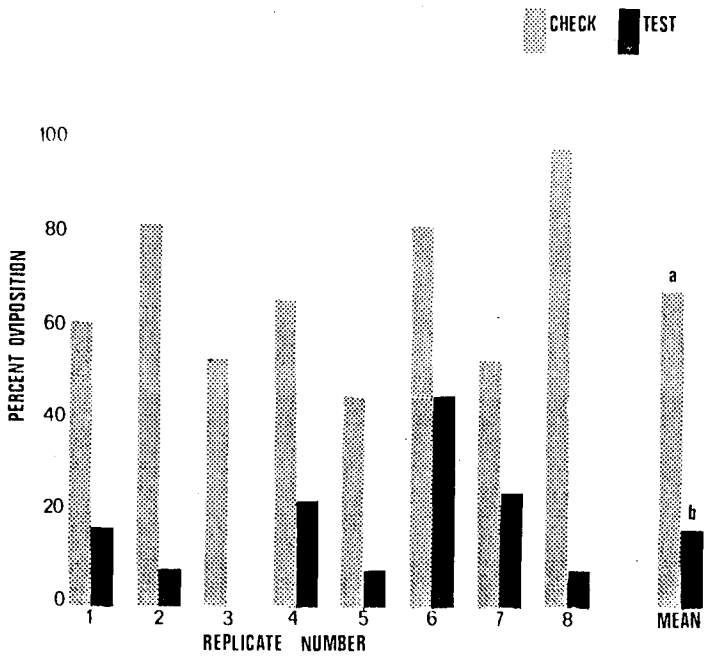

Figure 11. Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (methanol washings coated) artificial cherries under laboratory conditions at 26.7°C, 45 to 55% RH and 300 to 400 FC light intensity

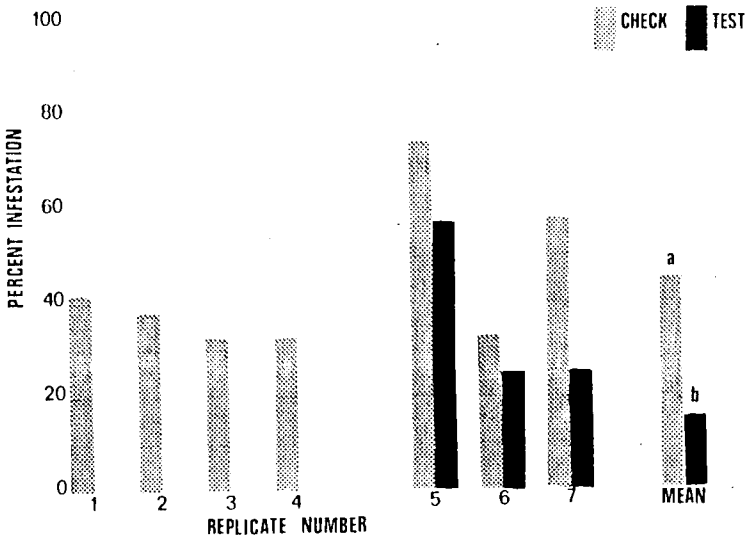

Figure 12. Field studies comparing oviposition of western cherry fruit fly in check (fresh natural cherries) vs test (natural cherries coated with water washings) cherries conducted at Entomology Farm, Oregon State University, Corvallis, Oregon

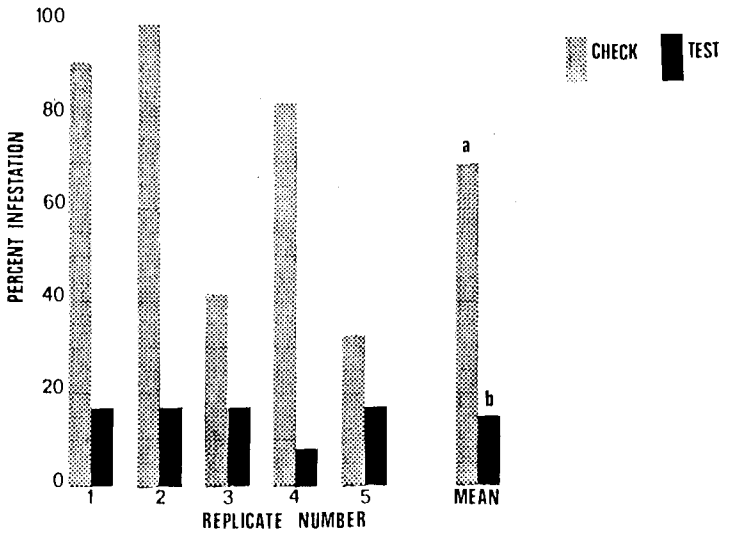

Figure 13. Field studies comparing oviposition of western cherry fruit fly in check (fresh unoviposited) vs test (methanol washings coated) natural cherries conducted at Entomology Farm, Oregon State University, Corvallis, Oregon

cherries sprayed with water extracts. In another experiment where cherries were treated with methanolic extracts (Figure 13, Appendix, Table VII), flies oviposited in 70% of the untreated cherries compared to 15% of the treated cherries indicating the effectiveness of water and methanol extracts containing the oviposition deterrent pheromone.

STABILITY AND PERSISTENCE OF THE PHEROMONE(S)

Results of bioassays with treated and untreated artificial cherries stored in a cold room at $3^{o}C$ showed no apparent decrease in the effectiveness of the pheromones even after two weeks. Oviposited cherries which were stored for two weeks and then bioassayed, showed that females oviposited in 26% of the treated cherries compared to 84% in the untreated cherries (Figure 14, Appendix, Table VIII) indicating a significant difference between these two groups of cherries. Preliminary studies indicated that at room temperature the oviposition deterrent pheromone was stable for more than two weeks.

Another interesting aspect of this study was the stability of the pheromone at $80^{o}C$. When artificial cherries with many oviposition draggings were heated to $80^{o}C$ and the new cherries made from this wax were bioassayed, the flies were still able to discriminate between these cherries and cherries made from unused wax. For example, females oviposited in 18% of the reconstituted cherries compared to 67% in cherries made from unused wax (Figure 15).

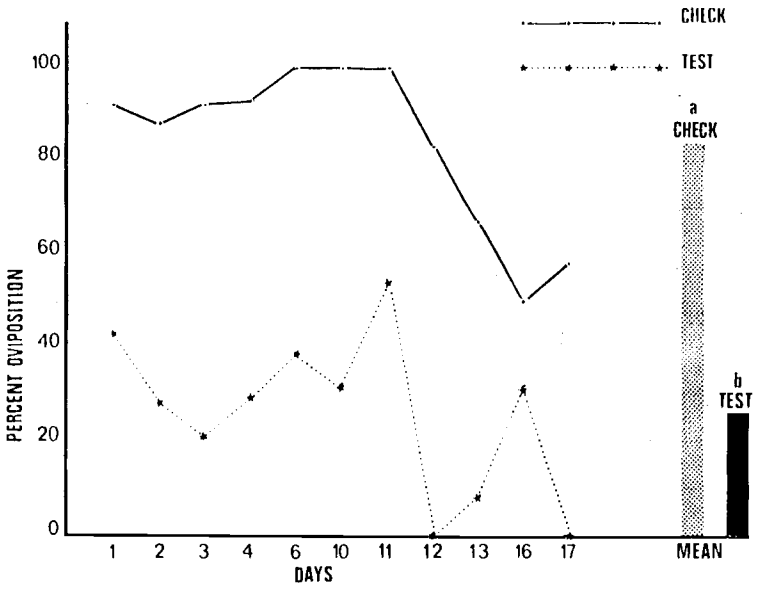

Figure 14. Rate of oviposition of the western cherry fruit fly on check (fresh unoviposited) and test (previously oviposited and stored at 3°C) cherries under laboratory conditions at 26.7°C, 45 to 55% RH and 300 to 400 FC light intensity

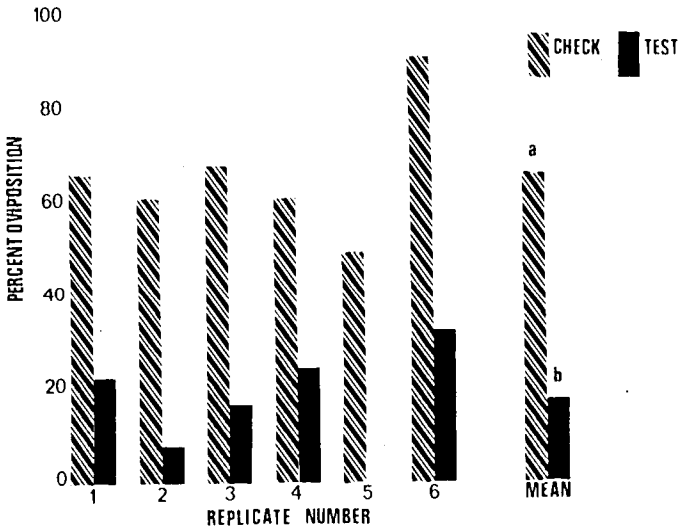

Figure 15. Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (highly oviposited wax) cherries under laboratory conditions at 26.7°C, 45 to 55% RH and 300 to 400 FC light intensity

This indicates that the pheromone(s) was extremely stable even at 80°C, which is unusual because most biologically active compounds are broken down by high temperatures. It is possible that the pheromone(s) may have been absorbed into the wax, thus retaining the biological activity. If this were the case, heating to 300°C or more should release the diffused or entrapped compound(s), but at such high temperatures precaution should be taken to prevent oxidation of waxes.

PROPERTIES OF THE OVIPOSITION DETERRENT

Data presented here indicated that the females of R. indifferens deposited certain chemical(s) after oviposition which affected the oviposition behavior of the incoming flies (Figure 7). The substance(s) applied to the cherry during the marking process possessed the characteristics of a pheromone, since it was produced by one individual and served as a chemical messenger to other individuals of the same species affecting their behavior in a predictable manner (Karlson and Butendant, 1959; Karlson and Luescher, 1959). The pheromone seems to be highly specific, as it is released only for a limited time, and affects a specific behavior of the flies. The release of pheromone is triggered with oviposition of the eggs, suggesting that it is associated with the reproductive system rather than the digestive or excretory systems, which usually release generalized compounds like metabolic waste products common to several insect groups or species.

Prokopy (1975b) indicated the highly specific action of such pheromone(s) in the apple maggot. In his experiments, the females of R. pomonella were not deterred by pheromone released by R. fausta females, and vice versa. Certainly, a pheromone released under very limited conditions, passing information for such a long period of time to control a particular biological action should be fairly specific in action.

Perception of the pheromone: It is not known how the pheromone is perceived by the incoming flies. It was quite obvious during many field and laboratory observations that the presence of pheromone on the cherry was not perceived by the ovipositing females unless they landed on the fruit with pheromone present on the surface. Upon alighting on such an oviposition surface, the females vigorously examined the fruit surface by touching it with their proboscises; within 12 seconds, they left cherries previously oviposited. If no pheromone were present, they tried to oviposit. Apparently the pheromone is perceived only at a very close proximity or upon contact, and is not perceived at a distance.

Effectiveness of the pheromonal compound(s): The pheromone is stable for at least two weeks at cold temperatures (Figure 14). Information on its stability in the field can be derived from the results of field studies. For example, infested cherries were picked and held for two days and then used in the experiment for two days as described in the materials and methods section. Results indicated

that the pheromone was effective for at least four days. In fact, it was effective for a much longer period. For example, the fruit infestation level studies from June 28 to July 28 (Figure 16) showed that most of the cherries had only one hatched or unhatched egg per cherry, yet there were many mature flies searching for oviposition sites in the sleeve cages.

These results indicate that the compound is very stable for at least four days and probably up to two to three weeks. Prokopy (1975b) has also shown that the oviposition deterrent pheromone of R. fausta is stable for at least four days. Katsoyannos and Boller (1976) have shown that in R. cerasi the pheromone is stable for about four weeks.

Cirio (1973), working with the walnut husk fly which oviposits more than one egg at each ovipuncture, proved that the larvae bring about chemo-physical alterations which deter repeated oviposition. Prokopy (1975b) on the other hand, showed that the first and second instar larvae of R. fausta do not contribute to the deterrence of oviposition.

No specific experiments were designed to separate the factors of deterrence in the field studies, but the results in the laboratory clearly demonstrated that the pheromone released is strong enough by itself to deter repeated oviposition for extended periods.

The water solubility of the pheromone is critical and might reduce its effectiveness in rainy conditions, but the high surface

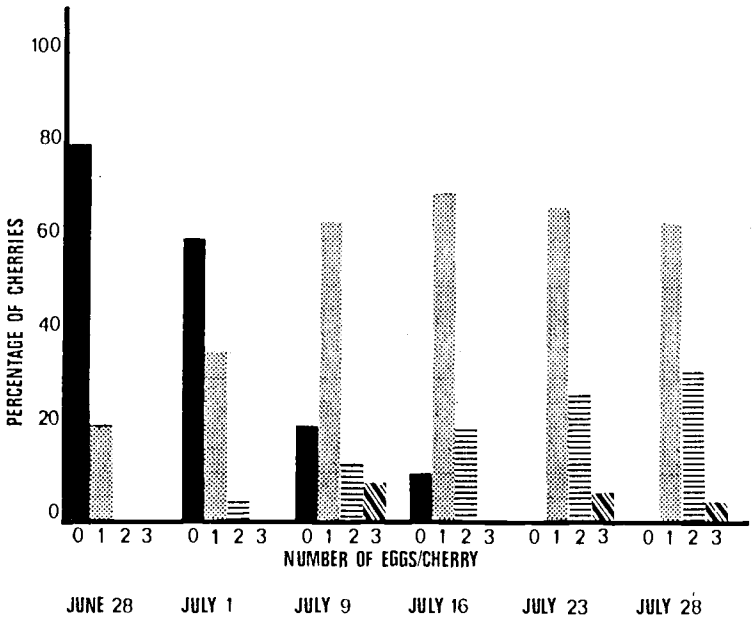

Figure 16. Infestation studies in field from June 28 to July 28 showing number of eggs/cherry and relative percentages

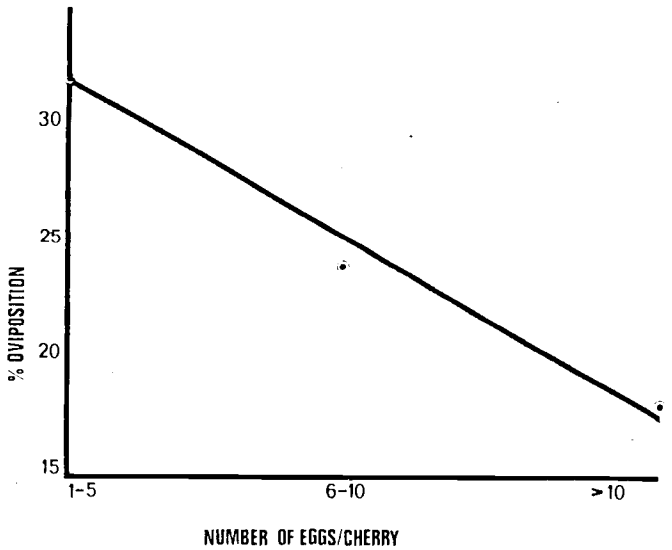

Figure 17. Rate of oviposition of the western cherry fruit fly showing discrimination within oviposited cherries under laboratory conditions at 26.7°C, 45 to 55% RH and 300 to 400 FC light intensity

tension of water might not wash off the pheromone as thoroughly (from the cherry surface) as in the laboratory. Therefore rain may not destroy all of the biological activity. The laboratory and field studies demonstrated that the flies can perceive the pheromone in very low concentration and the amount of pheromone released may exceed the amount needed, particularly in R. indifferens where the dragging and release time of pheromone is twice that of any other recorded Rhagoletis species. Katsoyannos (1975) proposed that other factors such as very short optimum time for oviposition, protection of cherries in clusters or by foliage, and the waxy surface of cherries might decrease the washability of the pheromone.

Influence on mating behavior: It was observed in the laboratory and field that mating occurred when the males approached females from behind while they were engaged in one of the three phases of ovipositional activities described earlier (Exp. 1 - Lab). Most mating was initiated on the fruits, and the flies apparently did not realize the presence of another individual unless they were in a close proximity. It is not known how males recognize females, but they rarely exhibit aggressive behavior toward them as they do towards other males (AliNiazee, 1974b). Apparently, the wing markings play an important role in the recognition process. Males of some species produce a substance on the fruit which either attracts female flies or arrests males (Prokopy and Bush, 1972; Feron, 1962, Katsoyannos,

1976; Prokopy, 1975a). Such compounds might be useful in initiating the mating process.

Prokopy and Bush (1972) reported that pheromone deposited by mature females on cherries arrested male flies. They also found that the pheromone dissipated within a few hours after deposition. It is not known whether the oviposition deterrent pheromone of R. indifferens is also responsible for such arresting action. If it is, the marking pheromone deposited by a female has two or more independent components which affect behavior; one that deters oviposition, and the other, a more volatile component, which arrests the males on to the cherry. The other possibility is that the pheromone has two components that vary in proportion over time. For example, soon after deposition the proportion is such that the males are arrested, but at a later stage the proportion changes so that oviposition by females is deterred. A third possibility is that in its original form the pheromone attracts and arrests male flies, but is not very stable in this form and soon oxidizes to a more stable compound which acts as an oviposition deterrent pheromone. The stability of the compound at very high temperatures ($80^{o}C$) supports the third hypothesis since a compound in its oxidized form will not change its properties as easily even after heating.

Significance and limitation of the pheromone: Data obtained during this study and an earlier study (AliNiazee, 1974b) show that the eggs are uniformly distributed in available fruits, with a

maximum of one egg per cherry (Figures 5, 8 and 16). In comparative studies with cherries heavily oviposited by females, the females showed preference for cherries with one to five eggs/cherry compared to cherries with six to ten eggs/cherry, or more than ten eggs/cherry (Figure 17). Though this is a very artificial situation which might not exist in the field, it does show that the gravid females are sensitive to the amount of pheromone deposited on the cherry surface (which is proportional to the number of eggs that have been oviposited). The response of flies to the pheromone is apparently based on its concentration (amount).

It is also interesting to note that under confined conditions, with limited fruit availability and with most of the fruit already oviposited, the flies laid more than one egg/cherry, although they preferred not to. A similar response was noticed by Prokopy (1972a) in the apple maggot, where flies attempted to bore 52% of the time into fruits that had one drag, compared to 12% into fruits which had 10 drags. Thus, the pheromone played a very significant role in the uniform distribution of eggs in available fruits for maximum exploitation of the host plant. In the case of R. indifferens, this may be related to the survival of the species, as the wild host, Prunus emarginata produces very small fruit which may not support the growth of more than one maggot.

It was observed that the female flies were deterred by the pheromone as long as fresh untreated cherries were available.

However, if no choice were available, they started ovipositing in cherries previously oviposited in by females. Thus, as seen in Figure 18 (Appendix, Table X), once 100% oviposition is reached in the untreated cherries the percent oviposition increases rapidly in the treated cherries (compare with Figure 10). Extrapolation of these results to a natural field situation raises questions as to the feasibility of using the pheromone in direct suppression of fruit fly populations. However, field studies by Katsoyannos and Boller (1976) with R. cerasi indicated a 76% reduction in fruit infestation when trees were treated with the deterrent pheromone mixed with a wetting agent. Apparently, female confusion was achieved by over abundance of the deterring pheromone.

The implications of current findings in a pest management program need further investigations. The pheromone could be used for suppressing fruit fly populations in more than one way. A direct method is spraying an aqueous solution of the pheromone on all but a few trees which in turn could serve as "trap trees" and receive a major share of the fly infestation in an orchard. These trees then can be sprayed with an insecticide to control the flies. Various visual or bait traps can be combined with a pheromone control system. If identified and synthesized, the pheromone could also be used as a direct fly confusion agent under low fly population conditions, followed by an insecticide, thus reducing the number of organophosphate treatments in cherry orchards.

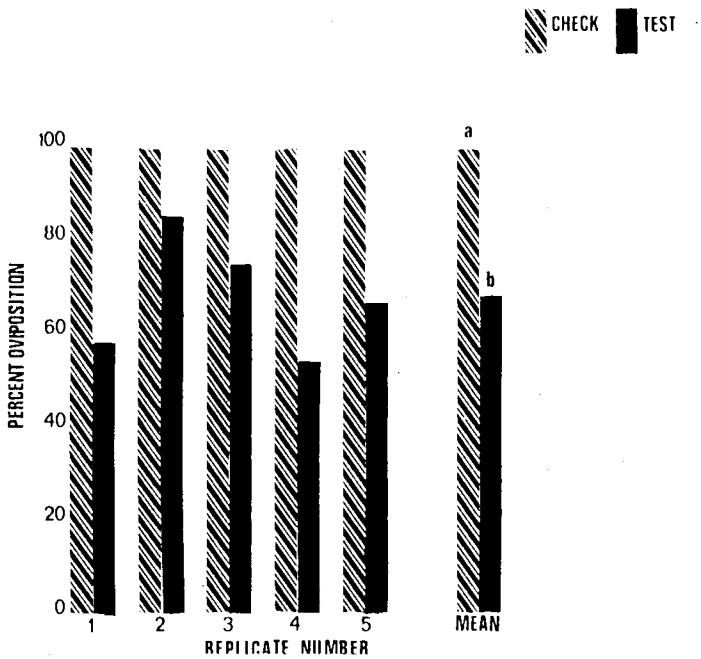

Figure 18. Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (water washings coated) cherries under laboratory conditions at 26.7°C, 45 to 55% RH and 300 to 400 FC light intensity

SUMMARY

The oviposition behavior of Rhagoletis indifferens Curran was studied under field and laboratory conditions during 1975 and 1976. Successful oviposition occurred in three steps; pre-penetration, penetration and post-penetration. During the last phase, the female rapidly circled around the cherry surface dragging her extended ovipositor for about 51 seconds and deposited a marking pheromone. If no egg were laid, no dragging followed. The pheromone deterred other females from ovipositing in an already-infested cherry. However the pheromone was perceived only after the flies arrived on marked cherries.

Laboratory studies on the distribution of eggs revealed that most oviposited cherries contained only one egg. Eighty percent of the artificial cherries had only one egg per cherry, while 16% had two eggs per cherry and four percent had three eggs per cherry. A similar situation was observed under field conditions, and a negative correlation between number of scars and number of egg(s) per scar was observed.

Studies in the laboratory indicated that the female flies were capable of discriminating between the previously oviposited and unoviposited cherries. An average of 77% of the previously unoviposited cherries were oviposited when compared to 24% of oviposited cherries when offered together in a cage. Field

results also were quite similar.

The pheromone was soluble in methanol and water. After the cherries were washed with either of the solvents, the flies were unable to discriminate between unoviposited and oviposited cherries. A mean of 73% oviposition occurred in checks compared to 65% in washed cherries.

The pheromone was stable in both solutions and retained its biological activity. When aqueous washings of pheromone were sprayed on unoviposited cherries, 25% of the treated cherries compared to 73% of check cherries were oviposited. When methanolic washings were sprayed on unoviposited cherries, 17% of the treated cherries were oviposited compared to 69% of untreated cherries. Field data substantiated these findings. The pheromone was stable on the cherry surface for at least two weeks at $3^{o}C$.

The pheromone has a potential of being used in fruit fly control programs. Future research should be conducted to determine the structure and activity of this pheromone and its application in a pest management program. Studies of the physical and chemical properties of the pheromone, leading to identification, should be given a high priority.

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APPENDIX

TABLE I

Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test(previously oviposited) artificial cherries under laboratory conditions at 26.7 ± 1°C, 45-55% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	78	22	19	3	72
2	100	31	35	8	69
3	62	17	12	3	73
4	100	54	42	10	46
5	85	8	18	1	77
6	100	77	25	14	23
7	62	17	11	4	73
8	23	0	3	0	100
9	83	38	15	7	54
10	92	17	29	2	82
11	83	0	13	0	100
12	77	33	16	5	57
13	92	8	15	1	91
14	92	33	27	4	64
15	67	8	16	1	59
16	31	0	5	0	88
17	85	42	21	6	51
18	100	15	30	2	85
19	85	42	32	6	51
20	100	38	28	6	62
21	69	42	18	5	39
22	50	31	13	7	38
23	58	0	12	0	100
Means[1]	a	b	a	b	
	77	24	20	4	68
Standard deviation	22	20	8	4	21

[1] Means in the same column followed by a different letter are significantly different at 5% level.

TABLE II

Oviposition of the western cherry fruit fly adults on check (fresh uninfested) vs test (previously infested) natural cherries at Entomology Farm, OSU, Corvallis, Oregon. July, 1976.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	58	15	7	2	74
2	100	50	30	6	50
3	83	46	22	8	44
4	67	8	8	1	88
5	80	12	26	5	85
Means[1]	a	b	a	b	
	78	26	19	4	68
Standard deviation	16	20	11	3	20

1/ Means in the same column followed by a different letter are significantly different at 5% level.

TABLE III

Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (water washed oviposited) artificial cherries under laboratory conditions at 26.7 ± 1°C, 44 - 45% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	77	67	13	9	13
2	67	69	15	11	0
3	54	50	9	7	7
4	77	75	11	9	3
5	92	62	18	10	33
Means[1]	a	b	a	b	
	73	65	13	9	11
Standard deviation	14	9	4	2	13

1/ Means in the same column followed by a different letter are significantly different at 5% level.

TABLE IV

Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (water washings coated) artificial cherries under laboratory conditions at 26.7°C, 45 - 55% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	50	23	9	4	54
2	77	33	11	5	57
3	67	15	10	3	78
4	83	31	13	4	63
5	83	15	17	2	82
6	77	33	17	4	57
Means[1]	a	b	a	b	
	73	25	13	4	65
Standard deviation	12	9	4	1	12

1/ Means in the same column followed by a different letter are significantly different at 5% level.

TABLE V

Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (methanol washings coated) artificial cherries under laboratory conditions at 26.7°C, 45 - 55% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	62	17	9	2	73
2	83	8	10	1	90
3	54	0	13	0	100
4	67	23	10	5	66
5	46	8	8	1	83
6	83	46	25	9	45
7	54	25	7	3	54
8	100	8	21	1	92
Means[1]	a	b	a	b	
	67	17	13	3	75
Standard deviation	19	15	7	3	20

1/ Means in the same column followed by a different letter are significantly different at 5% level.

TABLE VI

Field studies comparing oviposition of western cherry fruit fly in check (fresh natural cherries) vs test (natural cherries coated with water washings) cherries conducted at Entomology Farm, Oregon State University, Corvallis, Ore.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	42	0	5	0	100
2	38	0	3	0	100
3	33	0	6	0	100
4	33	0	6	0	100
5	75	58	9	8	23
6	33	25	5	3	24
7	58	25	7	3	57
Means[1]	a	b	a	b	
	45	15	6	2	72
Standard deviation	16	22	2	3	37

1/ Means in a column followed by different letter are significantly different at 5% level.

TABLE VII

Field studies comparing oviposition of western cherry fruit fly in check (fresh unoviposited) vs test (methanol washings coated) natural cherries conducted at Entomology Farm, Oregon State Univ., Corvallis, Ore.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	92	17	19	1	82
2	100	17	26	2	83
3	42	17	8	3	60
4	83	8	19	1	90
5	33	17	5	2	49
Means[1]	a	b	a	b	
	70	15	15	2	73
Standard deviation	30	4	9	0.8	18

1/ Means in a column followed by a different letter are significantly different at 5% level.

TABLE VIII

Rate of oviposition of the western cherry fruit fly on check (fresh unoviposited) and test (previously oviposited and stored at 3°C) cherries under laboratory conditions at 26.7°C, 45 - 55% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	92	43	22	8	53
2	88	28	22	5	68
3	92	21	21	3	77
4	93	29	31	4	69
6	100	38	28	6	62
10	100	31	35	8	69
11	100	54	42	10	56
12	83	0	13	0	100
13	67	8	16	1	88
16	50	31	13	7	38
17	58	0	12	0	100
Means[1]	a	b	a	b	
	84	26	23	5	71
Standard deviation	17	18	9	3	19

1/ Means in a column followed by a different letter are significantly different at 5% level.

TABLE IX

Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited wax) and test (highly oviposited wax) cherries under laboratory conditions at 26.7°C, 45 - 55% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	67	23	9	4	66
2	62	8	9	1	87
3	69	17	12	2	75
4	62	25	12	3	60
5	50	0	11	0	100
6	92	33	27	5	64
Means[1]	a	b	a	b	
	67	18	13	3	75
Standard deviation	14	12	7	2	16

1/ Means in a column followed by a different letter are significantly different at 5% level.

TABLE X

Rate of oviposition of the western cherry fruit fly adults on check (fresh unoviposited) and test (water washings coated) cherries under laboratory conditions at 26.7°C, 45 - 55% RH and 300 - 400 FC light intensity.

Replicate number	Cherries with eggs (%)		Total number of eggs		Percent reduction in oviposition
	check	test	check	test	
1	100	58	30	13	42
2	100	85	32	22	15
3	100	75	31	8	25
4	100	54	26	9	46
5	100	67	40	13	33
Means[1]	a	b	a	b	
	100	68	32	13	32
Standard deviation	-	13	5	6	13

1/ Means in a column followed by a different letter are significantly different at 5% level.