EVALUATIONS OF QUALITY OF IRRADIATED MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA* (WEIDEMANN) (DIPTERA:TEPHRITIDAE), AT THE RELEASE SITE IN MIAMI, FLORIDA DURING AN ERADICATION PROGRAM IN 1985

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Weidemann), was introduced accidentally into southern Florida in March 1985. Eradication efforts included four bait-sprays followed by releases of sterile flies four times per week for 12 weeks. The flies to be released were reared and sterilized in Hawaii and shipped as pupae by commercial air freight to Miami. Quality evaluations consisting of pupal weight, sex ratio, emergence rate, flight ability and mating propensity of the flies were made before release. In general, the quality of the flies received in Miami was found to be satisfactory throughout the shipment period.

RESUMEN

La mosca del mediterráneo, *Ceratitis capitata* (Weidemann), se introdujo accidentalmente en el sur de la Florida en Marzo de 1985. Esfuerzos para erradicarla incluyó cuatro rocios-cebo seguido por la liberación de moscas estériles cuatro veces por semana por 12 semanas. Las moscas liberadas se criaron y esterilizaron en Hawaii y transportadas a Miami como pupa por via aérea comercial de carga. Antes de liberar las moscas, se evaluaron sus cualidades por el peso de las pupas, la proporción de sexos, taza de salida de los adultos, la abilidad de vuelo, y la propensidad al acoplamiento de las moscas. En general, la calidad de las moscas recibidas en Miami fue satisfactoria durante el período de transports.

The Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (medfly), is an agricultural pest in Europe, Southern Africa, Central and South America, Australia and

Hawaii. Occasionally it has been found in the contiguous United States. In such cases, the pest usually has been eradicated by bait spray programs (Christenson 1958). The sterile insect technique (SIT) has been used for eradication of medflies in certain experimental situations and most recently in Mexico and Central America (Schwarz et al. 1985). It is now being used as part of eradication efforts for small introductions in the United States (Florida 1985, 1987, California 1987).

The SIT involves a coordinated program to rear, sterilize, and release male flies in sufficient numbers to "overflood" the wild population while retaining their ability to deliver their load of sterile sperm to fertile, wild females. A rearing and handling procedure resulting in the production of effective sterile flies is needed for the technique to successful (Calkins et al. 1982).

The medfly was accidentally introduced into Miami, Florida in March 1985. After four aerial sprays of malathion and Staley's sauce bait, (A. E. Staley Manufacturing Co., Atlanta, Ga), the sterile insect technique was used to ensure eradication of the pest. Approximately 5 million sterile flies, reared in and shipped from Hawaii each of 4 days per week, were released over an 208 km^2 area. A total of 200 million flies were released in the control area from May 5 to July 16, 1985.

Reported here are results of laboratory tests conducted to evaluate the quality of sterile medflies shipped from Hawaii to Miami, Florida during 1985 for release in the medfly eradication project in Dade County, Florida.

Materials and Methods

The Medflies used in this project were reared at the California State-USDA,-APHIS Medfly Laboratory in Honolulu, Hawaii (Tanaka et al. 1970). Two days prior to emergence, pupae were mixed thoroughly with neon red dye powder (Day-Glo Color Corp., Cleveland, OH) to mark emerging flies and packaged in 4-litre polyethylene bags creating a state of hypoxia. The flies then were irradiated with 12 Krad from a Co^{60} source (Ohinata et al. 1977). No adverse effect on mating competitiveness of flies treated with Day-glo dye has been found (Holbrook & Fujimoto 1970). Nine polyethylene bags containing pupae were then placed in cardboard boxes and six artificial ice packages (Blue Ice®) were included to keep the pupae cool during shipping. The boxes were shipped by commercial air carrier to Miami International Airport. When the boxes of pupae arrived at the laboratory after about 14 h, they were opened and a dial thermometer was inserted into each bag to measure the temperature of the pupae. A 50-ml sample of pupae was taken from each bag. These samples were combined and thoroughly mixed before being used for the following tests: sex ratio, eclosion rate, flight ability, and mating propensity, as called for by standard quality control protocols.

The pupae from each shipment were removed from the bags and placed in 35x60x30 cm cardboard boxes and moved into a 12 m long house trailer for emergence. The trailer was kept dark with the temperature maintained at $24 \pm 1°C$. Each box contained ca. 30,000 pupae from which flies began to emerge about 24 h later. Flies were provided with cakes of a mixture of sugar and yeast hydrolysate (3:1) or sugar cubes coated with yeast hydrolysate. Water was supplied by an agar block made of Gelcarin (G.P. 812) placed on the screened lid. Three days later, the boxes were transferred to a cooling trailer (4.5°C) where flies were immobilized. They were then removed from the boxes and placed into a machine designed especially for aerial release of medflies. The processes of removing flies from boxes and releasing them from the air each required 45-60 min. The temperature was maintained at 4.5°C during both operations.

A house trailer was modified to serve as the quality control laboratory. Rooms had individual heating, air conditioning and lighting systems to provide suitable conditions for holding and testing the insects.

To determine sex ratios, five sub-samples of 100 pupae were taken from the combined sample from each shipment and placed in petri dishes. The pupae were held in the quality control (QC) laboratory at 27 ± 1°C and 14L:10D. Four days later, the number of flies that emerged was counted and the sex ratio calculated.

Percent emergence and percent fliers were determined from each shipment by using a combined test. An individual flight test unit consisted of a petri dish (9 cm diam) and a tenite butyrate cylinder 10 cm high and 9 cm (outside diam.). The cylinder was painted black on the outside so that light could enter only at the top. Before each use, the inside of each cylinder was lightly coated with unscented talcum powder to prevent the flies from crawling out. This powder was removed from a 1 cm band around the inside bottom of the tube and the tube was placed into the petri dish. A sample of 100 pupae was placed in the petri dish, and the flight cylinder was put in place. A 1x10 cm paper strip folded accordion-like was placed against one side of the cylinder in the petri dish as a resting place for newly emerged flies. The test was replicated five times (100 pupae each) for each shipment. All five cylinders were placed inside a large screen cage (60x60x50 cm). Flies were aspirated out of the screen cage daily to prevent them from falling back into the tubes at death. After 60 h, the number of unemerged, partially emerged, and deformed and normal appearing flies that remained in the cylinder were counted. The percent emergence and percent fliers were calculated by the following formula:

$$\frac{\%\ \text{emergence} = 100 - (\text{unemerged pupae} + \text{partially emerged adults})}{\%\ \text{fliers} = \%\ \text{emergence} - \text{flies remaining in the tube}).}$$

The mating propensity test measured the ability of unmated sterile male flies, produced in a mass rearing laboratory, to copulate successfully with virgin females. This test was conducted weekly on shipments 8, 13, 19, 23, 28, 33, 38, 43, 48, 50, & 51 with a modification of methods described by Boller et al. (1977, 1981). About 3,000 to 4,000 pupae from the sample described above were placed in an emergence plexiglass cage (20x40x30 cm) together with sugar cubes and water. Males and females were separated and placed into holding cages within 4 h after emergence. The holding cage consisted of a 360 ml waxed paper cup with a single hole punched in the bottom through which a piece of cotton dental wick was passed. This paper cup was then placed over another 180 ml plastic cup filled with water. For aeration, a circular opening 10 cm in diam was cut in the lid of the cup and covered with a 14-mesh nylon screen. A small cup (30 ml) containing a mixture of sugar and yeast hydrolysate (3:1) was placed on the bottom of the holding cage to provide food for flies. Twenty-five male or 25 female flies were introduced into each holding cage. Each test consisted of five replicates. However, an additional cage for each sex were prepared to provide extra flies if needed, or if a male was inadvertently placed in a virgin female cage. The cages of males or females were inspected periodically and any cage that was discovered to contain mating pairs was discarded.

All of the cages with flies were held at 27 ± 1°C and 14L:10D. The percent relative humidity varied from 60 to 80%. The flies were held for 7 days; day 1 was counted as the day of emergence. The flies were placed in the dark room the night prior to the test. In the morning, 25 females and 25 males were introduced into a mating cage (20x30x40 cm) under a dim light in the dark room during a 5-min period. After five cages were thus filled, they were transferred back to the holding room. A timer was started, mating pairs were removed in 10 dram snap-cap vials at 10-min intervals.

The number of mating pairs that formed within 10 min, and subsequently for each 10-min interval, was recorded for 60 min. The test was then terminated and the number of remaining males and females (not including the dead ones) were counted. The mating index (MI) for each replication was calculated by summing the number of mating pairs

formed within a 10-min period and multiplying by a weight factor (Boller et al. 1981). The number of pairs in th test may not necessarily be 25, because some flies may have died during the test. The MI for five replicates was then averaged to get the mean mating index (MMI) for each shipment.

There were several experimental variables affecting mating propensity that were tested at the release site. A test was designed to study the influence of age of the flies on mating propensity. The test was conducted in the same manner as described above, but flies were held in containers for 14 days before being tested.

The effects of low temperatures on mating propensity was tested. Flies used for this test emerged in the QC laboratory and were placed in holding containers as described. About 60 h after emergence and at the same time flies from the same shipment were being released in the field, holding containers with flies were placed in the refrigerator trailer for 1 h at 4°C. The flies were then transferred back to the QC laboratory and the mating propensity test was performed. This was to verify whether flies that were treated similarly to those that were being released, would retain a high mating index.

An experiment was designed to determine whether the current procedure of aerial release affected the mating propensity. The aerial release took place in the morning between 0600-0900 hrs and lasted ca. 1 h. About 3,000 flies remaining in the release unit at the end of the release flight were collected and placed in a 20x30x40 cm plexiglass cage. As soon as flies recovered from the low temperature, they were sexed and placed in holding cages. The cages were placed in the QC laboratory and the mating test was conducted at the age of 7 days, 4 days after flies were collected from the aerial release.

A test was designed to study the influence of light on mating propensity. Flies used for this test from shipments 33, 38 and 43 emerged in the QC laboratory and were placed in holding containers as described above. The mating propensity test was conducted on the proper day. The treatment exposed the flies for 4 h to normal daylight, before being tested, whereas the other treatment was tested immediately following removal from the dark room, as is the standard procedure.

Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC) was used to analyze data of these tests and to compare them with data from the mating propensity tests conducted under specified conditions from the same shipment.

A charting method for means was used on data from shipments involving emergence rates, flight ability and mating propensity (Boller et al. 1981). The baseline in each case was developed from the specific data from all of the shipments to show correlations among tests that deviated from the average evaluation score. The control limits were determined by three standard deviations of the means.

Results and Discussion

A quality control manual developed by USDA in 1986, revised in 1988, gives the specifications and tolerances for pupal weight, sex ratio, emergence, flight ability and mating propensity at the release site (Brazzel et al. 1986). These specifications and tolerances are: *Pupal weight*—7 mg, if below 6.5 mg for more than 5-10 lots, a problem exists. *Sex ratio*—variation of 45-55% of either sex is considered normal. A trend of 40:60 or greater indicates potential problems and possible rejection by the client. *Percent emergence*—eclosion in excess of 80% is considered satisfactory, below 75% is not acceptable. *Percent fliers*—if less than 60% of the adults escape from the flight tubes, it is not considered satisfactory. *Mating index*—a mean mating index of 50 or greater is considered satisfactory.

The average weight of pupae, 8 days or older, from shipment numbers 15 to 51 was 7.9 ± 0.09 mg measured in Hawaii prior to shipment (Kobayashi, personal communica-

tion). The temperatures at the time the shipments arrived at the Miami laboratory varied from 20-22°C.

The sex ratio of flies shipped to Miami in 1985 was approximately 50:50 (male:female), and varied less than 5% from the mean.

The average percentages of emergence and percent fliers were 87.32 ± 0.78 (Mean ± SE) and 71.19 ± 1.37, respectively. Two of the first seven shipments had emergence rates below 85%. Five of the first seven shipments had flight ability rates below 75% and a total of seven shipments consisted of less than good fliers. The reason for the low percentages could have been that the personnel who conducted the quality control tests were not familiar with the new techniques, or the rapid increase in production in Hawaii may have resulted in procedures that were detrimental to the flies. In one case, talcum powder was not wiped from the bottom of the tube, and therefore, flies became coated with powder and could not fly from the tube. After the technique was corrected and the room temperature was adjusted, the percent emergence and percent fliers increased.

The MMI of flies emerging in the QC laboratory in Miami from all lots tested were higher than 63 except for shipment 48, which had an index of 50. The average MMI for 11 shipments tested in Miami was 67.8. Boller et al. (1981) studied the mating propensity of seven laboratory-reared strains of medfly from Europe and Africa, and reported that the MMI of the unirradiated flies from these strains varied from 17.1 to 62.4. The MMI of *C. capitata* has been improved by selection, and it could reach a plateau of 60-70 after four generations (Boller & Calkins 1984). They considered that any strain with an MMI higher than 60 would be classified as fast mating flies.

The influence of age on mating propensity apparently was not significant (MMI of 7- and 14-day-old flies were 63.10 ± 6.45 and 62.69 ± 9.39, respectively). The mean mating index of the laboratory reared *C. capitata* increases with age and reaches a plateau about 7-8 days after emergence (Boller et al. 1981).

The effect of low temperature (4.5°C) on mating propensity also was not significant for exposure of 1 hr. (MMI of control flies and treated flies were 70.66 ± 3.06 and 68.68 ± 2.14, respectively). Mating propensity of flies emerged in the QC laboratory versus flies taken from the airplane at the end of the aerial release were not significantly different (MMI 67.8 ± 8.6 for control flies and 67.3 ± 4.2 for the flies taken from the airplane).

The exposure of the test insects to light for 4 h before initiation of the mating tests reduced mating propensity by 22 to 32%. Those held in darkness until just before the test had an MMI of 75.85, while those exposed to the extended light period had an MMI of only 54.57. The quality of flies received in Miami was considered satisfactory for pupal weight, sex ratio, emergence rate, flight ability and mating propensity.

The specifications of fly performance at the factory door are more stringent, because at that point, the pupae had not undergone the stress of many hours in hypoxia or of shipping. Unfortunately, quality control data taken prior to shipment were not available to us.

The combination of bait-spray followed by release of sterile flies appears to be a biorational approach for eradication of small introductions of medflies. The release of sterile flies, although more expensive than numerous bait spray applications initially, effectively reduces the number of bait sprays required, eases the public reaction to pesticides being sprayed over populated areas and reduces the number of complaints for damage to automobile paint.

End Note

Mention of a commercial or proprietary product does not constitute an endorsement of that product by the USDA.

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