# PROCEEDINGS

# OF THE

# 26th ANNUAL MEETING

July 29 to August 4, 1990

Mayaguez, Puerto Rico

Published by:

Caribbean Food Crops Society

with the cooperation of the USDA-ARS-TARS

Mayaguez, Puerto Rico

# THE EFFECTS OF X-RAY RADIATION ON LARVAE OF THE CARIBBEAN FRUIT FLY, Anastrepha suspensa (Loew)

D.F. Davis, D.E. Weidhaas, and J.D. Cox

AgriCon National, P.O. Box 1702, Gainesville, FL 32602
Weidhaas Consultants, 1330 NW 25th Terr., Gainesville, FL 32605
Citrex Technologies, 901 NW 8th Ave., Gainesville, FL 32601

## ABSTRACT

EPA cancellation of the use of the fumigant EDB gave rise to research of alternative methods for disinfestation of agricultural commodities susceptible to infestation by various species of fruit flies. One potential alternative is X-ray radiation. Three series of tests were conducted with 'Marsh' white grapefruit artificially infested with larvae of the Caribbean fruit fly, Anastrepha suspensa (Loew), and exposed to 5, 10, 20, 30 and 35 krads of X-ray radiation. Percent reduction of insects ranged from 82.8-100, with Probit 9 mortality estimated above 35 krad. No adult flies emerged from larvae exposed to any level of X-ray tested. The rate of deterioration increased in fruit exposed to 30 and 35 krad.

## INTRODUCTION

Cancellation of the use of the fumigant ethylene dibromide (EDB) by the U.S. Environmental Protection Agency (EPA) gave increased emphasis to research on alternative methods for the disinfestation of commodities susceptible to infestation by various species of fruit flies (Family: Tephritidae). Such research had a high priority in Florida to protect the Japanese market for Florida grapefruit which by 1979 had attained a record 168,000 metric tons annually (Kitagawa and Kawanda, 1979). By 1989, the Japanese market was the major outlet for exports of 23.5 million cartons of Florida grapefruit or about twice the 1979 exports to Japan (Thompson, 1989). Florida grapefruit is subject to quarantine restrictions due to the presence in Florida of the Caribbean fruit fly, Anastrepha suspensa (Loew).

Alternatives considered as candidates for replacement of EDB included the following:

1. Fumigation with methyl bromide.
2. Conditioning the fruit at 60°F and shipping at low temperature (from 33-36°F).
3. Irradiation, using gamma radiation from cobalt or cesium sources.
4. Combinations of alternatives, such as low temperature plus fumigation with methyl bromide.
5. Hot water dips.

6. Hot air.
7. Establishment of "Fly Free" areas from which all export fruit would be selected at certain times of the year.
8. Eradication of the Caribbean fruit fly from Florida using a combination of insecticide and the sterile insect technique (SIT).

None of these alternatives appear to be the "perfect" treatment to replace EDB. Each one has some kind of drawback such as expected short life of alternative fumigants from future cancellation by EPA, extensive management requirements, time-consuming handling, high cost and potential consumer resistance.

Radiation has been of interest for disinfestation of fruit flies from fruits for a long time. Balock et al. (1956) proposed the use of gamma irradiation for fruit shipped from Hawaii to mainland U.S. Since then, numerous investigators have studied the use of ionizing radiation. Included were Balock et al. (1963), Benschoter and Telich (1964), Shipp and Osborne (1968), Cavalloro and Delrio (1971), Seo et al. (1973) and many others. These studies were conducted using various species of fruit flies. Considerable work has been done in Florida on the use of radiation against the Caribbean fruit fly and this work was reviewed and summarized by Spalding and Davis (1985) for the International Conference on "Radiation Disinfestation of Food and Agricultural Products" held in Honolulu, HI, November 14-18, 1983 (See Proceedings, pp. 160-165, published by the University of Hawaii, 1985).

Radiation appears promising as a quarantine treatment for fruits and other commodities susceptible to infestation by various species of fruit flies. Increased interest in radiation occurred after approval by the U.S. Food and Drug Administrarion (FDA) about 5 years ago for up to 100 krad of radiation for application to human foods. There appear to be "windows" of applicability or radiation between levels that are lethal to infesting fruit flies and levels that affect the quality of the fruit. On Florida grapefruit, a window appeared to be in the range from 30-35 krad for "Probit 9" security mortality of larvae inside the fruit. Lower dosages prevent the emergence of adult flies from the immature stages. From the data reviewed by Spalding and Davis (1985), no Caribbean fruit fly pupae were recovered from infested grapefruits irradiated at 60 or 90 krad. Of 9,707 insects irradiated at 30 krad, only 4 pupae were recovered, giving a weighted mean mortality of 99.87 percent. One adult female emerged but died before any ovipositioning. Of 13,226 insects irradiated at 15 krad, 149 pupae were recovered for a weighted mean mortality of 98.22 percent. One male adult that emerged died within one day. These data also showed that earlier stages of the Caribbean fruit fly were more sensitive to gamma radiation than were later stages. A dosage of 10 krad did not prevent pupation of 20,000 seven-day-old, laboratory reared larvae in agar medium, but did prevent adult emergence. Other

research cited showed that irradiation at 8 krad sterilized both male and female adult flies when irradiated as ten- and twelve-day-old pupae or one-day-old adults. It seems clear, therefore, that irradiation at doses below 15 krad assures that only sterile adults could develop from immature stages of the Caribbean fruit fly in irradiated fruit, if any adults emerged at all.

Only a few published reports include data from the use of radiation by X-rays. It appears X-rays have not been investigated extensively, primarily because available X-ray machines are not easily adapted for applications to agricultural commodities resulting in slow throughput. Obtaining data on large numbers of insects with X-rays is quite time consuming. However, X-rays should be evaluated to a greater extent because this source of ionizing radiation could represent one of the most viable alternatives to EDB fumigation. X-rays compare favorably with other sources of radiation in cost, safety, shielding, portability, availability and consummer resistance. In addition, if a suitable X-ray machine were available, applications to several commodities susceptible to infestation by fruit flies and other insect pests appear to hold considerable promise. X-ray radiation also could be used in producing sterile insects for use in the application of the sterile insect technique.

MATERIALS AND METHODS

Determining the effects of X-rays on the larval stage of the Caribbean fruit fly internally infesting 'Marsh' white grapefruit, Citrus paradisi Macf., was performed in four discrete parts: (1) exposing the grapefruits to large numbers of caged adult flies; (2) exposing the grapefruits to X-rays; (3) collecting and counting all larvae and pupae emerging from the grapefruits; and (4) determining the number of adult flies emerging from the collected larvae and pupae. Included in all tests were one pre-treatment untreated control and one post-treatment untreated control for comparison.

All four parts of the procedures except exposure of fruit to X-rays were carried out in a room measuring 43 feet long by 16 feet wide and 7 feet 7 inches high. Since the Caribbean fruit fly in Florida is under a state quarantine administered by the Florida Department of Agriculture and Consumer Services (FDACS), the room was completely sealed with polyethylene film on all inside walls. Sealed double door entry vestibules were on each end of the room. The room was examined and approved for use by a representative of FDACS before Caribbean fruit flies were introduced. Essentially, this room comprised a "Quarantine Laboratory". Maximum and minimum temperatures and relative humidity were recorded daily. Flies were maintained in sealed cages at all times throughout the three series of tests.

Three series of tests were conducted, each containing the experimental variables, as follows:

Series One

| Variable | Treatment |
|---|---|
| C-1 | Pre-treatment Untreated Control |
| K-1 | Exposed to 10 krad |
| K-2 | Exposed to 20 krad |
| K-3 | Exposed to 30 krad |
| C-2 | Post-treatment Untreated Control |

Series Two

| Variable | Treatment |
|---|---|
| C-1 | Pre-treatment Untreated Control |
| K-5 | Exposed to 5 krad |
| K-1 | Exposed to 10 krad |
| K-2 | Exposed tp 20 krad |
| C-2 | Post-treatment Untreated Control |

Series Three

| Variable | Treatment |
|---|---|
| C-1 | Pre-treatment Untreated Control |
| K-5 | Exposed to 35 krad |
| C-2 | Post-treatment Untreated Control |

The time schedule for the three series of tests was pre-determined by the life cycle of the Caribbean fruit fly. Adults become sexually mature, mate and females oviposit about 8 days after emergence. The egg stage is 3 days, the larval stage is 7 days and the pupal stage is 14 days. Therefore, about 32 days are required from adult to adult. These times may vary slightly depending on temperature, humidity and the quality of the adult food source.

The general procedures for infesting the fruit, exposing the infested fruit to X-rays, collecting the yield of immature insects from the fruit and collecting the emerging adults is represented in the following schedule of tasks:

| DAY(S) | TASK |
|---|---|
| 0 | 1 - Establish adult fly colony in Fruit Infestation Cage (FIC) |
| 1 | 2 - Place fruit in FIC |
| 7-10 | 3 - Remove fruit from FIC |
| Same as Task 3 | 4-5- X-ray fruit; Place fruit in Fruit Holding Cage (FHC) |
| 14-21 | 6 - Daily, collect and count immatures from sand at bottom of FHC |
| Same as Task 6 | 7 - Transfer pupae to Adult Emergence Cage (AEC) |
| 21-28 | 8 - Count number of adults emerging from each experimental variable |

Part 1. Exposing Grapefruits to Fruit Flies

A specially designed Fruit Infestation Cage (FIC) was used to expose grapefruits to adult Caribbean fruit flies. The cage measured 10 feet long, 4 feet wide and 4 feet high. The long sides were covered with aluminum window screen and 32-mesh plastic netting. The top and bottom were 3/4-inch plywood. The two ends were 3/4-inch plywood with doors and sleeved arm ports installed in each. Inside the cage, a 30-degree incline made of 1/4-inch wire cloth extended from one end to the other end of the cage. Grapefruits were entered through an arm port at the high end of the incline, rolled down the incline and positioned at some point at the lower end of the incline. Suspended on wire hangers from the top of the cage were small platforms containing protein adult diet, sugar cubes and water cups with wicks emerging through plastic lids. A one-tube fluorescent light fixture was suspended from the top of the cage and was controlled by a timer set for a 12-12 light-dark cycle. A large population of adult Caribbean fruit flies was maintained continuously in the FIC by placing from 7,000 to 15,000 pupae in cups of moist vermiculite inside the cage each week. The pupae were obtained from a laboratory-reared colony maintained by FDACS in Gainesville, Florida.

The experimental plan was to use 40 grapefruits for each treatment variable. The fruits were placed in the FIC on a time schedule calculated to provide fruit infested with eggs and larvae of the fly at the time the fruits were exposed to X-rays.

Part 2. Exposing Grapefruits to X-ray Radiation

In Series One and Two, grapefruits were transported in thermal chests from the Quarantine Laboratory to an X-ray facility at the University of Florida for treatment. The fruits were irradiated one at a time. Individual fruits to be irradiated were placed in a specially made tumbler. The tumbler continuously rotated the fruits on two axis during irradiation to ensure uniform dose deposition within the fruits. The variable radiation field intensity was due primarily to the $1/R^2$ attenuation of the point (X-ray) source. The X-ray generator used was a GE Maxitron Ortho Voltage Machine. During all irradiations, the X-ray spectra was 210 KVp, filtered with 2 mm of aluminum. A tube current of 10 milliamps and a source-to-fruit distance of 6-7 inches were used, producing a dose rate of about 1,000 R per minute. Dosimetry was performed using Fricke and thermoluminescent dosimeters (TLDs) which were placed in sealed ampules at various distances within the fruits. After exposure to X-rays, each fruit was returned to the thermal chest which contained a small quantity of sand. Upon return to the Quarantine Laboratory, the sand was sifted to collect any larvae that emerged from the fruit during transport. Immediately upon return to the Quarantine Laboratory, the X-rayed fruits were placed in specially designed Fruit Holding Cages (FHCs) in which

all larvae emerging from the fruit were collected in sand beneath the cages were also 210 KVp and 10 milliamps. The filtration used was 2mm of aluminum. The dose rate for these experiments was also about 1,000 R per minute.

Part 3. Collecting Fruit Flies From the Grapefruits

Following exposure to X-rays, the grapefruits were placed in the FHCs. Each experimental variable was placed in a separate cage. These cages measured 22 inches wide, 24 inches deep and 42 inches high. Three sides were covered with 32-mesh plastic netting. The front was a hinged door also screened with 32-mesh netting. The top was 3/4-inch plywood. The bottom of the cage consisted of a 14-inch-long sheet metal plenum that ended in a 4- by 6-inch rectangular opening. A plastic tube connected the plenum opening to an open screw-top glued to the top of a plastic food refrigerator dish containing clean white sand. The screw-top connection permitted easy disconnection daily to sift the sand and collect fly larvae and pupae. The grapefruits were divided equally into three 18-inch square plastic trays with open mesh bottoms. The trays rested on horizontal slides inside the cage. Beneath each tray was a piece of soft Celotex board that rested on a slide angled downward from the back of the cage to the front. The downward angle of the Celotex board utilized the negative geotropism of the larvae so that larvae leaving the fruit would crawl down the Celotex board and fall into the metal plenum at the bottom of the cage and then into the sand in the dish below the cage. Each day, the sand was sifted and the larvae and pupae collected, counted and transferred to moist vermiculite. Cups containing the immature insects in the vermiculite were placed in Adult Emergence Cages (AECs) in which emerging adults were collected and counted.

Part 4. Collecting Emerging Adults

After all larvae and pupae were collected from the sand from the FHCs and counted, they were placed in plastic cups containing moist vermiculite. The cups were placed in 18-inch cube AECs. The AECs were covered with aluminum window screen and 32-mech plastic netting on the top and two sides. The back and bottom of the cage were 1/2-inch plywood. Each day, the cages were examined for adult Caribbean fruit flies. When adults were observed, they were collected with a small handheld vacuum with a plastic collecting tube. The adults were immobilized by placement in a freezer for about 10 minutes and then counted.

RESULTS AND DISCUSSION

The results obtained for each experimental variable in Series One, Two and Three tests are summarized in Table 1. The critical measurement of dose-response effects are the percent reduction in insects leaving the fruit exposed to X-rays versus those leaving the fruit not exposed to X-rays (Untreated

Controls). Percent reduction due to X-ray treatment of infested grapefruits was calculated from the mean immature insect (larvae and pupae) yield per fruit at each treatment level compared to the mean number of immature insects per fruit recovered from the most appropriate untreated control for comparison.

There were some differences in the results obtained in the Series One and Series Two tests. In Series One, there was almost no immature insect yield from fruit exposed to any level of X-ray tested, with only 2 dead pupae from the 30 krad treatment. All X-ray treatments tested exceded 99.68 percent reduction, which is 0.2868 percent below the level considered equivalent to "Probit 9" quarantine security mortality. In Series Two, the 20 krad treatment attained 98.65 percent reduction which compared favorably with the 99.96 percent reduction for the 30 krad treatment in Series One.

One factor in the differences in the results obtained in Series One and Two tests was that the fruit used in Series One tests was "early season" fruit. Research has established clearly that grapefruit harvested in Florida in November and December is more resistant to infestation by the Caribbean fruit fly than is fruit harvested later in the season. This factor seems evident in the lowest count of "insects per fruit" in the C-1 untreated controls in Series One of all untreated controls in all three Series of tests. A significantly higher level of infestation was obtained in the fruit used in the post-treatment control (C-2) in Series One. This fruit had matured in the laboratory from the time the C-1 fruit was exposed in the FIC and obviously was more susceptible to infestation.

The 35 krad treatment tested in Series Three gave a slightly lower percent reduction in insects per fruit than did the 30 krad treatment in Series One tests. However, the fruit used in Series Three tests was "late season" fruit and was from a different harvest season. As noted, the highest infestation levels obtained in all tests was for the fruit used in the Series Three tests. In consideration of the fruit condition, the 99.54 percent reduction in insects per fruit compares well with the results of Series One tests.

It is important that no adult fruit flies emerged from the immature insects recovered from any of the grapefruits exposed to any X-ray treatment included in Series One, Two and Three tests.

A better view of the dose-effect responses can be seen in the data as presented in Table 2, where all of the experimental data from six untreated controls, two 10 krad and two 20 krad treatments, were accumulated and combined. In this presentation, percent reduction in insects per fruit was highly correlated with X-ray dose and ranged from 82.79 for 5 krad to 99.95 for 35 krad. The 30 and 35 krad treatments produced an insect reduction

Table 1. Total number of Caribbean fruit flies recovered from 'Marsh' white grapefruit after exposure to X-ray.

| Experimental Variable | Insects Recovered No. Larvae | Pupae | Insects per Fruit | Insect Reduction % | Adults Emerged No. | % |
|---|---|---|---|---|---|---|
| **Series 1 Tests** | | | | | | |
| C-1 | 412 | 133 | 13.6 | - | 335 | 61.5 |
| 10 krad | 0 | 0 | 0 | 100 | 0 | 0 |
| 20 krad | 0 | 0 | 0 | 100 | 0 | 0 |
| 30 krad | 0 | 2 | 0.05 | 99.96 | 0 | 0 |
| C-2 | 2776 | 893 | 122.3 | | 2055 | 56.0 |
| **Series 2 Tests** | | | | | | |
| C-1 | 966 | 181 | 29.4 | - | 816 | 71.1 |
| 5 krad | 682 | 200 | 22.05 | 50.32 | 0 | 0 |
| 10 krad | 37 | 17 | 1.35 | 96.96 | 0 | 0 |
| 20 krad | 20 | 4 | 0.60 | 98.65 | 0 | 0 |
| C-2 | 1988 | 371 | 58.98 | - | 1062 | 45.0 |
| **Series 3 Tests** | | | | | | |
| C-1 | 1294 | 256 | 77.5 | - | 300 | 19.4 |
| 35 krad | 0 | 1 | 0.5 | 99.54 | 0 | 0 |
| C-2 | 2141 | 665 | 140.3 | - | 567 | 20.2 |

All variables contained 40 fruits except (S-1, C-2) which had 30 fruits and (S-2, C-1) which had 39 fruits.
(S-1,30 krad) compared to C-2; all treatments in S-2 compared to combined controls (C-1 + C-2); 35 krad in S-3 compared to combined S-3 controls (C-1 + C-2).

Table 2. Accumulative number of Caribbean fruit flies recovered from 'Marsh' white grapefruit exposed to X-ray in Series 1, 2 and 3 Tests.

| Experimental Variable | Insects Recovered: No. Larvae | Pupae | Insects per Fruit | Insect Reduction: % 1/ | Adults Emerged: No. | % |
|---|---|---|---|---|---|---|
| Control | 8623 | 2180 | 63.92 | - | 5135 | 47.53 |
| 5 krad | 389 | 51 | 11.0 | 82.79 | 0 | 0 |
| 10 krad | 37 | 17 | 0.675 | 98.94 | 0 | 0 |
| 20 krad | 15 | 3 | 0.225 | 99.65 | 0 | 0 |
| 30 krad | 0 | 2 | 0.050 | 99.92 | 0 | 0 |
| 35 krad | 0 | 1 | 0.031 | 99.95 | 0 | 0 |

Grapefruits per Variable: Control = 169; 5 krad = 40; 10 krad = 80; 20 krad = 80; 30 krad = 40; and 35 krad = 32.

1/ Calculated as Control minus Treatment divided by Control, C-T/C.

slightly below "Probit 9" security mortality in the Caribbean fruit fly immatures internally infesting the grapefruits.

The data in Table 2 also emphasize the result that no adult Caribbean fruit flies emerged from the larvae and pupae that were exposed to any of the X-ray treatments tested, even the 5 krad treatment. This finding is in agreement with the literature for exposure of Caribbean fruit fly immatures to radiation from gamma sources; namely, adult emergence does not occur when immatures are exposed to radiation at any level at or about 10 krad.

Figure 1 presents the data in Table 2 as Probit transformations plotted against log of dose of X-rays. This presentation clarifies that the highest dose of X-rays included in the tests described herein did not give a percent reduction of insects per fruit that was equivalent to "Probit 9" mortality. However, the 35 krad dose produced a reduction that was only slightly below "Probit 9".

Florida grapefruit exposed to irradiation from a gamma source has been tested for flavor, aroma and composition effects (Moshonas and Shaw, 1982). Although some slight effects were shown, there have been no reports of serious changes in composition or formation of compounds that would be toxic relative to human food, even at the highest doses of radiation that would be practical for application to grapefruit or other fruits for control of fruit flies.

The results of fruit quality tests are being reported separately. Although exposure to 5 krad of X-rays seemed to reduce the rate of deterioration of grapefruit, exposure to 30 and 35 krad increased the rate of deterioration compared with unexposed fruit. Therefore, it is doubtful if X-ray radiation or radiation from any other source could be used if "Probit 9" mortality must be met as required by governmental quarantine regulatory agencies. But from a purely quarantine viewpoint, radiation appears promising considering exposure of immatures to 5 to 10 krad prevents emergence of adult insects. This finding would be important under the concept of quarantine security as discussed by Ouye and Gilmore (1985) which seems to be a more reasonable approach than "Probit 9" mortality. Therefore, the use of X-rays as a radiation source appears very promising considering the comparative costs, restrictions, shielding requirements, availability and consumer resistance in comparison with other radiation sources.

While low-energy X-ray irradiation shows promise as an alternative treatment method to fumigation, current low-energy X-ray generator technology is inappropriate for processing agricultural products. A novel X-ray generator designed specifically for the low-energy X-ray irradiation of agricultural products is currently under development by Citrex Technologies,

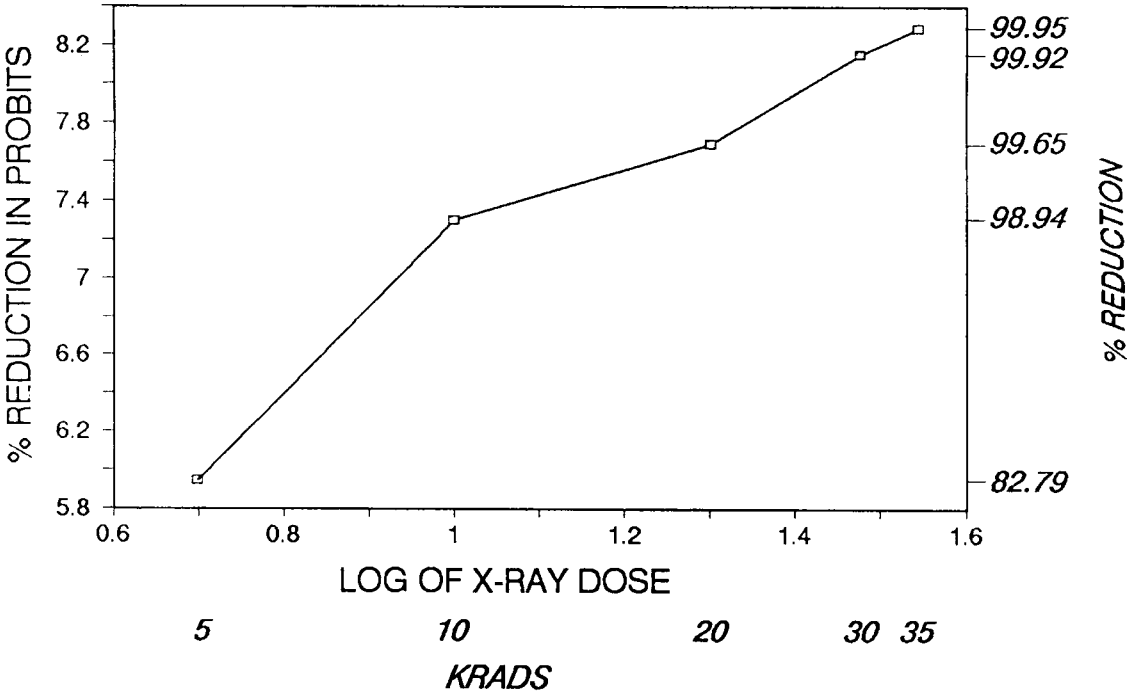


Figure 1. Percent reduction as probit transformation vs log of dose of x-rays for exposure of Caribbean fruit flies.

Inc., Gainesville, FL. Basically, it consists of a large anode X-ray source with modular tubes placed next to each other to produce a large area, high-dose and low-energy X-ray field. Calculations predict the generator will be capable of irradiating anywhere from 1 to 1,000 pounds of commodity per hour with absorbed doses in the 10 to 100 krad range.

ACKNOWLEDGEMENTS

The authors express their appreciation to Mr. Nelson Smith, Biological Technician, for his dedicated attention to duty in conducting the laboratory phases of the tests reported herein. Also, the authors are indebted to the Florida Department of Agriculture and Consumer Services, Gainesville, FL, for granting permission to conduct studies with the Caribbean fruit fly which is under quarantine restrictions, and for providing large numbers of the flies from their laboratory-reared colony. Dr. Carol Calkins, USDA-ARS, Gainesville, FL, provided adult fly food and technical advice regarding handling of the insects. This research was supported, in part, by a Small Business Grant from the U.S. Department of Agriculture.

REFERENCES

Balock, J.W., Christenson, L.D., and Burr, G.O. 1956. Effects of gamma rays from Cobalt-60 on immature stages of the Oriental fruit fly, Dacus dorsalis (Hendell), and possible application to commodity treatment problems. Proc. 31st Annual Meeting Hawaiian Acad. Sci., p. 18.

Balock, J.W., Burditt, A.K., and Christenson, L.D. 1963. Effects of gamma irradiation on various stages of 3 fruit fly species. J. Econ. Entomol. 56:42-46.

Benschoter, C.A., and Telich, C.J. 1964. Effects of gamma rays on immature stages of the Mexican fruit fly. J. Econ. Entomol. 57:690-691.

Cavalloro, R., and Delrio, G. 1971. Biological effects of gamma irradiation on the life stages of two fruit fly species. Proc. 3rd Nat. Symp. on Radioecology, Oak Ridge, Tenn. 2:1179-1190.

Kitagawa, H., and Kawanda, K. 1979. Marketing of Florida grapefruit in Japan. Proc. Fla. State Hort. Soc. 92:241-245.

Moshonas, M.G., and Shaw, P.E. 1982. Irradiation and fumigation effects on flavor, aroma and composition of grapefruit products. J. Food Sci. 47(3):958.

Ouye, M.T., and Gilmore, J.E. 1985. The philosophy of quarantine treatment as related to low-dose radiation. Proc. Int. Conf. on Radiation Disinfestation of Food and Agricultural Products, Honolulu, HI, pp. 67-69.

Seo, S.T., Kobayashi, R.M., Chambers, D.L., Dollar, A.M., and Hanaoka, M. 1973. Hawaiian fruit flies in papaya, bell peppers and egg plant: quarantine requirements. J. Econ. Entomol. 66:937-939.

Shipp, E., and Osborne, A.W. 1968. Irradiation of Queensland fruit fly pupae to meet quarantine requirements. J. Econ. Entomol. 61:1721-1726.

Spalding, D.H., and Davis, D.F. 1985. Potential for gamma radiation as a quarantine treatment for Caribbean fruit fly in citrus. Proc. Int. Conf. on Radiation Disinfestation of Food and Agricultural Products, Honolulu, HI, pp. 160-165.

Thompson, T. 1989. Florida citrus meets foreign demand. The Grower 22(9):20-22.