Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities

PROCEEDINGS OF THE FINAL RESEARCH CO-ORDINATION MEETING
KUALA LUMPUR, MALAYSIA, 27–31 AUGUST 1990
ORGANIZED BY THE JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES
IN FOOD AND AGRICULTURE

IAEA

USE OF IRRADIATION AS A QUARANTINE TREATMENT OF FOOD AND AGRICULTURAL COMMODITIES

PANEL PROCEEDINGS SERIES

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AND HELD IN KUALA LUMPUR, MALAYSIA,
FROM 27 TO 31 AUGUST 1990

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1992

VIC Library Cataloguing in Publication Data

Research Co-ordination Meeting on Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities (1990 : Kuala Lumpur)

Use of irradiation as a quarantine treatment of food and agricultural commodities : proceedings of the Final Research Co-ordination Meeting on Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Kuala Lumpur, Malaysia, from 27 to 31 August 1990. — Vienna : International Atomic Energy Agency, 1992.

p. ; 24 cm. — (Panel proceedings series, ISSN 0074–1876)

STI/PUB/873

ISBN 92–0–102992–6

Includes bibliographical references.

1. Radiation preservation of food. 2. Plant quarantine. I. International Atomic Energy Agency. II. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. III. Title. IV. Series.

VICL 92-00028

Printed by the IAEA in Austria
August 1992

FOREWORD

Trade in fresh fruits and vegetables from tropical and subtropical countries is hampered seriously by insect infestation, especially by fruit flies of the Tephriditae family. Major importing countries such as Australia, Japan and the United States of America require that such commodities be certified free of quarantine pests or that they be subjected to approved quarantine treatments prior to importation. Up to 1984, fruits and vegetables from fruit fly infested areas were fumigated by ethylene dibromide (EDB) to meet the quarantine regulations of national and international trade. However, EDB was banned by the United States Environmental Protection Agency in September 1984. Many other countries have either followed the ban or restricted the use of this fumigant in food. The deregistration of EDB has deprived the trade in fruits and vegetables of an effective, broad spectrum fumigant to overcome quarantine barriers. Other treatments such as methyl bromide and phosphine have not proved to be as effective as EDB, while physical processes such as heat or cold treatment, which are capable of insect disinfestation in these commodities, also have their limitations.

In 1985, the Co-ordinated Research Programme (CRP) on the Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities was initiated by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The purposes of the CRP were to determine the radiation doses required to provide quarantine security from insects and other pests infesting food and agricultural commodities in trade, to assess the tolerance of host commodities to the radiation dose(s) required to provide quarantine security and to distribute information on the possible use of irradiation as a quarantine treatment of food and agricultural commodities to interested parties.

The results of the work carried out under this CRP over the past 5 years by laboratories in several countries have demonstrated that irradiation is a viable alternative to EDB fumigation of food. Neither the radiation dose required for fruit fly disinfestation (0.15 kGy) nor the dose required to provide quarantine security against all stages of other anthropod pests (0.3 kGy) causes significant changes in the physicochemical and organoleptic properties of most fruits and vegetables.

These Proceedings include the final reports of work carried out by the scientists who co-operated in this CRP, which were presented at the Final FAO/IAEA Research Co-ordination Meeting held in Kuala Lumpur, Malaysia, from 27 to 31 August 1990. Some review papers, presented at the Second Task Force Meeting on Irradiation as a Quarantine Treatment of Fresh Fruits and Vegetables held in Bethesda, Maryland, USA, in January 1991, are also included.

Initially, the Scientific Secretary of this CRP was C.J. Rigney. On his return to Australia in 1989, co-ordination of the programme was undertaken by P. Loaharanu, Head, Food Preservation Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.

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REVIEW

Co-ordinated Research Programme on Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities

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The potential role of irradiation as a quarantine treatment of fruits and vegetables infested by various insect pests, especially fruit fly of the Tephriditae family, has been recognized by the Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency since 1970 when they convened a Panel of Experts to address this topic in Honolulu, Hawaii, United States of America. Research on this topic was given further impetus when the United States Environmental Protection Agency prohibited the use of ethylene dibromide (EDB) as a food fumigant in 1984. The ban of EDB jeopardized trade in fruits and vegetables from countries endemic to fruit fly, since no other quarantine treatments were as effective as EDB nor were they available at that time.

The Co-ordinated Research Programme (CRP) on Use of Irradiation as a Quarantine Treatment of Food and Agricultural Commodities was implemented by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in early 1986. The objectives were:

(1) To determine the radiation doses required to provide quarantine security from insects and other pests infesting food and agricultural commodities in trade
(2) To assess the tolerance of host commodities to the radiation dose(s) required to provide quarantine security
(3) To distribute information on the possible use of irradiation as a quarantine treatment of food and agricultural commodities to interested parties.

Research Co-ordination Meetings (RCMs) were convened at Chiang Mai, Thailand, in 1986 and at Orlando, Florida, USA, in 1988 to review the progress made under the scope of this CRP. The conclusions of the work carried out from 1986 to 1990 were made at the Final RCM held at Kuala Lumpur, Malaysia, from 27 to 31 August 1990.

1. EFFECTIVENESS OF IRRADIATION AS A QUARANTINE TREATMENT AGAINST FRUIT FLY SPECIES

Participants attending the Final RCM presented reports of the research carried out with the support of or in association with the IAEA under Research Contracts and Research Agreements conducted over 5 years. The results of studies on the effect of irradiation on some 10 species of fruit fly were covered. In general, the data served to confirm laboratory studies that had previously been presented. Confirmatory tests involving populations in excess of 100 000 eggs and larvae were conducted for most of the species. They showed that a dose of 150 Gy, or less, provided probit 9 quarantine security based on the prevention of emergence of normal adults. This dose (150 Gy), adopted at the Task Force Meeting on Irradiation as a Quarantine Treatment convened by the International Consultative Group on Food Irradiation (ICGFI) in Chiang Mai in February 1986, was the minimum to be used against fruit fly of the Tephritidae family.

The data presented at the RCM showed that doses lower than 150 Gy would be adequate to provide quarantine security for several species of fruit fly. For example, a dose of 75 Gy was shown to be adequate for the Queensland fruit fly; *Bactrocera (Dacus) tryoni*; a dose of 100 Gy, or less, was reported to be adequate for fruit infested by the Mexican fruit fly, *Anastrepha ludens*, the West Indian fruit fly, *A. obliqua*, and a third species, *A. serpentina*. This dose was also adequate for the Western Cherry fruit fly, *Rhagoletis indifferens* [1]. The subject of quarantine efficacy, as related to treatment schedules, was considered. However, quarantine treatment is not a substitute for good agronomic practice.

The Group concluded that optimum use of pest management must continue in order to provide high quality commodities with a minimum population of pests present at the time of harvest. It also considered whether irradiation at a minimum absorbed dose of 150 Gy would result in the survival of larvae that could continue to feed and damage the fruit quality. It was agreed, however, that in commercial practice irradiation at this minimum dose would result in the majority of fruit in a container receiving doses higher than 150 Gy. Such a treatment would provide an additional safeguard to fruit quality and minimize survival and/or reduce the feeding activity and life span of the treated larvae.

Continued revision of the family Tephritidae has resulted in a proliferation of the fruit fly species and a regrouping of the species. Testing each of these species in line with established protocols may not be feasible. A review of the differences in radiation sensitivity is needed to determine if these differences are due to the species or to the age or stage of development of the insects being tested. The dose calculated to give 95% mortality could serve as a basis for comparisons of egg hatch, larval pupation or adult emergence. Similarities between species may be useful in reducing the amount of testing required to meet the research protocols.

The Group considered that the criterion for quarantine effectiveness should be the inability of the pest to reproduce in the new environment. However, this could result in finding adult flies in traps that could initiate a quarantine action such as increased trapping, larval surveys or implementation of a spraying programme. Therefore, it was recommended that the criterion for effectiveness of irradiation as a quarantine treatment should be the non-emergence of a normal adult capable of flight.

2. EFFECTIVENESS OF IRRADIATION AS A QUARANTINE TREATMENT AGAINST MITES AND INSECTS OTHER THAN FRUIT FLIES

The research programmes carried out under this CRP included the effectiveness of irradiation against a number of insects and mites[1], in addition to fruit flies. These pests infest a range of produce from fruits and vegetables to cut flowers and stored products.

The protocols developed for determining the treatment efficacy for quarantine purposes for fruit flies were found to be generally inapplicable to these other pests. This may be due to the non-availability (often impossibility) of laboratory culture methods; to the extended life cycles of the pests, often beyond the post-harvest life of the commodity; to the size of the pests, especially mites, thrips and microhymenoptera; and to the multiplicity of stages that may be present in the produce.

These problems were recognized by the ICGFI Task Force that met at Chiang Mai in 1986, and a generic dose of irradiation treatment ensuring at least sterility in the pest population treated was recommended. This generic dose, set at a minimum of 300 Gy, would ensure quarantine security against any stage of any arthropod pest and should not require a minimum of further efficacy data.

In all instances, the results of studies by members of the CRP supported the integrity of the 300 Gy generic dose. Mortality of the mango seed weevil was shown to be complete when estimated at the end of the life cycle (7 months). Irradiation is very important as a quarantine disinfestation treatment, since there are currently no alternative measures available for this pest. The results of studies on *N. xan-*

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[1] Coleoptera (beetles) — *Naupactus xanthographus*, *Sternochaetus mangiferae*, *Sternochaetus olivieri*, *Sitophilus oryzae*, *Sitophilus granarius*, *Acanthoscelides obtectus*
Hemiptera (bugs, scale insects) — *Quadraspidiotus perniciosus*
Isoptera (termites) — *Neotermes chilensis*

thographus, a pest of export grapes from South America, were more preliminary in nature but in complete accord with those of the mango seed weevil.

The radiosensitivity of mites falls between those of beetles and moths. A dose of 250–300 Gy sterilized adult mites and stopped development of the immature stages. Therefore a generic dose of 300 Gy would achieve quarantine security while irradiation for immediate kill would require a dose of 2 kGy.

Although quarantine security can be achieved in all cases, individual pests not yet dead at inspection after treatment would create a problem for quarantine inspectors. This has been overcome in a number of countries (Australia, Chile, New Zealand, etc.) by introducing a quality assurance process during production. This can minimize the likelihood of the presence of pests in export commodities. It is achieved through integrated pre-harvest pest management programmes and surveillance during the packing and grading procedures. It would be desirable if such a practice led to the registration of producers.

Residues of chemicals are becoming increasingly unacceptable to consumers. This could lead to the early loss of alternative disinfestation treatments such as methyl bromide fumigation. While non-persistent chemicals can be used in the pre-harvest phase to reduce residues at harvest, residue free post-harvest methods are essential. Currently, the only methods which meet this criterion are cold, heat and irradiation. No one treatment is applicable to all uses, but there are some where irradiation is the only option if export of a commodity is to be achieved. Certainly, irradiation is more broadly effective and less phytotoxic than either heat or cold.

3. ASSURANCE OF PRODUCT QUALITY AND ADMINISTRATIVE CONTROL PROCEDURES

Ample and comprehensive literature is available from over 30 years of research on the effects of irradiation on various horticultural commodities. Experimental data have shown the negligible phytotoxic effects of irradiation on many products treated at doses higher than those necessary to ensure quarantine security.

However, in order to put into practice irradiation as a quarantine treatment procedure with assurance of quality, a comprehensive and harmonized programme should be formulated and implemented to facilitate international trade in fresh produce.

3.1. Product tolerance to irradiation

The results generated from this CRP have shown that many commodities tolerate irradiation at doses above those necessary to ensure quarantine security. The experimental results obtained showed no phytotoxicity at a dose level of 150 Gy, the

recommended quarantine treatment for fruit fly control in most tropical, subtropical and temperate fruits. These results agree with previous research findings.

It was found that most fruit tolerated doses much higher than 150 Gy. For example, papaya, longan, Kiwi fruit, etc. tolerate a dose of 1000 Gy, or higher. However, the tolerated dose range varies, depending on the product origin, cultivar, handling practices and local conditions. This is evidenced in mangoes from different regions (e.g. Australia, Mexico, the Philippines and Thailand).

3.2. Need for transport trials and market testing

The Group concluded that there were insufficient data on transport trials and market testing of irradiated fruit. These data are essential to the industry in order to evaluate the use of irradiation as a quarantine treatment for export.

3.3. Quality control and assurance

To ensure the high quality of irradiated produce, interested industries should consider an integrated approach by having good agronomic practices, including adequate pest and disease control; efficient handling and transport to minimize post-harvest stress; proper inspection, conditioning, processing and culling; good manufacturing practices in the packing house, irradiation treatment facility and loading areas; and internationally standardized dosimetry.

3.4. Potential for developing a technique to confirm treatment of irradiated insects

Preliminary data were generated on the use of part of an insect found infesting a fruit on arrival in an importing country (e.g. the size of the supraoesophageal ganglion in the fruit fly) as a marker of the insect's having been irradiated. These experiments suggest a potential technique that can be developed to aid a quarantine inspector in determining if an insect discovered in a supposedly treated fruit will progress to an adult, at which stage it may become a quarantine problem.

3.5. Benefits of combination treatments

Exported fruit is subject to decay from post-harvest organisms and disorders other than insects (e.g. fungi, bacteria and chilling injury). Treatments combining irradiation, heat and perhaps others such as non-toxic chemicals may help to maintain the highest quality attainable for each product.

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IONIZING RADIATION AS A QUARANTINE TREATMENT FOR CARIBBEAN FRUIT FLIES IN GRAPEFRUITS, CARAMBOLAS AND MANGOES*

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Abstract

IONIZING RADIATION AS A QUARANTINE TREATMENT FOR CARIBBEAN FRUIT FLIES IN GRAPEFRUITS, CARAMBOLAS AND MANGOES.

Grapefruit infested with Caribbean fruit fly, *Anastrepha suspensa* (Loew), eggs and larvae were exposed to ionizing radiation from research and commercial ^{60}Co sources to determine the effects of radiation on pupation and adult emergence. A dose of 50 Gy, followed by 5 days of cold storage, resulted in a quarantine security dose based on the prevention of adult emergence. A dose of 150 Gy was required without cold. A dose of 40 Gy prevented the hatch of 1-25 hour old naked eggs, but increasing doses were required for older eggs and 1000 Gy were required for 60 hour old eggs. When larvae were treated in rearing medium, no adults emerged following treatment at 50 Gy. In large scale tests, no adults emerged at 60 Gy and those emerging at 40 Gy were abnormal. When infested mangoes were treated, a dose of 75 Gy was estimated to give quarantine security. When uninfested fruits were treated at doses of up to 1500 Gy, 'Tommy Atkins' mangoes were more susceptible to injury than were 'Keitt'. A dose of 50 Gy, applied to infested carambolas, prevented the emergence of adult Carribbean fruit flies. No damage was observed when 'Arkin' carambolas were treated at 600 Gy.

1. INTRODUCTION

Caribbean fruit fly (CFF), *Anastrepha suspensa* (Loew), larvae infest citrus and other subtropical fruits. When mature, the larvae leave the fruit, pupate in soil and adults emerge. Extensive research has been conducted on the effects of gamma radiation on this species at Miami, Florida, United States of America, using small ^{60}Co sources, or elsewhere using a larger, commercial irradiator. Most of our

* Research carried out in association with the IAEA under Research Agreement No. 2967/CF.

research has been to determine the effects of irradiation on naked insects, eggs or larvae in rearing medium, grapefruits, mangoes or other fruits. Research on the effects of irradiation on fruit quality and storage has been conducted at Miami and elsewhere in Florida.

2. MATERIALS AND METHODS

The grapefruits, carambolas and mangoes required for this research were obtained from commercial packing houses and infested by placing the fruits in large, outdoor cages containing several thousand adult flies that had been reared in the laboratory. The infested fruits were held for various periods of time to permit the eggs to develop and hatch and to allow for larval development. For laboratory scale tests, the infested fruits were treated in our ^{60}Co research irradiators. For large scale tests, the fruits were shipped to and treated in a commercial ^{60}Co irradiator. Following treatment, the fruits were placed in holding cages where the surviving larvae could complete their development, leave the fruit and form puparia. These were collected periodically and held for adult emergence. The population of fruit fly eggs or larvae present in the treated fruits was estimated on the basis of the population present in untreated fruits held under comparable conditions. Tests to evaluate the effects of irradiation on fruit quality were done on high quality fruit obtained from commercial packing houses.

The two ^{60}Co research irradiators at our laboratory had dose rates of 4–5 and 145–250 Gy/min, respectively, during the period of these experiments. The dose rate of the commercial irradiator was 2.1 Gy/min.

3. RESULTS AND DISCUSSION

The CFF eggs collected at 5 hour increments were irradiated on moist towels in Petri dishes. A dose of 40 Gy prevented the hatch of 1–25 hour old eggs. Doses of 125, 175, 400 and 600 Gy were required to prevent the hatch of 35, 40, 50 and 55 hour old eggs, respectively, and 1000 Gy were required for 60 hour old eggs. When the eggs were placed under the skin of grapefruit for treatment, and subsequently transferred to rearing medium, no adults emerged after treatment at 25 Gy.

When the CFF larvae were treated in rearing medium at ages from 1 to 7 days, no adults emerged following treatment at 50 Gy. In large scale tests, approximately 20 000 larvae were treated at doses of 20, 40 and 60 Gy. At 20 and 40 Gy, 5500 and 15 adults emerged, respectively. No adults emerged at 60 Gy and all of those emerging at 40 Gy were abnormal.

Research on the effects of irradiation of infested grapefruits followed by cold storage showed that some larvae were able to survive and form puparia when treated

TABLE I. EFFECTS OF COMBINED IRRADIATION–COLD TREATMENT ON THE MORTALITY OF CARIBBEAN FRUIT FLIES IRRADIATED IN GRAPEFRUITS

Dose (Gy)	Days at cold	No. of insects treated	% mortality	
			Immatures	Adults
0	0	7567	0	0
	2	3766	60.92	
	4	3766	93.07	
	6	3766	98.65	
	8	3766	99.87	
5	0	3808	3.05	42.70
	2	3097	48.47	82.79
	4	1153	90.11	98.27
	6	2828	99.15	99.89
10	0	3808	3.57	75.42
	2	3097	72.26	96.32
	4	1153	76.93	97.31
	6	2828	99.58	99.93
20	0	3808	77.07	98.69
	2	3097	86.34	99.97
	4	1146	98.78	99.91
	6	2845	99.79	99.96
40	0	3808	88.31	100
	2	3097	82.76	100
	4	1153	97.40	100
	6	2845	99.89	100
80	0	3808	93.38	100
	2	3097	87.92	100
	4	1153	97.74	100
	6	2863	100	100

TABLE II. EFFECTS OF IRRADIATION ON THE MORTALITY OF CARIBBEAN FRUIT FLIES IRRADIATED IN GRAPEFRUITS [3]

Mean dose (Gy)	No. of insects treated	% mortality	
		Immatures	Adults
43	2877	79.1	100
77	2877	92.5	100
112	1931	98.2	100
117	2877	98.9	100
197	2877	99.97	100
225	1966	99.85	100
406	1959	100	100
415	2877	100	100

TABLE III. EFFECTS OF IRRADIATION ON THE MORTALITY OF CARIBBEAN FRUIT FLIES IRRADIATED IN CARAMBOLAS [6]

Dose (Gy)	No. of insects treated	% mortality	
		Immatures	Adults
5	1119	18.2	60.59
10	6233	9.9	99.48
15	1119	17.6	99.64
20	1119	16.3	100
25	10373	30.1	99.97
50	6423	1.3	100
100	5304	43.7	100
200	337	74.5	100

at doses of 40 and 80 Gy (Table I). However, no adults emerged from these puparia. Cold storage for 2, 4 or 6 days increased the mortality, based on the pupation of larvae treated at all the doses. Cold storage also increased the mortality, based on the adult emergence at doses of 5, 10 or 20 Gy [1]. On the basis of these data, the dose required for quarantine security (99.9968% mortality) [2] was calculated to be 50 Gy, followed by cold storage for 5 days at 1.1°C.

When cartons of infested grapefruit were irradiated in a commercial ^{60}Co irradiator at doses ranging from 34 to 533 Gy, no larvae formed puparia at doses in excess of 300 Gy and no live adults emerged at any of the doses tested (Table II) [3]. On the basis of these data, a dose of 150 Gy was proposed for quarantine security.

Infested Florida mangoes were treated at doses ranging from 25 to 200 Gy [4]. No adults emerged at any of the doses applied. The effects of ionizing radiation on the quality, decay and storage of the mangoes were determined at doses ranging from 150 to 1500 Gy for the two cultivars 'Tommy Atkins' and 'Keitt' [5]. These effects varied with dose and with cultivar. For 'Tommy Atkins', ripening was delayed for 2–3 days by 150 and 250 Gy, but accelerated by 1500 Gy; the pH of the juice decreased and the titratable acidity increased as the dose increased and the internal breakdown increased at or above 250 Gy. For 'Keitt', the internal breakdown decreased at 1500 Gy. For both cultivars, scald like peel injury increased at or above 500 Gy, and hollow pockets and darkening of the flesh increased when treated at 1500 Gy. A dose of 750 or 1500 Gy was required to reduce fruit decay caused by anthracnose or stem end rot. However, these doses caused unacceptable injury, especially to the 'Tommy Atkins' fruit.

When carambolas of the cultivars 'Arkin', 'Star King' and 'Golden Star' were infested by the CFF larvae and irradiated at doses ranging from 1 to 250 Gy, no adults emerged at doses of 50 Gy or above (Table III) [6]. However, some of the larvae survived and formed puparia at 250 Gy. No adults emerged in a large scale test when over 100 000 immature fruit flies infesting the 'Arkin' fruits were tested at a dose of 50 Gy. No phytotoxicity was observed when the 'Arkin' fruits were treated at doses of up to 600 Gy.

ACKNOWLEDGEMENT

The co-operation received from the IAEA is gratefully acknowledged.

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GAMMA IRRADIATION AS A COMMODITY TREATMENT AGAINST THE QUEENSLAND FRUIT FLY IN FRESH FRUIT*

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Abstract

GAMMA IRRADIATION AS A COMMODITY TREATMENT AGAINST THE QUEENSLAND FRUIT FLY IN FRESH FRUIT.

Third instars of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), were more tolerant to gamma irradiation than other stages that infest fresh fruit from Australia. A dose of 75 Gy prevented the development of adults when the eggs or larvae were irradiated in apples (*Malus domestica* L.), oranges (*Citrus sinensis* Osbeck), avocados (*Persea americana* Mill.), mangoes (*Mangifera indica* L.), tomatoes (*Lycopersicon esculentum* Mill.) and cherries (*Prunus avium* L.). The proventriculus of the treated larvae developed normally, while development of the supraoesophageal ganglion was retarded. All the fruits, with the exception of avocados, tolerated 100 Gy without developing injury symptoms. The quality of 'Ron's Seedling', 'American Bing' and 'Lambert' sweet cherry drupes was not affected by doses of up to 1000 Gy. Peduncle discoloration increased in 'Ron's Seedling' cherries irradiated at 600 and 1000 Gy. When 'Lisbon' lemons (*Citrus limon* (L.) Burm. f.) were treated at doses of up to 1000 Gy and stored at 15°C for up to 6 weeks, irradiation reduced the total titratable acidity and the total soluble solids, while the juice and pH increased. Irradiation accelerated the yellow colour formation in green lemons, as well as flesh and peel softening and button senescence in both yellow and green lemons. Tissue damage in the form of flesh and albedo discoloration, albedo toughness and flesh cavitation occurred in the irradiated lemons. Irradiation increased pericarp browning in 'Bengal' lychees (*Litchi chinensis* Sonn.) stored at 4°C or 10°C at 85–90% relative humidity, but had no effect on lychees stored at 20°C in a constant flow of ethylene free air at 95–100% relative humidity. Irradiation had no effect on the ethylene production, but lychees dosed at 75 and 150 Gy evolved more carbon dioxide than did untreated lychees or those dosed at 300, 600 or 1000 Gy. Lychees treated with a combination of polyvinyl chloride wrapping plus irradiation at 75–300 Gy and stored at 4°C

* Research carried out in association with the IAEA under Research Agreement No. 3059/CF.

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experienced no adverse effects on the fruit quality, taste or mould development compared with wrapped, dipped, untreated lychees. The ascorbic acid content of 'Valencia' oranges was not affected by doses between 25 and 300 Gy.

1. INTRODUCTION

Fruit flies of the family Tephritidae are major quarantine pests throughout the world and strict measures are taken to reduce the risk of introducing them into uninfested areas. Export requirements are generally very stringent and any trace of infested produce can result in the rejection of entire shipments.

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is a serious pest of quarantine significance for the export of fresh Australian produce. Until recently, the fumigant ethylene dibromide (EDB) was the most widely used commodity treatment against the Queensland fruit fly. However, alternative disinfestation treatments have been studied. One such alternative is gamma irradiation. A dose of 150 Gy, or less, has satisfied quarantine requirements for mangoes infested with the oriental fruit fly, *D. dorsalis* Hendel [1], grapefruit infested with the Caribbean fruit fly, *Anastrepha suspensa* Loew [2], and papayas and other fruits infested with the eggs or larvae of the oriental fruit fly, the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, and the melon fly, *D. cucurbitae* Coquillett [3]. Rigney and Wills [4] found that a dose of 75 Gy provided quarantine security against the eggs and larvae of the Queensland fruit fly in oranges. In the United States of America, the United States Department of Agriculture has recently approved irradiation as a quarantine treatment for Hawaiian papaya against fruit flies [5] and a food irradiation plant is currently under construction in Florida, USA, for the irradiation of oranges against fruit flies [6].

Irradiation at a dose of 150 Gy has been recommended as an effective quarantine treatment against fruit flies of the family Tephritidae [7]. At these low dose rates, death is generally not immediate. This can be a disadvantage if the product is for immediate export and there is a nil insect requirement. Thus, there is an urgent need for an accurate technical procedure able to determine that irradiation has taken place and been administered at the correct dose. Rahman et al. [8] found that irradiation reduced the size of the supraoesophageal ganglion in larvae of the Mediterranean fruit fly, but that there was no significant effect on the size of the proventriculus. They proposed that the proventriculus be used as a basis for the comparison of sizes of the supraoesophageal ganglion between irradiated and non-irradiated fruit fly larvae. It is hypothesized that their findings be extrapolated to *B. tryoni.*

Many Australian fruits are subject to quarantines that restrict their movement, both interstate and overseas. Japan bans the importation of cherries from Australia because of possible infestation by several pests, including the Queensland fruit fly [9]. Australian cherries may gain entry to the USA, providing they have been treated

with cold storage or a combination of cold storage and methyl bromide fumigation [10]. Fruits from most cultivars are tolerant of methyl bromide, although some researchers cite deleterious effects on the flavour and external appearance [11, 12].

Restrictions have been placed on the export of Australian 'Lisbon' lemons to many countries because of possible infestations of the Queensland fruit fly and the Mediterranean fruit fly. Quarantine disinfestation of these insects in Australia had been carried out with EDB [13] but, with its banning in the USA [14] and worldwide concern over the usage of toxic chemicals, alternative quarantine treatments are being sought. Lemons can be stored at 1 °C for 16 days to provide quarantine security against fruit fly infestations with negligible phytotoxic effects on the fruit [15]. However, a significant decrease in the shelf-life occurs due to the treatment. Also, Japanese authorities require that quarantine treatments be completed before the shipments leave Australia [13].

Studies on the phytotoxic effects of irradiation on lemons have shown that they tolerated doses over 1000 Gy, followed by storage at 25 °C [16]. However, when irradiation was followed by storage at 15 °C undesirable internal browning and cavitation developed [17]. Studies on lemons irradiated at low doses showed that at 200–800 Gy they degreened more rapidly than the controls during the week following treatment, but thereafter complete degreening was quicker in the control fruits [18]. When irradiated at 750–1000 Gy and then stored at 7 °C there were no adverse effects on the quality of 'Eureka' lemons [19].

Researchers in the USA [20] studied the effects of gamma irradiation on the levels of total soluble solids (TSS), acidity and reducing sugars in lychees and found no significant differences between the lychees irradiated at 300, 500 and 1000 Gy and the fresh, untreated lychees when the fruits were stored at 15.6–21.1 °C for 6 days. Irradiation at doses of up to 2000 Gy caused no significant change in the nutritional value of the lychees or in the concentration of radiation sensitive vitamins [21] and, although there was a loss of up to 17% in the levels of ascorbic acid in lychees irradiated at 3000 Gy, this figure was computed to indicate an insignificant change relative to losses of 37 and 88% ascorbic acid in canned and frozen lychees, respectively [22]. Moy et al. [23] irradiated six varieties of lychee at 500 and 1000 Gy, followed by storage at 7.2 °C for 1–3 weeks, and found no detectable deterioration in the aroma, flavour, texture or pulp colour and a decrease in storage decay. Akamine and Goo [24] indicated that 250 Gy was the maximum dosage tolerated by fresh lychees packed in polyethylene film. When combined with a dose of 250 Gy, they suggested storage at 7.2 °C. Ross and Brewbaker [25] showed that the pericarp colour of lychees treated at 500–1000 Gy was significantly browner than untreated lychees, which remained red, but there were no deleterious effects on the flavour or pulp colour.

Concern has been expressed that irradiation may cause serious nutritional losses in food [26]. Some studies have shown that the vitamin C content of oranges is significantly reduced after irradiation [27], while others have found that it is not

[28, 29]. It has been suggested that reports showing decreases in vitamin C investigated the ascorbic acid content only and not the dehydroascorbic acid content [27, 30]. Roberts [30] suggested that irradiation may cause the reduction of ascorbic acid to dehydroascorbic acid, undetectable by conventional methods of vitamin C analysis but still active in the human body as a precursor to vitamin C.

This paper reports on studies made on the effects of irradiation on the Queensland fruit fly and some of its host fruits conducted by researchers in New South Wales (NSW), Australia, between 1980 and 1990, some of which have been published elsewhere [4, 31].

2. MATERIALS AND METHODS

2.1. Effects of irradiation on the Queensland fruit fly

Fruits for infestation were purchased at the Flemington Markets in Sydney, NSW, or harvested in orchards around the central coast of NSW, dipped in fungicide, allowed to dry and placed in nylon mesh cages (0.2 m^3 volume) housing 10 000–15 000 fertile Queensland fruit fly adults [32]. After infestation, the fruits were held at 26°C and 75% relative humidity to allow development of the insect to the target life stage: egg, young larva (first or second instar) or old larva (third instar), at which stage they were irradiated. After treatment, the fruits were held for up to 6 weeks over sand at 26°C and 75% relative humidity for pupation. The puparia were collected and held for an additional 6 weeks in sand to allow adult emergence.

The fruits tested were: 'Valencia' oranges (*Citrus sinensis* Osbeck) from the central coast of NSW, 'Granny Smith' apples (*Malus domestica* L.) from Orange, NSW, 'Fuerte' avocados (*Persea americana* Mill.) and 'common' mangoes (*Mangifera indica* L.) from the north coast of NSW, 'Floradade' tomatoes (*Lycopersicon esculentum* Mill.) from the central coast of NSW and 'Ron's Seedling' cherries (*Prunus avium* L.) from Young, NSW. All the fruits were at the market mature stage at harvest. They were treated within 2 days of harvest.

To test the efficacy of irradiation, five replicates, each of 160 'Ron's Seedling' cherries, were laboratory infested with *B. tryoni* eggs by placing the fruits on mesh cages housing 3–5 week old flies and allowing oviposition through the mesh [32]. Infested fruits were stored at 26°C and 70% relative humidity, the optimum conditions for development of *B. tryoni*, until the larvae had attained first, second and third instars. The infested fruits were treated when the insects were at the egg stage and the three larval stages, separately. The doses were 0, 75 and 100 Gy. After irradiation, the infested fruits were stored at 26°C over dry sand in which the surviving larvae pupated. Puparia were collected and the least susceptible stage was determined on the basis of the relative number of puparia formed.

In a follow-up experiment, the fruits were infested with fruit fly eggs and irradiated at 0, 75 and 100 Gy when the larvae had developed to the most tolerant stage. The surviving puparia were collected and stored at 26°C. The efficacy of the treatment was based on the number of emergent adult flies recorded.

2.2. Identification of irradiated larvae

2.2.1. Treatment of larvae

Three hundred and sixty oranges were punctured, placed on gauze covered cages containing adult colonies of *B. tryoni* and infested over a 6 hour period. The oranges were stored until the larvae had reached the appropriate ages for irradiation. Eggs and first, second and third instars were exposed to doses of 0, 25, 50, 75 and 100 Gy, using a two way factorial: immature life stage and irradiation dose. Twenty fruits were randomly allocated to each treatment; 40 infested fruits were used as the control. The fruits were irradiated as described elsewhere, and returned to the Gosford Horticultural Postharvest Laboratory, placed on trays over sand and stored at 26°C and 70–75% relative humidity. Development of the larvae was monitored daily.

In the second part of the experiment, eggs were collected in plastic oviposition cups placed in the fruit fly cages, removed from the cups and placed on an artificial larval medium based on finely milled oaten chaff. Eggs and first or third instars were irradiated at a dose of 75 Gy. Larvae were dissected at the mature third instar.

2.2.2. Experimental preparation

To measure the supraoesophageal ganglion and proventriculus it was necessary to adopt a simple and accurate dissecting method. D.T. Anderson, Zoology Department, University of Sydney, recommended the following: place the living specimen on a slide in a drop of saline solution under a dissecting microscope, place a pin in the eighth abdominal segment to hold the larva in place and place a second pin in the prothoracic segment directly behind the mouth hooks. The rapid forward movement of the anterior pin severs the larva, revealing the organs of interest.

Ten mature third instars were removed and dissected per treatment. The measurements made were those described by Rahman et al. [8] using a binocular microscope with ocular graticule. Figure 1 illustrates a typical dissection of *B. tryoni* at the third instar. The length and breadth of each lobe of the supraoesophageal ganglion and the proventriculus were measured. The area was calculated using the formula for an ellipse (πab, where a and b represent the major and minor radii, respectively). The first part of the experiment was replicated four times and the second part twice.

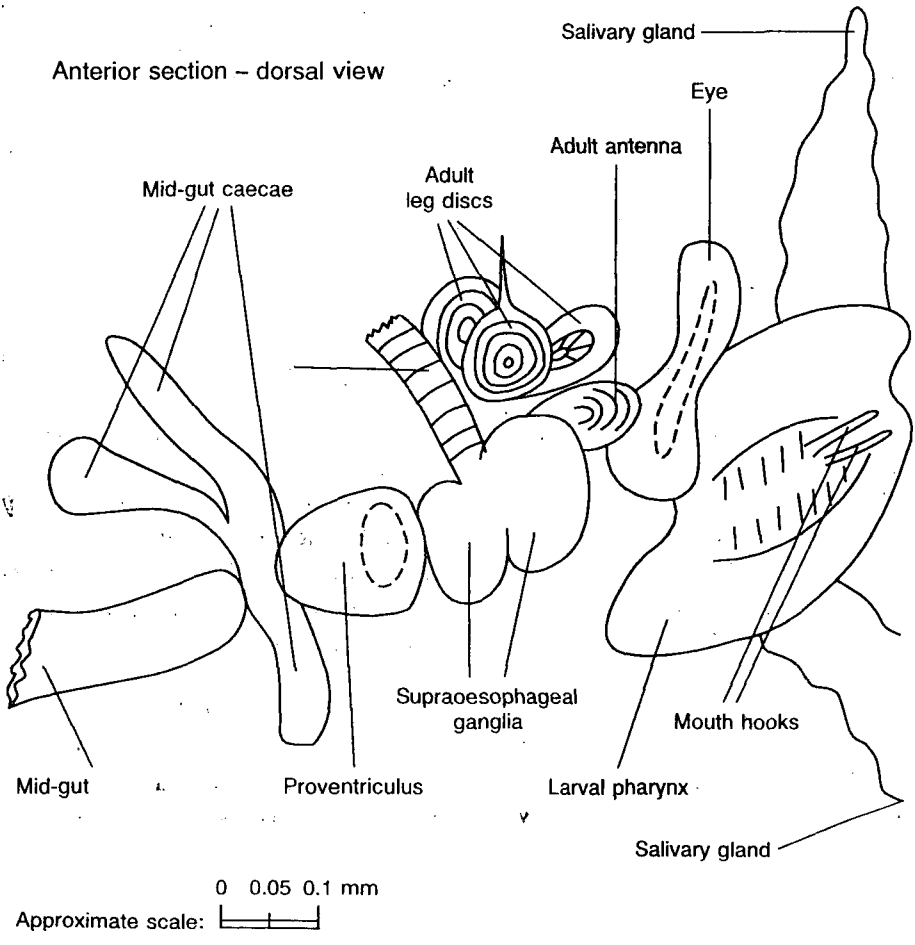

FIG. 1. Dissection of a third instar Queensland fruit fly larva to show the proventriculus and supraoesophageal ganglion.

2.3. Tolerance of fruits to irradiation

The maximum/minimum ratio of the dose received by a product in a commercial food irradiator varies according to the product, package thickness and density, and configuration of the irradiator [33]. Therefore, tests of commodities for adverse effects from irradiation must take into account the possibility that the maximum dose received may be three times the minimum effective dose.

2.3.1. *Cherries*

Cherries were harvested and treated as described elsewhere [31]. The fruits were irradiated at the Australian Nuclear Science and Technology Organisation (ANSTO). After treatment, the fruits were returned to Gosford, stored at 10°C for 10 days or at 1°C for 20 days and assessed for evidence of damage.

In the first experiment, the 'Ron's Seedling' fruits were tested to determine if an increase in relative humidity or a reduction in ethylene (C_2H_4) concentrations would reduce peduncle discoloration [34]. The fruits were placed in plastic containers, half of which were covered with a plastic wrap and half with a wrap plus vermiculite impregnated with potassium permanganate [31]. The fruits were irradiated at 0, 75, 150, 300, 600 and 1000 Gy and stored at 10°C for 10 days. The fruits were assessed for TSS (°Brix), firmness, peduncle discoloration, external damage and fruit weight.

In the second experiment, 'American Bing' and 'Lambert' cherries were irradiated at 0, 75, 150 and 300 Gy and stored at 1°C for 20 days to study the effects of a combination of irradiation and cold storage treatment. The fruits were assessed for peduncle abscission, external damage, skin browning and skin colour [31].

2.3.2. *Lemons*

Three times during June 1987, 1500 'Lisbon' lemons from the Somersby growing region of NSW were harvested at two stages of maturity. Half were green fruits, showing the first signs of a colour break, and half were completely yellow, except for the region around the stylar end. Two hours after harvesting, the lemons were dipped in a fungicidal solution of 0.1% benomyl and 250 ppm guazatine, allowed to dry, treated with a commercial citrus wax and sorted according to fruit length: 60–70, 71–80 and 81–90 mm. Fruits of each size and stage of maturity were randomly packed in single layer trays of 24–32 lemons and allocated for irradiation at 0, 75, 150, 300, 600 and 1000 Gy. Two hundred and fifty green and 250 yellow fruits were irradiated at ANSTO in each of three replicate treatments 1 day after the harvest date. After irradiation, the treated and control fruits were stored at 15°C, the recommended temperature for long term storage of lemons [35].

Samples of 12 fruits were taken from each batch after 2, 4 and 6 weeks of storage. The fruits were weighed and peel firmness was determined as the force required for penetration of the point of a stainless steel conical probe with a maximum diameter of 5 mm, from the point to a depth of 7 mm, at three positions around the equator of the lemon. Subjective assessments were made on each of the lemons for peel colour — scored from 1 (deep yellow) to 5 (silver-green); peel damage — scored from 1 (no damage) to 5 (over 50% of the peel brown or black); albedo toughness — scored from 1 (no toughening) to 5 (severe toughening) determined by the ease of penetration of the point of a stainless steel knife blade; albedo discoloration

— scored as 1 (white), 2 (pink) or 3 (dark pink to brown); flesh browning — scored from 1 (no pinkness or browning) to 5 (severe browning); and flesh cavitation — scored as 1 (no cavitation), 2 (slight cavitation totalling < 1 cm^2 of cut surface when the lemon is bisected) or 3 (severe cavitation).

The juice from each lemon was extracted using an electric citrus reamer, then strained through a 1 mm^2 mesh stainless steel sieve. The volume of juice was determined and measurements made of the TSS, pH and total titratable acidity (TTA) expressed as grams of citric acid per 100 mL of juice. The average value for 12 lemons from each treatment unit was calculated.

2.3.3. Lychees

Lychees (cultivar 'Bengal') were picked when harvest mature at Rosebank, via Lismore, on the north coast of NSW, air freighted to Sydney (about a 2 hour flight southwards) and stored at 10°C. Half the fruits were dipped in a hot benomyl solution (0.5 g/L at 52°C) for 2 minutes. The other half was not dipped. Half of the dipped fruits and half of the non-dipped fruits were then packed in plastic punnets (fruit baskets), 12–15 fruits per punnet, overwrapped in a polyvinyl chloride 'cling' film and placed in retail lychee trays, measuring 47 cm × 36 cm × 10 cm, each tray containing six punnets of dipped fruits and six of non-dipped fruits. The remaining fruits were not wrapped, but were placed in retail mango trays (47 cm × 32 cm × 10 cm) divided into 10 partitions by cardboard strips, with 12 lychees in each partition. Five partitions held dipped fruits and five non-dipped fruits. In total, there were 12 trays of wrapped fruits and 12 trays of non-wrapped fruits.

The fruits were irradiated at ANSTO 2 days after harvest at doses of 0, 75, 150, 300, 600 and 1000 Gy. Two trays of wrapped and two of non-wrapped lychees were treated at each of the six doses. After irradiation, the fruits were transported by road to Gosford, 2 hours to the north of Lucas Heights, and stored at 4°C or 10°C and a relative humidity of 70–80% prior to quality assessments.

Samples of 10 lychees from each treatment unit were removed, weighed and placed in separate plastic respiration jars, which were continuously ventilated with humidified C_2H_4 free air at a rate of approximately 1 L/h at 20°C, to determine the C_2H_4 production and respiration, as measured by carbon dioxide (CO_2) evolution, from the enclosed lychees. Wrapped lychees were removed from their plastic wrapping 8 hours after irradiation. Samples of air were taken once daily for 7 days after irradiation by syringing gas samples through the self-sealing outflow tubing of the respiration jar. The concentrations of CO_2 and C_2H_4 in the effluent gas were determined using a gas liquid chromatograph. The lychees were scored for browning and mould development after 9 days of storage.

Visual scoring of lychees commenced once it was obvious that colour changes were taking place. The non-wrapped lychees stored at 10°C for skin colour (brown-

ing) were scored after 3, 5 and 7 days. The wrapped lychees stored at 10°C were scored (without removing the film) for mould development and pericarp browning after 21, 23 and 26 days in storage. The non-wrapped fruits stored at 4°C were scored for browning after 12 days in storage and then taste tested. The wrapped lychees stored at 4°C were scored for browning after 34, 37 and 42 days in storage and after 42 days at 4°C plus 2 days at room temperature (15–21°C). Scoring for mould development was done after 42 days at 4°C and after 42 days at 4°C plus 2 days at room temperature.

Skin colour scoring was based on a scale of 1–10, where 1 indicated that $<10\%$ of the fruit's pericarp was brown or black and 10 indicated that 90–100% of the fruit's pericarp was similarly discoloured. Mould development was scored from 1–5, where 1 indicated that $<20\%$ of the pericarp was covered in mould and 5 indicated 80–100% cover. Sensory evaluations were conducted using a triangle test where each panellist was presented with three unlabelled specimens (one irradiated and two identical controls) and asked to choose by taste and sight one specimen that differed from the others [36]. Fruits irradiated at 0, 75, 300, and 1000 Gy were taste tested.

2.3.4. Oranges

'Valencia' oranges from the Murrumbidgee irrigation area of southern NSW were selected from packaged Grade 1 fruit intended for export to Japan. The oranges originated from several orchards but were packaged together. Three replicates of 40 fruits per dose were irradiated in air at ANSTO. The fruits were irradiated at 0, 25, 50, 75, 100, 150 and 300 Gy. The oranges were then stored at 10°C for 4 weeks to simulate the maximum likely export shipping time. Six fruits from each treatment and replicate were randomly selected and juiced. The juice was stored in stoppered glass vials and frozen for analysis. The juice was analysed for its ascorbic acid content using the indolphenol titration method [37]. Each sample was halved for replicate titrations. Titrations were performed for a second time on the control (0 Gy) and 300 Gy samples 2 weeks after the first series of analyses in order to detect possible deterioration of the samples in frozen storage.

2.4. Statistical analysis of the data

Whenever appropriate, data were treated by analysis of variance using the Genstat programs [38]. The least significant difference (LSD) values were calculated at $k = 100$ using the Duncan–Waller Bayesian k ratio (LSD) rule [39]. The homogeneity of variance was tested using the Bartlett's test and the mean separation was tested using the LSD test at the 5 and 1% levels. If there were no statistical differences due to replicating the irradiation treatments and if the data appeared to be normally distributed, the data were pooled.

2.5. Irradiation facility

Irradiation was carried out in air in a 100 kCi ^{60}Co gamma ray plaque source at the Lucas Heights Gamma Technology Research Irradiator, ANSTO.[1] Dosimetry was routinely carried out using ferrous ammonium sulphate. In most cases, the maximum/minimum ratio of the dose received was less than 1.5. For the treatment of some fruits, the maximum/minimum ratio of the dose received was 1.8–2.0. The dose rate was about 9.25 Gy/min.

3. RESULTS AND DISCUSSION

3.1. Effects of irradiation on the Queensland fruit fly

Second and third instar Queensland fruit fly larvae treated in infested 'Ron's Seedling' cherries were more tolerant than eggs or first instars to irradiation based on the number of puparia formed after treatment (Table I) [31]. No insects survived to eclosion when third instars were irradiated at 75 or 100 Gy (Table II) [31]. These results indicated that a dose of 75 Gy could provide quarantine security against fruit fly eggs and larvae infesting cherries by breaking the life cycle before adult eclosion.

The data given in Table III indicate that, based on adult emergence, old (third instar) larvae of the Queensland fruit fly were more tolerant to gamma irradiation than eggs or young larvae when treated in infested 'Valencia' oranges, 'Granny Smith' apples, 'Fuerte' avocados, 'Floradade' tomatoes, 'common' mangoes and 'Supreme' cherries. A dose of 75 Gy completely prevented the emergence of adults from the puparia formed after the treatment of over 90 000 eggs, 50 000 young larvae and 550 000 old larvae infesting these fruits. These trials confirmed quarantine security at the probit 9 level (99.9968% mortality), which is the level required for the importation of fresh fruits and vegetables into the USA [40].

3.2. Identification of irradiated larvae

The results of the experiment in which eggs or larvae were treated in oranges showed that gamma irradiation had a significant effect on the ratio of the approximate transectional area of the proventriculus to that of the supraoesophageal ganglion (P/G ratio) in mature larvae (Table IV). It appeared that there was no significant effect of irradiation dose on the P/G ratio, which remained unchanged despite the dose received. However, the life stage at which irradiation treatment took place had a significant bearing on the P/G ratio. When treated at the third instar, little time was allowed between treatment and dissection (approximately 2 days). The P/G ratio after third instar larvae were irradiated at 25 Gy was 2.77, while the ratio for

[1] 1 Ci = 3.70×10^{10} Bq.

TABLE I. EFFECTS OF GAMMA IRRADIATION OF 'RON'S SEEDLING' CHERRIES INFESTED WITH IMMATURE STAGES OF THE QUEENSLAND FRUIT FLY ON SUBSEQUENT FORMATION OF PUPARIA
(Adapted from Ref. [31])

Stage treated	Dose (Gy)				
	No. of puparia[a]			Mortality (%)[b]	
	0	75	300	75	300
Eggs	460.3	0	0	100	100
Larvae (instar)					
First	389.3	0	1.3	100	99.7
Second	576.7	429.7	419.0	25.5	27.7
Third	505.3	458.3	494.7	9.3	2.1

[a] Each figure represents the mean number of puparia formed from five replicates of 160 fruits each.
[b] The mortality is based on the formation of puparia from untreated 'Ron's Seedling' cherries infested with immature life stages of *B. tryoni*.

TABLE II. EFFECTS OF GAMMA IRRADIATION OF 'RON'S SEEDLING' CHERRIES INFESTED WITH THIRD INSTAR QUEENSLAND FRUIT FLY LARVAE ON SUBSEQUENT ADULT ECLOSION
(Adapted from Ref. [31])

Dose (Gy)	No. of puparia formed[a]	No. of adults emerged[b]	Emergence (%)
0	1450	1389	95.8
75	1080	0	0
100	1238	0	0

[a] The total number of puparia from five replicates of 100 fruits each.
[b] The total number of emerged adults from five replicates of 100 fruits each.

TABLE III. EFFECTS OF GAMMA IRRADIATION OF VARIOUS FRUITS INFESTED WITH IMMATURE QUEENSLAND FRUIT FLIES ON SUBSEQUENT ADULT EMERGENCE

Dose (Gy)	Stage and No. treated/No. of adults emerged					
	Eggs		Larvae			
			Young		Old	
	Treated	Adults emerged	Treated	Adults emerged	Treated	Adults emerged
			'Valencia' oranges			
12.5	31 452	481	40 218	995	27 553	846
25	31 452	4	40 218	19	27 553	136
50	31 452	3	40 218	0	27 553	47
75	31 452	0	23 839	0	220 328	0
			'Granny Smith' apples			
12.5	12 063	37	14 225	502	11 866	2251
25	12 063	26	14 225	97	11 866	325
50	12 063	25	14 225	75	11 866	165
75	12 063	0	12 225	0	128 373	0
			'Fuerte' avocados			
12.5	38 463	1289	20 376	760	20 018	2115
25	38 463	27	20 376	73	20 018	88
50	38 463	1	20 376	0	20 018	1
75	38 463	0	20 376	0	213 638	0
			'Floradade' tomatoes			
12.5	10 791	1	11 383	521	2 891	759
25	10 791	3	11 383	1	2 891	85
50	10 791	0	11 383	0	2 891	0
75	10 791	0	11 383	0	2 891	0
			'common' mangoes			
12.5	40	20	681	168	504	361
25	40	0	681	6	504	152
50	40	0	681	0	504	65
75	40	0	681	0	504	0
			'Supreme' cherries			
75	1 389	0	2 898	0	1 484	0

TABLE IV. RATIO OF PROVENTRICULUS TO ONE LOBE OF SUPRA-OESOPHAGEAL GANGLION OF MATURE QUEENSLAND FRUIT FLY LARVAE IRRADIATED AS IMMATURE LARVAE IN ORANGES

Stage treated (instar)	Dose (Gy)				
	0	25	50	75	100
First	1.90	3.57	4.95	5.40	4.74
Second	1.90	3.74	4.12	4.22	4.36
Third	1.90	2.77	3.40	3.67	3.16

TABLE V. RATIO OF PROVENTRICULUS TO ONE LOBE OF SUPRA-OESOPHAGEAL GANGLION OF MATURE QUEENSLAND FRUIT FLY LARVAE IRRADIATED AT 75 Gy AS EGGS OR IMMATURE LARVAE IN REARING MEDIUM

Stage treated	Dose (Gy)	
	0	75
Eggs	1.28	3.66
Larvae (instar)		
First	1.22	3.73
Third	1.36	2.03

untreated larvae was 1.90. This difference, while significant at the 1% level, may be difficult to detect in a quarantine inspection situation.

The second part of the experiment, in which eggs or larvae were treated in rearing medium, confirmed that gamma irradiation affected the P/G ratio of mature Queensland fruit fly larvae when eggs and first and third instars were dosed at 75 Gy (Table V).

TABLE VI. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 1°C FOR 20 DAYS ON THE QUALITY ASPECTS OF 'AMERICAN BING' AND 'LAMBERT' CHERRIES
(From Ref. [31])[a]

Attribute	Dose (Gy)			
	0	75	150	300
			'American Bing'	
Peduncle discoloration[b]	2.05b	2.28a	2.27a	2.37a
Skin colour[c]	1.83a	1.84a	1.72b	1.76b
External damage	1.52	1.39	1.57	1.47
Force required to remove the peduncle (g)	10.7	10.3	10.4	10.1
			'Lambert'	
Peduncle discoloration[b]	2.34a	2.17b	2.21a,b	2.12b
Skin colour[c]	1.77a	1.63b	1.68b	1.67b
External damage	1.31	1.27	1.49b	1.40b
Force required to remove the peduncle (g)	7.1a	5.7b	6.6a,b	6.7a,b

[a] The mean separation in each row was calculated using the Duncan–Waller Bayesian k ratio (LSD) rule (k = 100) [39]; each figure represents the mean of five replicates of 50 fruits each.

[b] Scored from 1 = completely green to 10 = completely discoloured.

[c] Scored from 1 = completely black to 5 = completely red.

3.3. Tolerance of fruits to irradiation

At doses of 12.5–100 Gy, no adverse effects on external appearance were noted in 'Valencia' oranges, 'Granny Smith' apples, 'Fuerte' avocados, 'Floradade' tomatoes, 'common' mangoes and 'Supreme' cherries. No adverse internal effects were observed at these doses, except in avocados, where some browning of vascular tissue was noted.

3.3.1. Cherries

The results of tests on cherries have been reported elsewhere [31]. Following irradiation of 'Ron's Seedling' cherries at doses of up to 1000 Gy and storage at 10°C, there were no significant effects on the TSS, firmness, external damage or fruit weight. Peduncle discoloration was correlated with dose and averaged 3.40 and 3.58 in fruits irradiated at 600 and 1000 Gy, respectively. Therefore, the fruits were classified as commercially acceptable. Addition of potassium permanganate did not reduce the extent of external damage or peduncle abscission.

There were no significant differences in the firmness, the force required to remove the peduncle from the drupe or the external damage when 'American Bing' or 'Lambert' cherries were irradiated at doses of up to 300 Gy and stored at 1°C for 20 days (Table VI) [31, 39]. There were significant differences in colour, with irradiated fruits tending to remain redder than the controls. Irradiation adversely affected the peduncle discoloration of 'American Bing' but not 'Lambert' cherries. The differences, however, would not be expected to render the fruit unmarketable.

Irradiation at a dose as low as 75 Gy delayed the normal skin coloration of the red skinned cherries 'Lambert' and 'American Bing'. Irradiation may, therefore, have some benefit in prolonging the shelf-life of some cherry cultivars. Further research is required to test this hypothesis.

Other researchers have found significant, though small, deterioration in firmness, flavour and appearance in 'Bing' cherries dosed at 600–800 Gy [41] and adverse texture effects have been reported when the fruits were irradiated [42]. Treatment at 2000–4000 Gy, in combination with refrigeration, enhanced the storage life of 'American Bing' cherries [43].

3.3.2. Lemons

3.3.2.1. Physical attributes

Irradiation did not affect the peel colour of yellow lemons but did affect that of green lemons, with acceleration of yellow coloration at higher doses (Table VII). A significant ($P < 0.01$) interaction between the irradiation dose and the storage duration was due to the stored green lemons yellowing more quickly as the irradiation dose increased. The colour of the non-irradiated green lemons did not change over the 6 week storage period at 15°C.

The calyces of all the irradiated lemons became brown within 2 weeks of storage at 15°C, reminiscent of the phytotoxic response of lemons to C_2H_4 during the commercial degreening processes. The calyces of the non-irradiated lemons remained green throughout the 6 week storage period.

The peel firmness of all the batches decreased over the storage period (Table VII). The green lemons remained firmer than the yellow fruits, even when

TABLE VII. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 15°C FOR 2, 4 OR 6 WEEKS ON THE VARIOUS ATTRIBUTES OF 'LISBON' LEMONS TREATED AS YELLOW OR GREEN FRUIT

Dose (Gy)	Maturity of fruit when treated and weeks in storage					
	Yellow			Green		
	2	4	6	2	4	6
	Peel colour (5% LSD = 0.47)[a]					
0	1.6	1.6	1.4	3.5	3.5	3.4
75	1.8	1.8	1.8	3.5	2.5	2.7
150	1.5	1.7	1.6	3.0	1.9	1.6
300	2.3	1.5	1.2	3.7	2.0	1.6
600	2.0	1.5	1.5	3.7	1.9	1.9
1000	2.4	1.6	1.5	3.7	1.9	1.7
	Peel firmness (5% LSD = 0.66)[b]					
0	5.1	4.5	4.4	6.8	6.7	6.5
75	5.1	4.4	4.3	7.0	4.8	5.6
150	3.8	2.5	2.5	5.9	4.2	4.6
300	4.1	2.6	2.3	5.9	4.9	3.6
600	3.6	2.6	2.3	5.9	4.2	3.5
1000	3.5	2.3	2.0	5.9	3.6	3.3
	Juice volume (mL/lemon) (5% LSD = 6.7)			**Yellow versus green (5% LSD = 4.9)**		
0	43.5	49.9	46.2	46.5	37.4	
75	37.2	37.1	31.2	36.8	28.7	
150	32.8	25.7	19.0	25.8	24.6	
300	33.2	23.5	18.0	24.9	23.9	
600	34.6	32.1	24.1	30.3	23.7	
1000	35.1	28.0	23.5	28.9	19.2	

[a] Peel colour scored from 1 (yellow) to 5 (green).

[b] Peel firmness was measured by a Chatillon penetrometer with a conical probe through the peel.

TABLE VIII. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 15°C FOR 4 OR 6 WEEKS ON DAMAGE TO 'LISBON' LEMONS[a]

Dose (Gy)	Type of damage to fruit and weeks in storage					
	Peel damage		Flesh discoloration		Cavitation	
	4	6	4	6	4	6
(5% LSD)	(0.51)		(0.48)		(0.23)	
0	1.0	1.0	1.0	1.0	1.0	1.0
75	2.0	2.0	1.0	1.2	1.0	1.0
150	2.6	2.9	1.9	2.2	1.0	1.0
300	2.8	3.4	2.0	2.8	1.0	1.2
600	2.4	3.2	1.8	3.0	1.2	1.3
1000	3.7	3.3	2.9	3.1	1.5	1.4

[a] Scored from 1 (no damage) to 5 (severe damage).

TABLE IX. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 15°C ON DAMAGE TO 'LISBON' LEMONS TREATED AS YELLOW OR GREEN FRUIT[a]

Dose (Gy)	Type of damage to fruit and maturity when treated					
	Peel damage		Flesh discoloration		Cavitation	
	Yellow	Green	Yellow	Green	Yellow	Green
(5% LSD)	(0.42)		(0.45)		(0.23)	
0	1.0	1.0	1.0	1.0	1.0	1.0
75	1.8	2.3	1.1	1.0	1.0	1.0
150	2.4	3.1	2.0	2.1	1.0	1.0
300	2.6	3.6	2.7	2.1	1.2	1.0
600	2.5	3.1	2.6	2.3	1.3	1.2
1000	3.0	4.0	3.5	2.5	1.7	1.2

[a] Scored from 1 (no damage) to 5 (severe damage).

TABLE X. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 15°C FOR 4 OR 6 WEEKS ON ALBEDO DISCOLORATION AND TOUGHNESS OF 'LISBON' LEMONS TREATED AS YELLOW OR GREEN FRUIT[a]

Dose (Gy)	Maturity of fruit when treated and weeks in storage					
	Albedo discoloration				Albedo toughness	
	Yellow		Green		Yellow	Green
	4	6	4	6	4	6
(5% LSD)	(0.52)		(0.67)		(0.50)	
0	1.1	1.0	1.0	1.0	1.6	1.0
75	1.1	2.1	1.3	2.0	2.9	3.7
150	2.3	2.8	1.8	3.8	2.8	4.0
300	2.3	4.4	1.3	3.3	3.9	4.1
600	1.7	4.3	1.8	3.8	3.3	4.1
1000	2.8	4.4	3.2	4.2	3.3	4.1

[a] Scored from 1 (no damage) to 5 (severe damage).

the lemons irradiated at the green stage had coloured to the yellow stage after 6 weeks of storage. An increase in irradiation dose caused a significant decrease in the peel firmness.

The amount of juice present in the lemons irradiated at both green and yellow maturities decreased significantly with increasing irradiation dose (Table VII). The irradiated green lemons had less juice than the irradiated yellow lemons up to 4 weeks of storage, but there were no significant differences in the juice quantities after storage for 6 weeks. Prior to treatment, the yellow lemons weighed more than the green lemons. The yellow lemons weighed, on average, 138 g and the green lemons 118 g. After treatment and storage there was no significant decrease in the weight of the green or yellow fruits with irradiation dose or time in storage. The observed decrease in juice volume after irradiation, without a corresponding decrease in fruit weight, suggests that irradiation may have increased the water content of the peel or albedo. The decrease in peel firmness may reflect an increase in the water content.

TABLE XI. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 15°C FOR 2, 4 OR 6 WEEKS ON THE CHEMICAL ATTRIBUTES OF 'LISBON' LEMONS

Dose (Gy)	Chemical attributes of fruit following weeks in storage					
	Total titratable acidity (g of citric acid/100 mL of juice)			Total soluble solids (°Brix)		
	2	4	6	2	4	6
(5% LSD)		(0.42)			(1.10)	
0	6.8	5.8	5.4	8.0	6.7	6.8
75	4.2	4.0	4.2	5.6	5.5	5.5
150	4.2	3.7	3.4	4.9	4.8	5.0
300	3.8	3.0	3.0	5.3	4.3	4.3
600	3.7	3.0	2.1	5.0	4.1	3.6
1000	3.3	2.6	1.8	4.8	4.4	3.2
Maturity (5% LSD = 0.38)						
Yellow	4.3	3.2	3.1			
Green	4.1	3.6	3.5			

3.3.2.2. Damage

Peel damage increased significantly with increasing irradiation dose (Table VIII). The green lemons were more severely affected than the yellow lemons; a dose as low as 75 Gy caused a 25% increase in the severity of damage in the green as compared with the yellow lemons (Table IX).

Irradiation caused severe flesh discoloration and cavitation (Tables VIII and IX) as well as albedo discoloration and toughness (Table X). Flesh discoloration and albedo discoloration occurred to an equal extent in lemons irradiated at either stage of maturity, while albedo toughness and cavitation were more pronounced in the irradiated yellow lemons than in the green lemons (Tables IX and X). Cavitation and flesh discoloration increased in severity as the storage period increased (Table VIII).

TABLE XII. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 15°C FOR 2, 4 OR 6 WEEKS ON THE pH OF 'LISBON' LEMONS TREATED AS YELLOW OR GREEN FRUIT

Dose (Gy)	Maturity of fruit when treated and weeks in storage					
	Yellow			Green		
	2	4	6	2	4	6
(5% LSD = 0.08)						
0	2.27	2.28	2.29	2.31	2.40	2.40
75	2.42	2.43	2.61	2.45	2.43	2.45
150	2.59	2.62	2.61	2.62	2.62	2.60
300	2.57	2.74	2.74	2.60	2.57	2.56
600	2.67	2.65	2.89	2.65	2.61	2.70
1000	2.66	2.75	2.96	2.65	2.72	2.86

3.3.2.3. Chemical attributes

The TTA decreased with increasing time in storage (Table XI). There were no significant differences in the TTA due to lemon maturity after irradiation and storage for 2 weeks at 15°C. However, after 6 weeks the differences were significant, with the TTA of the irradiated yellow lemons being less than that of the irradiated green lemons.

The TSS decreased significantly following irradiation and storage (Table XI). There were no differences in the TSS between maturities following storage at 15°C for 2 and 4 weeks, but after storage for 6 weeks the TSS was greater in the irradiated green lemons than in the irradiated yellow lemons.

The TSS/TTA ratio increased significantly over time for lemons whether they were irradiated or not, but the increase was more marked in the yellow lemons. The increase in the TSS/TTA ratio reflects a normal trend, where the acidity levels decrease more rapidly during storage than do the TSS levels [44]. The irradiated lemons showed a significantly higher TSS/TTA ratio than the non-irradiated control fruits, even though both the TTA and TSS in the irradiated lemons were less than those in the non-irradiated lemons.

The pH of the treated lemons increased with irradiation dose and storage time (Table XII). The highly significant maturity × dose × storage time interaction

appeared to be mostly due to the dose effects on the differences in pH of yellow fruit following storage.

Irradiation would not be a viable quarantine treatment for the disinfestation of green or yellow 'Lisbon' lemons at doses between 75 and 1000 Gy followed by storage at 15°C because of the severe phytotoxicity. In contrast, other researchers have shown that irradiation in combination with storage at 7°C satisfactorily preserved 'Eureka' lemons for 6–7 weeks [19]. Irradiation and storage at the lower temperature may have some application for the disinfestation of 'Lisbon' lemons and should be investigated, particularly in combination with curing the fruits before treatment [45]. Additionally, other studies have shown that 'Eureka' lemons irradiated at 1000 Gy and then stored at 25°C showed no serious phytotoxic symptoms [16]. Studies on the effects of lower doses in combination with storage at 25°C for 'Lisbon' lemons could lead to a quarantine disinfestation treatment for warm climate regions where the costs of refrigeration are prohibitive.

3.3.3. Lychees

3.3.3.1. Pericarp browning

Dipped, non-wrapped lychees irradiated at 0, 75, 150 and 300 Gy and stored at 4°C for 12 days were significantly redder than those dosed at 600 and 1000 Gy. Wrapped lychees stored at 4°C for 34, 37 and 42 days and for 42 days at 4°C plus 2 days at room temperature showed less browning when dipped in benomyl than when not dipped. When irradiated at 0, 75, 150 and 300 Gy, whether dipped or not, the pericarp of the wrapped fruits was redder than that of the fruits treated at 600 and 1000 Gy (Fig. 2) [39].

Non-wrapped lychees irradiated at 75, 150 and 300 Gy and stored at 10°C for 3 and 7 days, whether dipped in benomyl or not, were significantly redder than those irradiated at 0, 600 and 1000 Gy. Wrapped lychees irradiated at 0, 75, 150 and 300 Gy and stored at 10°C for 21, 23 and 26 days showed less skin browning than the wrapped lychees treated at 600 and 1000 Gy (Fig. 2).

Lychees stored at 20°C in a constant flow of humidified pure air for 9 days were significantly browner when not dipped than when dipped. There were no significant irradiation dose effects on pericarp discoloration. Fruits which had been stored and irradiated under a plastic wrap before being removed from their wrapping for C_2H_4 and CO_2 evaluation were redder than those that were stored and irradiated without wrapping (Fig. 3) [39].

3.3.3.2. Mould development

Significantly less mould developed in fruits dipped in a hot benomyl solution than in those not dipped. For lychees stored at 10°C and 20°C, there was no fungi-

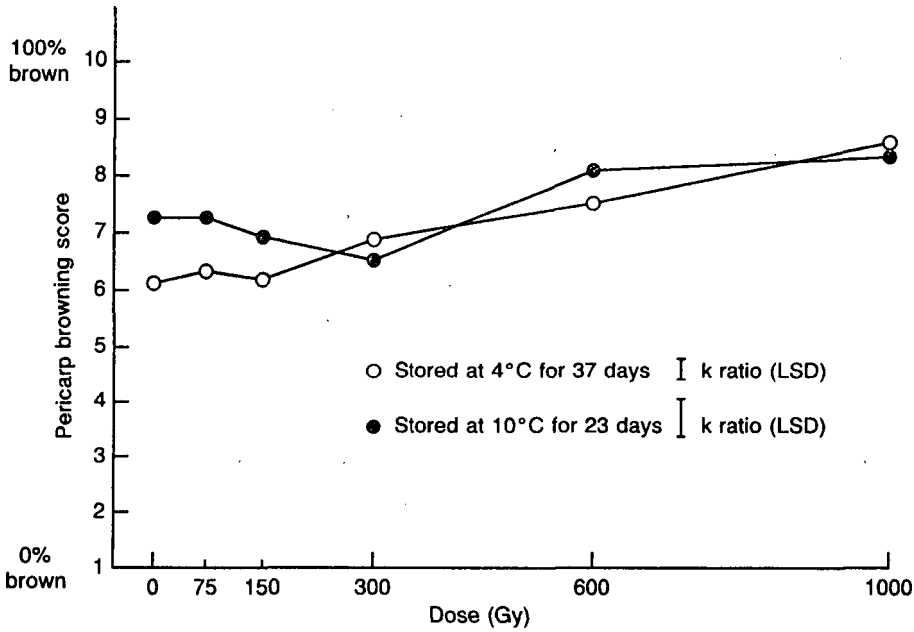

FIG. 2. Effects of irradiation on the pericarp browning of lychees wrapped in plastic at two storage temperatures. The mean separation at k = 100 was calculated using the Duncan–Waller Bayesian k ratio (LSD) rule [39].

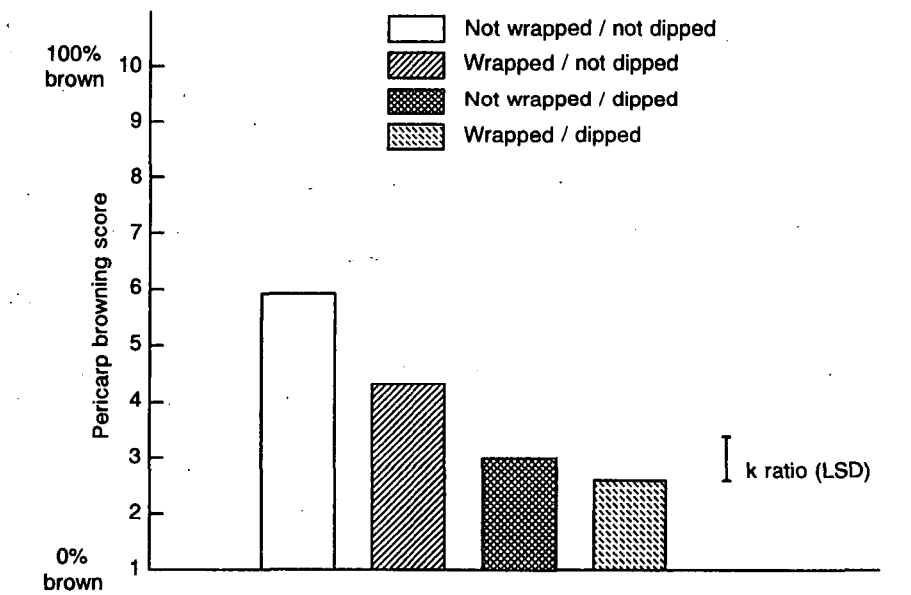

FIG. 3. Effects of a plastic wrap and benomyl antifungal dips on the pericarp browning of lychees treated at 300 Gy and stored for 9 days at 20°C in a constant flow of humidified pure air. The mean separation at k = 100 was calculated using the Duncan–Waller Bayesian k ratio (LSD) rule [39].

TABLE XIII. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 4°C FOR 34, 37 OR 42 DAYS ON MOULD DEVELOPMENT OF WRAPPED 'BENGAL' LYCHEES DIPPED OR NOT DIPPED IN BENOMYL FUNGICIDE[a]

Dose (Gy)	Storage period (days) of fungicide treated lychees					
	34		37		42	
	Dip	No dip	Dip	No dip	Dip	No dip
(5% LSD = 0.38)						
0	1.00	1.08	1.08	1.97	2.14	3.31
75	1.00	1.02	1.12	2.28	2.57	3.59
150	1.00	1.00	1.15	1.89	2.39	3.61
300	1.02	1.05	1.31	1.76	3.56	3.62
600	1.01	1.02	2.02	2.11	3.61	4.00
1000	1.00	1.02	2.06	2.10	4.81	4.32

[a] Mould development rated from 1 (<20% of the surface damaged by mould) to 5 (>80% damaged); fruits that scored >1 were commercially unacceptable.

cide interaction with irradiation on mould development. However, lychees stored at 4°C for 42 days and those stored at 4°C for 42 days plus 2 days at room temperature developed more surface mould when treated at 600 and 1000 Gy than when treated with 0, 75, 150 and 300 Gy (Table XIII). Non-wrapped lychees from the 4°C and 10°C treatments developed browning to 100% of the pericarp before developing mould and were discarded as being commercially unacceptable.

3.3.3.3. Sensory evaluation

There were no detectable taste differences in the wrapped or non-wrapped lychees stored at 4°C for 30 days between the untreated lychees and those receiving doses of 75, 300 or 1000 Gy. There were no significant taste differences between the wrapped fruits stored at 10°C for 26 days, dipped or not dipped, as a result of the irradiation dose.

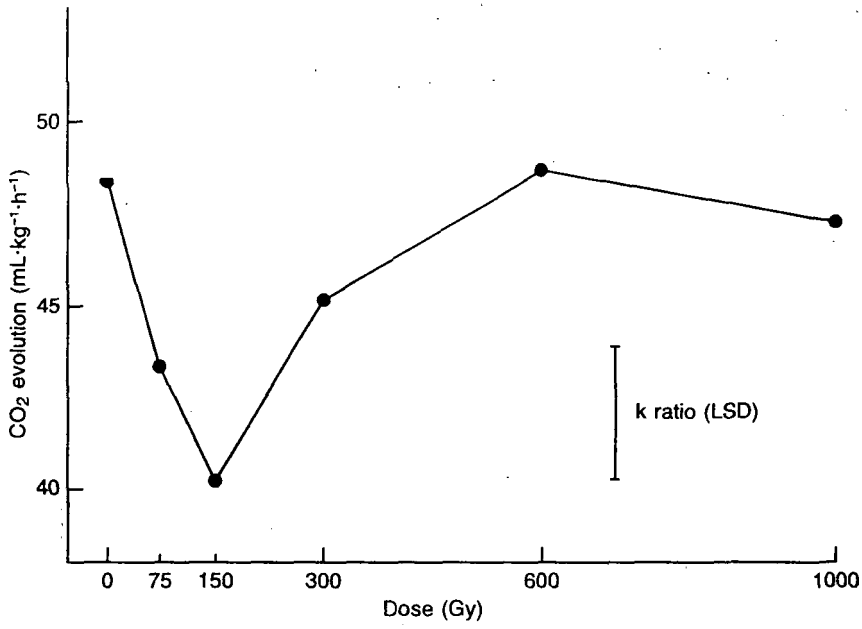

FIG. 4. Effects of irradiation on the carbon dioxide (CO_2) evolution by benomyl dipped lychees stored for 9 days at 20°C in a constant flow of humidified pure air. The mean separation at k = 100 was calculated using the Duncan–Waller Bayesian k ratio (LSD) rule [39].

3.3.3.4. Ethylene production and respiration

There were no dose effects on the C_2H_4 production over a period of 7 days after irradiation. A significant increase in the C_2H_4 evolution was seen from days 4 to 6 in non-dipped fruits compared with dipped fruits, but the levels of C_2H_4 remained very low (< 1 L·kg^{-1}·h^{-1}) throughout irradiation.

There were significant effects of the wrap and dip on the CO_2 evolution from lychees over a period of 7 days after irradiation, where both the presence of a wrap for 2 days before and during irradiation and a fungicidal dip 2 days prior to irradiation caused a decrease in the level of CO_2 production. Analysis of variance on the results of dipped fruits for the period 3–6 days after irradiation showed that there was an effect of irradiation dose on the CO_2 evolution where fruits treated at 75 and 150 Gy produced significantly less CO_2 than the control fruits and those treated at 300, 600 and 1000 Gy (Fig. 4) [39].

3.3.3.5. Discussion of lychee research

Although there were statistical differences in pericarp discoloration and mould development between wrapped lychees stored at 4°C and either dipped or not

dipped, it is doubtful whether the differences between the mean scores would be economically appreciable. In these trials, it appeared that when wrapped lychees were stored at 4°C the fungicidal dip treatment was not crucial to the maintenance of pericarp colour and mould control. Storage of lychees at 4°C was more useful in preserving the skin colour in non-wrapped lychees (by about 2 days) than storage at 10°C, but when wrapped and dipped there was little difference in the levels of pericarp discoloration between fruits stored at 4°C and 10°C. Additionally, there was no difference in rot development in fruits stored at 10°C compared with those stored at 4°C if they were dipped in a benomyl solution and wrapped in plastic film.

Because of the differing effects of irradiation on lychees stored under normal atmospheric conditions at 4°C and 10°C and under the modified atmosphere utilized for respiration studies, it was difficult to correlate the irradiation effects on CO_2 production with skin colour development between the two atmospheric systems. For lychees stored under normal atmospheric conditions, doses of 600 and 1000 Gy caused an acceleration of pericarp browning and mould development. However, when the lychees were stored under a modified atmospheric regime (a constant flow of humidified air free of C_2H_4), the fruits treated at 600 and 1000 Gy were not significantly different from the non-irradiated fruits or those dosed at 75–300 Gy after 9 days storage at 20°C, with no significant difference in the level of mould contamination. The possibility that C_2H_4 may have mediated irradiation induced damage [46] appeared to be discounted by the very low levels of C_2H_4 produced during storage, there being no major differences in the C_2H_4 production between the control and the irradiated fruits. An irradiation dose of 75–300 Gy had no effect on the pericarp colour or the mould development when compared with the untreated fruits. Such a dose range would be beneficial as a post-harvest treatment for disinfestation against several fruit fly species of quarantine importance [3, 4, 47].

Storage of fruits in the modified atmosphere contained under the plastic wrap for 2 days prior to irradiation, then during irradiation and for 8 hours thereafter, appeared to reduce the level of pericarp discoloration seen in the irradiated, non-wrapped fruits. Several researchers [48–50] have indicated that oxygen tension at the time of irradiation may reduce the tissue browning which is normally catalysed by oxidase enzymes. The plastic wrap used in the trials reported in this paper was impermeable to CO_2 and thus allowed a build-up of CO_2 under the wrap, thereby creating an oxygen tension which may have reduced the adverse effects of irradiation seen under normal atmosphere storage. If this is the case, then research is required to determine whether this amelioration would also extend to insect pests, thus requiring higher disinfestation doses, as is the case in irradiation of fruit fly puparia under a nitrogen atmosphere for the production of aggressive adults for sterile insect release programmes [51, 52].

TABLE XIV. EFFECTS OF GAMMA IRRADIATION AND SUBSEQUENT STORAGE AT 10°C FOR 4 WEEKS ON THE ASCORBIC ACID CONTENT OF 'VALENCIA' ORANGES. ANALYSES WERE PERFORMED 12 DAYS (AND AT 26 DAYS FOR THE 0 AND 300 Gy TREATMENTS) AFTER THE SAMPLE WAS TAKEN AND FROZEN

Dose (Gy)	No. of samples[a]	Ascorbic acid content (mg/100 mL of juice)	Variance
0	18	5.43	0.37
0	18	5.18	0.30
25	18	5.36	0.20
50	18	5.07	0.35
75	18	4.93	0.19
100	18	5.26	0.24
150	18	4.97	0.29
300	16	5.41	0.34
300	18	5.43	0.07

[a] Pooled data for nine paired titrations in three replicate irradiations.

3.3.4. *Oranges*

No differences were found in the ascorbic acid content of non-irradiated oranges and those treated at 25–300 Gy (Table XIV). It was therefore unnecessary to analyse for dehydroascorbic acid, since gamma irradiation, in the dose range studied in these experiments, did not cause the reduction of ascorbic acid to dehydroascorbic acid. It is probable that the reduction reaction occurs after irradiation at higher doses, although reports are inconsistent. At 800 Gy, there was no decrease in ascorbic acid in carrots [53], but there was at 10 000 Gy, when a corresponding increase in dehydroascorbic acid was noted [27]. There were no changes in the ascorbic acid content of papaya dosed at 1000 Gy [54] or of papaya, mangoes, strawberries or lychees dosed at 2000 Gy [21]. It appears that if irradiation does decrease the vitamin C content of fresh produce, it occurs at a higher dose than that required for quarantine security (300 Gy) against pests [7]. Gamma irradiation used as a quarantine disinfestation treatment will not adversely affect the vitamin C content of 'Valencia' oranges.

ACKNOWLEDGEMENT

The co-operation received from the IAEA is gratefully acknowledged.

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EFFECTS OF IONIZING ENERGY ON FRUIT FLIES AND SEED WEEVIL IN AUSTRALIAN MANGOES*

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Abstract

EFFECTS OF IONIZING ENERGY ON FRUIT FLIES AND SEED WEEVIL IN AUSTRALIAN MANGOES.

Irradiation was evaluated as a quarantine measure for the disinfestation of Australian mangoes against two species of fruit fly, *Bactrocera* (= *Dacus*) *tryoni* (Froggatt) and *B. jarvisi* (Tryon), and the mango seed weevil, *Sternochaetus mangiferae* (Fabricius). For the fruit flies, the third instar stage was determined as the most tolerant of irradiation, but disinfestation trials were also undertaken against mature eggs as the stage most likely to be present in any infested fruit at the time of treatment. A dose range of 74–101 Gy on the 'Kensington' variety of mango prevented the emergence of adult flies, but large numbers of treated larvae and some eggs developed to the pupal stage. This places important emphasis on the field control of fruit flies if detection of still living larvae in fruit at inspection is to be avoided. Disinfestation trials on the mango seed weevil were complicated by the inability to culture this insect in the laboratory. This necessitated the use of naturally infested fruit of the 'common' variety and precluded trial work on specific stages of known age. A dose range of 298–339 Gy (nominally a minimum of 300 Gy) prevented adult emergence and ensured 100% mortality by 8 months on the samples treated. The slow life cycle of the seed weevil requires that assessment of mortality be delayed by a minimum of 1 month, and for full mortality up to 6–8 months. The presence of live insects in irradiated fruit causes problems if detected at inspection unless export fruit is obtained from weevil free orchards. No significant fruit damage would be expected at a fruit fly treatment range of 100–200 Gy, but the maximum/minimum ratio for weevil treated fruit would need to be minimized as the treatment level is near the threshold for damage to the 'Kensington' variety of mango.

* Research carried out in association with the IAEA under Research Agreement No. 4055/CF.

1. INTRODUCTION

The deregistration of ethylene dibromide by the authorities in the United States of America in 1984 [1] for quarantine disinfestation purposes and the announced intention of most other countries to phase out its use has stimulated research on alternatives. Irradiation is particularly appropriate as a disinfestation procedure with high levels of security possible at low doses. It is appropriate across a wide range of fresh fruits and vegetables and has the added advantage of leaving no residues.

In Queensland, and elsewhere in Australia, use of irradiation has been examined for a number of purposes [2–5], the most important of which is as a quarantine disinfestation treatment against fruit flies (Tephritidae) and the seed weevil, *Sternochaetus mangiferae* (Fabricius), in mangoes [6]. In the Co-ordinated Research Project between the International Atomic Energy Agency and the Queensland Department of Primary Industries (QDPI), we specifically examined the possible use of irradiation as a disinfestation treatment against the Queensland fruit fly, *Bactrocera* (= *Dacus*) *tryoni* (Froggatt), and Jarvis' fruit fly, *B. jarvisi* (Tryon), together with the seed weevil, as pests of mangoes for export from Australia. Some export markets have mandatory disinfestation requirements against all three species, others against only the fruit flies. Therefore, development of separate disinfestation schedules is warranted.

2. OBJECTIVES

The overall objectives of this research programme are:

(1) To determine the comparative tolerance to irradiation of the life stages of relevant Australian tephritid fruit flies

(2) To undertake disinfestation trials against fruit flies and other relevant pests on fruits of export significance to determine the dose needed to achieve quarantine security.

3. MATERIALS AND METHODS

3.1. Test insects

The two species of fruit fly are maintained in culture at the Indooroopilly entomology laboratories of the QDPI using the culture methods detailed by Heather and Corcoran [7] for *B. tryoni*. Comparative tolerance studies were undertaken on eggs and larvae of known ages and stages of development [8].

3.2. Test fruits

The mangoes used for disinfestation testing against the fruit fly were obtained exclusively from north Queensland. Low levels of insecticide residue were a major requirement. The mangoes used for seed weevil disinfestation were also obtained in north Queensland from old trees with high natural levels of infestation.

3.3. Infestation

The fruits used for fruit fly disinfestation were cage infested. They were pinholed in accordance with our laboratory practice in order to facilitate oviposition in each fruit and exposed for 20–30 minutes in cages containing about 25 000 flies, approximately half of which were gravid females at peak levels of fecundity. One fruit in every six was segregated and grouped as an untreated control. The number of puparia which developed from the larvae infesting these fruits was used to estimate ($\times 5$) the number of individuals present in fruits at the time of treatment. The infested fruits were held for emergence of any adult fruit flies at 26°C and 65% relative humidity. Eclosion of adults from the untreated fruits was estimated from the subsamples.

Infestation of the mango seed weevil could not be undertaken in the laboratory. We do not have a laboratory culture method for this insect and the insect's habit of infesting partly grown fruit on the tree is difficult to emulate. It is known that oviposition by this weevil can occur at any time up to fruit maturity, but these later infestation times may not achieve sufficiently developed larvae to effectively test the quarantine security conferred by the treatment. On this basis, we used field infested mangoes exclusively, even though the available 'common' variety differs from the commercial variety 'Kensington'.

3.4. Treatment

Comparative studies on the stages of the two fly species were undertaken using graded doses of irradiation applied to the eggs or larvae in vitro using a Gammacell-220 (Atomic Energy of Canada Limited, Ottawa, Ontario) available near our Indooroopilly laboratories. The dose was calculated by extrapolation from a calibration measurement [9]. To reduce the heterogeneity of the data, irradiation was undertaken in a short term nitrogen atmosphere achieved by enclosing the insects to be irradiated in a sealable plastic bag triple flushed with pure nitrogen. Disinfestation treatments were undertaken in the pilot scale batch irradiator GATRI operated by the Australian Nuclear Science and Technology Organisation, Sydney. Because of the distance from our laboratories (1000 km), samples were transported by air overnight or directly by road within 1 day.

For fruit flies, we used a minimum nominal dose of 75 Gy. In practice, this resulted in a dose range of 74–101 Gy and maximum/minimum ratios between 1.05 and 1.3, achieved by careful calibration.

For the mango seed weevil, we used the International Consultative Group on Food Irradiation recommended minimum generic dose of 300 Gy [10]. This resulted in a dose range of 298–339 Gy and narrow max/min ratios between 1.06 and 1.13.

3.5. Dosimetry

The dosimetry for Gammacell calibration, GATRI calibration and treatment monitoring was undertaken by the Fricke method.

3.6. Estimation of mortality

For fruit flies, the efficacy of treatments was estimated on the basis of adult survival. The puparia that developed from larvae treated in the infested fruits were held at 26°C and 65% relative humidity for the possible eclosion of normal adults. The number treated was estimated on the basis of the number of puparia from the untreated fruits.

Mango seed weevil treated fruits were held for the possible emergence of adults from the seed. A subsample of fruit was dissected at treatment to determine the proportion of each stage present and the remaining seeds, treated and untreated, were dissected 7–8 months after treatment.

3.7. Data

The results of multiple dose irradiation treatments on fruit fly eggs and larvae were subjected to log dose probit analysis using an unpublished programme [11] based on Finney [12]. Use of this method to predict probit 9 values tends to lead to overestimation of the dose needed because of non-linearity with these transformations. Omissions of the log transformation can improve linearity in some instances, but this was not undertaken on the data reported on here.

4. RESULTS

4.1. Fruit flies

Log dose probit data for the egg and larval stages of both species showed 6 day old mature third instars to be the most tolerant stage assayed (Table I). Within the egg stage, tolerance increased with development time. These results confirm that, as for other species, the third instar is the most tolerant stage found in fruit. Because

TABLE I. MORTALITY OF 2 AND 30 HOUR OLD EGGS AND 6 DAY OLD LARVAE (THIRD INSTAR) OF *B. tryoni* AND *B. jarvisi* TO IRRADIATION IN A NITROGEN ATMOSPHERE ON THE BASIS OF ADULT EMERGENCE

Species	Stage	Age	LD_{50} (Gy) (95% fiducial limits)	$LD_{99.9}$ (Gy) (95% fiducial limits)	Slope (±SE)
B. tryoni	Eggs	2 h	0.137 (0.120–0.153)	1.839 (1.308–2.973)	2.64 (0.43)
	Eggs	30 h	7.149 (6.076–7.941)	16.612 (13.206–28.527)	8.44 (1.60)
	Larvae	6 d	41.380 (40.867–51.875)	62.705 (60.807–64.996)	17.12 (0.72)
B. jarvisi	Eggs	2 h	0.078 (0.066–0.089)	1.060 (0.782–1.624)	2.73 (0.24)
	Eggs	30 h	39.016 (34.684–47.883)	166.655 (95.491–1169.258)	4.88 (1.28)
	Larvae	6 d	30.009 (24.710–37.241)	73.934 (49.707–786.818)	7.89 (2.46)

of the heterogeneity of response, LD_{50} is judged to be the best point of comparison of stages and species. On the basis of the non-overlap of 95% fiducial limits, *B. jarvisi* is significantly less tolerant of irradiation than *B. tryoni* at both the immature egg and third instar stages. The 30 hour old eggs of *B. jarvisi* were significantly more tolerant than those of *B. tryoni*, but did not differ significantly from the third instars of either species.

Confirmatory trials on 100 000 eggs and larvae of each species in fruit (three trials against the eggs and larvae of each species) showed that probit 9 security was achieved with the doses used, namely 74–101 Gy (Table II). Large numbers of puparia resulted from these treatments, but no adults eclosed, despite the favourable holding conditions. Eclosion from the puparia of untreated eggs and larvae averaged 75% for *B. tryoni* and 82% for *B. jarvisi*.

4.2. Seed weevil

The results of multiple sampling over an 11 week period after irradiation showed that mortality is incomplete for 4 weeks (Fig. 1). Preliminary observations

TABLE II. RECOVERY OF PUPARIA AND ADULTS FROM 1 DAY OLD EGGS AND 5 DAY OLD THIRD INSTARS OF *B. tryoni* AND *B. jarvisi* TREATED IN 'KENSINGTON' MANGOES AT A GAMMA RADIATION DOSE OF 74–101 Gy. THE MEAN ADULT EMERGENCE FROM THE UNTREATED FRUITS WAS 75% for *B. tryoni* AND 82% FOR *B. jarvisi* *(Totals for three trials)*

Species	Stage	No. of fruits		No. of insects treated[a]	Recovery		
		Not treated	Treated		Untreated puparia	Treated	
						Puparia	Adults
B. tryoni	Eggs	269	1316	208 604	42 147	475	0
	Larvae	162	810	138 635	27 727	11 099	0
B. jarvisi	Eggs	179	895	110 935	22 187	482	0
	Larvae	215	1083	153 814	30 277	17 851	0

[a] The number treated was estimated from the ratio of treated to control (untreated) fruits times the number of puparia recovered from the control fruits.

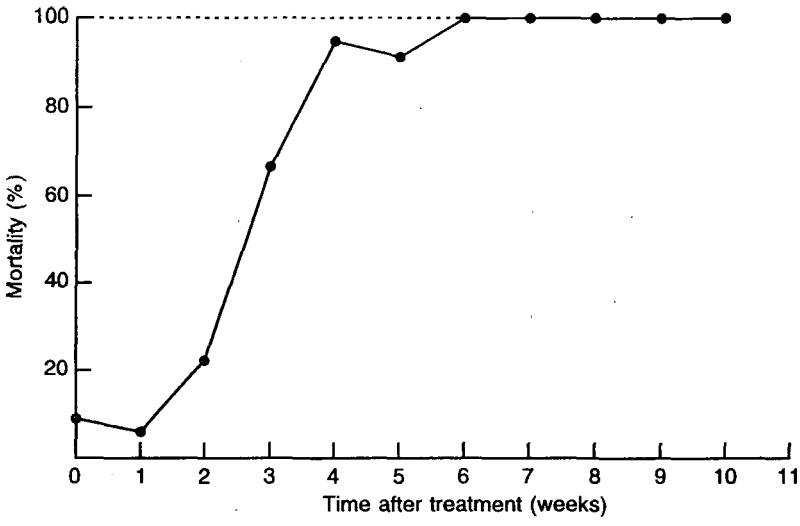

FIG. 1. Mortality (%) of seed weevil in samples at weekly intervals after irradiation at 300 Gy.

had shown occasional surviving larvae after 3 months. However, in subsequent trials with large numbers of fruit no emergence occurred from the infested fruits treated at 298–339 Gy (nominally a minimum of 300 Gy), and dissection at 7 months showed live adult survivors in the untreated fruits but none in the treated fruits (Table III).

5. DISCUSSION

The fruit fly irradiation trials achieved probit 9 security (99.9968% mortality) demonstrated at the 95% probability level as no adult survivors from 100 000 treated individuals. The dose used was a minimum of 75 Gy and was based on the treatment level shown to be effective in oranges and avocados by Rigney and Wills [5]. However, because the actual dose ranged from 74–101 Gy, probit 9 security was only proven at the 101 Gy level. On this basis, some quarantine authorities may require 100 Gy, although this would overestimate the dose needed for probit 9. On this basis, a commercial treatment could apply 200 Gy to some fruits, assuming a 2.0 max/min ratio.

McLauchlan et al. [3] undertook a series of related trials with mango in which the physiological characteristics of the fruit were assessed at a range of possible quarantine doses. They concluded that a minimum dose of 75 Gy with a max/min ratio of 1.28 would not adversely affect the marketability of the 'Kensington' mango,

TABLE III. MORTALITY OF THE MANGO SEED WEEVIL (7 MONTHS AFTER IRRADIATION AT 298–339 Gy) OF INFESTED FRUITS CONTAINING LARVAE, PUPAE AND ADULTS. THE NUMBERS TREATED WERE ESTIMATED FROM A SUBSAMPLE DISSECTED AT THE TIME OF TREATMENT

Fruits	No. of fruits	No. of insects treated			No. of survivors (7 months)		
		Larvae	Pupae	Adults	Larvae	Pupae	Adults
Untreated	522	32	354	108	0	0	110
Treated	1956	1065	725	161	0	0	0

the predominant commercial variety in Australia. Doses up to a minimum of 300 Gy at a max/min ratio of 1.3 and dose rates of 6.2–6.6 Gy/min were tested and judged not to cause unacceptable fruit damage, although some lenticel enlargement and minor skin scalding could be present.

Boag et al. [13], also participants in the QDPI fruit irradiation programme, investigated the conditions under which unacceptable damage might result from disinfestation doses of irradiation. They concluded that for the 'Kensington' mango maturity at irradiation would be a major factor as to whether the fruit might be damaged. Fruit picked as immature as possible in an effort to maximize the time available for transport to distant markets was most prone to damage. Other participants in the QDPI fruit irradiation programme [14] examined the effect of irradiation on post-harvest disease control. They concluded that at the disinfestation dose used no significant post-harvest disease control occurred in mangoes and that a hot water + fungicide treatment would still be required.

The outcome of these supplementary studies was that 'Kensington' mangoes would withstand irradiation doses for fruit fly disinfestation, but that the doses for mango seed weevil disinfestation would be marginal for damage. Since only a very few markets are concerned with the mango seed weevil as a quarantine pest, most export trade can be developed on the basis of fruit fly disinfestation. For Australia, the major market concerned with seed weevil is the USA. Currently, mangoes do not have sufficient post-harvest life to be transported to the USA by sea; air freight is the only practicable means of transport. If irradiation were to be approved for both fruit fly and seed weevil disinfestations for this market, relatively mature fruits could be used; this would minimize damage.

A major concern with irradiation disinfestation is the continued development of many eggs and larvae to the pupal stage, where death invariably occurs. For the fruit fly, this means that field pest management must be of a high order if the detection of larvae in fruit at post-treatment inspection is to be avoided. In any event, it is an unwise policy to rely on disinfestation treatments to make up for shortfalls in field control.

The mango seed weevil presents a different problem, since field control has been shown to be difficult. Neither hygiene programmes nor insecticide sprays can ensure freedom of fruits from seed weevil in any year [15], although they can reduce infestations to a low level.

However, because new plantations of mango established in isolation from other older trees are generally free of seed weevil for the first 10 years, export fruits could be taken from these areas. It is likely that a well executed plantation quarantine programme could extend the weevil free period indefinitely. Plantations can be monitored for the presence of seed weevil by destructive sampling at levels commensurate with the level of risk deemed acceptable. Irradiation could then provide quarantine assurance without complications of larval interception and concern about whether the fruits had been irradiated.

ACKNOWLEDGEMENT

The co-operation received from the IAEA is gratefully acknowledged.

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GAMMA IRRADIATION AS A QUARANTINE TREATMENT FOR CARAMBOLA, PAPAYA AND MANGO*

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Abstract

GAMMA IRRADIATION AS A QUARANTINE TREATMENT FOR CARAMBOLA, PAPAYA AND MANGO.

Preliminary experiments carried out on the effects of irradiation on carambola (*Averrhoa carambola* L.), papaya (*Carica papaya* L.) and mango (*Mangifera indica* L.) with regard to fruit fly treatment, fruit injury and the physicochemical and organoleptic properties showed that irradiation can be successfully developed and should be investigated further as a quarantine treatment for these fruits. Emergence of normal adult fruit flies of the *Dacus dorsalis* complex did not occur when infested carambolas were treated at doses as low as 100 Gy. Carambola showed external symptoms of injury at irradiation doses in excess of 200 Gy. There appeared to be some reduction in sugar content at doses exceeding 100 Gy. Papaya, cv. 'Eksotika', tolerated irradiation up to 300 Gy. Irradiation at this dose did not alter

* Research carried out with the support of the IAEA under Research Contract No. 5041/RB.

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the ripening behaviour, nor did it cause any injury or alter the organoleptic properties of the fruit. An additional benefit was that doses above 250 Gy significantly reduced freckling of the fruit and enhanced its cosmetic value. 'Eksotika' is an ideal candidate for quarantine treatment using gamma irradiation. Mango, cv. 'Harumanis', tolerated irradiation fairly well. Exposure of fruit to doses of up to 750 Gy did not produce significant injury.

1. INTRODUCTION

The fruit industry in Malaysia is important from an economic, social and nutritional standpoint [1]. Apart from the pineapple, fruit cultivation has traditionally been confined to smallholders and it is estimated that there are about 135 000 such holdings widely scattered throughout Peninsular Malaysia [2]. Malaysia has a rich diversity of native tropical fruits with an excellent potential for commercial development. It is also endowed with a favourable climate that enables cultivation of a variety of introduced fruits. However, in the past fruit production did not receive the attention it deserved because the mainstream of development was directed towards rubber, palm oil and cocoa. Realizing the vast unexploited potential of tropical fruits, the government is now strongly supporting the systematic expansion of the fruit industry and the cultivation of fruits on a commercial scale, particularly for the export market.

Three fruit types, carambola (*Averrhoa carambola* L.), papaya (*Carica papaya* L.) and mango (*Mangifera indica* L.), are now being exported in significant quantities to Hong Kong, Singapore and several European countries where the plant quarantine regulations are not restrictive to the fruit trade. For example, in 1988 exports of carambola amounted to 6000 tonnes valued at about Malaysian $15 million (US $5.5 million), while in 1988 exports of Malaysia's 'Eksotika' papaya rose to 24 000 tonnes valued at Malaysian $12 million (US $4.4 million) [3]. These export figures are expected to increase rapidly over the next few years, since demand currently exceeds the supply of fruit available [3].

As with many other crops, these fruits are not spared the ravages of pests. Tephritid fruit flies, which are endemic to Peninsular Malaysia, attack and damage all three of these fruit types. Besides causing losses in the field [4], these flies are also of major quarantine importance and prevent the free trade and movement of these fruits. However, lucrative markets such as Australia, Japan and the United States of America are not open to these fruits, which are being successfully exported to other countries. This is primarily due to the quarantine restrictions placed on the fresh fruits imported into the three above mentioned countries because of fruit fly infestation [5]. To overcome such barriers, Malaysia is currently investigating a number of post-harvest disinfestation procedures that would meet the quarantine and regulatory requirements of importing countries. For example, the vapour heat treatment procedure is being investigated for export of the fruits to Japan, while high tem-

perature forced air, low temperature storage and other methods are also being looked into [4].

Currently, Malaysia mainly exports fruit to Singapore, Hong Kong and some countries in Europe where no quarantine treatments are required [3]. In the past, research into the post-harvest disinfestation of fresh fruits received little attention. Apart from some preliminary work on the use of dimethoate dips for tomato and carambola [6], no other local data are available on post-harvest disinfestation or quarantine treatments.

Some studies have been carried out on the effects of gamma irradiation on the storage life of papaya [7] and its chemical effects on papaya and mango in Malaysia [8]. However, there is a dearth of information on the use of irradiation as a quarantine treatment for Malaysian fruits. Low dose irradiation is being investigated as a post-harvest quarantine treatment to permit export of fruits to markets currently restricted by fruit fly quarantine regulations. The objective of a Research Contract with the International Atomic Energy Agency was to generate basic data on the response of insects to irradiation dose and associated fruit injury and the physico-chemical changes in irradiated carambola, papaya and mango. These data would be used as the basis for negotiations with the regulatory authorities of various countries with a view to considering irradiation as an acceptable quarantine treatment for the export of carambola, papaya and mango from Malaysia. With the completion of a 2 MCi commercial scale irradiation plant (Sinagama) at the Unit Tenaga Nuklear, Dengkil, Malaysia, in late 1988, commercial application of irradiation as a quarantine treatment is currently available.[1]

The paper outlines the experimental data generated from studies carried out on the effects of gamma irradiation on fruit flies of the *Dacus dorsalis* complex that infest carambola [9], together with the physical, chemical, physiological and sensory studies conducted on irradiated carambola, papaya and mango.

2. EXPERIMENTAL METHODS

2.1. Irradiation facilities

A ^{60}Co gamma cell irradiator (GC 4000A, Bhabha Atomic Energy Research Centre, Bombay, India) located at the Tun Ismail Atomic Research Centre (Puspati) was used for most of the experiments at a dose rate of 76.5 Gy/min (November 1985). The dose uniformity (maximum/minimum ratio) for these experiments was determined as 1.2. The Sinagama facility was used for experiments where a large number of fruits was treated.

[1] 1 Ci = 3.70×10^{10} Bq.

2.2. Fruit fly rearing

Fruit flies were reared on a carrot medium diet [10] in a 4 m × 3 m × 3 m room at a temperature of 27°C ± 2°C and 65–75% relative humidity. Fluorescent lighting (320 W) provided illumination, with a photoperiod of 12:12 (light:dark) achieved through electric timers.

2.3. Fruit fly mortality

2.3.1. Immature insects treated in carrot medium

Fruit fly eggs were collected over a 4 hour period from a colony of laboratory reared adults, placed in carrot based rearing medium and held in an incubator at 27°C ± 1°C until the egg or larval stage was attained:

(a)	Eggs	:	20 hours
(b)	First instar	:	30 hours
(c)	Second instar	:	2–3 days
(d)	Third instar (early)	:	4–5 days
(e)	Third instar (late)	:	6–7 days

The eggs were collected and suspended in water thickened with carboxy methyl cellulose and dispensed with a calibrated syringe on to about 60 g of the carrot medium in a Petri dish. Each treatment consisted of four such Petri dishes, to give four replicates. The doses used for this experiment were 0, 25, 50, 100, 250 and 500 Gy.

Since the irradiation treatment facility was located 28 km from the rearing facility, a set of mobile controls was used for each immature stage tested.

Following irradiation, all the treated fruits, including the controls, were placed in an incubator at 27°C ± 1°C and 65–75% relative humidity and held over sterilized sawdust for larval pupation and subsequent adult emergence.

2.3.2. Immature insects treated in carambola

Pest and pesticide free carambola fruits were purchased from selected farms for this study. The fruits chosen were at Harvest Index 2 (colour less than 25% yellow on the skin) [11]. Each fruit was inoculated with 100 fruit fly eggs in the laboratory and held at 27°C ± 1°C in a walk-in constant environment chamber until the required developmental stages (eggs and first, second and later third instars) were reached.

The infested fruits, together with the uninfested carambola that served as the mobile controls, were then transported to the irradiation facility and treated at doses of 0, 25, 50, 100 and 250 Gy. Four fruits were used for each dose, with each fruit

representing one replicate. Following treatment, the infested fruits were transported back to the controlled environment chamber where they were held for larval pupation and adult emergence.

2.3.3. Small scale tests of third instars treated in carambola

Carambola fruits (Harvest Index 2) were purchased from selected growers and exposed to mature flies in a cage for approximately 40 minutes. Following oviposition, the fruits were held at 27°C ±1°C for 5 days until the larvae reached the third instar. Twenty per cent of the infested fruits were held as controls, while the remaining 80% were irradiated. Twenty fruits were used for each dose tested. The fruits were irradiated at 0, 10, 20, 40 and 80 Gy and held for larval pupation and adult emergence, as described previously.

2.3.4. Large scale tests of third instars treated in carambola

These experiments were conducted following the recommendations of the Task Force Meeting of the International Consultative Group on Food Irradiation, which recommended a minimum dose of 150 Gy to prevent the emergence of normal fruit fly adults from the pre-adult stages [12]. Carambola fruits (Harvest Index 2) were purchased from selected farms for these tests. Each fruit was inoculated with 100 early third instars (5 days old, reared on artificial medium). The fruits were stored for 1 day at 27°C ± 1°C and transported to the gamma cell irradiator (including the mobile controls) where they were treated at 150 Gy. Ten per cent of the fruits were held as controls, while the rest received irradiation treatment. Following treatment, the fruits were held over sawdust at 27°C ± 1°C and the number of puparia formed and adults emerging was recorded.

2.4. Fruit disease incidence, injury and quality

2.4.1. Carambola

Premium quality carambolas (cv. 'B10'), free of pests and blemishes, were selected at Harvest Index 2 for evaluation of the effects of irradiation on disease incidence, phytotoxicity, quality and other responses.

In the first experiment, the fruits were treated at 0, 25, 50, 100, 250, 500 and 1000 Gy in the gamma cell irradiator. Eight fruits were used for each dose. The fruits were then divided into two lots and stored separately at either 7°C for 6 weeks or at 23°C for 1 week. After storage, the fruits were observed for differences in skin colour and discoloration (using a scale of 1–5), disease, rust, dark ribs and other signs of irradiation injury (each using a scale of 0–3).

A second experiment was conducted to determine more accurately the irradiation levels that cause injury, particularly in the 100–250 Gy range. The fruits were treated with 0, 100, 150, 200 and 250 Gy at the Sinagama facility and stored at 23°C for 1 week. After storage, the fruits were observed for differences in skin colour, disease, rust and dark ribs, as described above. Twelve fruits were used at each dose.

In a third experiment, premium quality carambola fruits were exposed to 0, 25, 50, 100, 250, 500 and 1000 Gy in the gamma cell irradiator. Eight fruits were treated at each dose. The following parameters were measured 1 day after irradiation: weight, colour (using a scale of 1–5) [11], firmness (using an Instron textural machine), total soluble solids (°Brix), pH and per cent moisture (oven drying). The sugar composition (fructose, glucose and sucrose) was also measured using high performance liquid chromatography.

2.4.2. Papaya

Premium quality papayas, cv. 'Eksotika', free of pests and blemishes, were selected for evaluation of the irradiation effects on disease incidence, quality, phytotoxicity and other responses.

Papayas were irradiated in the gamma cell irradiator at doses of 0, 50, 100, 200 and 300 Gy. Five fruits were treated at each dose. All the fruits used were of uniform ripeness, with a tinge of yellow, and were stored at 20°C. The respiration rate, ethylene evolution and skin colour changes were determined daily for 9 days.

In a second experiment, the papayas were treated with 0, 250, 500, 750 and 1000 Gy at the Sinagama facility. Eight fruits were treated at each dose. After irradiation, half the fruits were stored at 23°C for 1 week and the remainder at 7°C for 18 days. The following parameters were measured: skin colour (using a scale of 1–6) [8], disease incidence (surface rot caused by various fungal organisms, using a scale of 0–3) and irradiation injury (skin discoloration using a scale of 0–3).

The papayas were treated at 0, 250, 500, 750 and 1000 Gy using the Sinagama facility and subsequently kept at 20°C for 7 days. Five fruits were treated at each dose. Organoleptic evaluations were conducted by an untrained panel of 19–25 persons. Starting with the highest dose, 1000 Gy, the panel was provided with fruits treated at progressively lower doses until no difference was detected between the treated and control fruits. Fruits were rated on a 1–5 score for odour, skin and flesh colour, flavour, texture, sweetness and overall acceptance.

'Eksotika' papayas exhibit a characteristic freckling of the skin, which increases as the fruit ripens and is considered to be cosmetically undesirable. The effects of irradiation on cosmetic freckling were rated on a score of 1–5, with 1 having the least and 5 the most freckles.

2.4.3. Mango

Premium quality mangoes, cv. 'Harumanis', were selected for evaluation of the effects of irradiation on disease incidence, quality, phytotoxicity and other responses.

Mangoes were irradiated with 0, 250, 500, 750 and 1000 Gy at the Sinagama facility. Eight fruits were treated at each dose. Following treatment, half the fruits were stored at 15°C for 4 weeks and the other half at 23°C for 2 weeks. After storage, the fruits were observed for changes in skin colour (green, yellowish green or yellow), incidence of surface rot diseases (using a scale of 0–3), firmness (using a scale of 0–3), flesh colour (green, yellow or orange) and irradiation injury as indicated by skin discoloration (using a scale of 0–3).

3. RESULTS

3.1. Fruit fly mortality

3.1.1. Immature insects treated in carrot medium

The effects of gamma irradiation on the subsequent pupation of fruit fly eggs and larvae that had been treated in carrot medium are summarized in Table I. These

TABLE I. DEVELOPMENT OF PUPARIA AFTER IRRADIATION OF FRUIT FLY EGGS OR LARVAE IN CARROT MEDIUM

Stage treated	No. of puparia formed at dose (Gy)					
	0	25	50	100	250	500
Eggs	625	275	152	31	0	0
Larvae						
First instar	500	670	275	245	140	0
Second instar	708	595	610	550	210	13
Third instar (early)	682	594	545	559	475	336
Third instar (late)	301	346	304	353	297	306

TABLE II. ADULT EMERGENCE FROM PUPARIA FORMED AFTER IRRADIATION OF FRUIT FLY EGGS OR LARVAE IN CARROT MEDIUM

Stage treated	No. of adults emerged at dose (Gy)					
	0	25	50	100	250	500
Eggs	495	0	0	0	0	0
Larvae						
First instar	386	0	0	0	0	0
Second instar	566	0	0	0	0	0
Third instar (early)	545	3	0	0	0	0
Third instar (late)	265	4	0	0	0	0

TABLE III. DEVELOPMENT OF PUPARIA AFTER IRRADIATION OF FRUIT FLY EGGS OR LARVAE IN CARAMBOLA FRUITS

Stage treated	No. of puparia formed at dose (Gy)				
	0	25	50	100	250
Eggs	290	13	1	0	0
Larvae					
First instar	362	90	30	24	0
Second instar	233	161	175	129	36
Third instar	266	197	[a]	138	43

[a] Data not available due to equipment malfunction.

TABLE IV. ADULT EMERGENCE FROM PUPARIA FORMED AFTER IRRADIATION OF FRUIT FLY EGGS OR LARVAE IN CARAMBOLA FRUITS

Stage treated	No. of puparia formed at dose (Gy)				
	0	25	50	100	250
Egg	178	0	0	0	0
Larvae					
First instar	285	0	0	0	0
Second instar	190	0	0	0	0
Third instar	221	33[a]	[b]	0	0

[a] Eighteen males and fifteen females.
[b] Data not available due to equipment malfunction.

TABLE V. PUPATION AND ADULT EMERGENCE AFTER IRRADIATION OF THIRD INSTAR FRUIT FLY LARVAE IN CARAMBOLA FRUITS

Dose (Gy)	No. from 80 fruits	
	Puparia	Adults
0	1432	1299
10	1298	776
20	714	100
40	933	10
80	867	1[a]

[a] Died 1 day after emergence.

TABLE VI. EFFECTS OF IRRADIATION ON SKIN COLOUR, FORMATION OF DARK RIBS, DISEASE AND RUST INCIDENCE IN CARAMBOLA FRUITS STORED AT 7°C FOR 6 WEEKS

Dose (Gy)	Initial skin colour[a] (±SD)	Fruit condition after 6 weeks at 7°C			
		Skin colour[a] (±SD)	Disease[b] (±SD)	Rust[b] (±SD)	Dark ribs[b] (±SD)
0	4.0 (0)	4.5 (0.6)	1.5 (1.3)	0.8 (0.5)	1.0 (0)
25	3.8 (0.5)	4.8 (0.5)	0.8 (0.5)	0.3 (0.5)	0.5 (0.6)
50	4.0 (0)	4.5 (0.6)	0.5 (0.6)	0.0 (0)	0.3 (0.5)
100	4.0 (0.8)	4.8 (0.5)	0.3 (0.5)	1.0 (0.8)	0.5 (0.6)
250	5.0 (0)	5.0 (0)	0.0 (0)	2.8 (0.5)	1.8 (0.5)
500	5.0 (0)	5.0 (0)	0.3 (0.5)	3.0 (0)	2.0 (0)
1000	5.0 (0)	5.0 (0)	0.3 (0.5)	3.0 (0)	2.0 (0)

[a] Colour score: 1 = green; 2 = <25% yellow; 3 = 25–75% yellow; 4 = >75% yellow; 5 = full orange.

[b] Disease, rust and dark ribs: 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.

results show that the egg stage was most susceptible to irradiation, since no puparia were formed at 250 Gy, and above. Tolerance to irradiation increased with the age of the larvae and the late third instar was observed to be the most resistant. Even at the highest dose tested, 500 Gy, 100% of the treated late third instars formed puparia.

Data for the emergence of adult flies from the puparia obtained in this study are summarized in Table II. It is seen that, despite the high number of puparia formed, adult emergence from puparia produced by the irradiated immature fruit fly stages was extremely low. Again, tolerance to irradiation increased with the age of the immatures at the time of treatment. No adults emerged from the eggs, or from the first and second instars treated at 25 Gy. When larvae of the early and late third instars were irradiated at 25 Gy, adult emergence was 0.5 and 1.5%, respectively. No adults developed from naked eggs or larvae treated at higher doses.

3.1.2. *Immature insects treated in carambola*

Studies of fruit fly eggs and larvae infesting carambola fruits confirmed that the developmental stage most resistant to irradiation was the third instar. Data in Table III show that the pupation of eggs and first instars was reduced at 25 Gy and prevented at 100 and 250 Gy, respectively. Data for adult emergence from puparia, collected from irradiated carambola fruits containing fruit fly eggs or larvae, are summarized in Table IV. A total of 33 adults emerged from third instar fruit fly larvae infesting carambola fruits that had been irradiated at 25 Gy. Eggs collected from these flies did not hatch into larvae when placed on carrot medium. No adults emerged from the puparia collected from fruits infested with eggs or first and second instars that were treated at 25 Gy, or from fruits infested with third instars treated at 100 Gy, and above. Equipment malfunction in the test of third instars at 50 Gy invalidated data for that portion of the experiment.

TABLE VII. EFFECTS OF IRRADIATION ON SKIN COLOUR, FORMATION OF DARK RIBS, DISEASE AND RUST INCIDENCE IN CARAMBOLA FRUITS STORED AT 23°C FOR 1 WEEK

Dose (Gy)	Initial skin colour[a] (±SD)	Fruit condition after 1 week at 23°C			
		Skin colour[a] (±SD)	Disease[b] (±SD)	Rust[b] (±SD)	Dark ribs[b] (±SD)
0	2.8 (1.0)	4.3 (0.5)	0.3 (0.5)	0.0 (0)	0.0 (0)
25	2.3 (0.5)	4.3 (0.5)	0.3 (0.5)	0.0 (0)	0.0 (0)
50	2.5 (0.6)	4.8 (0.5)	0.0 (0)	0.0 (0)	0.0 (0)
100	2.5 (0.6)	4.5 (0.6)	0.0 (0)	0.0 (0)	0.0 (0)
250	3.3 (1.0)	3.3 (1.0)	0.0 (0)	0.0 (0)	2.0 (0)
500	3.8 (0.5)	5.0 (0)	0.0 (0)	0.0 (0)	1.8 (1.0)
1000	2.3 (0.5)	5.0 (0)	0.0 (0)	3.0 (0)	3.0 (0)

[a] Colour score: 1 = green; 2 = <25% yellow; 3 = 25–75% yellow; 4 = >75% yellow; 5 = full orange.

[b] Disease, rust and dark ribs: 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.

TABLE VIII. EFFECTS OF IRRADIATION ON SKIN COLOUR, FORMATION OF DARK RIBS, DISEASE AND RUST INCIDENCE IN CARAMBOLA FRUITS STORED AT 23°C FOR 1 WEEK
(Second experiment)

Dose (Gy)	Initial skin colour[a] (±SD)	Fruit condition after 1 week at 23°C			
		Skin colour[a] (±SD)	Disease[b] (±SD)	Rust[b] (±SD)	Dark ribs[b] (±SD)
0	2.8 (0.5)	4.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
100	2.5 (0.6)	4.8 (0.5)	0.0 (0)	0.0 (0)	0.0 (0)
150	2.0 (0)	4.8 (0.5)	0.0 (0)	0.0 (0)	0.0 (0)
200	2.5 (0.6)	5.0 (0)	0.0 (0)	0.0 (0)	1.0 (0)
250	2.5 (0.6)	5.0 (0)	0.3 (0.5)	0.0 (0)	2.0 (0)

[a] Colour score: 1 = green; 2 = <25% yellow; 3 = 25–75% yellow; 4 = >75%yellow; 5 = full orange.
[b] Disease, rust and dark ribs: 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.

3.1.3. Small scale tests of third instars treated in carambola

The results of this experiment are summarized in Table V. A total of 1432 puparia were obtained from larvae infesting fruits held as controls and 1299 adults emerged from these puparia. When the third instars in infested fruits were irradiated at 20 or 40 Gy, both larval pupation and adult emergence were reduced. At the highest dose used, 80 Gy, 867 puparia were obtained, but only one adult emerged. This fly died 1 day after emergence, despite the availability of water, sugar and protein.

3.1.4. Large scale tests of third instars treated in carambola

A total of 18 000 third instars were treated at 150 Gy. These larvae produced 2379 puparia, with no adults emerging. The 1800 untreated third instars produced 1047 puparia and 176 adults. Since emergence from the controls was rather low in this trial, it is proposed that these experiments be repeated after determinination of the causes of the high control mortality.

TABLE IX. EFFECTS OF IRRADIATION ON THE PHYSICOCHEMICAL CHARACTERISTICS IN CARAMBOLA FRUITS

Dose (Gy)	Weight (g) (±SD)	Skin colour[a] (±SD)	Firmness (kgf)[b] (±SD)	Total soluble solids (°Brix) (±SD)	pH (±SD)	Moisture (%) (±SD)
0	197 (55)	3.0 (0)	2.2 (0.3)	9.0 (0.0)	3.9 (0.1)	90.9 (0.1)
25	180 (23)	3.0 (0)	2.2 (0.4)	9.2 (0.1)	4.0 (0.2)	90.7 (0.1)
50	173 (27)	3.0 (0)	2.2 (1.7)	9.2 (0.3)	3.9 (0.1)	90.9 (0.2)
100	179 (36)	3.0 (0)	2.0 (0.5)	8.4 (0.1)	4.0 (0.2)	90.8 (0.1)
250	184 (41)	3.0 (0)	2.0 (0.4)	9.2 (0.0)	4.1 (0.1)	90.2 (0.1)
500	207 (43)	3.4 (0.6)	1.8 (0.2)	8.4 (0.1)	3.9 (0.1)	90.0 (0.4)
1000	167 (36)	4.0 (0.4)	1.7 (0.2)	8.8 (0.1)	3.9 (0.1)	89.8 (0.1)

[a] Colour score: 1 = green; 2 = <25% yellow; 3 = 25–75% yellow; 4 = >75% yellow; 5 = full orange.
[b] 1 kgf = 9.807×10^0 N.

TABLE X. EFFECTS OF IRRADIATION ON THE SUGAR COMPOSITION IN CARAMBOLA FRUITS 1 DAY AFTER TREATMENT

Treatment (Gy)	Fructose (%) (±SD)	Glucose (%) (±SD)	Sucrose (%) (±SD)	Total sugar (%) (±SD)
0	3.24 (0.95)	3.14 (1.23)	0.95 (0.31)	7.33 (1.83)
25	3.34 (0.81)	3.28 (1.08)	0.95 (0.24)	7.57 (1.65)
50	2.51 (0.25)	2.37 (0.08)	1.13 (0.42)	6.01 (0.76)
100	2.58 (0.16)	2.46 (0.28)	0.89 (0.00)	5.93 (0.43)
250	2.65 (0.34)	3.01 (0.98)	1.12 (0.43)	6.78 (1.75)
500	2.42 (0.01)	2.32 (0.03)	0.86 (0.01)	5.60 (0.01
1000	2.57 (0.20)	2.34 (0.01)	0.89 (0.31)	5.80 (0.52)

TABLE XI. EFFECTS OF IRRADIATION ON SKIN COLOUR, DEVELOPMENT OF DISEASE AND IRRADIATION INJURY IN PAPAYAS STORED AT 23°C FOR 1 WEEK AFTER TREATMENT

Dose (Gy)	Initial skin colour[a] (±SD)	Fruit condition after 1 week of storage		
		Skin colour[a] (±SD)	Disease[b] (±SD)	Irradiation injury[c] (±SD)
0	2.0 (0)	4.0 (0)	0.0 (0)	0.0 (0)
250	2.0 (0)	4.8 (0.5)	0.0 (0)	0.0 (0)
500	2.0 (0)	4.5 (1.0)	0.3 (0.5)	0.0 (0)
750	2.0 (0)	4.8 (0.5)	0.0 (0)	0.0 (0)
1000	2.0 (0)	5.0 (0)	0.3 (0.5)	0.0 (0)

[a] Colour score: 1 = green; 2 = tint of yellow; 3 = green more than yellow; 4 = yellow more than green; 5 = yellow with a little green; 6 = yellow.
[b] Disease score (surface rot): 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.
[c] Irradiation injury (skin discoloration): 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.

TABLE XII. EFFECTS OF IRRADIATION ON SKIN COLOUR, DEVELOPMENT OF DISEASE AND IRRADIATION INJURY IN PAPAYAS STORED AT 7°C FOR 18 DAYS

Dose (Gy)	Initial skin colour[a] (±SD)	Fruit condition after 18 days of storage		
		Skin colour[a] (±SD)	Disease[b] (±SD)	Irradiation injury[c] (±SD)
0	2.0 (0)	2.0 (0)	0.0 (0)	0.0 (0)
250	2.0 (0)	2.5 (0.6)	0.0 (0)	0.0 (0)
500	2.0 (0)	2.0 (0)	0.0 (0)	0.0 (0)
750	2.0 (0)	2.0 (0)	0.0 (0)	0.0 (0)
1000	2.0 (0)	2.0 (0)	0.3 (0.5)	0.0 (0)

[a] Colour score: 1 = green; 2 = tint of yellow; 3 = green more than yellow; 4 = yellow more than green; 5 = yellow with a little green; 6 = yellow.
[b] Disease score (surface rot): 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.
[c] Irradiation injury (skin discoloration): 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.

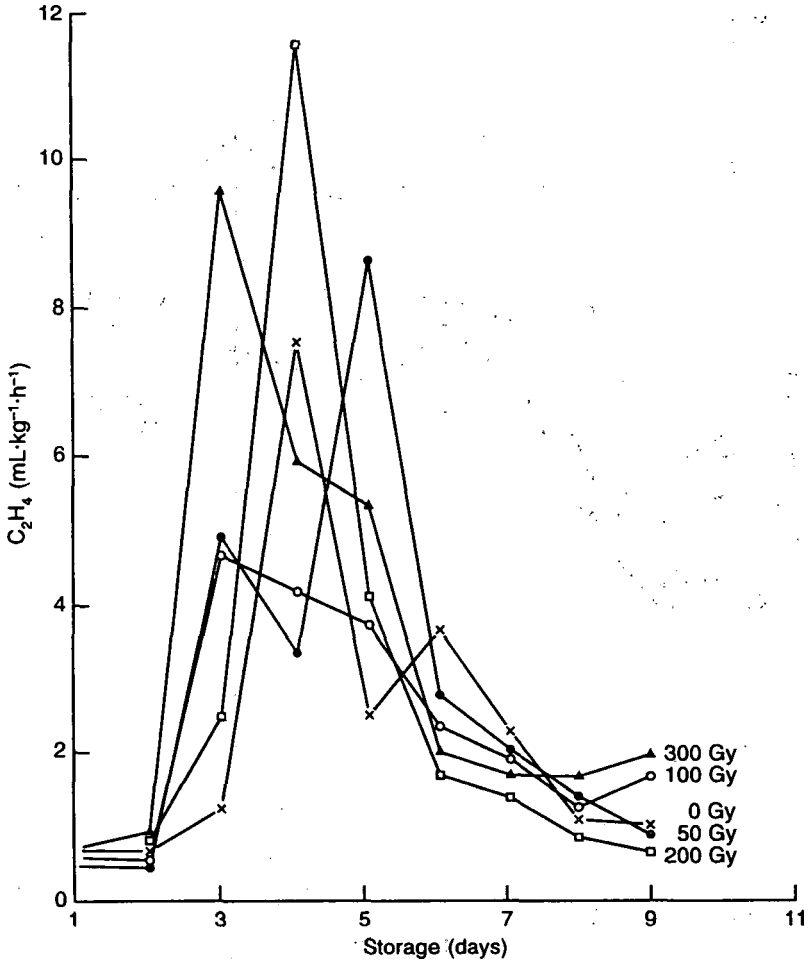

FIG. 1. Production of ethylene following irradiation of 'Eksotika' papayas at 0, 50, 100, 200 and 300 Gy and storage at 20°C for up to 9 days.

3.2. Fruit disease incidence, injury and quality

3.2.1. Carambola

The results of the first experiment to determine the effects of irradiation on carambola fruits are summarized in Tables VI and VII. Irradiation injury was observed as darkening of the ribs or outer edges of fruits that had been stored at either 7°C or 23°C following treatment at doses of 250 Gy, and above. The rust

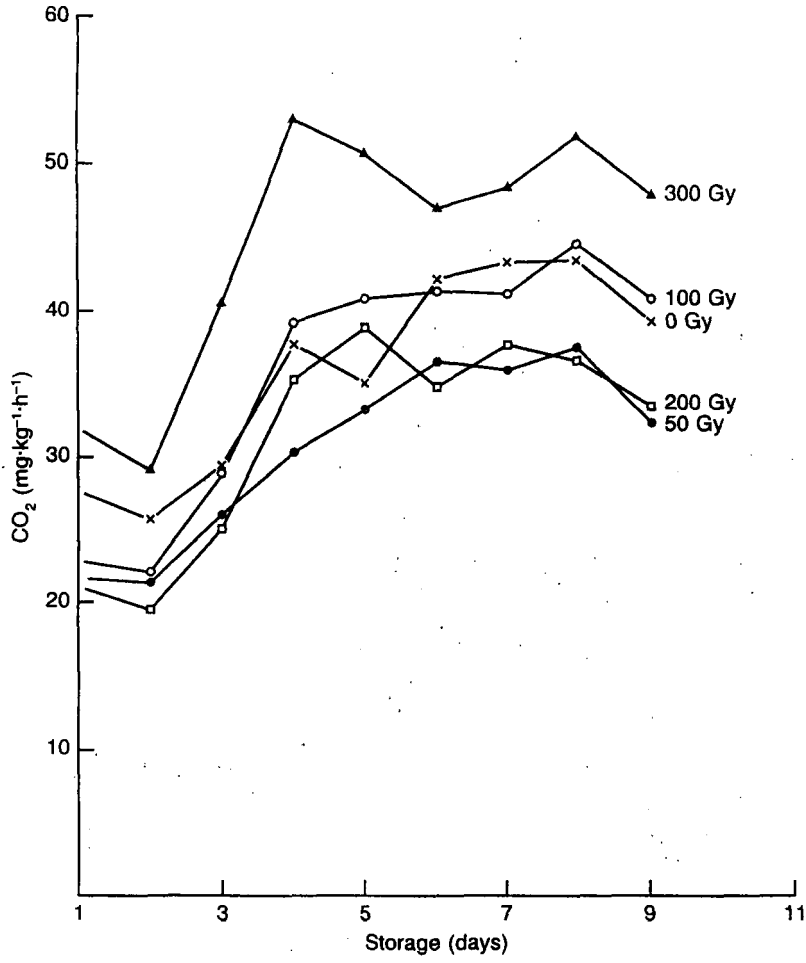

FIG. 2. Respiration rate of 'Eksotika' papayas following irradiation at 0, 50, 100, 200 and 300 Gy and storage at 20°C for up to 9 days.

score increased when fruits were irradiated at 250 Gy, or above, and subsequently stored at 7°C for 6 weeks (Table VI), or were treated at 1000 Gy and stored at 23°C for 1 week (Table VII). At the highest dose tested, 1000 Gy, the treated fruits were observed to lose their glossiness and become dehydrated.

The results of the second experiment to determine the effects of irradiation on disease incidence, colour and injury are summarized in Table VIII. When fruits were stored at 23°C for 1 week after irradiation, injury (25% darkening of the ribs) occurred at a dose of 200 Gy, and above. Doses above 200 Gy accelerated ripening, as indicated by the skin colour.

The results of the studies carried out on the effects of irradiation on the physicochemical characteristics of carambola are shown in Table IX. Doses of up to 1000 Gy did not show major changes in fruits with respect to the weight, total soluble solids and pH. However, with regard to firmness and moisture content, there appeared to be some softening of the fruit and dehydration at a dose of 1000 Gy. The total sugar levels, as measured by the fructose, glucose and sucrose contents (Table X), appeared to decrease with increasing doses of irradiation. This needs to be investigated further to determine if such reductions are significant.

3.2.2. Papaya

Papayas irradiated at up to the highest dose tested, 1000 Gy, ripened normally when stored for 1 week at 23°C (Table XI) with no signs of irradiation injury (skin discoloration). The flesh colour and texture also appeared to be normal. Storage at 7°C prevented colour development, regardless of dose (Table XII).

TABLE XIII. EFFECTS OF IRRADIATION ON THE SKIN COLOUR IN PAPAYAS STORED AT 20°C FOR 9 DAYS

No. of days	Colour[a] at irradiation dose (Gy)				
	0	50	100	200	300
1	2.0	2.0	2.0	2.0	2.0
2	2.0	2.0	2.0	2.0	2.0
3	2.0	2.0	2.0	2.3	2.0
4	2.8	2.3	2.5	2.8	2.8
5	2.8	3.0	3.5	3.0	3.8
6	3.3	3.5	4.0	3.5	4.5
7	4.0	4.0	4.3	4.3	5.3
8	4.3	4.5	4.5	4.5	5.3
9	5.3	5.3	5.5	5.5	6.0

[a] Colour score: 1 = green; 2 = tint of yellow; 3 = green more than yellow; 4 = yellow more than green; 5 = yellow with a little green; 6 = yellow.

TABLE XIV. EFFECTS OF IRRADIATION ON THE ORGANOLEPTIC PROPERTIES IN PAPAYAS STORED AT 20°C FOR 7 DAYS[a]

Trial/ dose (Gy)	Odour	Skin colour		Flesh colour		Flavour	
		Green/ yellow	Like/ dislike	Yellow/ red	Like/ dislike	Off/ not off	Like/ dislike
Trial 1							
0	3.69a	2.75c	2.75b	3.31a	3.69a	3.94a	4.04a
750	3.21ab	4.21a	3.87a	3.02ab	3.21b	3.40ab	3.52b
1000	3.11b	3.70b	3.84a	2.75b	3.05b	3.24b	3.23b
Trial 2							
0	3.66a	2.96b	3.08b	3.06a	3.51a	4.07a	3.86a
500	3.37a	3.70a	3.69a	3.18a	3.74a	3.65b	3.73a
Trial 3							
0	3.68a	3.28b	2.92b	3.61a	3.65a	3.80a	3.66a
250	3.87a	3.73a	3.36a	2.93b	3.45a	3.32a	3.58a
Trial 4							
0	3.41a	3.00c	2.83b	3.25a	3.72a	3.93a	3.99a
250	3.51a	3.86ab	4.01a	3.28a	3.59a	4.06a	3.81ab
500	3.51a	4.18a	4.14a	3.17a	3.38a	3.37a	3.15b
750	3.44a	3.55b	3.38b	3.28a	3.59a	3.66a	3.63ab

Trial/ dose (Gy)	Texture		Sweetness		Overall acceptance
	Soft/ hard	Like/ dislike	Sour/ sweet	Like/ dislike	
Trial 1					
0	2.96b	3.79a	3.80a	3.69a	3.76a
750	2.99ab	3.39a	3.36ab	3.43ab	3.19b
1000	3.41a	3.31a	3.22b	2.96b	3.02b
Trial 2					
0	2.86b	3.45a	3.76a	3.95a	3.91a
500	3.46a	3.63a	3.62a	3.63a	3.56a
Trial 3					
0	2.76a	3.40a	3.44a	3.40a	3.36a
250	3.38a	3.70a	3.75a	3.55a	3.52a
Trial 4					
0	2.89b	2.34a	3.47a	3.75a	3.63a
250	3.07ab	3.38a	3.81a	3.58a	3.45ab
500	3.37a	3.28a	3.03b	3.13a	2.95b
750	3.45a	3.51a	3.39ab	3.46a	3.58ab

[a] Means (based on a range of 1–5) within trials and columns followed by the same letter are not significantly different (P = 0.05) [13].

TABLE XV. EFFECTS OF IRRADIATION ON PAPAYA FRECKLES

Dose (Gy)	Score for freckles[a]
0	4.9a
250	3.1b
500	2.5b
750	1.6c

[a] Means (based on a score of 1–5) within columns followed by the same letter are not significantly different (P = 0.05) [13].

The ethylene evolution and respiration rate of papayas irradiated at up to 300 Gy and stored at 20°C for 9 days were normal (Figs 1 and 2). The fruits exhibited the normal pattern of respiration expected of climacteric fruit. However, colour development was accelerated (Table XIII).

The results, summarized in Table XIV [13], show that the organoleptic properties of 'Eksotika' papayas were not significantly altered at doses of up to 750 Gy, but were altered at a dose of 1000 Gy. A dose of 250 Gy enhanced some of the organoleptic properties. It is recommended that this dose be used for commercial treatment purposes.

Untreated 'Eksotika' fruits exhibit an undesirable freckling of the skin, which increases in intensity as the fruit ripens. Such freckling was significantly reduced following irradiation at doses of 250, 500 and 750 Gy (Table XV) [13].

3.2.3. Mango

The results of the study on mangoes, cv. 'Harumanis', irradiated at doses of up to 1000 Gy and stored at either 15°C for 4 weeks or at 23°C for 2 weeks are summarized in Tables XVI and XVII, respectively. Fruits treated at doses of up to 1000 Gy and stored at 15°C did not show any irradiation injury (skin discoloration). All the treated fruits appeared to be normal. However, the flesh of fruits treated at 750 or 1000 Gy and stored at 23°C was rotten.

Diseases, especially anthracnose and stem end rot, were not controlled when fruits were stored at either 15°C or 23°C. The firmness of the treated fruits did not differ from that of the untreated fruits at either storage temperature.

TABLE XVI. EFFECTS OF IRRADIATION ON SKIN COLOUR, DEVELOPMENT OF DISEASE, FIRMNESS, FLESH COLOUR AND IRRADIATION INJURY IN MANGOES STORED FOR 4 WEEKS AT 15°C

Dose (Gy)	Initial skin colour[a] (±SD)	Fruit condition after 4 weeks of storage				
		Skin colour[a] (±SD)	Disease[b] (±SD)	Firmness[c] (±SD)	Flesh colour[a] (±SD)	Irradiation injury[d] (±SD)
0	1.0 (0)	1.0 (0)	1.0 (0)	3.0 (0)	5.0 (0)	0.0 (0)
250	1.0 (0)	2.8 (1.5)	1.0 (0)	3.0 (0)	5.0 (0)	0.0 (0)
500	1.0 (0)	2.0 (1.2)	1.0 (0)	3.0 (0)	5.0 (0)	0.0 (0)
750	1.0 (0)	2.0 (1.2)	1.0 (0)	3.0 (0)	5.0 (0)	0.0 (0)
1000	1.0 (0)	2.3 (0.5)	1.0 (0)	3.0 (0)	5.0 (0)	0.0 (0)

[a] Colour score: 1 = green; 2 = shrivelled green; 3 = yellowish green; 4 = yellow green; 5 = yellow.
[b] Disease score (surface rot): 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.
[c] Firmness score: 0 = hard; 1 = firm; 2 = slightly soft; 3 = soft.
[d] Irradiation injury (skin discoloration): 0 = nil; 1 = <25%; 2 = 25–50%; 3 = >50%.

TABLE XVII. EFFECTS OF IRRADIATION ON SKIN COLOUR, DEVELOPMENT OF DISEASE, FIRMNESS, FLESH COLOUR AND IRRADIATION INJURY IN MANGOES STORED FOR 2 WEEKS AT 23°C

Dose (Gy)	Initial colour[a] (±SD)	Fruit condition after 4 weeks of storage				
		Skin colour[a] (±SD)	Disease[b] (±SD)	Firmness[c] (±SD)	Flesh colour[a] (±SD)	Irradiation injury[d] (±SD)
0	1.0 (0)	2.5 (1.0)	2.3 (0.5)	3.0 (0)	5.0 (0)	0.0 (0)
250	1.0 (0)	2.8 (0.5)	1.0 (0)	3.0 (0)	5.0 (0)	0.0 (0)
500	1.0 (0)	3.0 (0) (+ 1 B)	1.8 (1.0)	2.5 (1.0)	5.0 (0) (+ 1 R)	0.0 (0)
750	1.0 (0)	2.8 (1.3)	0.8 (0.5)	2.0 (0)	R	0.0 (0)
1000	1.0 (0)	2.0 (1.2)	0.8 (0.5)	2.8 (0.5)	R	0.0 (0)

[a] Colour score: 1 = green; 2 = brownish green; 3 = yellowish green; 4 = orange green; 5 = orange; R = rotten; B = black.
[b] Disease score (surface rot): 0 = nil; 1 = <25%; 2 = 25-50%; 3 = >50%.
[c] Firmness score: 0 = hard; 1 = firm; 2 = slightly soft; 3 = soft.
[d] Irradiation injury (skin discoloration): 0 = nil; 1 = <25%; 2 = 25-50%; 3 = >50%.

4. CONCLUSIONS

In the small scale tests, emergence of normal fruit fly adults from infested carambola did not occur at doses as low as 100 Gy, indicating that treatment of the fruits at this dose would provide the quarantine security required. This needs to be confirmed in tests using 30 000–100 000 third instar fruit fly larvae. In the large scale tests, using the recommended minimum dose of 150 Gy [12], no adult emergence was observed. However, low adult emergence in the controls necessitates that these experiments be repeated.

From the preliminary experiments carried out on the effects of irradiation on carambola, papaya and mango, with regard to fruit injury and the physicochemical and organoleptic properties, it appears that irradiation can successfully be developed and should be investigated further as a quarantine treatment for these fruits.

Carambola showed external symptoms of injury at irradiation doses in excess of 200 Gy. There appeared to be some reduction in the sugar content at doses exceeding 100 Gy, but this needs to be confirmed by further experimentation. Carambola may not be a suitable candidate for irradiation at doses exceeding 200 Gy. Use of irradiation as a quarantine treatment is only applicable if quarantine security can be demonstrated at a maximum treatment dose of less than 200 Gy.

Papaya, cv. 'Eksotika', showed good tolerance to irradiation at up to 300 Gy. At this dose, irradiation did not alter the ripening behaviour, nor did it cause any injury or alter the organoleptic properties of the fruit. An additional benefit was that doses in excess of 250 Gy significantly reduced freckling of the fruit and enhanced its cosmetic value.

Mango, cv. 'Harumanis', tolerated irradiation fairly well and doses of up to 750 Gy did not produce significant injury.

Large scale quarantine treatment experiments using third instar fruit fly larvae can proceed.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from the IAEA as well as the assistance provided at the Sinagama facility at Dengkil.

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IRRADIATION OF MANGOES AS A QUARANTINE TREATMENT*

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Abstract

IRRADIATION OF MANGOES AS A QUARANTINE TREATMENT.

This research project was conducted following the guidelines of research protocols for post-harvest treatments developed by the United States Department of Agriculture. Laboratory bioassays included the irradiation of mangoes (*Mangifera indica* L.) infested with third instar larvae of *Anastrepha serpentina* (Wiedemann), *A. ludens* (Loew), *A. obliqua* (Macquart) and *Ceratitis capitata* (Wiedemann) at doses of 10–250 Gy. Irradiation doses were applied using a ^{60}Co Atomic Energy of Canada Limited Model JS-7400 irradiator. The design was chosen to obtain a maximum/minimum ratio equal to, or less than, 1025. *C. capitata* was the species most tolerant to irradiation. A dose of 60 Gy, applied to third instar fruit fly larvae in the infested fruits, sterilized this species and prevented the emergence of adults of the other three species. A dose of 250 Gy was required to prevent emergence of *C. capitata*. In fertility tests using emerged adults of *A. ludens* and *A. obliqua*, a dose of 30 Gy gave 45 and 27% fertility, respectively. The adults of *A. serpentina* that emerged died before reaching sexual maturity. Confirmatory tests, at the probit 9 security level, were done at 100 Gy for the three species of *Anastrepha* and at 150 Gy for *C. capitata*. The quality of fruits irradiated up to 1000 Gy was evaluated by chemical, physiological and sensorial tests. Determination of vitamin C indicated that there was no loss in the nutritive value of the fruit. It also was observed that fruit metabolism was not accelerated, since no significant increase in respiration or transpiration was registered and consumers accepted both the treated and untreated fruits in the same way.

* Research carried out with the support of the IAEA under Research Contract Nos 5150/RB and 5350/RB.

1. INTRODUCTION

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA) has established rigorous quarantine restrictions to control the movement of fresh fruits from tropical countries into the United States of America [1]. In Mexico, mangoes (*Mangifera indica* L.) and citrus (*Citrus* spp.) have exportation restraints, since they are attacked by fruit flies of the genus *Anastrepha*, especially by *A. ludens* (Loew) and *A. obliqua* (Macquart) and, occasionally, by *A. serpentina* (Wiedemann). The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is established in Guatemala and appears sporadically in the border region of Mexico, where it is considered a species of quarantine importance [2]. Since the ban of ethylene dibromide as a fumigant, citrus for export has been treated with methyl bromide and mangoes with hot water [3]. However, it is important to have alternative treatments available. Such treatments should be efficient and economically feasible. A promising alternative is the use of gamma irradiation.

In Mexico, studies on the application of irradiation as a quarantine treatment for mango and orange (*Citrus sinensis* (L.) Osbeck) were started in 1984, following an economic study [4] and a study carried out by scientists from the Instituto Nacional de Investigaciones Nucleares (ININ), the Fruit Growers National Commission (CONAFRUT) and the State University of Chapingo (UACH), that showed the technical feasibility of irradiation. They found that minimum doses of 450 and 630 Gy would disinfest mango and orange, respectively, based on first instar larval mortality. The quality of fruits irradiated at up to 1000 Gy was evaluated by chemical, physiological and sensorial tests [5].

2. OBJECTIVES

(1) To conduct laboratory tests, using ^{60}Co, to determine the minimum dose of irradiation required to prevent the emergence or reproduction of adults from third instar *A. serpentina, A. ludens, A. obliqua* and *C. capitata* larvae infesting mangoes

(2) To conduct laboratory tests to confirm the efficacy of the dose required for quarantine security (probit 9) using the stage of each species of fruit fly most tolerant to radiation

(3) To determine the effects of irradiation on the quality and storage of mangoes.

3. METHODOLOGY

This project was conducted following the guidelines of research protocols for post-harvest treatments developed by the Agricultural Research Service (ARS) of the

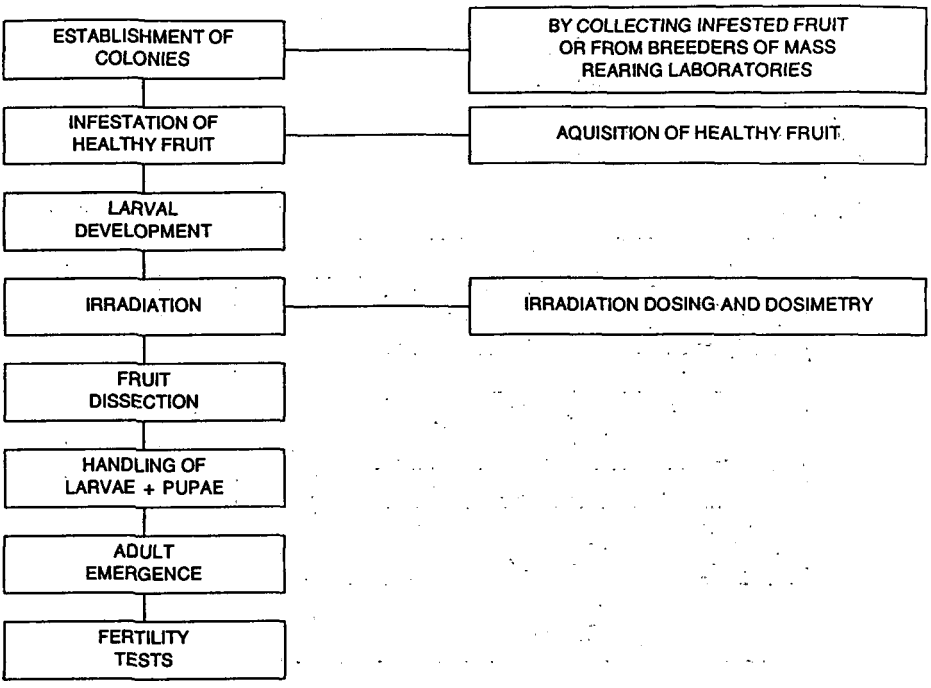

FIG. 1. Flow chart of activities during laboratory and confirmatory tests.

USDA to provide the data required to support a proposed quarantine treatment using irradiation [6]. Figure 1 presents a flow chart of the activities carried out in order to reach the objectives of the research.

3.1. Establishment of insect colonies

The *A. serpentina* colony was formed after collecting 3500 kg of the mammee apple (*Mammea americana* L.) from which 165 650 larvae were recovered. From 3000 kg of mangoes, 156 600 larvae of *A. obliqua* were obtained. Mature larvae were placed in humid soil at 28°C ± 1°C and 70 ± 5% relative humidity and allowed to pupate. Two or 3 days before emergence of the adults, the puparia were separated from the soil and placed in infestation cages. To form the *A. ludens* and *C. capitata* colonies, fruit fly puparia were obtained from the Mass Rearing Laboratory of the Programa Moscamed (SARH-USDA).

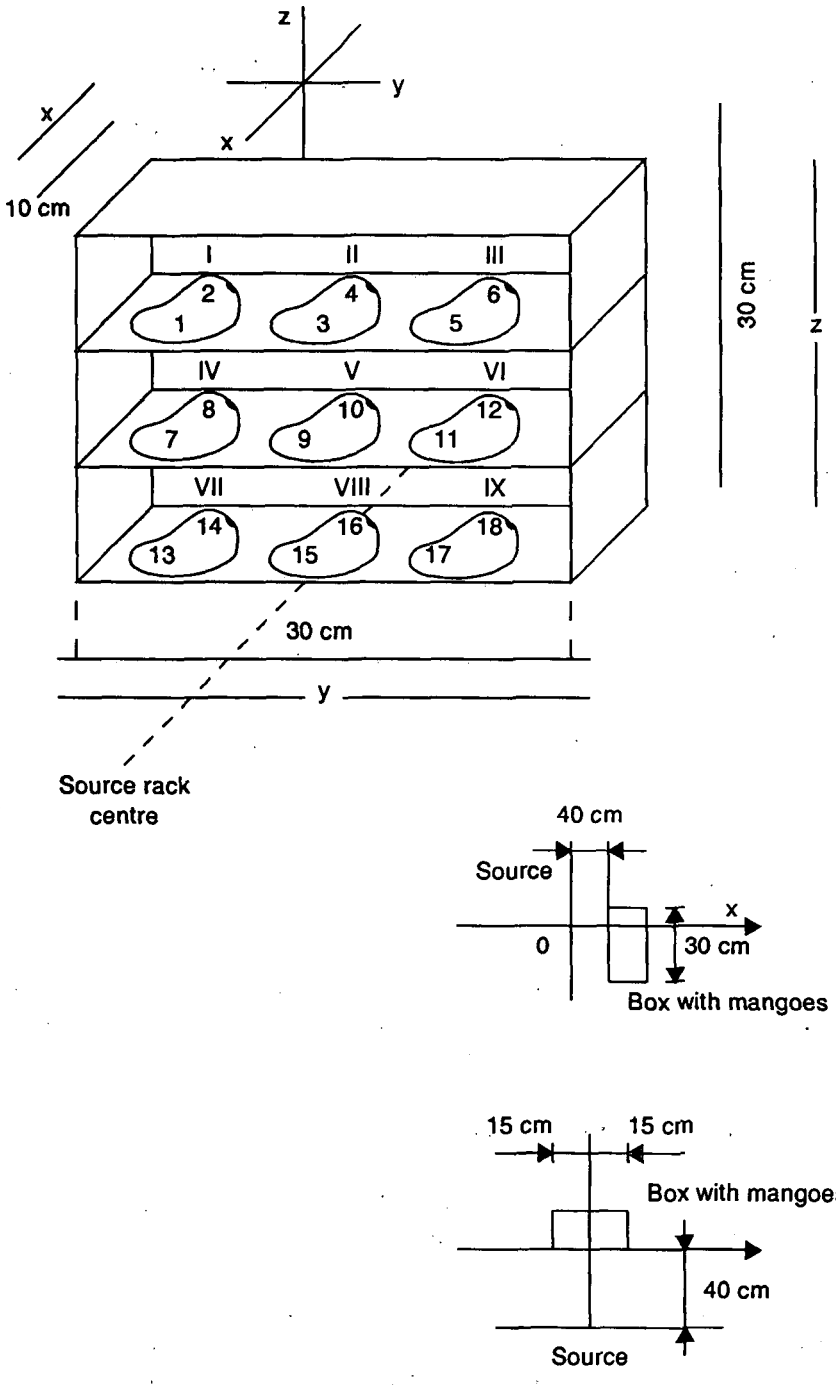

FIG. 2. Position of dosimeters on each mango for dose rate determination.

3.2. Fruit infestation

For this study, several varieties of mango ('Ataulfo', 'Kent' and 'Keitt', as well as wild mangoes) were used, with a weight of 250–300 g each, sizes 12 and 13. Wooden cages, 70 cm × 70 cm × 70 cm, with a capacity for 2000 pairs of flies, were used for fruit infestation. The flies were fed a mixture of sugar and protein (3:1) and water. The cages were equipped with two metallic grills at 30 and 60% of the cage height, respectively, on which the fruits to be infested were placed.

To obtain 20–50 larvae per fruit, the time of infestation varied from 6 to 48 hours, depending on the biological characteristics of the species under study (sexual aggressiveness, fecundity and fertility). Once the fruits were infested, they were removed from the cage, placed in plastic trays and stored at 26°C ± 2°C and 80 ± 5% relative humidity to allow development of the larvae to the third instar, which was the most resistant to irradiation [7, 8]. Development of the *Anastrepha* species and the *C. capitata* larvae was completed in 15 ± 1 and 9 ± 1 days, respectively.

3.3. Irradiation and dosimetry process

The fruits were treated using a ^{60}Co Atomic Energy of Canada Limited (Nordion) Model JS-7400 irradiator, with an activity of 23 740 Ci on 1 January 1989.[1] The absorbed dose was determined using vials with 4 mL of Fricke solution. These vials were placed on the mangoes and on the wooden frames (10 cm × 30 cm × 30 cm). Each frame was divided into three levels and three mangoes were placed on each level, with a total of nine fruits per sample. The mango containers were placed 40 cm from the irradiation source in such a way that the centre of the container coincided with the centre of the ^{60}Co frame (Fig. 2). Table I shows the dose rates applied at each position. The frames with mangoes were rotated 180°C on the Z axis halfway through the exposure time to reduce the maximum/minimum ratio. The average value on the X axis was 1.005 (Table II). The mean dose rate on 2 May 1989 was 14.07 Gy/min. The exposure times were calculated to give doses of 10, 20, 30, 40, 60, 80, 100, 120, 150 and 250 Gy.

3.4. Laboratory tests for irradiated fruits and larvae

In the laboratory tests, the infested fruits were dissected 1 day after treatment. The live larvae were counted and placed in containers with humid soil in an environment of 26°C ± 1°C and 80 ± 5% relative humidity to allow pupation of the larvae. Eight days after pupation, the number of normal and malformed puparia, as well as the number of larvae which did not reach pupation, were determined. Normal

[1] 1 Ci = 3.70 × 10^{10} Bq.

TABLE I. DOSE RATES FOR THE DOSIMETERS PLACED AMONG THE MANGOES IN WOODEN CONTAINERS EXPOSED TO GAMMA RADIATION AT 40 cm FROM THE SOURCE

No. of dosimeter	Position (cm)			Dose rate (Gy/min)
	X	Y	Z	
1	40	10	6	16.6
3	40	0	6	17.3
5	40	−10	6	16.7
7	40	10	−11	17.1
9	40	0	−11	16.9
11	40	−10	−11	16.3
13	40	10	−22	16.6
15	40	0	−22	16.7
17	40	−10	−22	16.9
2	45.4	10	6	11.1
4	45.5	0	6	11.4
6	45.5	−10	6	11.3
8	45.5	10	−11	10.9
10	45.0	0	−11	11.3
12	46.0	−10	−11	11.4
14	45.0	10	−22	11.3
16	46.5	0	−22	11.2
18	46.5	−10	−22	11.0

puparia were left in the medium to allow them to continue their development until emergence as adults. These laboratory tests ended when approximately 5000 insects per treatment had been tested for each species.

In treatments where the adults emerged, they were separated by sex 24 hours after emergence. When they reached sexual maturity (approximately 10 days for the three *Anastrepha* species and 3 days for *C. capitata*), the treated females were paired

TABLE II. MAXIMUM/MINIMUM RATIO FOR EXPOSURE OF THE MANGOES TO IRRADIATION

No. of mango	Maximum/minimum ratio	
	Without rotation	With rotation on X axis
1	1.49	1.01
2	1.51	1.00
3	1.47	1.01
4	1.56	1.00
5	1.49	1.01
6	1.42	1.00
7	1.45	1.01
8	1.49	1.00
9	1.52	1.01
Mean	1.48	1.005

with untreated males and vice versa for the purpose of measuring the fecundity, fertility and longevity of the adults, as well as their flight ability and morphological condition.

3.5. Confirmatory tests

The minimum absorbed dose of radiation required for quarantine security was estimated on the basis of probit analyses [9] of the laboratory data for each of the four species. According to the ARS/USDA protocol, the confirmatory tests have to be done using a sample of 100 000 individuals [6]. The fruits were infested with each species, as previously described. For each lot of infested fruits, 75% were chosen at random for treatment at the proposed minimum effective dose. The remaining 25% of the fruits were used as the control. Two days after treatment, the fruits were dissected and the number of live larvae from both the treated and the control fruits was counted. The recovered larvae were placed in humid soil to pupate and, later, the number of emerged adults was also counted. This process was repeated for additional lots of fruit until at least 100 000 individuals were tested.

TABLE III. PUPATION AND EMERGENCE OF ADULTS AFTER IRRADIATING MANGOES INFESTED WITH THIRD INSTAR *A. serpentina* LARVAE AT DIFFERENT DOSES

Dose (Gy)	Larvae		Puparia		% adult emergence
	Total No. tested	No. died	No. normal	No. malformed	
0	5106	102	4842	162	88.64
10	4719	258	3964	497	8.62
20	5255	296	4176	783	3.31
30	5841	399	4715	727	0.38
40	5537	276	3964	1297	0.18
60	4025	393	2429	1203	0
80	4136	357	2666	1113	0
100	4317	730	2383	1204	0
120	4845	886	2517	1442	0
150	4640	831	2065	1744	0
250	6067	2632	1277	2158	0

4. RESULTS AND DISCUSSION

4.1. Laboratory tests

Tables III–VI report the data on the effects of irradiation on the larval development, pupation and emergence of adults for *A. serpentina, A. ludens, A. obliqua* and *C. capitata,* respectively, when third instars were treated in mangoes. An increase in the mortality of *A. serpentina* larvae was observed at 100 Gy. At 250 Gy, 43% of the treated larvae were dead when the fruits were dissected. This compared with 2% larval mortality for the controls and 5–10% mortality at doses of 10–80 Gy. For the other three fruit fly species, less than 4% of the larvae were dead in both the treated and the untreated fruits. At least 90% of the larvae infesting the untreated fruits produced normal puparia. At 250 Gy, 21, 56, 77 and 85% of the larvae of *A. serpentina, A. ludens, A. obliqua* and *C. capitata,* respectively, produced normal

TABLE IV. PUPATION AND EMERGENCE OF ADULTS AFTER IRRADIATING MANGOES INFESTED WITH THIRD INSTAR *A. ludens* LARVAE AT DIFFERENT DOSES

Dose (Gy)	Larvae		Puparia		% adult emergence
	Total No. tested	No. died	No. normal	No. malformed	
0	5342	51	4979	312	84.29
10	5450	94	5048	308	23.52
20	5441	119	4806	516	6.52
30	4808	122	4044	642	1.10
40	5517	76	4733	708	0.11
60	5513	209	4395	909	0
80	5260	120	4113	1027	0
100	5800	219	3880	1701	0
120	4842	126	3223	1493	0
150	6337	156	4081	2100	0
250	5617	208	3123	2286	0

puparia. None of the larvae of the three species of *Anastrepha* treated at 60 Gy were able to complete their development and emerge as adults. At this dose, emergence of *C. capitata* was 0.34%; a few adults emerged even at a dose of 150 Gy.

The results of the laboratory tests were used to calculate the dose/mortality curves for each of the four species of fruit flies, based on probit analysis [9, 10]. These data (Table VII) [10] showed that the dose required for 95% mortality of *A. serpentina, A. ludens, A. obliqua* and *C. capitata* was 15, 21, 19 and 36 Gy, respectively, and that the dose required for quarantine security of 99.9968% mortality, based on the prevention of adult emergence, was 136, 106, 133 and 113 Gy, respectively. Although the statistics from probit 9 analyses of these data gave doses of over 100 Gy for the three *Anastrepha* species, the confirmatory tests were done at 100 Gy, since the laboratory tests at 60 Gy had resulted in a lethal dose.

TABLE V. PUPATION AND EMERGENCE OF ADULTS AFTER IRRADIATING MANGOES INFESTED WITH THIRD INSTAR *A. obliqua* LARVAE AT DIFFERENT DOSES

Dose (Gy)	Larvae		Puparia		% adult emergence
	Total No. tested	No. died	No. normal	No. malformed	
0	6625	160	6022	443	83.50
5	4057	44	3639	374	38.30
10	4112	89	3924	99	20.50
20	4426	109	4093	224	3.70
30	4491	141	3880	470	0.99
40	4872	212	3971	689	0.14
60	4194	65	3841	288	0
80	4662	66	3941	655	0
100	4307	107	3837	363	0
120	3827	121	3390	316	0
150	4239	260	3339	640	0
250	2427	53	1862	512	0

The results of studies on the reproductive ability of emerging adults are summarized in Table VIII. In the case of *A. serpentina*, emergence of adults occurred at doses of 10–40 Gy but all the flies died before reaching sexual maturity. The fertility for *A. ludens* adults that emerged from the larvae treated at a dose of 30 Gy and reached sexual maturity was 45%. The response of *A. obliqua* adults was similar to that observed in *A. ludens*, except that there was a 21% hatch at a dose of 40 Gy. For *C. capitata*, although a dose of 250 Gy was required to prevent adult emergence, a dose of 60 Gy inhibited their reproduction.

4.2. Confirmatory tests

According to data obtained in the laboratory tests at 60 Gy, emergence of adults of the three species of *Anastrepha*, as well as the ability of *C. capitata* adults

TABLE VI. PUPATION AND EMERGENCE OF ADULTS AFTER IRRADIATING MANGOES INFESTED WITH THIRD INSTAR *C. capitata* LARVAE AT DIFFERENT DOSES

Dose (Gy)	Larvae		Puparia		% adult emergence
	Total No. tested	No. died	No. normal	No. malformed	
0	6089	49	5797	243	90.39
10	3220	26	3037	157	75.06
20	3369	79	2726	564	37.69
30	3740	79	3358	303	4.54
40	5117	78	4781	258	2.07
60	4450	53	4030	367	0.34
80	5146	63	4872	211	0.156
100	8536	280	7630	626	0.093
120	5806	79	5171	556	0.103
150	5268	242	4343	683	0.095
250	5192	138	4426	628	0.0

TABLE VII. STATISTICS FROM PROBIT ANALYSES FOR EMERGENCE OF ADULTS DEVELOPING FROM THIRD INSTARS OF FOUR SPECIES OF FRUIT FLY LARVAE INFESTING IRRADIATED MANGOES [10]

Species	No. tested	Intercept (±SE)	Slope (±SE)	Dose (Gy)	
				LD_{95} (95% FL)[a]	$LD_{99.9968}$
A. serpentina	25 377	3.8 (0.5)	2.4 (0.4)	14.6 (11.8, 17.7)	135.9
A ludens	26 729	2.2 (0.4)	3.4 (0.3)	21.2 (18.9, 24.6)	106.4
A. obliqua	26 152	3.8 (0.2)	2.7 (0.2)	19.3 (16.6, 23.2)	133.4
C. capitata	38 770	−0.7 (1.9)	4.8 (1.4)	36.1 (27.3, 67.0)	112.7

[a] FL = fiducial limit.

TABLE VIII. EMERGENCE AND FERTILITY OF ADULTS DEVELOPING FROM THIRD INSTARS OF FOUR SPECIES OF FRUIT FLY LARVAE INFESTING MANGOES IRRADIATED AT DIFFERENT DOSES

Dose (Gy)	Adult emergence (%) and egg hatch (%) by species							
	A. serpentina		*A. ludens*		*A. obliqua*		*C. capitata*	
	Emergence	Hatch	Emergence	Hatch	Emergence	Hatch	Emergence	Hatch
0	79.4	84.0	85.6	92.0	85.0	85.0	90.4	95.0
5	—	—	—	—	38.3	72.6	—	—
10	10.3	0	25.4	51.8	20.5	49.2	75.1	89.0
20	4.2	0	7.4	50.6	3.7	37.6	37.7	88.0
30	0.5	0	1.3	45.0	0.99	27.0	4.5	73.2
40	0.25	0	0.1	0	0.14	20.5	2.07	62.4
60	0	0	0	0	0	0	0.34	0

to reproduce, were completely inhibited. To add a margin of safety, confirmatory tests were done using a dose of 100 Gy with the *Anastrepha* species and a dose of 150 Gy for *C. capitata*. These tests showed that no adults emerged from over 100 000 treated larvae of each species (Table IX). More than 75% of the larvae dissected from the untreated fruits pupated and emerged as adults.

4.3. Effects on mango quality

According to regulations established by the Food and Drug Administration, ionizing radiation can be used on fresh fruits and vegetables at doses not greater than 1000 Gy [11]. The quality of 'Kent' mangoes irradiated at doses of up to 1000 Gy was evaluated by chemical (soluble solids, acidity, pH and vitamin C), physiological and sensorial tests [5]. Determination of vitamin C indicated that there was no loss in the nutritive value of the fruit. It was also observed that fruit metabolism was not accelerated, since no significant increase in respiration or transpiration was registered. Consumers accepted both the treated and the untreated fruits in the same way. These studies showed that the mango quality, as well as the respiration and transpiration rates, were not affected adversely when the fruits were treated at doses of up to 1000 Gy.

TABLE IX. EFFECT OF GAMMA RADIATION ON EMERGENCE OF ADULTS AFTER IRRADIATING MANGOES INFESTED WITH THIRD INSTAR *Anastrepha* LARVAE AT A DOSE OF 100 Gy AND *C. capitata* LARVAE AT A DOSE OF 150 Gy

Results of treatment	Fruit fly species			
	A. serpentina	*A. ludens*	*A. obliqua*	*C. capitata*
Untreated larvae				
Total	34 486	42 382	38 113	20 304
Dead larvae	909	277	940	116
Dead pupae	7 198	8 475	5 609	3 283
Emerged adults	26 379	33 905	31 564	17 221
Emergence %[a]	78.8	80.0	84.9	85.3
Treated larvae				
Total	105 252	101 794	100 400	100 854
Dead larvae	9 303	2 025	1 384	1 381
Dead pupae	95 949	99 769	99 016	99 473
Emerged adults	0	0	0	0
Emergence %	0	0	0	0

[a] Dead larvae were not considered when calculating emergence.

5. CONCLUSIONS

In laboratory tests, a dose of 60 Gy applied to mangoes infested with third instar *Anastrepha* larvae prevented the emergence of adult fruit flies. When a dose of 60 Gy was applied to mangoes infested with third instar *C. capitata* larvae, 15 adults emerged but their ability to reproduce was inhibited. In this species, five adults emerged from over 5000 larvae in fruits treated at 150 Gy, but 250 Gy prevented adult emergence.

The irradiation dose required for quarantine security as a treatment for infested mangoes was determined as 100 Gy for the three species of *Anastrepha* and 150 Gy for *C. capitata*. These doses prevented adult emergence from over 100 000 treated larvae of each species in confirmatory tests, and the fruit quality was not adversely affected.

The recommended dose for quarantine security is 150 Gy for mangoes infested with any fruit fly species of economic importance in Mexico.

ACKNOWLEDGEMENTS

The authors acknowledge the financial and technical support received from the IAEA to carry out this study, as well as support from the Soconusco Region Fruit Growers and the National Fruit and Vegetable Growers Confederation. This Co-operative Research Project was carried out by the Programa Moscamed (SARH-USDA) and the Instituto Nacional de Investigaciones Nucleares.

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EFFECTS OF GAMMA RADIATION ON THE INSECT MORTALITY AND FRUIT QUALITY OF PHILIPPINE 'CARABAO' MANGOES*

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Abstract

EFFECTS OF GAMMA RADIATION ON THE INSECT MORTALITY AND FRUIT QUALITY OF PHILIPPINE 'CARABAO' MANGOES.

Research using gamma radiation for the disinfestation of oriental fruit fly, *Dacus dorsalis* Hendel, larvae in 'Carabao' ('Manila Super') mangoes and its effect on the overall quality and acceptability of the treated fruit was undertaken in the Philippines. The results showed that mature larvae of the fruit fly were the insect stage most tolerant to irradiation, with the young eggs being the most sensitive. Using more than 100 000 mature larvae in mangoes, a minimum dose of 100 Gy was required to prevent the emergence of adult fruit flies and to maintain quarantine security against the possibility of introducing this pest into the importing country. 'Carabao' mango fruits subjected to gamma radiation at 100, 150 or 250 Gy resulted in fruits of an acceptable quality. In contrast to vapour heat treatment, no internal breakdown

* Research carried out with the support of the IAEA under Research Contract No. 5000/RB.

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was observed, even in fruits irradiated at 350 Gy. At this dose, a low, but significant, incidence of pulp discoloration was found in one trial only. Both vapour heat treatment and gamma radiation needed to be supplemented with hot water treatment for effective and more consistent disease control. Although irradiation appears to delay ripening, its effect seems to be largely on the development of peel colour. The results of this study indicated that irradiation could be an appropriate quarantine treatment for the 'Carabao' mango. Therefore, use of irradiation at a minimum dose of 100 Gy as a quarantine treatment for the oriental fruit fly in mature green mango fruits can be recommended. However, when field infestation studies were conducted on 3200 mature green 'Carabao' mangoes obtained from different parts of the country, a very low field infestation of 0.031% was observed. A single fruit was found to be infested with eight larvae of the oriental fruit fly. With these findings, quarantine treatment may not be required, provided proper protection from infestation is applied after harvest or before export.

1. INTRODUCTION

Application of gamma radiation as a physical method of disinfestation against fruit flies was recognized as a potential quarantine treatment for tropical fruits by Balock et al. [1]. Since then, many investigations on the effects of gamma radiation on the different stages of fruit flies have been conducted [2–6]. This research work was aimed at finding an alternative quarantine treatment to ethylene dibromide (EDB), which was banned in 1984 by the United States Environmental Protection Agency because of safety problems [7].

The role of irradiation as a quarantine treatment for fresh fruits was enhanced when the Food and Drug Administration approved low dose irradiation of fruits and vegetables, up to 1 kGy (100 krad), with emphasis placed on insect disinfestation [8]. Irradiation has been considered as a replacement for EDB, particularly for commodities infested by fruit flies [9, 10]. In the Philippines, studies along this line were conducted at the Philippine Atomic Energy Commission (now the Philippine Nuclear Research Institute (PNRI)). Radiosensitivity studies on the different stages and ages of the oriental fruit fly, *Dacus dorsalis* Hendel, showed that mature larvae are the most resistant to radiation damage, with young eggs being the most sensitive [11]. Also, earlier studies showed that when fruits were irradiated at 250 Gy, 0, 2, 4 or 6 days after egg inoculation, the mean number of adults emerged per 25 fruits was 0.0, 0.1, 0.9 and 1.0, respectively [12]. Wholesomeness studies showed that at doses of 500–750 Gy decay was reduced [13], ripening was delayed without affecting the organoleptic qualities [14] and no carcinogenic or mutagenic effects were induced by irradiation [15].

Recently, use of EDB was banned in Japan and vapour heat treatment (VHT) is now the method of disinfestation required for mangoes imported from the Philippines. Studies in our laboratory [16, 17] have shown that VHT induces internal

breakdown (IB), a physiological disorder characterized by the presence of unsightly and tough spongy tissue. Although Japan has not approved irradiation as a quarantine treatment for Philippine mangoes, the USA, which is a potential market for this commodity, is considering irradiation as a possible method of disinfestation. This project was therefore undertaken to establish an irradiation treatment protocol for the quarantine disinfestation of Philippine mangoes through studies on the effects of radiation on the mortality of the oriental fruit fly and on the quality of Philippine mangoes. Specifically, the following were the objectives of the project:

(1) To establish dose mortality data for the egg and larval stages of the fruit flies
(2) To observe the effects of radiation on subsequent adult emergence and longevity of the adult fruit fly
(3) To validate the proposed treatment using 100 000 test insects per dose and stage of development for a probit 9 security level
(4) To conduct confirmatory tests on the effective dose obtained, using large samples of infested mangoes
(5) To determine the effects of radiation on the gonads of the immature stages of the fruit fly as an indicator of radiation damage
(6) To determine the effects of gamma radiation on the physiological and postharvest quality of mango fruits and to compare these effects with VHT
(7) To determine the natural field infestation of mature green mango fruits in different orchards.

2. MATERIALS AND METHODS

2.1. Natural infestation of fruits

The fruits used in these trials were obtained from an exporter's packing house and were classified as export quality (Japan grade). Only mature green, medium sized (250–300 g) fruits harvested during the peak season were used.

To determine the field infestation of 'Carabao' mangoes, samples of mango fruits were procured from the packing houses of two mango exporters: the Philippine Far East Agro-Products, Inc. and the Pelican Agro Products, Inc. The fruits were purchased by exporters from different parts of the country. Most of these fruits came from mangoes examined by Plant Quarantine Inspectors of the Japanese Government and the Bureau of Plant Industry, Philippine Department of Agriculture, assigned to the exporter's packing house and suspected of being infested with fruit fly eggs. In the laboratory, the fruits were placed in plastic jars lined with coir dust and stored for 2 weeks to allow any eggs or larvae to develop. During the third week, the fruits were dissected for any larvae present, while the coir dust was sieved for any puparia that had developed from the eggs or larvae in the fruit.

2.2. Radiation effects on the oriental fruit flies

For artificial infestation of mature green, export grade mangoes, the fruits were shallow punctured with a 'frog', or flower holder, which had about 16–18 minute nails. About 100 oriental fruit fly eggs were inoculated in the tiny holes and the fruits were held at room temperature in a screened cabinet until the insects had reached the desired age or stage for treatment, i.e. 20–24 hour old eggs, and 2 day old and 7 day old larvae, representing mature eggs, and young and mature larvae, respectively.

2.2.1. Larval survival and adult emergence

In the irradiation test, four artificially infested fruits were packed in a plastic jar (18 cm × 12 cm) and exposed in the dry gamma room of the Philippine Research Reactor I at radiation doses of 50, 100, 150, 200, 250, 300, 350 and 400 Gy delivered at a rate of 6.75 Gy/min. Six plastic jars were used to give a total of 24 fruits per dose and per stage of development. An untreated batch served as the control lot.

After irradiation, each fruit was placed individually in a plastic jar, at the bottom of which was sieved coconut coir dust, and covered with muslin cloth to prevent the insects from escaping. At 1–2 days before pupation, each fruit was dissected and the live larvae were counted. These larvae were allowed to pupate in the coir dust. Seven days later, the coir dust was passed through a sieve (12 mesh) and the puparia collected were placed in a fruit fly cage (20 cm × 18 cm × 18 cm) for adult emergence.

2.2.2. Dose mortality tests

Mature green mangoes were punctured using a frog and exposed for about 2 hours in a large cage (80 cm × 60 cm × 62 cm) containing about 20 000–22 000 adult flies for oviposition. The flies varied in age from 2 to 4 weeks after emergence. After infestation, the mangoes were stored in a screened cabinet maintained at ambient temperature. Then, 1 or 7 days after infestation, representing mature eggs and mature larvae, respectively, the mangoes were packed in a single layer in cartons and irradiated in air with 100 or 350 Gy at the dry gamma room facility. Three cartons were stacked, one on top of another, and rotated 180° after half of the predetermined time of exposure was attained in order to provide more uniform exposure. Each carton contained about 20 mango fruits of the same degree of maturity. Following treatment, the mangoes were held in beer cases containing coconut coir dust as the medium for pupation and covered with newspaper. Two weeks after treatment, the puparia were sieved from the coir dust, counted and placed in plastic cups kept in a small screened cage for adult emergence. A week later, the number of emerged adults was recorded.

2.2.3. Large scale tests

Mature fruit fly eggs were collected from 2–4 week old adults using punctured plastic receptacles. With the aid of a camel hair brush, about 100 eggs were counted and placed on a square of moist blotting paper. A total of five blotting paper squares were prepared and arranged in a plastic Petri dish. Four or five Petri dishes containing approximately 2000–2500 eggs were placed sideways in a mango carton.

About 200–250 mature (5–6 day old) larvae were counted and placed in a plastic cup half-filled with moist coconut coir dust and covered with muslin cloth. A total of 10 plastic cups was prepared and placed in the inner portion of the mango carton, with the remaining cells filled up by 10 mature, green, export grade mangoes for the fruit quality test. About 8000–10 000 eggs and larvae and 40 mangoes were irradiated at weekly intervals until a total of 100 000 test insects per dose and per stage of development was completed for the probit 9 test.

The insects and fruits were treated at 100, 150 and 350 Gy using the multipurpose gamma irradiator facility. An unirradiated lot was designated as the control batch. The dose rate ranged from 6.0 to 6.7 Gy/min and the maximum/minimum dose ratio averaged about 1.63.

After treatment, the number of larvae hatching from the treated eggs was determined and the surviving larvae were placed on a sweet potato–yeast diet [18] and kept under ambient conditions (22°C–29°C) for 7–8 days. The mature larvae developing from the treated eggs were sieved from the diet, counted, placed in moist coir dust and held for another 7–8 days, at which time the puparia were sieved, counted and held in plastic cups contained in small fruit fly cages (18 cm × 18 cm × 20 cm) for adult emergence. The mature larvae irradiated in the moist coir dust were held in plastic cups for adult emergence.

2.2.4. Confirmatory tests

To confirm the disinfestation dose for 'Carabao' mangoes, a total of 470 kg of fruits was purchased at an exporter's packing house at the rate of 60 kg/week. The mature green fruits to be infested were punctured with a frog and about 85 fruits were placed inside a large fruit fly cage (80 cm × 60 cm × 62 cm) containing approximately 20 000 2–4 week old adult flies. The fruits were exposed to gravid females for oviposition for 1–2 hours. After infestation, the fruits were removed from the cages and held in a single layer on trays containing coir dust. The fruits were held at ambient conditions in screened cabinets. On day 5–6 after infestation, the fruits were removed from the screened cabinet, placed in mango cartons and irradiated at 100 Gy using the multipurpose gamma irradiation facility. After irradiation, the treated fruits were returned to the screened cabinet and held on a tray containing coir dust for about 2 weeks. Each tray was labelled according to the batch number and the radiation dose received. The trays of treated fruits were placed in

a separate screened cabinet from that containing the control fruits. Two weeks after treatment, any larvae that had pupated in the coir dust beneath the infested fruits were sieved from the dust and counted. The fruits were dissected to ensure that any slow developing larvae were also counted and held in the coir dust for pupation. The puparia were placed on a paper boat and held in a small fly cage for about 1 week. Any adult flies emerging were recorded as survivors.

2.2.5. Microscopic examination of insects

Samples of 24–30 hour old eggs and 5–7 day old larvae were irradiated, using the Gammacell-220 irradiator, with doses of 0, 100, 150 and 350 Gy at an average dose rate of 9.2 Gy/min. The treated eggs were placed in a modified sweet potato-yeast diet and the surviving larvae were allowed to develop and mature. About 20 larvae from each treatment were examined and dissected in saline solution using a stereo microscope to observe and compare any effects of radiation on the internal organs, especially the gonads, of the larvae. This examination was undertaken to find an indicator of radiation damage, with sterility as the end result. Irradiation, dissection and microscopic examination of the larvae and puparia were repeated until a sufficient number of samples was observed and conclusions could be drawn.

2.3. Radiation effects on fruits

The mango fruits used in these studies were obtained from an exporter's packing house and five trials were conducted. The fruits in the first two trials were grown in Pampanga, those in the third trial in Bataan, while the fruits used in the fourth and fifth trials were grown in Bulacan. All these provinces are located on the Island of Luzon.

The fruits were placed in cell type corrugated cartons and irradiated at 0, 100, 150 and 350 Gy for the first three trials at the PNRI using the multipurpose gamma irradiator at an average dose rate of 6.6 Gy/min. In the last two trials, a dose of 250 Gy was used instead of 150 Gy in order to conform to the protocol for evaluating the effects of radiation treatments on fruit quality. There were three replicates per treatment, with a carton containing 20 fruits (5–6 kg per carton) serving as a replicate. After irradiation, the fruits were taken to the Postharvest Horticulture Training and Research Center (PHTRC) at the University of the Philippines at Los Baños for evaluation.

The fruits subjected to VHT were treated in a commercial chamber (12 tonne capacity) located at the Food Terminal Inc. The sample fruits were included in the lots to be treated commercially and the total treatment time ranged from 4 to 5 hours. The fruits were then packed in cartons and taken to PHTRC.

The fruits used in all the trials were subjected to the usual packing house operations for export mangoes; however, there was a lag period of about 48 hours between

harvesting and treatment. In the first three trials, the fruits were not subjected to the hot water treatment (HWT) for disease control. In the last two trials, however, all the test fruits were immersed in water at 52°C–55°C for 10 minutes [19] about 4 hours prior to either irradiation or VHT.

The fruits were stored at 10°C–12°C to simulate the shipping conditions for export. The development of peel colour was monitored using a peel colour index (PCI) of 1 (green) to 6 (full yellow) [20]. Prior to storage, the fruits were marked for continuous monitoring of peel colour development and weight loss.

At the end of the storage period, the visual quality of the fruits was determined following a rating of 9 (excellent) to 1 (inedible/unmarketable under ordinary conditions), with 3 as the limit of marketability. The incidence of disease was likewise noted. The fruits (still packed in cartons) were then transferred to 25°C for subsequent ripening.

Upon reaching the table ripe stage (TRS), the fruits were subjected to a two step sensory evaluation (intact fruits, followed by the cubed pulp) using untrained panellists. A continuous scoring system using a 10 cm line, with 10 representing the most favourable response, was employed. For hedonic rating, a scale of 1 (dislike extremely) to 9 (like extremely) was used.

The physicochemical characteristics of the ripe fruit were determined as follows: the chromaticity (yellowness) was measured with a Minolta chromameter; the total soluble solids of the water extracted pulp were measured with a hand held refractometer; the titratable acidity was determined by titration with a standard base (0.1N NaOH); and the pH with a pH meter. The total sugars were analysed by the phenol–sulphuric method [21], while the reducing sugars were determined by the Nelson–Somogyi method [22]. Ascorbic acid was determined with 2.6-dichlorophenol indophenol [23].

The visual quality and incidence of disease were again noted in the ripe fruits. The incidence of physiological disorder was evaluated upon reaching TRS. The fruits were cut longitudinally on both sides of the seed and the severity of IB was rated on the basis of the proportion of the cut surface affected. The indices used were: 0 = no IB, 1 = slight, up to 25%, 2 = moderate, 26–50%, and 3 = severe, more than 50% of the cut surface affected.

The respiration rate was measured by determining the total CO_2 evolved by the fruits after 1 hour of enclosure in jars (static system). The CO_2 was analysed using a Varian gas chromatograph (GC) equipped with a thermal conductivity detector. The internal gas was sampled under reduced pressure and the ethylene measured with a GC equipped with a flame ionization detector.

2.4. Radiation sources

Irradiation was accomplished using one of the three ^{60}Co facilities at the PNRI: as of 1971, the dry gamma room of the Philippine Research Reactor I contain-

ing a ^{60}Co source with an initial strength of 20 000 Ci; as of 1971, the Gammacell-220 irradiator containing ^{60}Co with an initial strength of 7770 Ci; and an irradiation chamber measuring 6 inches in diameter and 8⅛ inches in height.[1] The latest facility is the batch type multipurpose gamma irradiator (Gamma Beam 651 PT). The latter was loaded in January 1989 with 30 000 Ci of ^{60}Co. The irradiator has four turntables with about 500 kg capacity per turntable or a total of 1 m^3 for the four turntables. These sources came from Nordion International Inc. (formerly the Atomic Energy of Canada Limited). The dosimetry was carried out by the Irradiation Services Group of PNRI using Fricke dosimeters.

2.5. Statistical analysis of data

The data from each study were analysed separately using factorial experiments in a randomized complete block design and subjected to analysis of variance (ANOVA) at the 5% level of significance [24]. The number of insects recovered was transformed using log (x + 1) prior to ANOVA. If significant differences were shown to exist after the ANOVA results, the treatment means were further compared using the Duncan's multiple range test [25]. To have 95% confidence that the treatment provides 99.9968% mortality (probit 9), about 100 000 test insects were treated for each stage of development as a statistical basis of quarantine security [26].

3. RESULTS AND DISCUSSION

3.1. Natural infestation of fruits

Of a total of 3200 mature green mango fruits (800 kg) sampled between 1985 and 1989 from different parts of the country, only one mango was found to be infested with eight larvae of the oriental fruit fly, or 0.031% field infestation. This mango came from the Island of Cebu, which is located in the Visayan Islands, about 700 km south of Manila. The mango fruits are produced all year round on Cebu and are usually wrapped in newsprint while on the tree in order to prevent damage from fruit flies. The single infested fruit obtained from the 800 kg of fruits examined may not have been infested while on the tree but after harvest, before shipment from Cebu to Manila. It was observed that the fruit was soft and not fresh. It is highly possible that it had been harvested for quite some time and had been included in the pile of fruits shipped to Manila for packing at the Food Terminal, Inc. Our findings showed that harvesting fruits in the mature green stage may eliminate the need for any

[1] 1 Ci = 3.70×10^{10} Bq; 1 in = 2.54×10^{1} mm.

TABLE I. MEAN NUMBER OF ORIENTAL FRUIT FLY LARVAE RECOVERED FROM EGGS OR LARVAE IRRADIATED IN MANGOES

Dose (Gy)	Stage treated[a]		
	Eggs (20 hour old)	Larvae (2 day old)	(7 day old)
0	63.8a	54.0a	56.5a
50	29.4b	56.0a	49.4b
100	3.6c	47.8b	50.1ab
150	1.5c	47.8b	48.4b
200	0.9c	44.0bc	41.8bc
250	0.04c	48.4b	43.0bc
300	0.08c	40.4c	39.1c
350	0	39.6c	33.8cd
400	0	41.1bc	31.4d

[a] The means within columns followed by the same letter are not significantly different (P = 0.05) [25].

TABLE II. EFFECTS OF RADIATION ON THE SURVIVAL OF ORIENTAL FRUIT FLY ADULTS TREATED AS EGGS OR LARVAE IN MANGOES

Dose (Gy)	Stage treated	Age	Mean % adults alive at weeks after emergence	
			1	2
0	Eggs	20–24 hour	98.2	96.4
	Larvae	2 day	100	98.6
	Larvae	7 day	100	95.4
50	Eggs	20–24 hours	—	
	Larvae	2 day	—	
	Larvae	7 day	65.2	0

TABLE III. RECOVERY OF ORIENTAL FRUIT FLY PUPARIA AND ADULTS FOLLOWING IRRADIATION 1 OR 7 DAYS AFTER INFESTATION (AS EGGS OR MATURE LARVAE) IN MANGOES

Date treated	Stage treated	Dose (Gy)	No. of insects recovered	
			Puparia	Adults
23 Feb. 1988	Eggs	0	4532	4381
		100	106	0
		350	0	—
	Larvae	0	4358	4239
		100	362	0
		350	0	—
1 Mar. 1988	Eggs	0	4297	4073
		100	83	0
		350	0	—
	Larvae	0	4345	4221
		100	217	0
		350	0	—
8 Mar. 1988	Eggs	0	4219	3786
		100	113	0
		350	0	—
	Larvae	0	4086	3892
		100	417	0
		350	0	—

quarantine treatment of mango fruits, provided they are protected from infestation after harvest, in the field or in the packing house, before shipment to importing countries.

3.2. Radiation effects on the oriental fruit flies

3.2.1. Larval survival and adult emergence

Table I [25] summarizes the effects of different doses of radiation on the egg and larval stages of the oriental fruit fly. The egg was observed to be more sensitive to radiation damage than the larval stage. Significantly fewer larvae survived from mature eggs that had been treated with 100 Gy than from those treated with 50 Gy,

with means of 3.6 and 29.4 larvae per fruit, respectively (Table I). No larvae survived from the eggs treated at doses above 300 Gy.

The data in Table I show that larval survival was inversely related to radiation dose. When the larvae were irradiated at doses of up to 150 Gy, there were only slight differences in radiation sensitivity between the control and the treatments for both the younger and older larval ages. However, significantly fewer larvae survived at doses above 300 Gy than at doses below 200 Gy. Also, when compared with the control, the larval period of the treated larvae was prolonged by 2 or more days. Such an observation may be due to the effects of radiation on certain physiological processes such as hormone production. Similar findings have been reported by other workers, as cited by Burditt in Ref. [9], but there has not been a complete account of such research to explain this phenomenon.

In our tests, no adults developed from any of the irradiated eggs or 2 day old larvae, and very few adults (an average of 2.25) emerged from those treated with 50 Gy as 7 day old larvae. All these adults died within 2 weeks of emergence (Table II), indicating reduction in adult longevity as a possible consequence of radiation damage to the somatic cells, only manifested in the adult stage.

The mortality in our tests was much lower than that reported by Prasad and Sethi [27] on third instars of the oriental fruit fly. They found that at doses of 100, 150 and 200 Gy, 70, 64 and 59% of the larvae pupated and 42.5, 41.5 and 31.5% emerged as adults, respectively. In another work, Loaharanu [28] reported that adult emergence was prevented at 250 Gy when 2 and 6 day old larvae were irradiated in mangoes.

3.2.2. Dose mortality tests

The results of small scale tests using about 6000 insects per treatment are summarized in Table III. These data showed that when the fruits were treated 1 and 7 days after infestation, none of the egg or larval stages exposed to 350 Gy were able to pupate. When the infested fruits were treated at 100 Gy, 2.3% of the insects treated 1 day after infestation (as eggs) and 7.8% of those treated 7 days after infestation (as larvae) formed puparia. However, none were able to emerge as adults.

3.2.3. Large scale tests

Table IV shows the results of irradiation on a large number of 'naked' eggs and larvae of the oriental fruit fly treated at doses of 100, 150 and 350 Gy. Again, it was evident that the eggs were the most sensitive and that the mature larvae were the stage most tolerant to irradiation. When the egg stage was irradiated, treatment at 350 Gy resulted in 100% mortality, compared with 96.96 and 99.92% mortality as larvae in the 100 and 150 Gy treatments, respectively. Observation of the larvae hatching from the treated eggs showed that they were small and sluggish compared

TABLE IV. RECOVERY OF ORIENTAL FRUIT FLY LARVAE, PUPARIA AND ADULTS FOLLOWING IRRADIATION OF MATURE EGGS ON BLOTTING PAPER AND LARVAE IN CUPS OF COIR DUST

Stage treated	Dose (Gy)	No. of insects treated	No. of insects recovered		
			Larvae	Puparia	Adults
Eggs	0	100 000	64 256	61 785	58 942
(20–24 hour old)	100	100 000	3 042	406	0
	150	100 000	783	12	0
	350	100 000	0	0	0
Larvae	0	101 050		97 993	92 841
(5–6 day old)	100	100 477		95 164	0
	150	100 090		93 104	0
	350	100 400		92 449	0

TABLE V. RECOVERY OF ORIENTAL FRUIT FLY PUPARIA AND ADULTS IN MANGOES INFESTED WITH 5 DAY OLD LARVAE AND TREATED AT 100 Gy FOR CONFIRMATORY TESTS

Replicate	No. of fruits tested		No. of insects recovered			
			Control		Treated	
	Control	Treated	Puparia	Adults	Puparia	Adults
1	95	160	14 935	11 750	21 350	0
2	95	160	13 840	9 340	19 450	0
3	95	160	15 893	7 968	20 135	0
4	95	160	16 678	13 628	14 380	0
5	125	160	19 180	15 435	21 536	0
6	130	160	20 526	11 485	17 365	0
7	110	160	18 735	12 414	16 932	0
Total	745	1120	119 787	82 020	131 148	0

with the control batch. Also, the larval period was extended by 2 or more days beyond the normal duration of the stage. Further development of these larvae to the pupal stage was reduced and no adults emerged. When the mature larvae were irradiated in the coir dust as the medium for pupation, no adults emerged from more than 100 000 larvae treated with doses ranging from 100 to 350 Gy, although no difference in the percentage pupation was observed between the treated (92.08–94.71%) and the control (96.97%) lots.

3.2.4. Confirmatory tests

Using the emergence of adult insects as the standard of survival, our studies showed that a dose of 100 Gy achieved better than the probit 9 level of mortality (99.9968%) as a basis of quarantine security [29]. This is very evident in our confirmatory tests using more than 100 000 test insects in the host fruits. Complete mortality of the test insects was obtained after treating a total of 1120 infested mango fruits compared with 82 020 adults that emerged from 119 787 puparia in 745 untreated fruits (Table V). These results are similar to earlier small scale irradiation studies of infested mangoes using the 2500 Ci source of ^{60}Co.

In Thailand, where the mango variety used was different to that of the Philippines, a dose of 150 Gy was reported to have achieved 99.9993% mortality when a total of 138 538 larvae of the oriental fruit fly was irradiated [30]. The 'Nang Klangwan' variety is larger and much longer than the Philippine 'Carabao' ('Manila Super') mango; therefore, the density and thickness differ, which may have affected the dose required for quarantine security. Similar findings have been reported in other commodities where the minimum dose required to prevent the development of adult oriental fruit flies from irradiated eggs or larvae was 209–291 Gy [6].

3.2.5. Microscopic examination of insects

The results of microscopic examination of the internal organs of mature larvae that had been irradiated as eggs or larvae (76 samples) and their controls (103 samples) showed that the gonads of the fruit fly cannot be used as an indicator of radiation damage in the egg or larval stage of the insect. Our examinations revealed the absence of any definitive organs of the reproductive system in the larval stage, although the tracheal system, fat bodies, digestive system and nervous system could be differentiated from one another.

In all the microscopic examinations done of larvae, the gonads could not be differentiated from the rest of the internal organs; this indicates that the germ cells are still probably in the pre-definitive stage during the egg and larval stages, although at these stages most of the body or somatic cells are undergoing cell division and will most likely be affected by the treatment.

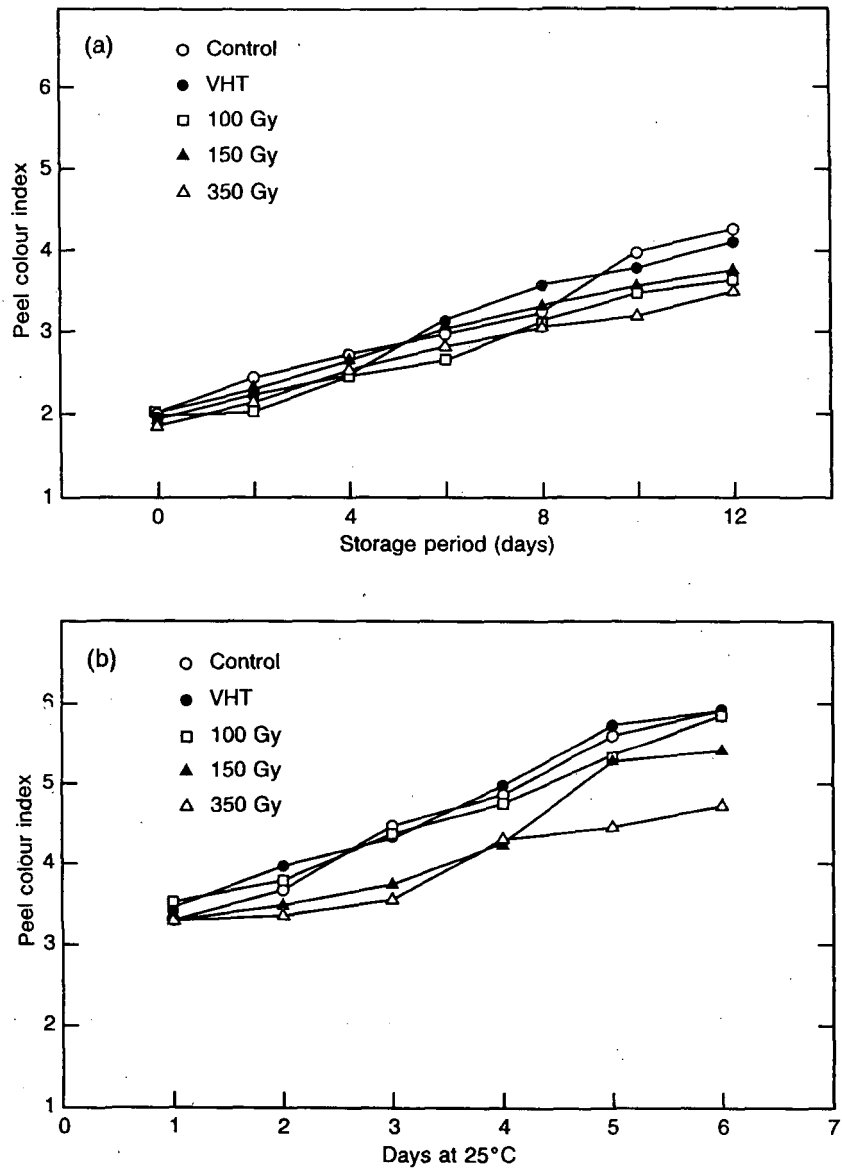

FIG. 1. Development of peel colour of irradiated and VHT 'Carabao' mangoes (a) during storage at 12 °C, and (b) upon transfer to 25 °C. Each data point represents the mean obtained from the first two trials using five fruits per replicate.

In the control, microscopic examination of the internal organs of the fruit fly was continued in the pupal stage at 2, 4, 6 and 8 days after pupation. It was only on 6 and 8 day old pupae that the gonads became distinct from the rest of the internal organs, with the testes assuming a pale yellow colour and the ovaries a somewhat flat white colour.

Irradiation treatment of the egg or larval stage of the fruit fly would not result in sterility in adults that may have survived the treatment, but would most likely result in mortality in the treated stage or in the stage following treatment, or abnormality in the surviving adult and reduced longevity, manifesting damage to the somatic cells during treatment. The idea of inducing sterility as a result of irradiation when the insect was treated in the egg or larval stage is quite remote, since microscopic examination revealed the absence of differentiated reproductive organs in the larval stage. It was only in the late pupal stage (6–8 days) that the gonads became distinct; the germ cells would be actively undergoing cell division and therefore be sensitive to damage by radiation, resulting in sterility in the adults [31]. The organs were more distinct and more easily recognizable in 8 day old than in 6 day old pupae. Therefore, sterility in the surviving adults could not be expected if irradiation was done in the egg or larval stage of the oriental fruit fly. However, mortality or abnormality would occur, most likely as a manifestation of irradiation injury to the somatic cells, similar to that observed in our study.

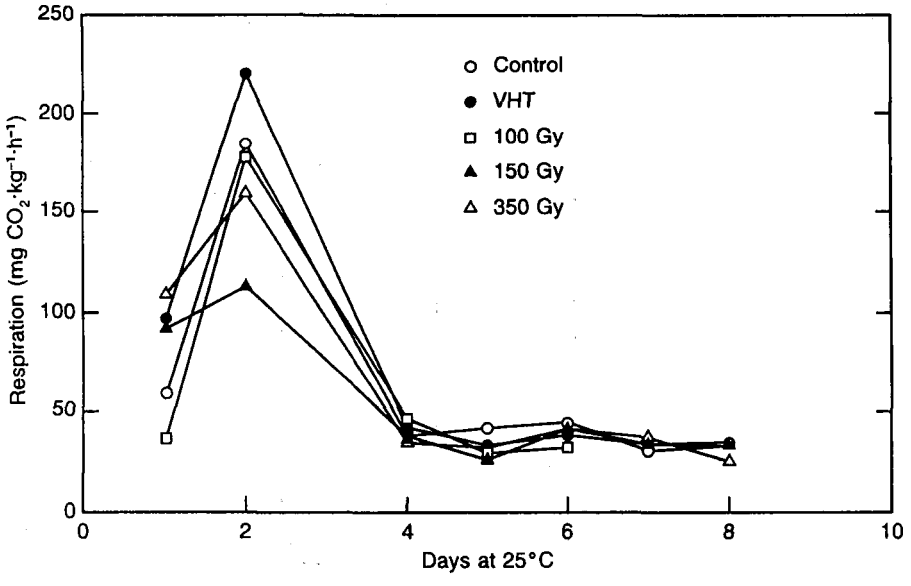

FIG. 2. Respiration rate at 25 °C of Pampanga grown 'Carabao' mangoes subjected to VHT and irradiation.

3.3. Radiation effects on fruits

At 12°C, the rate of peel colour development was retarded by irradiation at 350 Gy, although the delay was significant only at the end of storage (Fig. 1(a)). After the transfer from 12°C to 25°C at PCI 3, delayed peel colour development was likewise observed only in fruits irradiated at this dose (Fig. 1(b)). Baral [32] also reported an extension of storage life in 'Carabao' mangoes irradiated at 500 and 750 Gy. A 2–3 day delay in ripening was also reported for 'Tommy Atkins' [33] and 'Alphonso' [34] mangoes irradiated at 250 Gy. For the former, the delay was also evident at a dose of 150 Gy.

In 1984, Dubery et al. [35] reported an altered respiratory pattern during the climacteric in 'Haden' mangoes irradiated at 750 Gy. In the first three trials of our study, there was no significant change in the respiratory climacteric in irradiated fruits, as exemplified by the results shown in Fig. 2. The absence of a significant effect on the respiratory climacteric might be attributed to the lower doses used in this study.

TABLE VI. PHYSICOCHEMICAL CHARACTERISTICS AT THE TABLE RIPE STAGE OF 'CARABAO' MANGOES SUBJECTED TO VAPOUR HEAT OR IRRADIATION TREATMENT AT A PEEL COLOUR INDEX OF 1.0, STORED AT 12°C FOR 14 DAYS AND SUBSEQUENTLY TRANSFERRED TO 25°C[a]

Treatment	TSS[b] (°Brix)	TA[c] (%)	pH	Reducing sugars (%)	Total sugars (%)	Ascorbic acid (mg/100 g)
Control	14.7	0.15	4.75	3.47	14.84	47.76
Vapour heat	14.6	0.20	4.38	3.58	16.54	60.15
Irradiation dose (Gy)						
100	14.3	0.18	4.23	2.97	14.60	52.58
150	15.6	0.23	4.33	2.16	13.42	51.67
350	16.2	0.20	4.47	1.80	13.65	63.62

[a] Values represent the means obtained from six fruits.
[b] TSS = total soluble solids.
[c] TA = titratable acidity.

TABLE VII. VISUAL QUALITY RATING, DISEASE INCIDENCE AND PHYSIOLOGICAL DISORDER OF 'CARABAO' MANGOES SUBJECTED TO VAPOUR HEAT OR IRRADIATION TREATMENT AT A PEEL COLOUR INDEX OF 1.0, STORED AT 12°C FOR 14 DAYS AND SUBSEQUENTLY TRANSFERRED TO 25°C[a]

Treatment	Visual quality rating		Disease incidence (%)		Physiological disorder (%) at TRS
	After 14 days at 12°C	At TRS	After 14 days at 12°C	At TRS	
Control	7.7	4.3	26.67	45.45	0
Vapour heat	8.0	7.2	0	19.78	23.03[b]
Irradiation dose (Gy)					
100	8.0	5.2	0	29.52	0
150	8.0	4.2	0	27.08	0
350	7.9	4.3	6.67	16.52	5.51[c]

[a] Each value represents the mean obtained from 10 fruits/replication (trial); the fruits were evaluated following storage and at TRS.

[b] Internal breakdown.

[c] Browning of the pulp, area less than 10% of the fruit surface.

At the TRS, no significant differences in acids and sugars were observed between the irradiated and non-irradiated fruits (Table VI), although the fruits irradiated at 150 and 350 Gy had lower values for total and reducing sugars.

The visual quality of the irradiated fruits at TRS was comparable to that of the control but inferior to that of the fruits subjected to VHT (Table VII). The latter is consistent with earlier observations made in our laboratory, namely, that VHT enhances the quality of peel colour of 'Carabao' mangoes [17].

In trials where the fruits were not subjected to HWT for disease control, VHT was found to reduce significantly the disease incidence in the two trials, while irradiation resulted in a significant decrease in disease incidence relative to the control fruits only in one trial (Table VII). In 1978, a delay in disease development in fruits irradiated at 160–220 Gy was reported [36]. Since the most prevalent post-harvest diseases of mango, anthracnose and stem end rot, are latent diseases, the effect of irradiation on peel ripening may partly account for this inhibition of disease development.

TABLE VIII. SENSORY CHARACTERISTICS AT THE TABLE RIPE STAGE OF 'CARABAO' MANGOES SUBJECTED TO VAPOUR HEAT OR IRRADIATION TREATMENT AT A PEEL COLOUR INDEX OF 1.0, STORED AT 12°C FOR 14 DAYS AND SUBSEQUENTLY TRANSFERRED TO 25°C[a]

Sensory parameters	Control	Vapour heat	Irradiation dose (Gy)		
			100	150	350
Peel quality					
Uniformity of colour	8.2ab	9.0a	5.8b	6.4b	5.9b
Colour intensity	8.0a	8.6a	7.1a	7.8a	6.2a
Degree of shrivelling	9.9a	9.9a	9.8a	9.8a	9.9a
Pulp colour quality					
Intensity	7.6a	7.8a	7.6a	6.3b	5.5b
Uniformity of colour	7.6a	7.7a	7.4a	7.6a	6.9a
Pulp aroma					
Characteristic aroma	7.3b	8.3a	7.3b	7.4b	6.4c
Off odour	9.5a	9.6a	9.4a	9.5a	9.6a
Flavour					
Hedonic rating	7.0a	6.8ab	5.5b	5.4b	4.0c
Sweetness	7.0ab	7.7a	5.9bc	6.0ab	4.7c
Sourness	7.1a	7.4a	8.0a	7.9a	4.6b

[a] The means within the rows followed by the same letter are not significantly different (P = 0.05) [25]. Each mean was obtained from the results of three trials. The sensory evaluation involved 15 panellists per trial.

In the first three trials, VHT consistently induced IB, with the incidence ranging from 23 to 32%. In contrast, no IB was observed in any of the irradiated fruits. Pulp injury, specifically discoloration, was observed in fruits irradiated at 350 Gy only in one trial (Table VII); even so, the incidence was rather low. Pulp discoloration has been reported in other cultivars, but at much higher doses, e.g. 1500 Gy in 'Tommy Atkins' [33]; this has been associated with increased polyphenol activity [37]. It is interesting to note that, in the latter study by Thomas and Janave, catecolase was found to increase in 'Totapuri', starting at a dose of 150 Gy.

Irradiation at 150 or 350 Gy resulted in ripe fruits with a less uniform peel colour, a less intense pulp colour and a less perceptible mango aroma relative to those subjected to VHT, as judged by a sensory panel (Table VIII) [25]. It has also

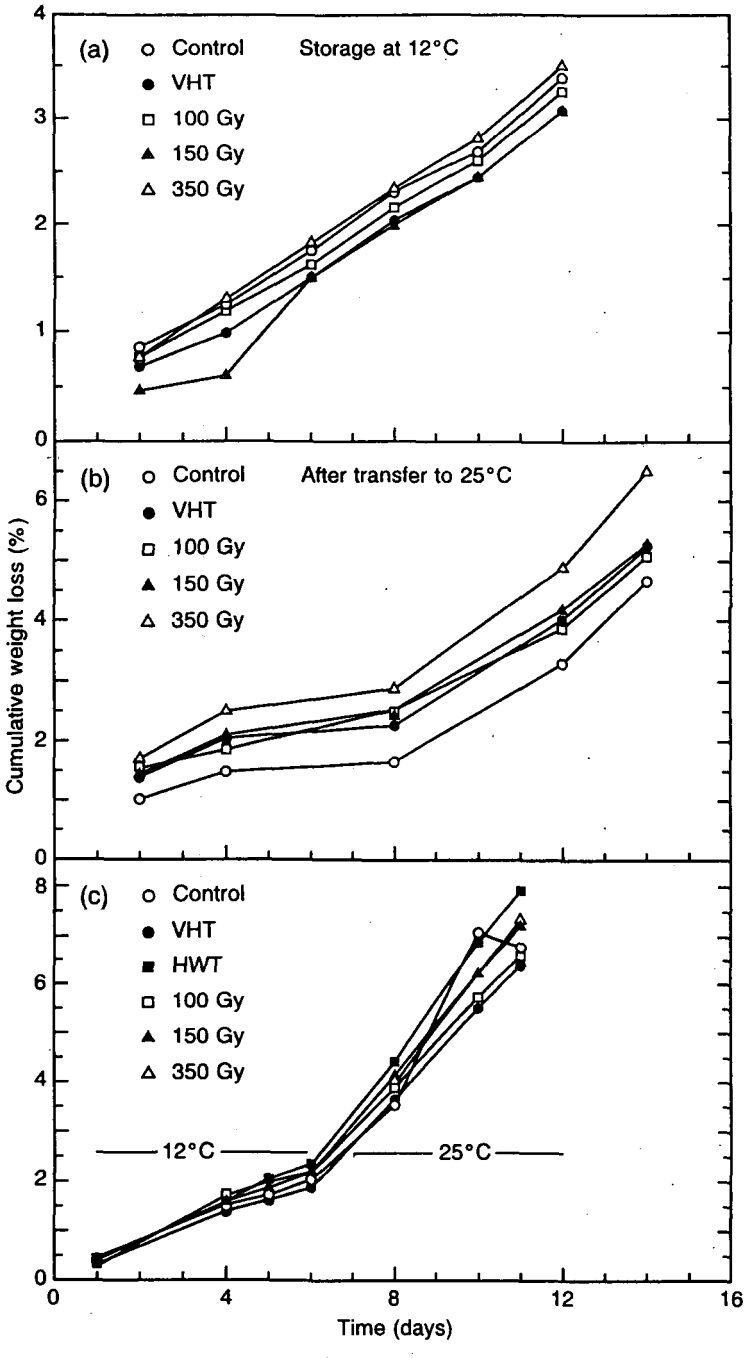

FIG. 3. Cumulative weight loss of (a) Pampanga grown, (b) Bataan grown, and (c) Bulacan grown 'Carabao' mangoes subjected to VHT and irradiation and then stored at 12°C. For (c), weight loss at 25°C is also included.

TABLE IX. PHYSICOCHEMICAL CHARACTERISTICS OF 'CARABAO' MANGOES SUBJECTED TO THE INDICATED TREATMENTS, STORED AT 12°C FOR 7 DAYS, TRANSFERRED TO 25°C AND EVALUATED WHEN THE CONTROLS REACHED THE TABLE RIPE STAGE[a]

Treatment	Peel colour index	Pulp firmness (kg/cm^2)	TSS[b] (°Brix)	TA[c] (%)	Yellowness	
					Peel	Pulp
Control	5.17ab	1.97b	11.45	0.16b	53.8b	65.9
Hot water	4.83b	2.09b	10.25	0.16b	52.7b	66.7
Hot water + vapour heat	5.50a	1.49b	10.56	0.21b	57.4a	66.9
Hot water + irradiation dose (Gy)						
100	4.92b	1.78b	10.46	0.17b	51.8b	66.9
250	4.92b	2.10b	9.77	0.22b	51.8b	66.7
350	3.25c	3.02a	8.92	0.34a	44.9c	65.7

[a] The means within columns followed by the same letter are not significantly different (P = 0.05) [25]. Each mean was obtained from three replicates consisting of six fruits.
[b] TSS = Total soluble solids.
[c] TA = Titratable acidity.

been reported that 'Carabao' mangoes irradiated at 750 Gy had a paler pulp colour [13]. Despite the present observations, the fruits subjected to 150 Gy were still quite acceptable. In contrast, fruits subjected to VHT had a distinctly better peel and pulp colour quality.

The effect of the treatments on weight loss was not consistent (Fig. 3). During storage at 25°C, irradiation at 350 Gy resulted in an increased weight loss only in fruits in the third trial (fruits from Bataan, Fig. 3(b)). In the fourth trial (Fig. 3(c)), where weight loss was monitored through ripening, no significant difference in terms of weight loss was observed between the irradiated and the VHT fruits.

A delay in peel colour development effected by irradiation at 350 Gy in the first three trials was also observed in fruits subjected to HWT prior to irradiation at 350 Gy. The results in Table IX [25] show that this combined treatment appears to delay ripening in the pulp as well as to indicate a greater pulp rupture force and a significantly higher titratable acidity. When Dhakar et al. [34] considered both fruit firmness and peel colour in 'Alphonso' mangoes, it was concluded that a 6 day delay in ripening was effected by irradiation at 250 Gy.

TABLE X. NUMBER OF DAYS REQUIRED TO REACH THE INDICATED PEEL COLOUR INDEX IN MANGOES SUBJECTED TO THE INDICATED TREATMENTS, STORED AT 12°C FOR 7 DAYS AND THEN TRANSFERRED TO 25°C

Treatment	No. of days to reach a peel colour index of				
	2	3	4	5	6
Hot water	3	4	5	8	9
Hot water + vapour heat	3	4	5	8	9
Hot water + irradiation dose (Gy)					
100	3	5	7	8	10
250	3	5	7	10	13
350	3	5	7	10	13

TABLE XI. COMPARISON OF PULP FIRMNESS AT A PEEL COLOUR INDEX OF 5 OR 6 IN MANGO FRUITS GIVEN THE INDICATED TREATMENTS

Treatment	Pulp firmness (kg/cm^2) at a peel colour index of	
	5	6
Hot water	0.29	0.32
Hot water + vapour heat	0.31	0.26
Hot water + irradiation dose (Gy)		
100	0.44	0.20
250	0.22	0.22
350	0.11	0.14

TABLE XII. INTERNAL GAS LEVELS IN MANGOES SUBJECTED TO VARIOUS TREATMENTS[a]

Treatment	Respiration (mg $CO_2 \cdot mg^{-1} \cdot h^{-1}$)	Co_2 (%)	C_2H_4 (ppm)
Control	25.3	—	—
Hot water	24.0	1.27b	0.07abc
Hot water + vapour heat	33.8	1.02c	0.06bc
Hot water + irradiation dose (Gy)			
100	47.3	1.47a	0.05c
250	54.5	1.52a	0.09ab
350	62.3	1.45a	0.10a

[a] The means within columns followed by the same letter are not significantly different (P = 0.05) [25]. The fruits were sampled for internal gas after equilibration at 12 °C. Each value represents the mean obtained from three fruits.

The chromaticity values taken from the peel with a chromameter confirm the pattern obtained with the subjective PCI, i.e. among the treatments, VHT combined with HWT resulted in enhancement of peel colour development, while irradiation at 350 Gy, even when combined with HWT, resulted in inhibition of yellowing. On the basis of PCI, all the irradiated fruits required more days to reach the full yellow stage than the non-irradiated fruits (Table X).

If the fruits at PCI 5 or 6 were compared in terms of pulp rupture force, however, the values obtained from fruits irradiated at 350 Gy were lower than those obtained from the other treatments, although statistical analysis rendered the differences insignificant (Table XI). Using a Humboldt penetrometer, Baral [32] observed that greater softening occurred in fruits irradiated at doses of 500 or 750 Gy, despite a delay in peel colour development. Moreover, this was associated with an increase in pectinesterase activity. These observations are reminiscent of those reported in irradiated 'Carabao' mangoes, which softened even while still green, and the irradiated fruits at the ripe stage, which were perceived to be softer than the non-irradiated fruits [13]. In 'Alphonso' mangoes, the effect of irradiation at 250 Gy on mango ripening was more pronounced in terms of the inhibition of chlorophyll degradation than ripening changes in the pulp [34].

In the last two trials, we did not observe any IB in fruits subjected to VHT. In a separate study, we have observed that subjecting mangoes to HWT prior to VHT significantly reduced the incidence of this disorder as long as the intervening period between the two treatments was about 4 hours.

In a study on the physiology of irradiated fruits, Dubery et al. [35] concluded that irradiation had two effects on fruit respiration: a more immediate stress response, indicated by increased respiration soon after treatment; and a reduction in the climacteric peak during ripening. In our study, the irradiated fruits showed higher initial respiration rates than the fruits subjected to VHT (Table XII) [25]. As a consequence, the initial levels of CO_2 in the irradiated fruits were higher than in the VHT fruits. In all the trials, we did not observe any significant and consistent effects on the respiratory climacteric.

4. CONCLUSIONS

From the quarantine point of view, our results showed that gamma radiation at a minimum dose of 100 Gy was sufficient to disinfest Philippine 'Carabao' ('Manila Super') mangoes infested with oriental fruit fly eggs or larvae. Radiation treatment of 100 Gy would provide quarantine authorities with a safe and effective level of the treatment's efficacy, since the emergence of adult oriental fruit flies was completely eliminated. In addition, irradiation at 100 or 250 Gy resulted in no adverse effects on the fruit quality. Irradiation at either dose offered a distinct advantage over VHT in that it did not induce internal breakdown. However, both treatments would need to be combined with hot water treatment in order to provide satisfactory control of disease. Moreover, the problem of internal breakdown associated with VHT can be minimized by timely application of the hot water treatment itself and by following recommendations with respect to maturity and other factors. However, with only 0.031% field infestation of mature green 'Carabao' mangoes, the need for any quarantine treatment may no longer be necessary, provided that proper protection of the commodity from infestation is imposed after harvest or before shipment of the fruits to importing countries.

ACKNOWLEDGEMENTS

Funding from the IAEA and the Philippine Nuclear Research Institute Grants-In-Aid Program is gratefully acknowledged. The authors wish to thank the Philippine Far East Agro-Products, Inc. and the Pelican Agro Products, Inc. for providing samples of mango fruits, and the San Miguel Corporation for providing several kilograms of brewer's yeast for the mass rearing of oriental fruit flies in the laboratory. The authors would also like to thank E.C. Cabalfin, L.G. Lanuza and the staff of the Irradiation Services Group, E.D. Tuazon of the Plant Quarantine Service, Bureau of Plant Industry, A.D. Cordero, R.B. Ilagan and D.M. Lazo of the Agricultural Research Group, PNRI, and K. Yaptenco, R. Rafols and the staff of the Philippine Far East Agro-Products, Inc. for the technical assistance provided.

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USE OF RADIATION IN AN EXPORT PLANT QUARANTINE PROGRAMME*

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Abstract

USE OF RADIATION IN AN EXPORT PLANT QUARANTINE PROGRAMME.

The oriental fruit fly, *Dacus dorsalis* Hendel, is recognized as a serious pest of many kinds of fruit. It causes losses in production and restricts trade between countries because of quarantine restrictions. Research was conducted to determine the natural infestation levels of fruit and the effects of gamma irradiation on fruit flies and fruits. When mangoes (*Mangifera indica* L.), longans (*Euphoria longana* Lamk.) and mangosteens (*Garcinia mangostana* L.) were collected from orchards, only one mango was naturally infested. Cracked or punctured mangosteens were infested in the laboratory. Irradiation of fruit fly eggs and larvae in medium showed that old larvae tolerated treatment better than young larvae or eggs. Pupation of younger larvae treated in mangosteens was reduced at a dose of 125 Gy and two males emerged from 1 day old larvae treated at 75 Gy. When 5 day old larvae were treated in mangoes, a dose of 150 Gy was effective as a quarantine treatment. Irradiation delayed the colour development of mangoes, decreased the vitamin C content and increased the reducing sugar and soluble solid contents. Treatment of mangosteens had no effect on the weight loss, freshness, firmness, pH, soluble solids and titratable acidity. However, for some treatments colour development was delayed and peel hardness and disease incidence were affected.

* Research carried out with the support of the IAEA under Research Contract No. 4278/RB.

1. INTRODUCTION

In general, all agricultural countries are concerned with the introduction of exotic organisms considered harmful to their agriculture. Plant protection laws and regulations are intended to prevent the introduction of many organisms specified as quarantine pests. Eradication is very difficult and costly, therefore importing countries only permit entry of pest free or treated commodities.

Thailand is one of many countries that exports plant materials such as field crops, vegetables and fruits. The government is trying to increase exports, for example, to ASEAN members, the Middle East market, the EEC market, the United States of America, Australia and Japan. Some countries are especially strict in their control of imported fruits because of fruit flies, e.g. the oriental fruit fly, *Dacus dorsalis* Hendel, and the melon fly, *D. cucurbitae* Coquillett. For a commodity to meet the laws and regulations of the destination country, the export country must treat such a commodity prior to shipment. Since 1980, Thailand has conducted a research project to achieve plant quarantine treatment by using ethylene dibromide (EDB). However, since the United States Environmental Protection Agency announced the ban on EDB [1], other alternative measures have had to be tried to take its place; formerly, EDB was used extensively worldwide. Irradiation is one of the experimental measures that may be applicable as a plant quarantine treatment for the fruit fly. It has recently been accepted by the Food and Drug Administration for this purpose [2].

The overall goal of the project was to evaluate irradiation as a quarantine treatment that could be effective in meeting the plant quarantine requirements of countries importing perishable commodities from Thailand and thus increase the national income. The specific objectives were:

(1) To establish the degree of natural fruit fly infestations present in fresh fruits considered for export
(2) To determine the survival of fruit flies treated at the egg or larval stage by irradiation
(3) To assess the minimum radiation dose or combinations of radiation and other treatments, such as hot water treatment used to control post-harvest decay, necessary to achieve levels of insect control that will satisfy the quarantine requirements of importing countries
(4) To determine the acceptability of products treated by radiation at previously established effective absorbed doses in collaborative studies with the co-operating quarantine authorities in importing countries
(5) To evaluate the effects of acceptable treatment combinations on the physical, physiological, chemical and sensory values.

2. MATERIALS AND METHODS

2.1. Infestation of fruits by fruit flies

2.1.1. Mango (Mangifera indica *L.*)

The degree of natural fruit fly infestation in fresh mangoes, cv. 'Nang Klangwan', was assessed by sampling 2400 fruits collected from three different locations (600, 600 and 1200 fruits from Chachoengsao, Ratchaburi and Chiang Mai, respectively). The fruits were kept in plastic trays covered with muslin to prevent reinfestation and placed in a controlled temperature room at 27°C ± 2°C for 5 days. The traces of damage done by fruit flies were examined. Each fruit suspected of being infested by the insects was kept in a plastic box (8 cm × 15 cm × 21 cm) in which sawdust had been placed and held for the development of any larvae, pupation of the larvae and adult emergence.

2.1.2. Longan (Euphoria longana *Lamk.*)

Eighty traps were set in four longan orchards (20 traps per orchard). The fruit flies were collected from the traps four times before harvesting the fruit. Eighty kilograms of longan were collected randomly four times from each orchard. Half of the longans were selected randomly each time for inspection under a dissecting microscope. The remaining fruits were kept in plastic boxes (1/2 kg/box) in which sawdust had been placed and held for observation, as above.

2.1.3. Mangosteen (Garcinia mangostana *L.*)

The mangosteens used for this study were selected randomly from three shops in the market. Sampling was done by collecting 15 kg of fruit from each shop. The traces of damage caused by the fruit flies were examined. The study was repeated three times.

A laboratory study was carried out on the efficiency of oriental fruit fly egg laying and survival in mangosteens. This experiment involved four treatments and four replications. Twenty fruits were used in each treatment. The treatments were carried out as follows:

(1) Control (healthy fruits)
(2) Shallow holes were made in the skin around the fruits (the holes did not reach to the pulp)
(3) Deeper holes were made in the skin around the fruits (the holes penetrated through the pulp)
(4) The fruits were cracked.

The fruits for each treatment were infested with the oriental fruit fly by placing the fruits in cages containing approximately 2000 adult flies for 15 minutes. The infested fruits were then kept in plastic boxes containing sawdust and maintained in the controlled temperature room at 27°C ± 2°C for observation, as above.

2.2. Effect of irradiation on immature oriental fruit flies

2.2.1. Immature stages treated in artificial medium

Oriental fruit fly eggs and larvae were irradiated in rearing medium. The experiment was conducted by using eggs (24 hour old) and first (1 day old), second (3 day old) and third (5 day old) instar fruit fly larvae in round (12 cm diameter) dishes of medium. Each dish contained approximately 1500 eggs (0.1 cm^3 of eggs). They were irradiated at doses of 0 (control), 20, 30, 40, 50 and 60 Gy in the centre of the irradiator. Each test was repeated five times. The control and the treated eggs and larvae were kept in controlled temperature rooms at 27°C ± 2°C for their development. Survival was determined by counting the larvae and puparia in the container 10 days after irradiation. The larvae and puparia were held for adult emergence.

2.2.2. Immature stages treated in mangosteens

Different stages of the larvae of the oriental fruit fly in mangosteens were treated with absorbed doses of 75, 100 and 125 Gy. Each fruit was artificially infested with 20 eggs. Fifty fruits were used for each stage of the larvae. Four stages of the larvae were used in each treatment; there were 1, 3, 5 and 7 day old larvae. The infested fruits were kept in controlled temperature rooms at 27°C ± 2°C. Before irradiation, 20% of each stage were selected randomly for the control. The irradiated and non-irradiated fruits were maintained in separated controlled temperature rooms at 27°C ± 2°C. These were examined by counting the larvae, puparia and adults.

2.2.3. Five day old larvae treated in mangoes at 150 Gy

The 'Nang Klangwan' mangoes used in this study were infested with the oriental fruit fly by being placed in cages containing approximately 25 000 adult flies for 6 hours. Puncturing of the skin allowed the female flies to oviposit directly into the fruits [3]. The infested fruits were packed in cardboard boxes (31 cm × 47 cm × 10 cm), 12 fruits per box, and kept in a controlled temperature room at 27°C ± 2°C for 6 days in order to obtain 5 day old larvae before treatment. Five day old larvae were used in this study because many scientists have reported that the resistance of fruit flies to irradiation increases as the flies develop [4–8]. Twenty per

cent of the infested fruits were used for the control and 80% for the treatments, which comprised irradiation at a minimum dose of 150 Gy by placing the boxes of fruit 103 cm from the ^{60}Co source. After treatment, the fruits were placed directly on shelves. The plastic boxes containing sand and sawdust were placed underneath the shelves to collect the puparia. The shelves and plastic boxes were covered entirely with muslin to prevent reinfestation. The treated fruits were maintained in the controlled temperature room at 27°C ± 2°C for 14 days. Examination was made by counting the number of puparia recovered. The puparia were then held for adult emergence.

2.3. Effect of irradiation on the disease and quality of fruits

2.3.1. Mango

Forty mango fruits were used in each treatment and 80 fruits were tested for the control. The treatments were:

(1) Irradiation at absorbed doses of 150, 300 and 600 Gy
(2) Hot water treatment by dipping at 55°C for 5 minutes
(3) A combination of irradiation and hot water treatment.

The mangoes were wrapped in sandwich paper and packed in the cardboard boxes. They were kept in the controlled temperature room at 16°C–18°C and 92–93% relative humidity. Observations were made after 12 days of storage.

2.3.2. Mangosteen

The mangosteens were carefully harvested on 11 and 12 August 1990 from different orchards in Choomporn in the Southern Province of Thailand. Stage 1 (turning stage) and stage 2 (pink peel) fruits were selected and wrapped separately in plastic bags before weighing and packing in fibre board cartons (2 kg per carton). They were then transported at 15°C by refrigerated truck to the Postharvest Technology Laboratory, Horticulture Research Institute, on 13 August 1990 and stored at 15°C. On 14 August 1990, the fruits were irradiated at the irradiation plant in Rangsit, Prathumthani, 30 km north of Bangkok, and returned to the cold room shortly after treatment.

Three boxes of fruits, with each individual box serving as a replicate, in each irradiation dose were checked every 4 days for weight loss, freshness of the calyx, colour development, firmness, pH, total soluble solids, titratable acidity and the incidence of disease and abnormality. Fruit freshness was represented by the score rating of the fruit stem in which 5 is the highest and 1 is the lowest. Colour development was scored by a rating of 1–5 for the peel colour: score 1 represented the turning

stage and scores 2, 3, 4 and 5 represented pink, red, dark red and purple, respectively.

2.4. Irradiation facility

The fruits were irradiated at the Office of Atomic Energy for Peace using an Atomic Energy of Canada Limited 650 ^{60}Co irradiation source. The dose rate during these experiments ranged from 4.54 to 5.13 Gy/min. Dosimetry was carried out using Fricke dosimeters. Some mangosteens were treated at the JS-8900 gamma irradiation facility in Rangsit, Prathumthani, where the dose rate was 10.97 Gy/min.

3. RESULTS

3.1. Infestation of fruits by fruit flies

3.1.1. Mango

The results of these tests revealed that only one mango of the 600 fruits collected from Chiang Mai was found to be infested by the oriental fruit fly. It had thirteen larvae.

3.1.2. Longan

The results from the trap collections in longan orchards showed that six species of fruit fly were present. In addition to 42 005 oriental fruit flies, 20 081 *D. correctus* (Bezzi), 116 *D. zonatus* (Saunders), 73 *D. cucurbitae* Coquillett, 39 *D. nigrotibialis* (Perkins) and 36 *D. caudatus* F. flies were collected. However, no fruit flies were found infesting the longans, which had been selected randomly from the orchards.

3.1.3. Mangosteen

The results of the collections of mangosteen from the three markets revealed that no fruit fly infestations were found.

The results of the laboratory experiment on infesting mangosteens with oriental fruit flies revealed that no puparia and adults developed on the healthy fruits or the fruits with shallow holes. It was found that the flies laid their eggs in these holes, but that the larvae died soon after hatching. However, larvae survived and formed puparia when there were deep holes in the fruits or when the fruits were cracked. A total of 1376 and 1808 adults developed from these larvae, respectively.

3.2. Effect of irradiation on immature oriental fruit flies

3.2.1. Immature stages treated in artificial medium

The results showed that old larvae were more tolerant to gamma radiation than young larvae or eggs.

3.2.2. Immature stages treated in mangosteens

The results are shown in Table I [9] for the development of puparia following irradiation of infested mangosteens. Pupation of 1 day old larvae was reduced by treatment at doses of 75 Gy, and above. However, treatment of older larvae at these doses did not have a consistent effect on the formation of puparia, since 5 and 7 day old larvae treated at 75 Gy were more susceptible to irradiation than those treated at 100 or 125 Gy. When 3 and 5 day old larvae were irradiated at 125 Gy, the formation of puparia was significantly lower than that for the untreated larvae. Two male adults were found from the 1 day old larvae that had been treated at 75 Gy.

3.2.3. Five day old larvae treated in mangoes at 150 Gy

The results of this study showed that a dose of 150 Gy achieved better than probit 9 mortality, based on the criterion of the non-emergence of adult flies, when 5 day old oriental fruit fly larvae were treated. On the basis of the emergence of

TABLE I. RECOVERY (%) OF ORIENTAL FRUIT FLY PUPARIA FOLLOWING IRRADIATION OF LARVAE IN MANGOSTEENS[a]

Dose (Gy)	Age of larvae at treatment (d)				Mean
	1	3	5	7	
0	88.17a	84.49a	84.92a	87.77a	86.34
75	60.40b	81.12ab	62.58b	47.13b	62.81
100	68.48b	76.45ab	89.37a	84.85a	79.79
125	40.94c	65.49b	68.26b	74.32a	62.25
Mean	64.50	76.89	76.28	73.52	72.80

[a] The means within the columns followed by a common letter are not significantly different (P = 0.05) [9].

TABLE II. EFFECTS OF GAMMA IRRADIATION AT AN ABSORBED DOSE OF 150 Gy ON 5 DAY OLD ORIENTAL FRUIT FLY LARVAE IN 'NANG KLANGWAN' MANGOES

Test	Control			Treated				
	No. of fruits	No. recovered		No. of fruits	No. tested		No. recovered	
		Puparia	Adults		Puparia	Adults	Puparia	Adults
1	124	27 423	24 117	496	109 715	96 472	100 823	1
2	84	9 515	6 400	336	38 060[a]	25 603	21 541	0
3	84	2 139	1 322	336	8 568	5 275	9 089	0
4	84	4 173	2 794	336	16 699	11 188	14 459	0
Total	376	43 250	34 633	1504	173 042	138 538	145 912	1

[a] Corrected from data reported previously [10].

34 633 adults from 376 untreated fruits, 138 538 flies should have developed from the 1504 treated fruits. However, only one adult fly was recovered (Table II) [10], resulting in a mortality of 99.9993%. Balock et al. [11] have reported that a dose of 100 Gy generally prevented immature stage fruit flies from developing into adults, while Prasad and Sethi [12] have reported that third instar maggots of *D. dorsalis*, when irradiated with 150 Gy, resulted in an adult emergence rate of about 41.5%. The dose of radiation obtained by Seo et al. [13] to prevent the development of the adult oriental fruit fly from irradiated eggs or larvae ranged from 214 to 291 Gy for infested papayas treated in cartons with thicknesses of 1.9–3.3 g/cm^2. They concluded that as the average density and thickness of the irradiated cartons of fruit increased, the minimum dose of gamma radiation required to prevent development of adult oriental fruit flies from irradiated eggs or larvae must be increased to compensate for the absorption by the additional mass about the centre of the irradiated volume. Ohta et al. [14] suggested that the minimum absorbed dose should be increased because when the treated eggs hatched the larvae could develop into third instars, and their feeding could reduce the marketability of any infested fruit.

When the criterion of efficacy based on the non-formation of puparia was used, the result was that only 25.9484% mortality was achieved. Balock et al. [11] have reported that doses over 1000 Gy failed to prevent pupation.

Text cont. on p. 130.

TABLE III. EFFECTS OF IRRADIATION AND HOT WATER DIPS ON THE CONDITION OF THE TREATED MANGOES

Hot water	Dose (Gy)	Vitamin C	g/100 g fresh weight			Soluble solid
			Moisture	Starch	Sugar	
No	0	3.91	89.7	2.56	9.38	12.6
No	150	2.76	89.2	1.45	10.08	13.0
No	300	2.26	88.6	1.34	9.80	14.0
No	600	0.24	88.5	1.61	11.27	14.0
Yes	0	2.69	89.2	2.26	10.92	13.0
Yes	150	2.68	89.7	1.59	9.08	12.4
Yes	300	1.21	88.9	1.93	10.29	13.4
Yes	600	1.73	88.8	1.04	10.73	13.4

TABLE IV. EFFECTS OF IRRADIATION DOSES ON THE WEIGHT LOSS OF MANGOSTEENS STORED AT 15°C FOR 4–28 DAYS[a]

Ripeness (stage)	Dose (Gy)	Weight loss (%) after storage (d)						
		4	8	12	16	20	24	28
1	0	0	0	0	0	0	32	0
	150	0	0	0	0	0	0	0
	300	0	0	0	0	0	0	0
	600	0	0	0	0	0	0	0
2	0	0	0	20	0	22	20	0
	150	0	0	0	0	0	0	0
	300	0	0	0	0	0	0	0
	600	0	0	0	0	0	0	0

[a] Fruits stored for 24 and 28 days were all infected by fungus.

TABLE V. EFFECTS OF IRRADIATION DOSES ON THE FRESHNESS OF MANGOSTEENS STORED AT 15°C FOR 4–20 DAYS[a]

Ripeness (stage)	Dose (Gy)	Freshness (score) after storage (d)				
		4	8	12	16	20
1	0	4.90a	4.87a	2.43a	1.50a	1.03a
	150	4.93a	4.83a	3.77a	1.70a	1.50a
	300	4.93a	4.73a	3.53a	1.80a	1.23a
	600	4.97a	4.80a	3.93a	1.73a	1.10a
2	0	4.93a	4.70a	2.67a	1.47a	1.00a
	150	4.73a	4.53a	2.73a	1.50a	1.00a
	300	4.70a	4.53a	2.83a	1.43a	1.43a
	600	4.73a	4.67a	2.77a	1.40a	1.13a

[a] The means within the ripeness stage and columns followed by a common letter are not significantly different (P = 0.05) [9].

TABLE VI. EFFECTS OF IRRADIATION DOSES ON THE COLOUR DEVELOPMENT OF MANGOSTEENS STORED AT 15°C FOR 4–20 DAYS[a]

Ripeness (stage)	Dose (Gy)	Colour (score) after storage (d)				
		4	8	12	16	20
1	0	1.83a	1.96a	2.80a	3.00a	4.33a
	150	1.90a	2.30a	2.50ab	2.80ab	3.00b
	300	1.96a	2.20a	2.40b	2.67ab	2.93b
	600	1.63a	1.90a	2.20b	2.40b	3.00b
2	0	2.73a	3.03a	3.17a	3.43a	4.00a
	150	2.47a	2.67a	3.10a	3.10a	4.00a
	300	2.30a	2.47a	3.00a	3.10a	3.67a
	600	2.23a	2.47a	2.67a	3.00a	3.33a

[a] The means within the ripeness stage and columns followed by a common letter are not significantly different (P = 0.05) [9].

TABLE VII. EFFECTS OF IRRADIATION DOSES ON THE PEEL HARDNESS OF MANGOSTEENS STORED AT 15°C FOR 24 AND 28 DAYS[a]

Ripeness (stage)	Dose (Gy)	Hardness (%) after storage (d)	
		24	28
1	0	4.37b	13.63b
	150	9.07a	29.03a
	300	10.07a	19.67a
	600	8.37a	20.07a
2	0	3.63b	12.73b
	150	12.73a	23.30a
	300	17.67a	23.33a
	600	11.17a	19.13a

[a] The means within the ripeness stage and columns followed by a common letter are not significantly different (P = 0.05) [9].

TABLE VIII. EFFECTS OF IRRADIATION DOSES ON THE FRUIT FIRMNESS OF MANGOSTEENS STORED AT 15°C FOR 4–20 DAYS[a]

Ripeness (stage)	Dose (Gy)	Fruit firmness (kg/cm^2) after storage (d)				
		4	8	12	16	20
1	0	766.93	745.63	686.43	595.03	580.20
	150	793.17	770.00	695.37	698.03	571.63
	300	762.70	718.47	681.77	663.17	587.70
	600	808.80	757.30	696.37	593.90	624.30
2	0	741.63	673.83	616.83	579.93	448.80
	150	753.33	720.63	629.67	648.10	530.63
	300	745.53	655.10	650.73	559.87	525.20
	600	705.27	687.77	668.93	624.93	500.53

[a] The means within the ripeness stage and columns were not significantly different (P = 0.05) [9].

TABLE IX. EFFECTS OF IRRADIATION DOSES ON THE pH OF MANGOSTEENS STORED AT 15°C FOR 4–20 DAYS[a]

Ripeness (stage)	Dose (Gy)	pH after storage (d)				
		4	8	12	16	20
1	0	3.07	2.23	2.97	3.07	3.33
	150	3.23	3.13	3.02	3.10	3.47
	300	3.17	3.20	3.07	3.17	3.27
	600	3.20	3.03	3.07	3.17	3.63
2	0	3.03	3.07	2.93	3.07	3.67
	150	3.03	3.07	3.00	3.10	3.37
	300	3.07	3.10	3.03	3.10	3.53
	600	3.07	3.10	3.00	3.13	3.43

[a] The means within the ripeness stage and columns were not significantly different (P = 0.05) [9].

TABLE X. EFFECTS OF IRRADIATION DOSES ON THE TOTAL SOLUBLE SOLIDS OF MANGOSTEENS STORED AT 15°C FOR 4–20 DAYS[a]

Ripeness (stage)	Dose (Gy)	Soluble solids (%) after storage (d)				
		4	8	12	16	20
1	0	18.73	18.67	18.70	19.07	17.67
	150	18.83	20.13	18.67	19.37	18.47
	300	18.10	19.97	19.14	18.76	18.80
	600	17.47	18.90	18.97	18.93	19.27
2	0	19.03	19.07	19.20	20.33	19.33
	150	18.90	19.67	19.73	19.87	19.40
	300	18.78	19.83	19.27	18.80	19.93
	600	19.53	19.07	20.53	19.33	19.87

[a] The means within the ripeness stage and columns were not significantly different (P = 0.05) [9].

TABLE XI. EFFECTS OF IRRADIATION DOSES ON THE TITRATABLE ACIDITY OF MANGOSTEENS STORED AT 15°C FOR 4–20 DAYS[a]

Ripeness (stage)	Dose (Gy)	Titratable acidity (%) after storage (d)				
		4	8	12	16	20
1	0	0.78	0.63	0.64	0.67	0.69
	150	0.66	0.68	0.72	0.67	0.67
	300	0.64	0.66	0.72	0.63	0.68
	600	0.70	0.64	0.68	0.66	0.69
2	0	0.83	0.72	0.67	0.75	0.70
	150	0.79	0.74	0.71	0.74	0.70
	300	0.81	0.70	0.70	0.69	0.75
	600	0.76	0.68	0.69	0.70	0.73

[a] The means within the ripeness stage and columns were not significantly different (P = 0.05) [9].

TABLE XII. EFFECTS OF IRRADIATION DOSES ON THE DISEASE INCIDENCE OF MANGOSTEENS STORED AT 15°C FOR 16–28 DAYS[a]

Ripeness (stage)	Dose (Gy)	Disease incidence (%) after storage (d)			
		16	20	24	28
1	0	0	84.07a	100.00a	100.00a
	150	0	77.73a	98.90a	100.00a
	300	6.9	87.03a	100.00a	100.000
	600	0	4.00b	92.50b	94.67b
2	0	0	47.17a	93.93a	100.00
	150	6.7	80.93a	100.00a	100.00
	300	7.8	78.53a	100.00a	100.00
	600	0	39.47a	87.27a	100.00

[a] The means within the ripeness stage and columns followed by a common letter are not significantly different (P = 0.05) [9].

3.3. Effect of irradiation on the disease and quality of fruits

3.3.1. Mango

The results indicated that irradiation, with or without hot water treatment, delayed the colour development of 'Nang Klangwan' mangoes, while hot water treatment alone had no effect on colour but did control anthracnose. When the mangoes were analysed to determine the vitamin C, total reducing sugar, soluble solid and water contents, the results showed that in all treatments the vitamin C content was reduced compared with the control, there was no difference in the water content between treatments and the total reducing sugar and soluble solids of irradiated mangoes tended to increase (Table III).

3.3.2. Mangosteen

The fruits in each carton of each irradiation dose were weighed when taken from the storage chamber and before checking for external and internal physiological changes. Table IV shows that the irradiation dose did not have any significant effect on the weight loss, apart from minor weight losses of the control fruits in stage 1 at 24 days and stage 2 at 12, 20 and 24 days.

Table V [9] shows that the irradiation doses did not have any effect on the freshness of mangosteens. The decline in fruit freshness was due to the storage time.

Irradiation had no effect on the colour development of fruits in the early storage times of stage 1 or in all the storage times of stage 2 (Table VI) [9]. However, treatment significantly delayed the development of fruit colour in stage 1 fruit stored for 12–20 days.

The effects of irradiation on the peel hardness of mangosteens did not appear until 24 days after storage. Table VII [9] shows that all the treatment doses then had a significant effect on the peel hardness when compared with the controls.

The fruit firmness results are shown in Table VIII [9]. The irradiation dose had no effect on the fruit firmness of mangosteens in two harvesting stages. The decline in fruit firmness was due to the storage time.

The results of the pH, total soluble solid and titratable acidity analyses are shown in Tables IX–XI [9], respectively. The irradiation doses had no effect on the pH or the percentage total soluble solids and titratable acidity in mangosteens.

The disease symptoms did not appear until after 16 days of storage. With the exception of the 600 Gy dose in stage 1, the irradiation dose had no effect on post-harvest disease control in mangosteens (Table XII) [9].

ACKNOWLEDGEMENTS

This project was supported in part by the IAEA. The co-operation of the staff of the Fruit Fly Disinfestation Project at the Department of Agriculture, the Post-harvest Technology Laboratory of the Horticulture Research Institute and the Office of Atomic Energy for Peace is gratefully acknowledged.

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ANATOMICAL CHANGES IN THE MATURE LARVAE OF TWO *Dacus* spp. FOLLOWING IRRADIATION*

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Abstract

ANATOMICAL CHANGES IN THE MATURE LARVAE OF TWO *Dacus* spp. FOLLOWING IRRADIATION.

Fruit flies of the Tephritidae family are regarded as one of the most important global pests of quarantine importance. Ethylene dibromide was widely used to disinfest the potential host fruits and vegetables in international trade, but the ban imposed on the use of this chemical fumigant led to contemplation of irradiation as an alternative treatment method. Before suggesting irradiation as a suitable quarantine treatment, emphasis should be placed on standardizing a method for identifying the treated insects to ensure that a particular fruit assignment has been irradiated with the required dose. A description is given of a distinct reduction in area of the supraoesophageal ganglion (about 72–76%) along with the non-responsive proventriculus in third instar melon fly, *Dacus cucurbitae* Coquillett, and oriental fruit fly, *D. dorsalis* Hendel, larvae treated at several stages of larval development with various doses of gamma irradiation. It is suggested that this reduction in size of the ganglion be used as a direct method for determining if any fruit fly larvae found in a fruit or vegetable shipment at the port of entry have been irradiated.

1. INTRODUCTION

Fruit flies of the Tephritidae (Diptera) family are a serious pest of citrus and deciduous fruits as well as fleshy vegetables in many countries of the world. A preliminary survey [1] has indicated that in Bangladesh a large amount of quality fruits (mangoes, lychees, pineapples, guavas, papayas, etc.) and seasonal vegetables is being lost due to fruit fly infestation, but so far no concrete estimation of such losses has been made available. The country has good prospects for exporting fruits and vegetables, especially to Middle East, South Asian Association for Regional Co-operation (SAARC) and some European countries. At present, about 250 tonnes

* Research carried out with the support of the IAEA under Research Contract No. 5033/RB.

of seasonal fruits are exported annually to these countries, but strict and effective quarantine measures are required to prevent the movement of fruit flies into areas where they are not present. Considering the current crisis in the international fruit trade due to the ban on the use of ethylene dibromide (EDB) by the United States Environmental Protection Agency [2], the lack of a reliable method of quarantine treatment has remained an obstacle to export and import between interdependent countries. In this situation, irradiation of fruits can be viewed as an effective alternative for quarantine purposes [3]. Before suggesting irradiation as a suitable quarantine treatment for the fruit trade, emphasis should be placed on standardizing a method for identifying the treated insects to ensure that any larvae found in a particular batch of fruit have been irradiated. Bearing in mind the above points, fruit flies were collected from infested fruit in various parts of the country and observations made on the post-irradiation changes in the total surface area of some target organs such as the supraoesophageal ganglion and the proventriculus of the larvae. The present study reports on the changes observed in larvae of the melon fly, *Dacus cucurbitae* Coquillett, and the oriental fruit fly, *D. dorsalis* Hendel.

2. MATERIALS AND METHODS

2.1. Test insects

To determine the effects of irradiation, various internal organs were measured at the early stages of the life cycle of the melon fly and the oriental fruit fly. The adult flies were reared in the laboratory in an aluminium framed nylon mesh cage (30 cm × 20 cm × 20 cm) at a room temperature of 25°C ± 2°C and about 80% relative humidity. The initial stock was raised from a collection of infested wild fruits and vegetables. The adults were supplied with a 3:1 yeast–sugar mixture after cooking at 60°C for 3 hours. Water was supplied in soaked cotton balls. The eggs were collected by placing bitter gourds in the cage for 2 hours. At this temperature regime, it took about 45–50 hours for the eggs to hatch and 12–15 days for the larvae to pupate. Larval rearing was maintained in the infested whole bitter gourds and supplemented with an artificial diet of a wheat bran medium following the procedures described by Rahman et al. [4, 5]. Colonies of about 5000 insects were maintained routinely in the laboratory for these experiments.

2.2. Irradiation procedure

An Atomic Energy of Canada Limited 650 ^{60}Co irradiation source (strength 38 579 Ci, February 1990), was used at a dose rate of 3.2 Gy/min.[1] The whole bit-

[1] 1 Ci = 3.70 × 10^{10} Bq.

ter gourds were treated 4, 24, 48, 72 and 96 hours after the eggs had been collected. Thus, the insects were treated as fresh, young and older eggs, and young and older larvae, respectively. The treatments were applied at doses of 0, 20, 50, 80, 100, 120 and 200 Gy. Usually, the irradiated gourds were placed on the larval diet (inside the aluminium framed nylon cages) for subsequent development.

2.3. Processing treated larvae

The insects were allowed to develop as larvae, and the mature third instars were collected for dissection and measurement of the area of the supraoesophageal ganglion and the proventriculus. The larvae were collected in water and dissected under a binocular microscope in a cavity slide with saline solution (pH6.8). The length and breadth of the supraoesophageal ganglion and the proventriculus were measured with a micrometer, using a minimum of five larvae from each treatment. The area was calculated (μm^2) using the formula for the area of an ellipse (πab, where a and b represent the major and minor radii, respectively).

3. RESULTS

It was observed that the fresh eggs of both species were more sensitive to the effects of irradiation than were older eggs or larvae. None of the 4 hour old eggs treated at, or above, 50 Gy survived to the third instar. The rate of pupation for insects treated as larvae was almost normal, even at the highest doses tested. However, no adults eclosed after a dose of 50 Gy, irrespective of the developmental stage at which they were irradiated.

Data obtained from the measurement of internal organs by dissecting the mature larvae, which had been irradiated 24, 48, 72 and 96 hours after egg laying, as young and older eggs, and young and older larvae, respectively, are presented in Table I. These data show that at doses of 50 Gy, and above, there was a consistent reduction in the area of the supraoesophageal ganglion with the increase in radiation dose. The reduction appeared to be consistent in both species. For example, at 150 Gy there was a 72–75% reduction in *D. cucurbitae*; the area of the ganglion measured in the mature larvae that had been irradiated was 0.171–0.191 μm^2 compared with 0.667–0.716 μm^2 in the control. For *D. dorsalis*, it was 0.172–0.182 μm^2 in the irradiated larvae compared with 0.679–0.725 μm^2 in the control.

The percentage reduction in the area of the supraoesophageal ganglion following irradiation was plotted as a probit against the applied dose on a log probit scale. The fitted regression lines were drawn for log dose and probit reduction in the supraoesophageal ganglion (Fig. 1(a) *D. cucurbitae* and 1(b) *D. dorsalis*). These

TABLE I. EFFECTS OF IRRADIATION ON THE AREA OF THE SUPRAOESOPHAGEAL GANGLION ($\mu m^2 \pm$ SE) OF MATURE THIRD INSTAR *D. cucurbitae* AND *D. dorsalis* LARVAE TREATED AT DIFFERENT TIME INTERVALS AFTER EGG LAYING[a]

Dose (Gy)	Time interval (h) after egg collection			
	24	48	72	96
		D. cucurbitae		
0	0.679 ± 0.019	0.716 ± 0.007	0.685 ± 0.042	0.667 ± 0.081
20	0.412 ± 0.006	0.523 ± 0.011	0.453 ± 0.009	0.425 ± 0.014
50	0.222 ± 0.008	0.258 ± 0.006	0.231 ± 0.007	0.224 ± 0.004
80	0.220 ± 0.002	0.244 ± 0.007	0.277 ± 0.049	0.221 ± 0.003
100	0.171 ± 0.005	0.183 ± 0.007	0.197 ± 0.006	0.173 ± 0.003
120	0.177 ± 0.003	0.203 ± 0.009	0.185 ± 0.008	0.192 ± 0.008
150	0.171 ± 0.005	0.173 ± 0.009	0.182 ± 0.007	0.191 ± 0.006
200	—	0.173 ± 0.010	0.174 ± 0.008	0.182 ± 0.005
		D. dorsalis		
0	0.725 ± 0.019	0.685 ± 0.019	0.679 ± 0.011	0.708 ± 0.008
20	0.425 ± 0.010	0.510 ± 0.009	0.429 ± 0.012	0.416 ± 0.008
50	0.201 ± 0.002	0.235 ± 0.006	0.224 ± 0.005	0.220 ± 0.006
80	0.207 ± 0.006	0.241 ± 0.007	0.231 ± 0.007	0.224 ± 0.004
100	0.179 ± 0.005	0.174 ± 0.003	0.192 ± 0.008	0.168 ± 0.028
120	0.171 ± 0.007	0.191 ± 0.006	0.173 ± 0.007	0.182 ± 0.007
150	0.172 ± 0.004	0.174 ± 0.004	—	0.182 ± 0.007
200	—	—	—	—

[a] The insects were treated 24, 48, 72 and 96 hours after the eggs had been collected as young and older eggs, and young and older larvae, respectively.

lines indicate that the pattern of reduction in the target organ with the increase in dose was similar for both species within the dose range applied.

Table II compares the percentage reduction in the area of the supraoesophageal ganglion for the two species when irradiated at a dose of 150 Gy, 24, 48, 72 and 96 hours after egg laying as young and older eggs, and young and older larvae, respectively. These data indicated that the pattern of reduction in the target ganglionic area was similar in these two species and also to that for larvae of the

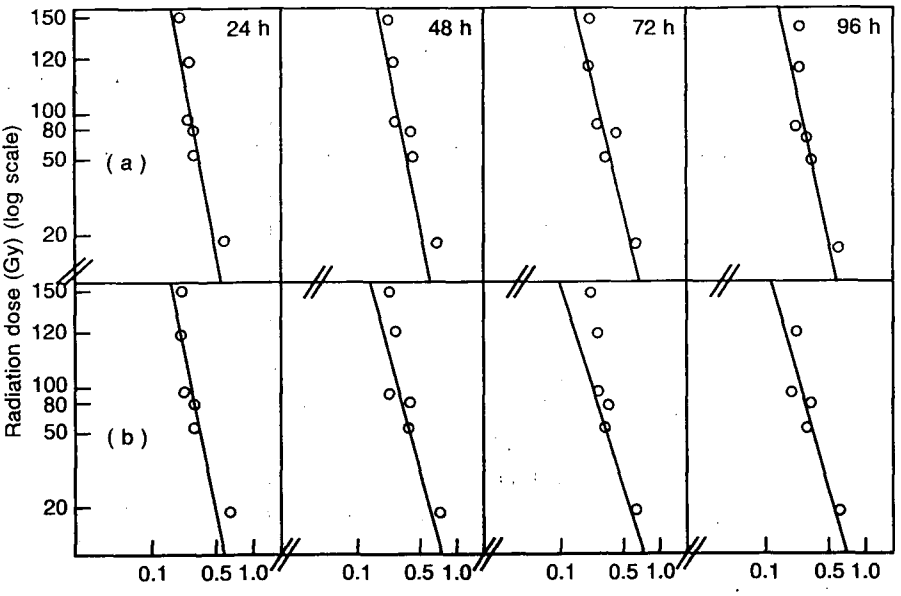

FIG. 1. Regression lines indicating the effects of radiation dose on the percentage reduction in the area of the supraoesophageal ganglion of mature third instar D. cucurbitae *(a) and* D. dorsalis *(b) larvae irradiated 24, 48, 72 and 96 hours after the eggs had been collected as young and older eggs, and young and older larvae, respectively.*

TABLE II. PERCENTAGE REDUCTION IN THE AREA OF THE SUPRAOESOPHAGEAL GANGLION OF MATURE THIRD INSTAR *D. cucurbitae* AND *D. dorsalis* LARVAE IRRADIATED AT 150 Gy AT DIFFERENT TIME INTERVALS AFTER EGG LAYING[a]

Age treated (h)	% reduction in the area of the supraoesophageal ganglion		
	D. cucurbitae	*D. dorsalis*	*C. capitata*[b]
24	74.8	76.3	69.0
48	74.7	76.1	57.3 (53 h)
72	73.2	76.2	74.6
96	71.8	74.9	68.5 (112 h)

[a] The insects were treated 24, 48, 72 and 96 hours after the eggs had been collected as young and older eggs, and young and older larvae, respectively.

[b] Taken from Ref. [6] for comparison purposes.

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) [5]. The radiosensitivity is distributed to a broad range of the life cycle, from the egg to larval development.

In contrast, the area of the proventriculus did not show much difference between the irradiated larvae and the non-irradiated control. For *D. cucurbitae*, the area measured 0.415–0.447 μm^2 in the irradiated larvae compared with 0.455 μm^2 in the control. For *D dorsalis*, it was 0.406–0.437 μm^2 in the irradiated larvae compared with 0.445 μm^2 in the control.

4. DISCUSSION

The results of the comparative measurements of the two target organs, the supraoesophageal ganglion and the proventriculus, from both species of the experimental flies, *D. cucurbitae* and *D. dorsalis*, were similar to our earlier findings for *C. capitata* [5, 6]. In each species, the sharp reduction in the area continued up to 80 Gy, and subsequently maintained an almost horizontal line. The view could be taken that within the dose range of 80 Gy, the majority of sensitive cells of the supraoesophageal ganglion reached at the peak of its degenerative process has yet to be elucidated.

Data in Table II indicate that the degree of reduction in the area of the supraoesophageal ganglion did not vary significantly among the species tested. This favours our previous suggestion that use be made of this direct method for identifying treated larvae of the Tephritidae family when proposing irradiation of fruits as an alternative to EDB for quarantine purposes [6]. However, representative species of other genera of the family have yet to be tested in order to formulate a generalized conclusion.

The decrease in size of the irradiated supraoesophageal ganglion provides direct information, especially when correlated with the size of the non-responsive proventriculus of a particular larva. This type of easy identification of irradiated larvae is an essential prerequisite for the implementation of radiation as a treatment for quarantine purposes [6, 7]. The radiation effect on the quarantine pest itself would be more reliable than the other indirect methods of identifying treated fruits used earlier [8].

Data obtained during the present studies in the laboratory require further confirmation under semi-pilot and pilot scale tests before practical implementation of the process is considered. At the same time, public acceptance, feasibility, appropriateness of food irradiation and other relevant aspects should be taken into account.

ACKNOWLEDGEMENTS

The support received from the IAEA and the Bangladesh Atomic Energy Commission is gratefully acknowledged.

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RADIATION DISINFESTATION OF FRUITS
*Effectiveness and fruit quality**

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Abstract

RADIATION DISINFESTATION OF FRUITS: EFFECTIVENESS AND FRUIT QUALITY.

The effectiveness of deactivating mature Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), eggs with gamma irradiation treatment at 500–600 Gy in several varieties of California peaches and nectarines was demonstrated. Small percentages of hatching and pupation occurred in plums, cherries and other varieties of peaches and nectarines after treatment at 600 Gy, but no adult eclosion was found. The higher dose may present an opportunity of making a difference in the marketability of some of the treated fruits, as compared with irradiation at a minimum of 150 Gy allowed as a quarantine treatment procedure. If larvae were found in the treated fruits upon arrival, quarantine officials may have to decide whether or not to release such a shipment. Combined treatment of papayas with heat, non-toxic chemical solutions and irradiation as a possible quarantine treatment did not result in synergism because of scalding of the fruit's skin. The quality of California stone fruits and 'Valencia' oranges was retained after irradiation at doses between 300 and 750 Gy, with minor differences in the controls, probably attributable to a certain degree of delayed ripening in some fruits.

1. INTRODUCTION

The effectiveness and technical feasibility of disinfesting various tropical and subtropical fruits by gamma radiation as a quarantine treatment procedure have been demonstrated with research studies conducted since the early 1960s by researchers in Hawaii [1–3] and Florida [4, 5], United States of America, Thailand [6, 7], Pakistan [8] and other tropical regions around the world [9, 10]. By the early 1980s, continuing studies on irradiation of fruits as an effective means of disinfestation had

* Research carried out in association with the IAEA under Research Agreement No. 3180/CF.

extended to citrus, especially California 'Valencia' oranges, and a number of stone fruits [11–14]. These studies were related to the major outbreaks of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), that occurred in 1975–1976 and 1980–1981 in southern California. In addition to stepping up the release of sterile flies in the infested and suspected areas, California agricultural authorities were considering other measures in the light of the possibility of a widespread infestation affecting the entire fresh fruit industry in California and elsewhere in the USA.

The ban on the use of ethylene dibromide as a quarantine fumigant in the USA by the United States Environmental Protection Agency in September 1984 [15] forced the tropical fruit industry, e.g. in Hawaii, to seek alternative methods of quarantine treatment for papayas and other fruits. Several thermal treatment methods adopted by the industry have led to fruit quality problems, costly harvesting and inspection schedules, discovery of insects in treated fruits at destination ports and loss of sales due to consumer dissatisfaction with thermally treated papayas. These thermal treatments include vapour heat, hot water double dip and, more recently, the high temperature forced air ('dry heat') method [16]. None of these methods has been found to be 100% effective in controlling the fruit flies or in preserving the quality of the fruits.

Irradiation, on the other hand, does not present these problems, and has consistently been shown to be the most effective and efficient method of disinfesting tropical fruits of fruit flies and meeting the quarantine requirements [17–19]. In April 1986, the Food and Drug Administration approved irradiation for treating fruits and vegetables at a dose of up to 1000 Gy for the purpose of disinfestation and delaying maturation [20]. In January 1989, the Animal Plant Health Inspection Service of the United States Department of Agriculture (USDA) approved the use of irradiation as a quarantine treatment procedure for Hawaiian grown papayas [21].

While there is general agreement among researchers and quarantine officials who have worked on and are familiar with irradiation that radiation disinfestation is efficacious and promising as a quarantine treatment for fruits, controversies over use of this technology have emerged over the past 6 years. These have arisen mainly from inadequate public education and misinformation spread by anti-nuclear activists on the purposes, benefits and safety of radiation technology for food preservation, plus confusion between an irradiator and a reactor, as well as publicity about nuclear reactor accidents and nuclear weapons development and deployment.

To help alleviate these problems, we envisioned that one of the technical approaches would be to combine heat, non-toxic chemical solutions and low dose gamma radiation as an alternative treatment with a view to providing an improvement in the required quarantine treatment of papayas and other tropical fruits. At the same time, there may be economic and psychological advantages in using such combined treatment if a synergism were to be found whereby the dose requirement for each of the treatments in a combined process (heat, chemical, and irradiation) was considerably lower than if each were used alone in a single process.

The objectives of our study were fourfold:

(1) To determine the effectiveness of low dose gamma radiation for disinfesting selected California stone fruits and citrus of Mediterranean fruit flies
(2) To explore the effectiveness of combining low dose gamma radiation with heat and a non-toxic chemical solution for disinfesting tropical fruits of oriental fruit flies
(3) To evaluate the effects of the treatments on the quality of these fruits
(4) To elucidate, as time and resources permitted, the interrelationships between the fruit properties (such as acidity, and water activity or content), the effectiveness of irradiation at a certain applied dose as a quarantine treatment method, the environmental factors (such as relative humidity, packaging, treatment temperature and post-treatment storage temperature), and the potential phytotoxicity on the treated commodity.

Detailed reports of some of these experiments have been published elsewhere [11–14]. These reports, as well as some unpublished material, are summarized.

2. MATERIALS AND METHODS

2.1. Disinfestation of the Mediterranean fruit fly in stone fruits

Research on gamma radiation for the disinfestation of stone fruits infested by fruit fly eggs and larvae [11, 12] used two Mediterranean fruit fly strains in the irradiation experiments on California fruits. A 'wild' strain was established from adults reared on Jerusalem Cherry (*Solanum pseudocapsicum* L.) fruits collected from the Volcanoes National Park on the Island of Hawaii. Another was a 'USDA strain', which has been reared in USDA research laboratories in Hawaii since the early 1950s.

Peaches, nectarines and plums were purchased from packing houses in California. Most of the fruits were packed 1 day after harvest and shipped to Hawaii within 7 days in refrigerated containers. Fruits for studying egg hatch were infested in cages in our laboratory 1–2 days after arrival, then irradiated at 21°C at doses of 100–1000 Gy. For studies on larval survival, the eggs were allowed to hatch on moist blotting paper and the larvae were implanted into the fruit [12].

2.2. Combined treatments for the oriental fruit fly in papayas

A USDA laboratory strain of the oriental fruit fly, *Dacus dorsalis* Hendel, was used for the study on combined treatments for the disinfestation of papayas. The 'Solo' variety of Hawaiian grown papayas at one-quarter to one-half ripeness were used for these experiments. The radiation doses applied were 260–850 Gy. All

papayas were hot water treated at 49°C for 20 minutes before infestation and irradiation to reduce fungal decay during storage. Some of the heat treatments were combined with chemical solutions, which included 2 and 5% Basic-H (containing 28% linear alcohol alkoxylates in undiluted form by the Shaklee Corporation, USA) and 2% Lauricidin (LC), a non-toxic, food grade, fatty acid derivative of saturated C_{12} lauric acid. The postulated mechanism of these chemicals was that the fruit fly eggs might be dewaxed by the solutions, hence the eggs might become more sensitive to subsequent gamma radiation treatment. LC is also bactericidal.

2.3. Quality and sensory evaluation of fruits

For sensory evaluation, the irradiated stone fruits were stored at 10°C and 21°C [11]. Oranges were stored at 7.2°C for 2, 4 or 7 weeks, or at 7.2°C for 4 weeks followed by 2 weeks at 21.1°C [13]. Triangle tests were used in taste panel evaluation of the stone fruits with 15–20 trained panellists from our departments. Replicate runs were conducted on alternate days. Established taste panel methodology was followed. A multiple comparison test was employed to determine the sensory qualities of 'Valencia' oranges, including the pulp colour, outer appearance, outer texture, aroma, flavour and texture of the pulp. For the purpose of maintaining uniformity throughout the quality ratings, a seven point scale was adopted for each quality in the study [14].

The following analytical methods were used in these tests:

(1) *Colour:* A HunterLab Tristimulus Colorimeter Model D25 M-9 was used, with L, a and b scales
(2) *Texture:* A shear press and the Magness–Taylor puncture test were used to measure the texture of the oranges
(3) *Ascorbic acid:* A Bausch and Lomb model Spectronic 88 spectrophotometer was used to measure the reduced ascorbic acid of the oranges; the values were expressed in mg/100 g
(4) *Total acidity:* The Association of Official Analytical Chemists (AOAC) direct titration method with 0.1N NaOH to an endpoint of pH8.1 [22] was used
(5) *Total soluble solids (TSS):* The TSS was measured in °Brix with a Bausch and Lomb Model 33-45-58 refractometer.

2.4. Radiation source

The Hawaii Research Irradiator with 100 ^{60}Co capsules of the National Brookhaven Laboratory, USA, design, located in the Food Technology Building, University of Hawaii, was used as the gamma radiation source for all the experiments. This source was upgraded to 42 500 Ci in January 1978.[1] The dose rate dur-

[1] 1 Ci = 3.70×10^{10} Bq.

ing our experiments was in the range of 25–55 Gy/min and the maximum to minimum ratio for the dose in the irradiator chamber was 1.09.

3. RESULTS AND DISCUSSION

3.1. Disinfestation of the Mediterranean fruit fly in stone fruits

3.1.1. Hatch of irradiated Mediterranean fruit fly eggs

The effects of gamma radiation on the hatch of Mediterranean fruit fly eggs which were oviposited on several varieties of stone fruits have been reported [12]. These data showed that 4.09, 1.69 and 16.31% of the mature eggs treated in several varieties of peaches, nectarines and plums, respectively, hatched at doses of up to 400–430 Gy.

In other experiments, eight varieties of nectarine, five of plum and two of peach, as well as one variety each of apricot and cherry, were irradiated at 400–600 Gy [12]. In most of the varieties tested, 600 Gy were sufficient to prevent 99% of the eggs from hatching. At 400 Gy, however, egg hatch in the infested nectarines ranged from 0.5% in 'Autumn Gold' to 46.7% in 'Sun Grand' (Table I) [12]. Also at 400 Gy, varietal differences in the hatch of eggs deposited in peaches ranged from 0.1% for 'O'Henry' to 12.3% for 'Fay Alberta' and in plums from 0.9% for 'President' to 45.4% for 'Casselman'. The hatchability of eggs oviposited in apricots and cherries was similar to that obtained for nectarines and peaches (Table I). The hatch rates were reduced at higher doses (between 400 and 600 Gy). While there were a few survivors of the eggs in irradiated apricots, cherries and peaches at 600 and 750 Gy [12], none of the larvae from the treated samples survived to the pupal stage. Data on five varieties of plum showed that hatchability was in the 15–16% range in two varieties after treatment at 600 Gy (Table I). The exact reason for the high hatch rate in plums is not understood. The high water content in plums as compared with that in other fruits might be a factor.

It is quite clear that the more mature the egg stage, the more resistant it is to radiation (Table II) [12]. These data showed that in nectarines none of the 0–4 and 24–28 hour old Mediterranean fruit fly eggs treated at 250 Gy hatched, while 17% of the 64–68 hour old eggs hatched. These data confirmed the findings of Balock et al. [1] that the highest dose level was required to prevent the hatching of eggs treated a few hours prior to hatching. Therefore, as an effective quarantine treatment, the radiation dose applied to fruits suspected of being infested should be that for the mature eggs.

Seo et al. [23] reported that minimum absorbed doses of 209–291 Gy in papayas, bell peppers and eggplants prevented adult emergence in Mediterranean fruit fly, oriental fruit fly and melon fly, *Dacus cucurbitae* Coquillett, larvae in

TABLE I. EFFECTS OF IRRADIATION ON THE HATCH OF MEDITERRANEAN FRUIT FLY EGGS TREATED IN DIFFERENT FRUIT SPECIES AND VARIETIES [12]

Fruit/variety	Hatch (%) of eggs treated at the specified dose (Gy)			
	0	400	500	600
Apricot				
Unknown	71.7	1.2	1.5	1.3
Cherry				
'Burlat'	86.7	6.8	3.7	0.3
Nectarine				
'Autumn Gold'	87.8	0.5	0.2	0.0
'Fairlande'	81.0	1.0	0.0	0.0
'Flamekist'	84.8	1.7	1.3	0.0
'Niagara Grand'	76.4	18.1	18.1	0.3
'Red Grand'	73.1	8.1	0.3	0.1
'Sam Grand'	78.1	0.6	0.1	0.1
'Summer Grand'	83.4	34.1	1.7	1.7
'Sun Grand'	82.3	46.7	58.6	0.2
Peach				
'Fay Alberta'	85.1	12.3	0.3	0.3
'O'Henry'	85.5	0.1	0.1	0.0
Plum				
'Casselman'	81.2	45.4	35.0	6.5
'Friar'	81.4	3.1	2.0	15.2
'Kelsey'	85.3	17.1	52.2	12.1
'Laroda'	81.2	10.5	10.9	16.5
'President'	72.0	0.9	3.5	1.2

TABLE II. EFFECTS OF THE AGE OF MEDITERRANEAN FRUIT FLY EGGS AT THE TIME OF IRRADIATION ON THE HATCH OF EGGS TREATED IN NECTARINES [12]

Dose (Gy)	Hatch (%) of eggs treated at the specified age (h)			
	0–4	24–28	48–52	64–68
0	79.2	80.8	83.6	82.1
250	0.0	0.0	0.5	17.2
500	0.0	0.0	0.0	0.0

TABLE III. EFFECTS OF THE SUBMERGENCE OF MEDITERRANEAN FRUIT FLY EGGS IN WATER DURING IRRADIATION ON EGG HATCH [12]

Dose (Gy)	Egg hatch (%)/treatment		
	Dry	Distilled water	Physiological water
0	90.3	90.8	
500	0.3	84.4	84.6

Hawaii. However, the results of our studies [11, 12] showed that at even higher doses a relatively high rate of egg hatch occurred. Some of the larvae which hatched might develop into large third instar larvae and thus could decrease the marketability of the fruits. Also, the question arises as to whether or not fruit importing countries would accept fruits with any living larvae in the shipment. Therefore, most of our experiments involved determining the minimum absorbed doses required to cause mortality in the mature eggs and larvae of the Mediterranean fruit fly in stone fruits rather than relying on the criterion of non-emergence of adult flies at the probit 9 security level.

The data reported in Table III [12] show results of an experiment in which the eggs were submerged in water. These data indicate that the eggs submerged in water were buffered from the effects of radiation. At 500 Gy, only 0.3% of the eggs hatched when treated under 'dry' (moist blotting paper) conditions. However, when

the eggs were placed in a small vial of water or physiological saline and then treated at 500 Gy, nearly 85% of the eggs hatched. This implies that the condition of the fruit, i.e. the high water content, over ripeness or bruised areas which can cause the eggs to be partially or wholly submerged in the liquid, may make a difference to the effectiveness of the radiation treatment.

3.1.2. Survival of irradiated Mediterranean fruit fly larvae

Eight varieties of nectarine, five of plum and two of peach, as well as one each of apricot and cherry, infested with fruit fly larvae were irradiated [12]. The survival rate for the mature larvae in the nectarines following treatment with gamma radiation at 600 Gy ranged from 8.9% pupation for larvae treated in 'Sun Grand' to 31.9% in 'Red Grand' (Table IV) [12]. Some of the variations in the survival rate and the percentage pupation of larvae in different varieties of fruit, such as 'Casselman' plums, appeared to be due to the high water content of the fruit. There was no eclosion from the puparia formed by the larvae treated in fruit at 500 and 600 Gy, with two exceptions. One female adult emerged from the larvae infesting a 'Kelsey' plum and one male from a 'Sun Grand' nectarine treated with 500 and 600 Gy, respectively. The female produced normal progeny when mated to a normal male; dissection and careful examination of the male fly showed that it was fully developed. It is suspected that the survivors were in fruits which were accidentally infested by flies after the irradiation treatment [12].

The data quite clearly suggest the effectiveness of irradiation in preventing adult eclosion. Since these experiments were conducted on the most resistant of the larval stages, larvae at any other stage of development which might be infesting the fruits would certainly be killed at doses of 500–600 Gy.

3.2. Combined treatments for the oriental fruit fly in papayas

Treating infested papayas with solutions of 2 and 5% Basic-H (at 49°C for 30 and 40 minutes, respectively) resulted in 99.9 and 100% mortality of oriental fruit fly eggs, respectively, and 2% LC (at 49°C for 20 minutes) resulted in 91% mortality of the eggs. Hot water for 20 minutes followed by gamma radiation treatment at 500 Gy reduced the hatchability of oriental fruit fly eggs in papayas to 1.4% and the hatched larvae died within 24 hours (Table V). Hot water plus 850 Gy completely prevented hatching.

Combined treatments with chemical solutions and gamma radiation, however, showed little synergistic effect (Table V). In some cases, it increased the mortality of oriental fruit fly eggs only slightly. The undesirable result was that the chemical solution caused some phytotoxicity on the papayas by scalding the fruits at the effective dose range. Possibly we did not use or find the proper chemical solution for the combined treatment. If other chemical solutions can be used without causing

TABLE IV. EFFECTS OF IRRADIATION ON THE PUPATION OF MEDITERRANEAN FRUIT FLY LARVAE TREATED IN DIFFERENT FRUIT SPECIES AND VARIETIES [12]

Variety	Pupation (%) of larvae treated at the specified dose (Gy)		
	0	500	600
Apricot			
Unknown	49.7	12.8	14.0
Cherry			
'Burlat'	64.9	26.5	12.9
Nectarine			
'Autumn Gold'	52.9	33.0	17.5
'Fairlane'	58.6	24.8	23.9
'Flamekist'	64.7	20.4	22.6
'Niagara Grand'	62.0	27.5	18.8
'Red Grand'	73.0	30.8	31.9
'Sam Grand'	24.8	21.0	19.0
'Summer Grand'	80.4	21.5	16.7
'Sun Grand'	63.8	28.3	8.9
Peach			
'Fay Alberta'	46.0	22.4	23.6
'O'Henry'	51.6	21.7	11.5
Plum			
'Casselman'	0.0	1.5	3.0
'Friar'	11.6	9.0	14.1
'Kelsey'	29.7	9.7	6.1
'Laroda'	39.3	14.2	11.2
'President'	1.6	1.5	1.3

TABLE V. MORTALITY OF ORIENTAL FRUIT FLY EGGS IRRADIATED IN PAPAYAS FOLLOWING A 20 MINUTE COMBINED HOT WATER AND CHEMICAL TREATMENT

Dose (Gy)	Egg mortality (%)			
	Hot water	Hot water + Basic-H (B-H)		
		2% B-H	5% B-H	2% B-H + 2% Lauricidin
0	45	84	97	93
260	68			95
400	80	100	71	
500	98.6	100	100	99
750	99			
850	100			

phytotoxicity to the fruit, the potential of combined chemical and irradiation treatments should be promising.

3.3. Quality and sensory evaluation of fruits

3.3.1. Stone fruits

The sensory qualities of plums and nectarines irradiated at 300 Gy were comparable to those of the untreated controls [11, 13]. The detectable differences between the irradiated plums and the controls were colour samples at 500 Gy, and the texture samples at 500 and 1000 Gy. On the irradiated nectarines, differences were found in the aroma samples at 500 Gy, and the colour, flavour and texture samples at 1000 Gy. On the irradiated peaches, differences were found in the colour samples between the controls and the samples irradiated at 300 and 500 Gy. Small differences were also detected in the flavour samples at 300 Gy and the texture samples at 1000 Gy. The differences in various sensory qualities between the irradiated samples and the controls could be due to some degree of delayed ripening in the irradiated fruits. Nevertheless, these results should be further confirmed because of the logistics in obtaining fruits for the experiments.

3.3.2. *'Valencia' oranges*

The results of studies on the sensory qualities of 'Valencia' oranges irradiated at up to 1000 Gy are summarized in Tables VI and VII [13, 14, 24]. The mean scores for the endocarp colour from each treatment of 'Valencia' oranges stored for 2–7 weeks showed that the colour of the endocarp was retained with radiation treatments up to 1000 Gy and storage at both refrigerated and air conditioned (7.2°C and 21.1°C, respectively) temperatures (Table VI). In spite of some significant differences in the outer appearance of the fruits between the treatments and the storage periods, the extent of injury was minimal. The highest dose of 1000 Gy caused no adverse change in the outer appearance of the irradiated fruits at both storage temperatures and storage periods (Table VI).

Generally, the quality of the outer and endocarp textures of the orange was preserved when irradiated up to 1000 Gy and stored at 7.2°C [13, 14]. Treatments at 750 Gy resulted in no adverse effects on the outer texture at either storage temperature. The mean scores for the endocarp texture of the irradiated oranges treated at up to 1000 Gy displayed no adverse quality change from the endocarp for both storage temperatures and periods (Table VI) [13]. Some of the fruit groups treated at 750 and 1000 Gy were significantly softer than the control.

The mean scores for the aroma and flavour of the irradiated oranges are shown in Table VII. The aroma of the oranges remained unaffected after irradiation at 500 Gy and storage for 6 weeks (the last 2 weeks at 21.1°C). Although the 1000 Gy treated fruits were rated low in aroma throughout the storage periods, fruits exposed to 1000 Gy exhibited no deleterious changes in aroma after 7 weeks of storage at 7.2°C. The average scores for the flavour of irradiated oranges treated at 750 and 1000 Gy were significantly lower ($P < 0.05$) than the control and the other irradiated samples [13]. The results indicated that increases in the dose, storage periods and temperatures resulted in some off flavour ratings. Fruits treated at 750 and 1000 Gy retained their flavour quality if the storage temperatures remained at 7.2°C. Treatments at up to 500 Gy resulted in no adverse changes in the flavour at any storage period or temperature. For the purpose of using irradiation as a quarantine treatment method, it seems that 500 Gy were quite adequate and, therefore, the flavour of the treated fruit should not be a problem.

Objective measurements of the colour and texture of various irradiated orange samples supported the taste panel results that the endocarp and epicarp (outer appearance) colours were retained throughout the storage periods and treatments, but that low temperature maintenance was necessary to retain the firm peels and pulp of the irradiated orange. The results also indicate the trend that increasing softening would occur at higher storage temperatures and increasing doses.

The ascorbic acid, total acidity and TSS of various irradiated orange samples and the controls were not significantly different from each other throughout the storage periods or as a result of the two storage temperatures [13, 14].

TABLE VI. AVERAGE SENSORY EVALUATION SCORES FOR THE ENDOCARP COLOUR AND TEXTURE AND THE OUTER APPEARANCE OF IRRADIATED ORANGES STORED AT 7.2°C FOR 2, 4 OR 7 WEEKS, OR AT 7.2°C FOR 4 WEEKS FOLLOWED BY 2 WEEKS AT 21.1°C [13, 14, 24]

Storage		Dose (Gy)	Average sensory evaluation score[a]		
			Endocarp		Outer appearance[d]
Temperature (°C)	Period (weeks)		Colour[b]	Texture[c]	
7.2	2	0	3.96a	3.85b	1.48c
		300	3.33c	4.35a	1.71b
		500	3.71b	3.94b	1.88a
		750	3.64b	4.40a	1.67b
		1000	3.85b	4.33a	1.33c
	4	0	4.15a	4.08c	1.46c
		300	3.94b	4.27c	1.42c
		500	3.92b	4.31c	2.42a
		750	3.96b	4.98a	1.83b
		1000	3.96b	4.56b	1.52c
	7	0	3.81b	4.17b	1.50b
		300	4.25a	4.10b	1.48b
		500	4.29a	4.33b	1.50b
		750	4.25a	4.63a	2.02a
		1000	4.15a	4.38b	1.42b
7.2 ± 21.1	4 + 2	0	3.92a	3.77c	1.56a
		300	3.73a	4.19b	1.36a
		500	3.90a	4.54b	1.71a
		750	3.50b	4.65a	1.50a
		1000	3.92a	4.77a	1.66a

[a] The means within columns and storage periods followed by the same letter are not significantly different (P = 0.05) [24].

[b] Colour scale: 1 = extreme light, 7 = extreme dark.

[c] Texture scale: 1 = extreme firm, 7 = extreme soft.

[d] Appearance scale: 1 = no injury, 7 = extreme injury.

TABLE VII. AVERAGE SENSORY EVALUATION SCORES FOR THE AROMA AND FLAVOUR OF IRRADIATED ORANGES STORED AT 7.2°C FOR 2, 4 OR 7 WEEKS, OR AT 7.2°C for 4 WEEKS FOLLOWED BY 2 WEEKS AT 21.1°C [13, 14, 24]

Storage		Dose (Gy)	Average sensory evaluation score[a]	
Temperature (°C)	Period (weeks)		Aroma[b]	Flavour[c]
7.2	2	0	1.19b	1.71b
		300	1.31b	1.69b
		500	1.75a	1.65b
		750	1.71a	2.40a
		1000	2.00a	2.33a
	4	0	1.48b	1.58b
		300	1.23b	1.77b
		500	1.60b	1.79b
		756	1.39b	2.33a
		1000	2.10a	2.50a
	7	0	1.54a	1.69b
		300	1.58a	1.81b
		500	1.52a	2.06b
		750	1.48a	1.96b
		1000	1.75a	2.29a
7.2 + 21.1	4 + 2	0	1.35c	21.5b
		300	1.60c	2.17b
		500	1.71c	2.48b
		750	2.38b	3.17a
		1000	2.92a	3.46a

[a] The means within columns and storage periods followed by the same letter are not significantly different (P = 0.05) [24].

[b] Aroma scale: 1 = no off aroma, 7 extreme off aroma.

[c] Flavour scale: 1 = no off flavour, 7 extreme off flavour.

Storage at 7.2°C and 21.1°C did not cause any major degree of external rot or spoilage. Blue mould rot and stem end rot were the decays observed [14]. Although the outer appearance remained unmarred in some samples, black rot caused by *Alternaria* progressed from the stem end of the orange, along the vascular tissues of the core after 2 weeks, or more.

3.3.3. 'Solo' papayas

The quality of 'Solo' papayas treated with up to 750 Gy was retained during storage at 25°C, as determined by several objective quality measurements. The respiration rates of the irradiated papayas were suppressed at 500 Gy, and above. Fruit firmness was retained at 260, 500 and 750 Gy. From these experiments, the obvious drawback of the combined treatment was that the skin of the papayas was scalded by the heat treatment, which was probably amplified by the chemical solutions treatment. Browning of different degrees slowly developed with or without irradiation.

4. CONCLUSIONS

Several conclusions can be drawn from these experimental findings:

(1) While 150 Gy is the suggested and approved minimum dose for treating fruits as a quarantine treatment procedure, the question of the marketability of fruits treated at this dose may arise. Somewhat higher doses, in the range of 260–500 Gy, would minimize the problem of possible fruit fly egg hatch. If a larva were found, either dead or alive, in an irradiated fruit at the port of entry the quarantine officials may have difficulty in deciding whether or not to release the shipment to the importer.

(2) The quality of various irradiated fruits (stone fruits, oranges and papayas) can be retained at radiation doses of 500–750 Gy, or even up to 1000 Gy. Proper control of the harvesting, shipping and handling procedures should allow some improvement or retention in the quality of the irradiated fruits. In addition, the storage temperature and relative humidity as well as the packaging are important factors for different types of irradiated fruit.

(3) Combining heat with a non-toxic chemical solution and irradiation has not been shown to be a workable method of quarantine treatment of fruits; at least the synergism has not been demonstrated from our experimental study. The idea may still be worth while pursuing. Of benefit is that a lesser dose of each treatment is used, thus decreasing treatment costs and increasing consumer acceptance of the irradiation of foods because of the very low doses used.

(4) The time and resources available did not allow us to pursue the fourth objective of our study. However, the observation that some fruit fly eggs and larvae survive better in fruits with a high water content suggests that the water content of a food, or its water activity, must be related to the effective dose at which a certain biological effect is to be achieved. Electron spin resonance signals reportedly linger longer in dried irradiated foods than in dried controls. However, with rather similar compositions among most of the fruits, the difference in survival rates of fruit fly eggs and larvae at the dose range of 400–600 Gy remains a mystifying question.

ACKNOWLEDGEMENT

The co-operation received from the IAEA is gratefully acknowledged.

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IRRADIATION AS A QUARANTINE TREATMENT FOR AGRICULTURAL PRODUCTS INFESTED BY ACARID MITES (ACARINA: ACARIDAE)*

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Abstract

IRRADIATION AS A QUARANTINE TREATMENT FOR AGRICULTURAL PRODUCTS INFESTED BY ACARID MITES (ACARINA: ACARIDAE).

Irradiation was considered as a quarantine treatment for acarid mites (Acarina: Acaridae) in agricultural commodities. For immediate mortality of the mites, doses higher than 2000 Gy of gamma radiation were required. Doses in the range of 1300–1500 Gy were sufficient if lethality within a few weeks was the goal. A dose of 250–300 Gy would be effective if the objective was to prevent the reproduction of living mites. At this dose, adult survivors of the acarid mites could be present in the treated commodities, but they would not give rise to offspring; thus, these pests would not be able to perpetuate in a new area. However, live mites in agricultural products could be of concern to quarantine personnel. Therefore, a simple test is needed to ensure that the mites have been irradiated and that they do not pose a quarantine risk. A test to verify that a pest has been irradiated and is incapable of reproduction may be based on the infecundity of the treated mites.

1. INTRODUCTION

Acarid mites (Acarina: Acaridae) are common pests of stored agricultural products. These mites not only cause economic loss of agricultural commodities in storage but, through quarantine, their presence restricts the export marketing of grains, dried fruits and vegetables, onions and other agricultural products. These restrictions may be eased by the application of a suitable disinfestation treatment: fumigation with chemicals such as methyl bromide or phosphine, heat treatment, cold treatment, or ionizing radiation [1].

Irradiation has been proposed as a quarantine treatment for various fruit flies, the mango weevil, *Sternochaetus mangiferae* (F.), and the codling moth, *Cydia pomonella* (L.), but little attention has been given to the possible use of irradiation as a potential quarantine treatment for the mites of stored products. The effects of

* Research carried out with the support of the IAEA under Research Contract No. 4659/RB.

ionizing radiation on different species of mite have not been studied in detail [2]. The objectives of this study were:

(1) To irradiate the immature stages of mites for determination of the dose required to prevent full development to normal adults
(2) To irradiate the adult mites of both sexes for determination of the dose required to prevent the production of viable offspring
(3) To devise a methodological approach for identification of the mites subjected to irradiation.

Stored agricultural products are rarely infested with a single species of acarid mite; most often, mixed infestations occur. An effective dose of radiation must sterilize or kill the most resistant of mites present within a commodity. At the same time, the lowest effective dose should be chosen because of economic savings. Because of this, the sensitivity to gamma radiation of the following mite species has been studied and compared: the mould mite, *Tyrophagus putrescentiae* (Schrank), the bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin) and a third species of mite, *T. neiswanderi* (Johnston and Bruce).

2. MATERIALS AND METHODS

All the mites used in the study were obtained from stock colonies maintained at the Department of Applied Entomology of the Agricultural University of Warsaw. The detailed methodology for these experiments has been reported elsewhere [3–10]. These colonies were maintained in rearing cages [11] at 25°C ± 1°C and 85 ± 5% relative humidity, and provided with wheat germ or yeast as food.

Samples of the mites within the medium were irradiated with ^{60}Co gamma rays using an irradiator of the type RChM-Gamma-20. The dose rate was about 20 Gy/min. A Fricke dosimeter was used for calibration [12].

2.1. Treatment of the immature stages of mites

Several 1–2 day old female and male mites were placed in each rearing cage with food. These mites produced eggs which were laid on the food, as well as on the whole inner surface of the cages. Every other day, the mites were transferred to new rearing cages, producing 'waves' of eggs left in the previously used cages. About 10 such transfers were made. The eggs were then incubated in darkness at 25°C ± 1°C and 85 ± 5% relative humidity.

After the last transfer, mites at different stages of development were obtained and then irradiated with gamma radiation. After treatment, they were stored in a thermostatically controlled cabinet until adult emergence. Then the adults were

sexed and paired. The number of eggs laid by these mites (fecundity), the viability of these eggs and the mortality of the males and females were recorded.

The effects of gamma radiation on mites at the older stages of development were also studied. Protonymphs and deutonymphs were isolated from the mass culture and treated with gamma radiation. Irradiated nymphs were allowed to reach the adult stage. Then the males and females that emerged were paired, and their fecundity, fertility and longevity observed.

2.2. Treatment of the adult mites

Studies on the irradiation of adults of both sexes were initiated by selecting inert deutonymphs from the stock colonies and holding them in separate cages. On the day following emergence, the adults were sexed and treated with ^{60}Co radiation. On the same day as the irradiation treatment, the irradiated mites were paired. During rearing and observation, the mites were kept in rearing cages supplied with food. The rearing cages were stored in darkness at 25°C ± 1°C and 85 ± 5% relative humidity.

Every 1–3 days, the number of eggs laid by females was determined, the eggs were removed from the cages and food was added. Fecundity was recorded by mounting the eggs laid by each female until her death. The viability of these eggs was observed and the mortality of the females and males recorded.

To determine the dose of gamma radiation that sterilized the males and females, the following methods were adopted. The treated and untreated mites were paired according to the following combinations:

(1) Untreated females and untreated males (control)
(2) Untreated females and treated males
(3) Treated females and untreated males
(4) Treated females and treated males.

The fecundity, fertility and longevity of these mites were recorded.

2.3. Identification of the irradiated mites

Cages containing food infested by the acarid mites were irradiated with 0 (control), 250, 500, 750 and 1000 Gy of gamma radiation. For each treatment, at least 50 females were isolated, placed in 10–12 rearing cages (five females per cage) and supplied with wheat germ or yeast as food. The treated cages were stored for 1, 2 and 3 weeks in darkness at 25°C ± 1°C and 85 ± 5% relative humidity. After these periods, the live adult mites were isolated, placed in separate rearing cages and supplied with food. Each rearing cage contained five females, or five females with 1–2 males. The number of eggs laid by the females was recorded on days 1, 2 and 3 after isolation of the mites.

Additionally, the irradiated females and the females from the stock colonies (not treated) were mounted on glass slides. Hoyer's modification of Berlese's medium was used. A drop of mountant was placed in the centre of the slide and the mite specimens were then transferred from the alcohol or directly from the culture using a needle. A cover glass was placed over the drop of mountant after orientation of the mite specimens under a stereoscopic binocular microscope. Generally, a single female was mounted on each slide. The females mounted on glass slides were studied under a microscope to determine the number of eggs visible within their bodies.

3. RESULTS

The results of the experiments are summarized. Details of the data obtained from these and other related experiments have been reported elsewhere [3–10].

3.1. Treatment of the immature stages of mites

3.1.1. Treatment of eggs

3.1.1.1. *T. putrescentiae*

The age of the mould mite eggs at the time of irradiation had a profound effect on their hatchability. The most susceptible stage was the 0–1 day old egg, exhibiting

TABLE I. EFFECTS OF RADIATION ON THE HATCH OF 0–4 DAY OLD BULB MITE, *R. echinopus*, EGGS AND THE SEX (% FEMALES) OF ADULTS DEVELOPING FROM TREATED 2 DAY OLD EGGS
(Modified from Refs [5, 6])

Dose (Gy)	Hatch (%) of 0–4 day old eggs				Females (%) from 2 day old eggs
	0–1	1–2	2–3	3–4	
0	87.7	92.3	85.8	94.8	51.8[a]
100	33.0	36.4[a]	100.0	100.0	1.0
200	25.2	25.8	98.3	99.6	—
300	0.0	0.0	100.0	100.0	5.4
500	0.0	0.0	98.1	100.0	2.4

[a] Revised data.

the greatest mortality after the radiation treatment. When older eggs of the mould mite were treated with doses of >260 Gy, some larvae emerged, but they soon died. With lower doses, sterile adults were occasionally obtained [3, 4].

3.1.1.2. *R. echinopus*

Eggs (0–2 day old) of the bulb mite were very sensitive to radiation; the sensitivity of the 0–1 and 1–2 day old eggs was similar (Table I) [5, 6]. A 100 Gy dose caused 63.6–67% mortality of these eggs, whereas irradiation at 200 Gy resulted in 74.2–74.8% embryonic deaths. Doses of ≥300 Gy prevented hatching following irradiation of 0–2 day old eggs. The older eggs (2–4 day old) were resistant to radiation. None of the doses tested (100–500 Gy) induced the mortality of the eggs. Because of the moderate mortality rates at the larval and nymphal stages, a significant portion of these eggs developed to the adult stage. However, the sex ratio in these adults was strongly male biased; only 1–5% females were recorded (Table I) [5, 6]. These males were sterile; the untreated females paired with males that emerged from the eggs treated with 500 Gy were found to produce no eggs.

3.1.2. Treatment of larvae

3.1.2.1. *T. putrescentiae*

No adults of the mould mite developed from larvae irradiated with ≥350 Gy, but at lower doses (180–260 Gy), sterile adults were occasionally obtained [3, 4]. Some of them appeared to be weak and sluggish.

3.1.3. Treatment of protonymphs

3.1.3.1. *T. putrescentiae*

Some sterile adults of the mould mite did develop from protonymphs treated with doses of ≥350 Gy [3, 4]. These males and females lived for a much shorter time than the controls. Of the inert protonymphs treated with 260 Gy, 85.3% reached the adult stage. The males and females that emerged were infecund; they lived 31.3 and 28.0 days, respectively. Treatment of inert protonymphs with 700 Gy caused them to have difficulty with moulting or deutonymphs and the adults that appeared soon died, often inside the moulting skin. The deutonymph stage usually lasted longer than in untreated mites. Two weeks after irradiation the mortality of the mites reached 87.8%. Following treatment, a few survivors lived on, but none lived longer than 40 days [3, 4].

3.1.4. Treatment of deutonymphs

3.1.4.1. *T. putrescentiae*

Inert deutonymphs of the mould mite irradiated at doses of 90–1320 Gy were able to moult and become adults [3, 4]. The fecundity and fertility of the adults that emerged from the 90 Gy treated deutonymphs was high, 172.8 eggs per fecund female, of which 78.0% hatched (Table II) [3, 10]. This was significantly lower than that of the control, which produced 415 eggs per female, of which 96.6% hatched. The adults that emerged from the 180 Gy treated deutonymphs produced 28.5 eggs per fecund female. The eggs laid by these mites during the first 2 weeks were dead; later, their viability increased to 63.8%. The eggs laid by mould mite adults that emerged from inert deutonymphs treated at doses of $\geq$260 Gy did not hatch (Table II). At 1320 Gy, the deutonymphs moulted with difficulty and the adults that emerged appeared to be weak and sluggish, produced no eggs and lived for a maximum period of 22 days.

When unsexed, intermediate aged inert deutonymphs of the mould mite were used, the sex ratio was about 1:1 in the emerging untreated adults (control) [7, 8]. No difference in sensitivity between the sexes was indicated in the adults that emerged from irradiated inert deutonymphs; the sex ratio was also about 1:1.

3.1.4.2. *R. echinopus*

Adults of the bulb mite obtained from irradiated deutonymphs were paired, and the production of eggs and their viability recorded [5, 6]. These data showed that the number of eggs laid decreased with the increase in dose (Table III) [5, 10]. Compared with the control, the fecundity of bulb mites that developed from irradiated deutonymphs was greatly reduced. For example, the adults that emerged from the deutonymphs treated with 350 Gy produced 93.6% fewer eggs than the control mites. The reduction in fecundity ranged from 90 to 100% when the deutonymphs were irradiated with doses of $\geq$250 Gy. Also, these mites produced significantly fewer eggs than the mites treated as adults (Table III). All the mites that emerged from the deutonymphs treated with 1000 Gy were infecund.

The adults that emerged from inert deutonymphs irradiated with 200 Gy produced eggs for 6 weeks after the treatment (Table IV) [6]. Those mites originating from the deutonymphs given $\geq$250 Gy produced eggs for only 2 weeks after the treatment and were infecund thereafter.

The viability of the eggs laid by mites that emerged from treated deutonymphs was very low (Table III). At 100 Gy, 37% of the eggs were dead, but at 200 Gy the mortality of the eggs was 86.5%. The eggs laid by mites irradiated with $\geq$250 Gy usually failed to hatch (a few hatched at 350 Gy).

TABLE II. FECUNDITY AND FERTILITY OF MOULD MITES, *T. putrescentiae*, TREATED WITH GAMMA RADIATION AS INERT DEUTONYMPHS OR 0–24 OR 24–48 HOUR OLD ADULTS
(Modified from Refs [3, 10])

Dose (Gy)	Infecund pairs (%)			Eggs/fecund female			Egg hatch (%)	
	Deuto-nymphs	Adult ages		Deuto-nymphs	Adult ages		Deuto-nymphs	Adult ages
		0–24 h	24–48 h		0–24 h	24–48 h		24–48 h
0	0.0	0.0	0.0	415.0	415.0	415.0	96.0	96.0
90	10.9	—	2.3	172.8	—	189.0	78.0	73.8
180	9.5	—	—	28.5	—	—	63.8	0.0
260	93.7	—	10.0	1.5	—	15.4	0.0	0.0
350	100.0	87.2	35.8	0	2.2	8.9	—	0.0
530	97.9	91.7	10.1	1	1.0	9.5	0.0	0.0
1320	100.0	97.0	33.8	0	1	1.7	—	0.0

TABLE III. FECUNDITY AND FERTILITY OF BULB MITES, *R. echinopus*, TREATED WITH GAMMA RADIATION AS INERT DEUTONYMPHS OR 24–48 HOUR OLD ADULTS
(Modified from Refs [5, 10])

Dose (Gy)	Infecund pairs (%)		Eggs/fecund female		Egg hatch (%)	
	Deutonymphs	Adults	Deutonymphs	Adults	Deutonymphs	Adults
0	0.0	0.0	335.6	335.6	99.2	99.2
100	0.0	0.0	248.6	261.0	62.6	30.6
200	0.0	0.0	83.9	104.8	13.5	0.4
250	0.0	0.0	14.4	43.6	0.0	0.2
300	2.7	0.0	12.5	30.5	0.0	0.0
350	2.4	—	21.8	—	1.0	—
400	0.0	0.0	11.0	65.6	0.0	0.0
500	2.6	0.0	18.4	59.7	0.0	0.0
900	15.0	3.7	6.5	25.6	0.0	0.0
1000	100.0	6.5	0	33.1	—	0.0
1200	—	96.7	—	5.0	—	0.0
1500	—	100.0	—	0	—	—

TABLE IV. EFFECTS OF GAMMA IRRADIATION ON THE PRODUCTION OF EGGS BY BULB MITE, *R. echinopus*, ADULTS TREATED AS DEUTONYMPHS OR AS 24-48 HOUR OLD ADULTS [6]

Dose (Gy)	Weeks post-treatment	Infecund pairs (%)		Eggs/fecund female	
		Deutonymphs	Adults	Deutonymphs	Adults
0	1	5.8	5.8	41.2	41.2
	2	0.0	0.0	56.7	56.7
	3	0.0	0.0	69.9	69.9
	4	0.0	0.0	43.6	43.6
	5	0.0	0.0	36.4	36.4
	6	23.5	23.5	23.5	23.5
200	1	0.0	0.0	31.7	79.2
	2	0.0	12.0	25.1	21.7
	3	11.5	32.0	15.7	8.8
	4	53.8	76.0	10.2	1.7
	5	92.3	96.0	17.5	1.0
	6	96.2	100.0	47.0	—
400	1	0.0	0.0	10.1	55.6
	2	74.1	14.3	2.1	8.6
	3	100.0	66.7	—	7.3
	4	100.0	100.0	—	—
	5	100.0	100.0	—	—
	6	100.0	100.0	—	—
600	1	0.0	0.0	18.2	54.7
	2	60.0	42.5	4.1	6.3
	3	100.0	92.5	—	1.0
	4	100.0	100.0	—	—
	5	100.0	100.0	—	—
	6	100.0	100.0	—	—
800	1	14.9	8.1	7.8	18.4
	2	87.2	10.8	2.0	10.1
	3	100.0	86.5	—	9.2
	4	100.0	100.0	—	—
	5	100.0	100.0	—	—
	6	100.0	100.0	—	—

3.1.4.3. *T. neiswanderi*

Irradiation also significantly reduced the fecundity and the hatch of eggs produced by adults of *T. neiswanderi* that developed from treated deutonymphs (Table V). Production of eggs was inhibited almost completely by a dose of 1200 Gy. At lower doses, the treated mites laid fewer eggs than the control. Adults that emerged from deutonymphs irradiated at 200 Gy produced only 61.8 eggs, of which 5.4% hatched. All the eggs were dead after treatment with ≥300 Gy [9].

3.2. Treatment of the adult mites

3.2.1. T. putrescentiae

The longevity of adults of the mould mite varied inversely with the radiation dose. Irradiation with 880, 1060, 1320, 1580 and 1850 Gy caused 100% mortality of the adults after 30, 26, 21, 10 and 8 days, respectively. Mites given a dose of 2110 Gy were dead after 3 days [3, 4, 10].

Sterility in acarid mites was achieved following irradiation of the adult females and males at much lower doses than needed to kill these pests. When both sexes of the mould mites were irradiated, some pairs ceased egg laying (Table II). Inhibition of egg laying was more pronounced in the mites irradiated

TABLE V. FECUNDITY AND FERTILITY OF *T. neiswanderi* MITES TREATED WITH GAMMA RADIATION AS INERT DEUTONYMPHS OR 24–48 HOUR OLD ADULTS

Dose (Gy)	Infecund pairs (%)		Eggs/fecund female		Egg hatch (%)	
	Deutonymphs	Adults	Deutonymphs	Adults	Deutonymphs	Adults
0	3.8	3.8	201.7	201.7	86.5	86.5
100	—	0.0	—	195.4	—	41.2
200	29.6	3.6	61.8	112.4	5.4	13.7
300	7.1	4.0	15.1	48.6	0.0	8.3
400	28.6	7.1	7.8	22.7	0.0	1.6
500	37.9	3.4	2.7	10.0	0.0	0.0
600	25.0	3.4	6.3	16.5	0.0	0.0
900	26.9	21.2	7.0	7.0	0.0	0.0
1200	95.0	95.0	2.0	2.0	0.0	0.0
1500	—	100.0	—	0	—	—

TABLE VI. EFFECTS OF 90 Gy OF GAMMA RADIATION ON THE PRODUCTIVITY OF THE MOULD MITE, *T. putrescentiae*, WHEN IRRADIATED AS 0–24 HOUR OLD ADULTS
(Modified from Ref. [3])

Days after treatment	No. of eggs incubated	Adult emergence	
		(No.)	(%)
2	45	2	4.4
3	166	21	12.7
9	361	26	7.2
16	415	244	58.8
17	113	66	58.4

as 0–24 hour old adults than as 24–28 hour old adults [3]. As noted earlier, untreated mites produced 415 eggs per fecund female, of which 96% hatched. When 24–48 hour old adults of both sexes were treated with a dose of 260 Gy, the mites produced 15.4 eggs per fecund female, all of which were sterile. Mites irradiated with a dose of 90 Gy oviposited 54% fewer eggs than the control. The hatchability of the eggs produced by these mites was variable in the subsequent 'egg waves'. Almost all the eggs that were laid during the first 2 weeks after treatment at 90 Gy by 0–24 hour old adults were dead (Table VI) [3]. Later, the viability of the eggs produced by these mites gradually increased to 73.8%. As a consequence, the percentage survival of eggs to the adult stage (termed "productivity") also increased. About 58% of the eggs produced on days 16 and 17 post-treatment developed into adults [3, 4].

3.2.2. *R. echinopus*

As shown in Table III, the fecundity of the bulb mite was greatly affected by gamma radiation at doses of ≥ 100 Gy. A significant portion of the eggs laid by 100 or 200 Gy treated mites hatched. However, the viability of the eggs was higher at 100 Gy than at 200 Gy (Table III). The viability of the eggs laid by 100 or 200 Gy treated mites was low during the first days after irradiation. Later, it attained rather stable levels. This indicates a quick post-irradiation recovery in fertility by mites irradiated with low doses of gamma radiation. Adult mites treated with 250 Gy produced 87% fewer eggs than the control (Table III). The treated pairs exhibited a great variability in sensitivity to radiation. Their fecundity ranged from 1 to 119, 24 to 114 and 1 to 64 eggs when the males and females were treated with 250, 500 and 900 Gy, respectively. A single larva hatched from an egg produced by mite

TABLE VII. FECUNDITY AND FERTILITY OF IRRADIATED OR NON-IRRADIATED FEMALE BULB MITES, *R. echinopus*, PAIRED WITH IRRADIATED OR NON-IRRADIATED MALES

Dose (Gy)	Treated (T) × untreated (U) combinations					
	Eggs/fecund female			Egg hatch (%)		
	T female × T male	T female × U male	U female × T male	T female × T male	T female × U male	U female × T male
U female × U male	335.6			99.2		
300	30.5	49.0	242.1	0.0	29.4	16.6
400	65.6	32.1	99.0	0.0	2.0	19.2
500	59.7	28.5	105.4	0.0	0.0	5.3
600	58.8	27.1	46.0	0.0	0.0	0.0

pairs treated with doses of 250, 300 and 400 Gy. In these cases, however, the lethality of the eggs was higher than 99.8%. All the eggs laid by mites irradiated with ≥500 Gy were sterile. The mites irradiated with 1000 Gy laid a few eggs and those treated with 1200 Gy occasionally produced eggs. All the pairs given ≥1500 Gy were infecund.

The bulb mites irradiated as adults produced eggs longer than the mites treated as inert deutonymphs. The mites given 600 or 800 Gy laid eggs during weeks 1, 2 and 3 after treatment and were infecund thereafter (Table IV).

The larvae hatched from the eggs laid by 100 and 200 Gy treated mites were allowed to develop to the adult stage, and the sex ratio in the progeny obtained was determined. A significant distortion of the sex ratio (preponderance of males) was found in progeny that developed from eggs laid during the first 5 days after the treatment [6]. This appears to be related to the lowered viability of the eggs reported for the same period after irradiation.

The females of the bulb mite were clearly more radiosensitive than the males, as shown by the differences in reproductive ability of irradiated adults mated with untreated opposites (Table VII). The sterilizing dose of gamma radiation was found to be 400–500 Gy for females, but between 500 and 600 Gy for males. Irradiated female bulb mites mated to untreated males were less fecund than those mated to treated males at doses of 400, 500 and 600 Gy. Untreated females mated to males irradiated at 300, 400 and 500 Gy were more fecund than either of the other mating combinations (Table VII). None of the eggs laid by irradiated females mated to irradiated males hatched and none of the eggs laid by mites where one sex had been treated at 600 Gy hatched. Females of the mould mite were also found to be more sensitive to the sterilizing action of the ionizing radiation than males.

3.2.3. T. neiswanderi

Irradiation of adults of *T. neiswanderi* with gamma rays significantly affected their egg production (Table V). All, or almost all, pairs were fecund when treated with doses of up to 600 Gy. However, at 900 Gy, 21% of the pairs were infecund; at 1200 Gy about 95% of the pairs failed to produce eggs. All the mites were infecund when treated with ≥1500 Gy.

The number of eggs laid per pair for mites irradiated with 100 Gy did not differ from that of the control, but at 200 Gy the fecundity was about half that of the control (Table V). The fecundity of mites irradiated with 300 Gy was a quarter that of the control. At doses of ≥500 Gy, the mites laid several eggs; at doses of ≥1500 Gy the mites were infecund. The viability of eggs produced by irradiated mites decreased with the increase in dose. At a dose as low as 100 Gy, more than 40% of the eggs hatched, but at 200 and 300 Gy the mortality of the eggs was 86 and 92%, respectively. At the higher doses, the eggs hatched only occasionally. Complete sterility of mites occurred with gamma radiation applied at ≥400 Gy [9].

TABLE VIII. EFFECT OF GAMMA RADIATION ON THE MORTALITY OF TREATED ADULT *T. neiswanderi* MITES

Dose (Gy)	Days required for mortality			
	LD_{50}		LD_{95}	
	Male	Female	Male	Female
0	42.5	57	75.0	89
100	49	—	77	—
300	50	—	77	—
600	41	—	54	—
900	37.5	—	49	—
1200	13.5	27	31	36.5
1500	2.5	3.2	17.5	24.6
1800	1.5	0.6	6.0	3.7

TABLE IX. VIABILITY OF EGGS LAID BY IRRADIATED *T. neiswanderi* MITES DURING WEEKS 1 AND 2 POST-TREATMENT

Dose (Gy)	Viability of eggs (%) laid in week	
	1	2
0	91.5	92.7
200	13.7	22.9
300	8.3	38.1

These data showed that the treated adults were more resistant to irradiation than the adults that developed from treated deutonymphs. Irradiation lowered the fecundity of adults that emerged from treated deutonymphs much more than in irradiated adults. For example, adults treated with 200 Gy laid 112.4 eggs during their life, whereas the adults that emerged from irradiated deutonymphs produced only 61.8 eggs. When mites were irradiated as deutonymphs directly, the viability of subsequent adults was much more affected than when the adults were treated. At a dose of 200 Gy, 13.7% of the eggs produced by the treated adults were viable, whereas only 5.4% of the eggs laid by mites irradiated as deutonymphs were viable.

TABLE X. FECUNDITY OF BULB MITE, *R. echinopus*, FEMALES ISOLATED RANDOMLY 1, 2 OR 3 WEEKS AFTER IRRADIATION OF INFESTED BREWER'S YEAST. FECUNDITY IS GIVEN AS THE MEAN FOR THE TOTAL NUMBER OF EGGS RECORDED FOR THE FIRST 3 DAYS AFTER ISOLATION

Dose (Gy)	Fecundity (eggs per five females per 3 days) in week		
	1	2	3
0	49.0	46.8	57.8
250	16.5	26.3	5.3
500	20.1	3.7	0.1
750	8.2	5.9	0.1
1000	3.8	3.4	0.0

Gamma radiation applied at doses of up to 600 Gy had very little effect on the longevity of male *T. neiswanderi* mites (Table VIII). Males treated with 100–300 Gy lived somewhat longer than the controls, and the number of days required for 50 and 95% mortality was higher than that for the control mites. Mites treated with 400–600 Gy lived as long as the untreated mites [9]. The longevity of males was shortened by doses $\geq$900 Gy. The mortality of both sexes increased as the dose of gamma radiation increased. In general, females were found to be more resistant than males. At a dose of 1200 Gy, 50% mortality of females and males occurred after 27 and 13.5 days, respectively. A dose as high as 1800 Gy did not cause the immediate mortality of the treated males and females. All the mites died (100% mortality) during the first week post-treatment [9].

The viability of eggs laid by mites irradiated with low doses of radiation (200–300 Gy) was not constant (Table IX). It increased with time following treatment, possibly indicating the differential response of the development stages of eggs to radiation. The mechanisms underlying this post-irradiation recovery have yet to be investigated.

3.3. Identification of the irradiated mites

It was found that adults of the bulb mite which emerged from inert deutonymphs irradiated with 200 Gy produced eggs for 6 weeks after treatment (Table IV). Those mites originating from deutonymphs given $\geq$250 Gy produced eggs for only 2 weeks after treatment and were infecund thereafter. However, mites

TABLE XI. NUMBER OF EGGS WITHIN THE BODIES OF IRRADIATED FEMALES OF *T. neiswanderi* MITES RECORDED DURING WEEKS 1 AND 2 POST-TREATMENT

Dose (Gy)	Post-treatment week	No. of females examined	No. of females with the indicated No. of eggs				
			0	1	2	3	4
0	1–2	20	2	12	5	4	1
300	1	15	9	4	1	1	0
	2	16	15	1	0	0	0
600	1	15	10	4	1	0	0
	2	16	15	1	0	0	0

irradiated as adults produced eggs longer than mites treated as deutonymphs. Mites given 600 or 800 Gy laid eggs during weeks 1, 2 and 3 after treatment and were infecund thereafter (Table IV) [6]. Therefore, a test to verify that a pest has been irradiated and is incapable of reproduction could be based on the infecundity of the treated bulb mites.

Since varying periods of time may elapse between the irradiation treatment and the quarantine inspection of the product, the live mites were isolated from the product 1, 2 and 3 weeks after treatment. The total number of eggs produced by these females on days 1, 2 and 3 after isolation is summarized in Table X. The fecundity of the females isolated 1 week after irradiation with 250 and 500 Gy was sometimes high; some groups of females produced more than 20 eggs during the 3 day period. Mites irradiated with doses higher than 500 Gy laid a few eggs, but always less than 20. Also, the females isolated for 2 weeks after treatment with 250 Gy often produced more than 20 eggs per five females during the 3 day period. In other treatment combinations, their fecundity was very low (Table X). The results obtained show that the test based on the infecundity of the treated bulb mites can be used for the identification of irradiated mites given ≥ 500 Gy. The same was found for the mould mite [4] and for *T. neiswanderi*.

The counting of any eggs visible within the bodies of females isolated from irradiated or untreated products also helped to identify the mites subjected to irradiation. The results presented in Table XI show that the untreated females of the *T. neiswanderi* mites had one to four eggs within their bodies; only 10% of the females had no eggs. Females treated with a dose of 300 Gy had 0–3 eggs (an average of 0.67). At week 2 post-treatment, only one of the 15 females checked contained an egg within its body.

4. CONCLUSIONS

When acarid mite eggs were treated at a dose of 300 Gy, some larvae emerged, but they soon died. With lower doses, sterile adults were occasionally obtained. The sensitivity of eggs to radiation appeared to depend on their age. Eggs irradiated from 0–1 day old mites were the most susceptible. No adults developed from larvae irradiated at ≥ 350 Gy, but some sterile adults did develop from treated protonymphs and deutonymphs.

The radiosensitivity of acarid mites decreased during their development. The adult stage was the most resistant to gamma radiation. Adults of the mould mite given a dose of 2110 Gy were dead after 3 days.

Sterility in mites was achieved following irradiation of the adults at much lower doses than needed to kill these pests. When both sexes of the acarid mite were irradiated with doses ≥ 250 Gy, the mites produced several eggs, all of which were sterile.

Normal mites produced eggs during their entire lives. Irradiated mites produced eggs during the first weeks and were infecund thereafter. The test based on the infecundity of the treated mites can be used for the identification of irradiated mites given 300–500 Gy, or higher, doses of gamma radiation. The counting of eggs visible within the bodies of females isolated from irradiated or untreated products could also help with the identification of mites subjected to irradiation.

Irradiation as a quarantine treatment for acarid mites in agricultural commodities can be considered in the following general terms. For immediate mortality of the mites, doses higher than 2000 Gy of gamma radiation are required. Doses in the range of 1300–1500 Gy would be sufficient if lethality within a few weeks is the goal. A dose of 250–300 Gy would be effective if the objective is sterility of the living mites.

ACKNOWLEDGEMENT

The author acknowledges the financial support received from the IAEA.

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EFFECTIVENESS OF CONVENTIONAL COMMODITY TREATMENTS (HEAT, REFRIGERATION, CHEMICAL, OTHERS) TO SATISFY QUARANTINE REGULATIONS

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Abstract

EFFECTIVENESS OF CONVENTIONAL COMMODITY TREATMENTS (HEAT, REFRIGERATION, CHEMICAL, OTHERS) TO SATISFY QUARANTINE REGULATIONS.

Quarantine treatments other than irradiation are discussed and examples given. Because of public concern about the use of chemicals to control insect pests in fruits and vegetables, more research emphasis by Agricultural Research Service, United States Department of Agriculture, scientists involved with developing quarantine treatments has been directed towards temperature manipulation and ways of eliminating the need for treatment. Single treatments include fumigation, temperature manipulation (heat/refrigeration), a modified atmosphere, physical barriers and an insecticide dip. Commodity quarantine treatment/certification research is concerned with killing pests and minimizing or eliminating the need for treatment. Future quarantine treatment research should be carried out along these lines.

1. INTRODUCTION

Quarantine treatments are designed to prevent the establishment of exotic pest species in areas where they are not already established. Specific quarantine treatments must be developed separately for each commodity and for control of the individual pest species infesting each commodity. Detection and/or exclusion of pest entry is the first line of defence and avoids expensive eradication programmes against quarantined insects. Commodities produced in quarantined areas can only be marketed after acceptabe treatments have been applied, or if the commodities have been certified free of the subject insect pest. The Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) is responsible for developing treatments that provide the required security against new insect introductions. The Animal and Plant Health Inspection Service (APHIS) of the USDA regulates commercial compliance with the prescribed commodity treatments.

Numerous quarantine treatments provide security against exotic pests in tropical and subtropical fruits and vegetables other than an irradiation treatment, such as the one which is approved for fruit flies in Hawaiian papayas. APHIS has authorized irradiation as a quarantine treatment for papayas intended for movement from Hawaii to continental USA, Guam, Puerto Rico and the Virgin Islands [1]. Many criteria must be met, including one that papayas receive a minimum absorbed ionizing radiation dose of 15 krad (150 Gy).

Irradiation is a single treatment and is grouped with other single treatments such as fumigation, temperature manipulation (heat or refrigeration), a modified atmosphere, physical barriers and an insecticide dip (which is an approved quarantine treatment in Australia). Apart from irradiation, each single treatment, combination or multiple treatments (including systems approach), and the ways of eliminating the need for treatment are discussed.

2. SINGLE QUARANTINE TREATMENTS

Individual treatments that, when used alone, provide quarantine security are single treatments. Residue data, as appropriate, should be included when the treatment is chemical, and phytotoxicity assessments should be included with the data that established the treatment.

2.1. Fumigation

This is defined as the act of releasing and dispersing a toxic chemical so that it reaches the organism wholly or primarily in the gaseous or vapour state. The fumigants available for quarantine use include methyl bromide, phosphine and hydrogen cyanide.

2.1.1. Methyl bromide

Methyl bromide is a gaseous fumigant marketed as a liquid under pressure in cylinders or cans. It has been used since the 1930s to disinfest agricultural warehouses and food plants, boxcars, processed commodities and bulk grain. It is the principal fumigant used for the post-harvest treatment of tree nuts, raisins and dry, edible beans. Also, it is used as the official quarantine treatment at atmospheric and reduced pressure for a wide range of food and non-food commodities by the USA and other countries throughout the world [2, 3]. Methyl bromide fumigation is often used for the following: (1) deciduous fruits, such as apples, cherries and pears from Chile, if any quarantine significant pests (other than fruit flies) are found on inspection; (2) cherries for the codling moth, *Cydia pomonella* L., the Western Cherry fruit fly, *Rhagoletis indifferens* Curran, the grape vine moth, *Lobesia botrana* (Schiffer-

mueller), and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); (3) tropical and subtropical fruits such as avocados where the Mediterranean fruit fly, the oriental fruit fly, *Dacus dorsalis* Hendel, and the melon fly, *D. cucurbitae* Coquillett, are found; (4) latania scale, *Hemiberlesia lataniae* Signoret, on avocados; (5) the *Anastrepha* species in grapefruits, oranges and tangerines from the West Indies, Puerto Rico, the Virgin Islands, Mexico and Belize, but not from Bermuda, where the Mediterranean fruit fly is found; (6) pineapples, oranges, grapefruits, tangerines and ethrogs from Mediterranean fruit fly countries, but not from countries south of Panama; and (7) citrus blackfly, *Aleurocanthus woglumi* Ashby. Methyl bromide is not approved for mangoes because it damages the fruit at the dosages needed to kill fruit fly infestations [4]. Avocados generally tolerate doses of methyl bromide fumigation that are approved for use against fruit flies [5]. If methyl bromide is not registered for use on food, its use in the treatment of such commodities is prohibited.

2.1.2. Phosphine

Phosphine gas is released from aluminium or magnesium phosphide fumigant preparation. Aluminium phosphide fumigant preparations are made as a powder, placed inside bags of a specific type, and as a solid that is shaped into pellets, tablets or rounds. Phosphine gas is released from the formulations whenever they are exposed to moisture such as that normally encountered in atmospheric air. Phosphine fumigation is used throughout the world for disinfesting a wide variety of stored products. Phosphine is slow acting, but even with this limitation it is internationally the predominant grain fumigant [6]. Phosphine may cause a stable chromosome rearrangement among applicators [7]. It is not approved for disinfesting fruits of fruit flies. It is corrosive to metals and produces severe damage to avocados and mangoes at doses effective against fruit flies in these hosts [4]. Perhaps phosphine could be developed as a treatment against some pests of quarantine importance, but considerable research would be required to determine its usefulness for specific commodities and pests.

2.1.3. Hydrogen cyanide

Hydrogen cyanide is one of the most toxic insect fumigants. It is formulated for fumigation as sodium cyanide, calcium cyanide or potassium cyanide. The fact that it is very soluble in water makes it unsafe to use on moist materials such as fruits and vegetables. A solution of hydrogen cyanide in water is a dilute acid, which renders fruits and vegetables unpalatable, possibly hazardous for human consumption, and unmarketable due to burn, wilt and discoloration. However, at certain dosages and under specific conditions, sodium cyanide is used to kill mites and surface insects such as scales, mealybugs and whitefly nymphs on California citrus for

shipment to Arizona and Japan [8]. Hydrogen cyanide is approved by APHIS for use on seeds, cotton and cotton products, khapra beetle, *Trogoderma granarium* Everts, in grains and seeds, and in broom corn and broom corn articles. Hydrogen cyanide is not approved by APHIS as a quarantine treatment for use on deciduous fruits, tropical and subtropical fruits, nuts and vegetables as well as Hawaiian fruits and vegetables.

2.2. Temperature manipulation

Temperature manipulation includes the use of heat and cold. The factors that must be considered in temperature manipulation are the effective temperature needed to kill the pest (efficacy), the tolerance of the commodity that is being treated (phytotoxicity) and the time of exposure. Examples of temperature manipulation used to control pests associated with tropical and subtropical fruits and vegetables include heated water, heated air, dry heat, vapour heat and refrigeration.

2.2.1. Heated water

Use of heated water (hot water dip) as a quarantine treatment is approved for fruit flies in mangoes and papayas from specified countries. Treatment for mangoes includes immersion in water at 46.1°C, or higher, for 65–90 minutes, depending on the cultivar, its origin and its weight [9]. For example, elongate mangoes such as 'Francis', weighing 570 g each, from Haiti would be treated for 75 minutes, and rounder, thicker mangoes such as 'Tommy Atkins', weighing 700 g each, from Mexico would be treated for 90 minutes. The treatment kills all the fruit fly stages in mangoes. Mangoes are not damaged by the treatment when they are treated properly. Papayas are affected adversely by the treatment and often have hard, unripened areas in the pulp. The treatment for papayas from Hawaii includes immersing them (less than one-quarter ripe) in water at 42°C for 30 minutes within 18 hours after picking and transferring them within 3 minutes to water at 49°C for 20 minutes. The treatment does not kill all the fruit fly stages in papayas. Dipping fruits in hot water or exposure of the fruits to saturated water vapour can cause water molecules to condense on the fruit, resulting in uneven ripening of the mature green fruits and complete interruption of the ripening process in some immature fruits. The hot water dip has limited use for quarantine purposes because most fresh commodities are damaged at the treatment schedule necessary to kill all the pests.

2.2.2. Heated air

The hot air treatment heats and humidifies the air in a closed system as the air is forced over the fruit surfaces. The dry bulb temperature is maintained at a few degrees above the dew point temperature so that condensation never occurs inside

the treatment area or on the commodities being treated. The relative humidity inside the treatment area may vary from ambient to greater than 80%, thereby preventing desiccation of the treated commodities. Hot air reduces post-harvest rots, can be applied to many commodities and has been used successfully to kill fruit fly immatures in grapefruits and other citrus, carambolas, mangoes and papayas. Research is under way in Weslaco, Texas, Hilo, Hawaii, and Miami and Orlando, Florida, where hot air is being developed for use on other commodities, including vegetables. The treatment has also erroneously been called dry heat treatment (discussed in Section 2.2.3). APHIS has approved a hot air (dry heat) treatment that disinfests Hawaiian grown papayas of fruit flies [10] and a commercial hot air treatment facility in Hawaii. Papaya fruits are exposed in an APHIS approved chamber to each air temperature in three steps for 2 hours, or until the indicated seed cavity temperature is reached. The treatment is completed at step 4, when the seed cavity reaches 47.2°C. The treated fruits may be hydrocooled immediately with tap water (20°C ± 5°C) when 47.2°C is reached:

Step	*Air temperature (°C)*	*Seed cavity temperature (°C)*
1	43.0 ± 1.0	41.0 ± 1.5
2	45.0 ± 1.0	44.0 ± 1.0
3	46.5 ± 1.0	46.0 ± 0.75
4	49.0 ± 0.5	47.2

A hot air treatment was developed to disinfest Florida grown carambolas of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) [11]. Carambolas are treated with air at 47.2°C for 90–120 minutes until the centre fruit pulp temperature reaches ≥45.5°C. The hot air treatment of 47.2°C does not damage carambolas unless the exposure to hot air is greater than 120 minutes [12].

2.2.3. Dry heat

Dry heat is an approved technology and is used where very high temperatures are tolerated by the treated product. The treatment is primarily used for plant disease control. For ear corn, dry heat at 75.5°C for 2 hours is approved by APHIS. For rice straw and hulls in groups of 11.34 kg (25 lb), or less, a dry heat treatment of 100°C for 1 hour is approved by APHIS. Basically, dry heat as a quarantine treatment is not applicable to the fresh fruit and vegetable market because the humidity is not controlled, phytotoxicity due to dehydration would be encountered and the fruit or vegetable would be damaged before the insects died.

2.2.4. *Vapour heat*

Vapour heat is an approved technology for the Mexican fruit fly, *A. ludens* (Loew), in grapefruits, oranges, tangerines and mangoes or for the Mediterranean fruit fly, the oriental fruit fly and the melon fly in bell peppers, eggplants, papayas, pineapples (other than 'Smooth Cayenne'), tomatoes and zucchini. For the Mexican fruit fly, the temperature of grapefruits, oranges, tangerines and mangoes is raised by vapour at 43.3°C until the fruit centre reaches this temperature within 8 hours, then held at 43.3°C for 6 hours. For the Mediterranean fruit fly, the oriental fruit fly and the melon fly, the treatment time is 8¾ hours with vapour at 44.4°C. Hallman et al. [13] reported that vapour heat at 43.3°C–43.7°C for 5 hours resulted in no damage to fresh 'Marsh' grapefruit and recommended that grapefruit infested with Caribbean fruit fly immatures be exposed to vapour at 43.3°C–43.7°C until the fruit pulp centres remain at 43.3°C for 50 minutes. In most instances, the actual killing temperature/time and the point at which phytotoxicity begins are very close. Physiologically, the vapour heat treatment (like hot water) affects functions such as respiration by preventing the free exchange of gases.

2.2.5. *Refrigeration*

Refrigeration (cold treatment) is approved for many pests in many different commodities such as the Mediterranean fruit fly, the Mexican fruit fly, the Caribbean fruit fly (and other *Anastrepha* species) in citrus; the Queensland fruit fly, *Dacus tryoni* (Froggatt), in apples, pears and kiwis; and the tortricid, *Cryptophlebia leucotetra* (Meyrick), in fruits and vegetables. The treatment temperatures range from 10 days at 0°C to 16 days at 1.66°C for the Mexican fruit fly and 13 days at 0°C to 22 days at 2.22°C for the Queensland fruit fly. The tortricid can be treated for 22 days at −0.55°C. A cold treatment developed for carambolas from Florida for shipment to Japan is being reviewed by Japanese officials. The refrigeration treatment for carambolas at 1.1°C for 15 days provided security against Caribbean fruit fly eggs and larvae in excess of 99.9968% mortality [14]. Cold treatment is useful for those commodities, such as apples, lychees and stone fruits, that can withstand temperatures near freezing and where the shelf-life is not sacrificed.

2.2.6. *Modified atmosphere*

Atmospheres are modified, or controlled, by either lowering the level of oxygen and raising the level of carbon dioxide, or by a combination of altered levels of the two gases. Modified atmospheres have been used successfully to control insects in stored grains and nut crops and to extend the storage life of fresh fruits such as apples, pears and strawberries [15]. Each commodity varies in its response to a particular modified atmosphere, so the exact combination of gases that can be

tolerated may differ for each kind of fruit or even each variety of a particular kind of fruit. Insect species also differ in their response to a particular modified atmosphere. Adult sweet potato weevils, *Cylas formicarius elegantulus* (Summers), were killed within 4–8 days when exposed to 8% oxygen plus 40–60% carbon dioxide at 30°C [16]. When Moffitt and Albano [17] treated codling moth life stages with 2.5% oxygen and 1–1.5% carbon dioxide, they found that this atmosphere did not effectively control the diapausing larval stage with a 133 day exposure at about 0°C. Modified atmosphere is not being actively investigated now, and relatively little research has been performed on insects found in harvested fresh commodities. It is useful in combination with another treatment such as methyl bromide for the codling moth in walnuts, or could be useful in combination with hot air for fruit flies in fruits.

2.3. Physical barriers

Physical barriers have not been used as a quarantine treatment method, although some research has been performed with the use of shrink wrapping to kill infestations of fruit flies in papayas [18], mangoes [19] and grapefruits [20]. The time required to kill all the life stages far exceeded the shelf-life of the commodity. There are opportunities of using this technology in combination with other treatment methods such as hot air.

2.4. Insecticide dips

Post-harvest dips using dimethoate or fenthion have been shown to be highly effective against the Queensland fruit fly in mangoes [21] and tomatoes [22] from Australia. Residue data should be included with data that establish such a treatment.

3. COMBINATION OR MULTIPLE TREATMENTS

Two or more single treatments, when used simultaneously or sequentially to provide quarantine security, are combination treatments. The combination treatments approved by APHIS include fumigation with methyl bromide plus refrigeration to kill Metiterranean fruit fly, Queensland fruit fly and oriental fruit fly immatures in ethrog, grapes, pears, plums, apples, apricots or peaches and for the light brown apple moth complex (*Epiphyas* species). Refrigeration for 21 days at 0.55°C, or below, plus fumigation with methyl bromide at 30–48 g/m^3, is approved for the *Epiphyas* species in apples, pears or grapes. The approved combination treatments currently used by industry include the use of reduced atmosphere with methyl bromide for the codling moth in walnuts, the degree of ripeness to eliminate fruit fly larvae and the double dip hot water treatment for eggs and larvae near the surface of Hawaiian papayas. Combination treatments also include a systems approach,

where the first pest control technology could be implemented in the field during production, such as an integrated pest management system, so that pest survival at the packing plant is limited by regulation. For example, one could require only fruits with 0.1% infestation, or less. If so, and if 1000 fruits were involved, 10 fruits would be infested. Assuming one larva per fruit, 10 larvae would be counted. If the fruit is held in refrigeration and the data show that 70% mortality would be expected, then only three larvae would remain. If the data show that culling fruits in the packing line eliminates 70% of the remaining larvae, one would end with 0.9 larva. This should be sufficient to provide quarantine security. If more larvae remained, some form of treatment such as refrigeration or heat could be included in the system somewhere so that, on average, less than one larva remained. Ideally, one would utilize those procedures normally used by industry that contribute to insect mortality. Biological control does not provide 100% mortality; however, it could be useful in combination with another treatment, such as releasing sterile males or using fruit fly attractants to reduce the insect populations.

4. ELIMINATION OF THE NEED FOR TREATMENT

Commodity quarantine treatment/certification research is concerned with killing pests and minimizing or eliminating the need for treatment. Before this approach is used, biological information must be gathered.

4.1. Resistant variety with no infestation

If a commodity is declared a host of a quarantine pest, but data show that a variety is free of infestation, the data should be submitted for analysis in order that it be considered a non-host. Currently, the 'Smooth Cayenne' pineapple has been declared a non-host of fruit flies from Hawaii [23]. Also, two varieties of tomatoes from Israel have been declared non-hosts of the Mediterranean fruit fly [24].

4.2. Non-host commodity at harvest time

Certain commodities, especially those that are harvested about, or slightly beyond, mature green, are free of infestation. Research has shown that the 'Sharwil' avocado, when harvested at the hard, mature green stage with the stem end attached, with no cuts or holes in the peel (peel intact) and packed in fly impervious cartons within 24 hours of picking, is not a host of the Mediterranean fruit fly, the oriental fruit fly or the melon fly in Hawaii [25]. On the basis of the data provided, APHIS has approved shipments from Hawaii to any destination in the USA [26].

Another method approved by APHIS is the double dip hot water treatment of Hawaiian 'Solo' papayas for three species of fruit flies. The treatment consists of

harvesting fruits less than one-quarter ripe combined with two hot water dips, one at 42°C for 30 minutes followed immediately by another at 49°C for 20 minutes. Selection by the stage of ripeness eliminates larvae, since none are found up to about one-fourth ripe [27]. The hot water dip kills all the eggs and larvae near the peel [28].

4.3. Pests that do not infest a host during part or all of the harvest season (preferred hosts are available), or where the population is extremely low or is not present during part of the year

The principle that hosts are free of infestation and require no treatment can be applicable under an APHIS certification system and may be due to one or more biological factors. An approved infestation free method utilizing this system is the Caribbean fruit fly in citrus from Florida. Early season grapefruits are free of infestation in a large production area of this state. The fruits require no treatment. A host free barrier is maintained. When populations increase later in the season, treatment may be required, depending on the trap catches of the adults. Also, APHIS has proposed that the fruit and vegetable regulation be amended by specifying definite provinces in Chile that are free of the Mediterranean fruit fly and where sand pears are grown which will allow the fruits to be imported into the USA without treatment [29].

4.4. Exclusion of a pest from a geographical area

A concept similar to the infestation free principle is currently being used in southern Texas for the Mexican fruit fly in citrus. The Mexican fruit fly population is thought to migrate into this area each year from Mexico. Therefore, a biological barrier, the release of sterile flies, is maintained by APHIS. As with the Caribbean fruit fly in Florida, when the trap catches in the barrier zone exceed a certain number, treatment is required.

4.5. Elimination of a pest from a geographical area

The ideal way of solving quarantine problems is to eliminate the pest(s) from a geographical area. In such a situation, the quarantine would be lifted. Japan used the male annihilation technique to eliminate the oriental fruit fly and is now eliminating the melon fly with the use of the sterile insect technique. In the USA, exotic pests, especially tephritid fruit flies, are eliminated whenever they invade. Candidate quarantine pests for elimination include the Mediterranean fruit fly, the oriental fruit fly, the melon fly, and *Dacus latifons* (Hendel), a recent introduction, from Hawaii, and the Caribbean fruit fly from Florida.

4.6. Inspection

Another example of eliminating the need for treatment is inspection in lieu of a treatment. Stone fruits from Chile present the risk of introducing various insect pests which do not normally feed on stone fruits but which may be present in shipments of the fruit as hitchhiking pests. APHIS allows the stone fruits into the USA in accordance with a preclearance programme involving their inspection in Chile and other requirements designed to ensure that they are free of insect pests [30].

5. CONCLUSIONS

The ARS has five laboratories where quarantine treatments are being developed. In the 1989 fiscal year, 8.8 scientist-years (SY) were devoted to research on heat (hot water, hot air and vapour heat); 3.7 SY to working with fumigants, primarily methyl bromide; 2.4 SY to eliminating the need for treatment; 2.1 SY to cold temperatures; 1.3 SY to combination treatments; 0.9 SY to irradiation; 0.4 SY each to physical barriers and modified atmosphere; and 1.3 SY to genetic engineering, physiology and miscellaneous work. Researchers performing market quality assessments required 1.4 SY. A quarantine treatment must kill all the pest infestations without damaging the quality of the treated commodity. Emphasis in quarantine research has been placed on temperature manipulation and fumigation. However, future research should be directed towards eliminating the need for commodity treatment.

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EFFECTIVENESS OF IRRADIATION AS A QUARANTINE TREATMENT AGAINST VARIOUS FRUIT FLY SPECIES

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Abstract

EFFECTIVENESS OF IRRADIATION AS A QUARANTINE TREATMENT AGAINST VARIOUS FRUIT FLY SPECIES.

Research on the effects of gamma irradiation on immature fruit flies has been conducted for over 30 years. The objectives of this research were to obtain the data required to develop dose mortality curves, to predict the dose required for quarantine security and to confirm that this dose was adequate. Studies on several species of fruit fly have shown that there is a variation in their sensitivity to radiation. Differences between species, differences in the age and stage of development of the flies being tested and differences in the experimental techniques used could account for much of the variation observed. The dose of radiation required to reduce egg hatch, larval pupation or adult emergence by 95% when applied to fruit fly eggs or larvae was used as a basis for comparison of the effects of irradiation on different species of fruit fly. Doses of <36 to >1000 Gy were required to reduce egg hatch, depending on the age of the eggs. Usually, <50 Gy applied to the eggs reduced pupation and <35 Gy reduced adult emergence by 95%. Doses of up to 700 and <50 Gy applied to immature larvae reduced pupation and adult emergence by 95%, respectively. For mature larvae, doses of up to 1600 Gy were required to reduce pupation by 95%, but less than 50 Gy reduced adult emergence by 95%. Higher doses, up to 80 Gy, were required to reduce adult emergence by 95% when mature larvae were treated in fresh medium or in medium with water added. In general, exposure of fruit fly eggs and larvae to a dose of less than 150 Gy prevented adult emergence, although in some cases the calculated dose for probit 9 (99.9968% mortality) security was estimated to be as high as 280 Gy. Theoretically, X rays or electron beams would have a similar effect on immature fruit flies. However, little research has been done using such sources of radiation.

1. INTRODUCTION

The family Tephritidae consists of about 4000 species of fly distributed throughout the world, most of which are not of economic importance. However, the family does include many important insect pests of fruit. Species found in warmer areas usually reproduce continuously, although at a slower rate during periods of adverse climatic conditions. Species from temperate climatic zones usually have only one or two generations each year, overwintering as pupae in diapause. Depending

on the species, temperature and other factors, the egg stage can take from 1 to 4 days, the larval stage from 7 to 15 days and the pupal stage from 10 to 20 days for species from tropical and subtropical areas, and up to 11 months, or longer, for those species that enter diapause. The egg and larval stages are present in fruits. The mature larva leaves the fruit in order to find a suitable location in which to pupate and eventually emerge as an adult.

Over 60 years ago, scientists found that when the eggs or larvae of *Drosophila melanogaster* Meigen were exposed to X rays their development was retarded, and pupation and adult emergence were reduced or prevented [1]. Koidsumi [2] found that X rays could be used to kill the larvae of the melon fly, *Dacus cucurbitae* Coquillett, and the oriental fruit fly, *Dacus dorsalis* Hendel, in fruits. Since 1956, there have been many studies to determine the sensitivity of immature fruit flies to radiation [3]. These studies have included basic and applied research to determine the feasibility of using irradiation as a treatment for fruits subject to quarantine restrictions because of fruit fly infestations. Most of this research has involved species of primary quarantine importance, although limited studies have been done on other species.

2. BASIS FOR QUARANTINE TREATMENT RESEARCH

2.1. Methodology

A quarantine treatment should either give complete kill of the pest against which the treatment is being applied or prevent establishment of the pest in new areas. Scientists must conduct basic and applied research to develop and evaluate quarantine treatments and to determine the level required for efficacy of the treatment. The objectives of this research were to obtain data in order to develop dose mortality curves, to use these curves to predict the dose required for quarantine security and to confirm that this dose was adequate.

Large quantities of infested commodities are required in order to conduct the tests necessary to establish the dose mortality curves needed to predict the doses required for quarantine security and to conduct confirmatory tests at these doses. Originally, naturally infested fruits were used for such tests, but infestations in these fruits were not consistent. Over the past 40 years, methods for rearing fruit flies have been adapted for the production of most species in numbers sufficient to conduct the research needed to evaluate quarantine treatments.

For quarantine treatment research, infested fruits must be held under suitable conditions until the eggs or larvae have reached the stage of development required for testing. These fruits are randomly divided into four or five groups of equal size. One group is selected at random to serve as a control in order to estimate the population of fruit fly eggs or larvae originally present in the fruits to be treated.

The remaining groups are exposed to treatment at selected doses, usually stepwise, and replicated several times. Following treatment, the fruits are placed in containers to permit the larvae to mature and leave the fruits. The larvae are collected and held for pupation and subsequent adult eclosion.

The number of insects surviving each dose is determined and compared with that of the control in order to calculate the resulting per cent mortality. These percentages are converted to probits in order to calculate the dose mortality curve and the dose necessary for quarantine security at the probit 9 (99.9968% mortality) security level.

Once the dose required to give probit 9 security has been predicted, it must be confirmed under large scale commercial or quasi-commercial conditions. A total of up to 100 000 fruit fly eggs or larvae must be treated using the selected quarantine treatment schedule.

2.2. Information required by regulatory agencies

The following research information is required to support regulatory acceptance of a proposed quarantine treatment:

(1) The quarantine significance of each species of fruit fly found naturally infesting the commodity must be established.

(2) The developmental stages and population level of each life stage of the fruit fly that may be present in the commodity at the time of treatment for export must be determined, since it is related to the step in the post-harvest sequence at which the treatment is applied.

(3) The response to treatment variables must be determined for fruit fly eggs and larvae at various stages of development in order to calculate the dose mortality curves and to propose a treatment dose which will provide adequate quarantine security against the stage present in the commodity that is most resistant to the treatment.

(4) The proposed dose that will provide an acceptable level of quarantine security based on dose mortality data must be subjected to confirmatory tests under actual or simulated commercial conditions using sufficient numbers in order to verify that the proposed treatment will provide adequate quarantine security. In the case of fruit flies of major quarantine importance, confirmatory tests should demonstrate that there are no survivors from a test population of 30 000–100 000 insects, depending upon the requirements of the regulatory agencies concerned.

(5) The criteria used for acceptance of a proposed quarantine treatment could be based on the dose required to prevent larval survival, pupation or emergence of normal adults capable of flight or reproduction. Acceptable techniques would be required to demonstrate that any adults emerging have been treated and are unable to reproduce and perpetuate the species.

(6) Data on phytotoxicity to commodities should be included with the data on treatment efficacy. Host commodities should be evaluated at 2–3 times the maximum dose encountered in order to determine the effects of the proposed quarantine treatments on the quality, phytotoxicity and storage of the fruits.

2.3. Treatment security

Baker [4] set forth the basis for evaluating proposed quarantine treatments of commodities subject to infestation by fruit flies. His criterion for the effectiveness of a quarantine treatment was the inability of the treated eggs or larvae to pupate. He used the population of insects recovered after an infested commodity had been treated, compared with the population of insects that would have been present if the commodity had not been treated, as a basis for evaluation of the effectiveness of a quarantine treatment. The latter was estimated from the recovery of insects in a comparable batch of untreated fruits. He calculated the per cent mortality at each dose, converted the percentages to probits and plotted these values against dose on a logarithmic scale. Statistically, the probit corresponds to the percentage of the normal deviate, increased by 5, and was used to transform the sigmoid mortality curve to a straight line. The resulting line could then be used to estimate the dose required for quarantine security. Since he had converted the per cent mortality to probits, he defined quarantine security as probit 9, which was equivalent to 99.9968% mortality.

Usually, infested fruits contain a mixture of eggs and larvae of several different ages. Calculation of dose response curves for such populations is complicated, since the resulting curve is a combination of curves for the different age populations present. As a result, the LD_{95} and other parameters may be much higher than if separate curves were calculated for each population age being treated.

Chew and Ouye [5] and Couey and Chew [6] applied statistics to estimate the confidence limits applicable to quarantine security tests. They found that an initial population of 93 616 larvae, with no survivors, must be treated at a given dose for 95% confidence of probit 9 security and that at least 148 247 larvae would need to be tested in order to be 95% confident of probit 9 security if there was a single survivor. Testing a population of 30 000 with no survivors will only meet the 95% confidence limits for 99.99% mortality, equivalent to a probit value of 8.72.

3. EXPRESSION OF RADIATION TREATMENT EFFECTS

The effects of irradiation on immature fruit flies can be expressed in various ways, depending on the dose and stage of development present at the time of treatment.

(1) Treatment of fruit fly eggs can result in the following responses:

(a) Failure of the egg to hatch
(b) The egg hatches, but the larva is not able to pupate
(c) The egg hatches, the resulting larva matures and forms a puparium, but an adult does not emerge
(d) The egg hatches, the resulting larva matures and pupates, but the adult that emerges is abnormal
(e) The egg hatches, the larva matures and pupates, and the adult that emerges appears to be normal.

(2) Treatment of immature fruit fly larvae can result in the following responses:

(a) The larva does not mature and form a puparium
(b) The larva matures and forms a puparium, but an adult does not emerge
(c) The larva matures and pupates, but the adult that emerges is abnormal
(d) The larva matures and pupates, and the adult that emerges appears to be normal.

(3) Treatment of mature fruit fly larvae can result in the following responses:

(a) The larva does not form a puparium
(b) The larva forms a puparium, but an adult does not emerge
(c) The larva pupates, but the adult that emerges is abnormal
(d) The larva pupates and the adult that emerges appears to be normal.

4. RESULTS OF RESEARCH

Usually, research on the effects of radiation on fruit flies has involved use of gamma rays from ^{60}Co or ^{137}Cs sources, although a few studies have used accelerators, X rays or fast neutrons. Scientists have used laboratory cultures of fruit flies reared on an artificial diet for many of the basic studies. However, fruits infested by laboratory reared flies or naturally infested fruits have been used in both basic and applied studies to demonstrate and confirm the effectiveness of irradiation as a quarantine treatment.

4.1. Dose mortality studies

Although many dose mortality studies have been made on various species of fruit fly, there is little consistency in the presentation of data. An attempt to develop a consistent method for evaluating the effectiveness of irradiation has been made by calculating the estimated dose required to give 95% mortality. In some instances, such data were readily available, but in others the author calculated the dose for 95%

TABLE I. DOSE (Gy) REQUIRED TO REDUCE HATCH, PUPATION AND SUBSEQUENT ADULT EMERGENCE BY 95% FOLLOWING EXPOSURE OF FRUIT FLY EGGS ON PAPER TO GAMMA RADIATION [7-11]

Species/reference	Age of eggs (h)	Dose (Gy) for 95% reduction		
		Egg hatch	Pupation	Adult emergence
Anastrepha ludens [7]	24	17		
Ceratitis capitata [8]	0.5	8.7	10	12
	18	12		8
	36	420	37	13
[9]	2	13		
	10	21		
	20	14		
	25	160		15
	35	380		
	45	1250		23
Dacus cucurbitae [9]	2	13		
	10	36		
	15	500		
	20	860		35
Dacus dorsalis [9]	2	13		
	5	24		
	15	27		
	20	370		
	25	570	130	28
	30	920		
Dacus oleae [8]	0.5	9	8	6
	18	15		8
	36	490	37	28
Dacus tryoni [10]	2-5	10		
	20-27	80	<30	<10
	<48		<80	10
Dacus zonatus [11]	4-5	<20		
	23-24	>120	>120	18
	29-30	>120	>120	12

mortality from the available data. Likewise, data are frequently presented on the dose required to prevent hatch of eggs, pupation of larvae or emergence of adults. However, data for such doses may be based on the only dose or on a few doses tested. Therefore, the following discussion is based on the sources of information available to the present author and on his interpretation of these data. Where data presented in the publications were not adequate to calculate the required doses, an attempt was made to contact the authors in order to obtain the necessary data.

4.1.1. Eggs

Basic research on the sensitivity of eggs to radiation has been done on naked eggs treated on moist filter or blotting paper (Table I) [7–11]. In general, a dose of <36 Gy, when applied to eggs that were $<50\%$ developed, reduced the hatch by 95%. Higher doses were required for eggs that were further developed. Over 1000 Gy were required to treat eggs when they were almost ready to hatch in order to reduce the hatch by 95%.

Usually, <50 Gy reduced pupation by 95% for larvae that had hatched from irradiated eggs (Table I). However, a dose of >120 Gy was required to reduce the pupation of larvae developing from treated eggs of the peach fruit fly, *Dacus zonatus* (Saunders), by 95% [11].

A dose of <35 Gy reduced the emergence of adults developing from irradiated fruit fly eggs that had been treated on paper by 95% (Table I).

4.1.2. Immature larvae

Basic research on the sensitivity of immature fruit fly larvae to radiation has usually been done by treating larvae in rearing medium (Table II) [7–10, 12–14]. In general, doses of <700 Gy reduced the pupation of irradiated immature larvae by 95%.

A dose of <50 Gy reduced the emergence of adults developing from immature larvae irradiated in rearing medium by 95%.

4.1.3. Mature larvae

Mature fruit fly larvae, those that had left the rearing medium, were more resistant to the effects of gamma radiation than were eggs or immature larvae (Table II). Doses of up to 1600 Gy were required to reduce the pupation of irradiated mature Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), the oriental fruit fly and the melon fly by 95% [9]. The reason that such a high dose was required was probably because the larva had initiated the metamorphosis process in order to form the puparium prior to, or during, treatment. However, even though the larva was able to form a normal puparium (the hardened, barrel like larval skin within which

TABLE II. DOSE (Gy) REQUIRED TO REDUCE PUPATION AND SUBSEQUENT ADULT EMERGENCE BY 95% FOLLOWING EXPOSURE OF FRUIT FLY LARVAE IN REARING MEDIUM TO GAMMA RADIATION [7–10, 12–14]

Species/reference	Age of larvae (d)	Host	Dose (Gy) for 95% reduction	
			Pupation	Adult emergence
Anastrepha ludens [7]	Mature	Air		<15
Ceratitis capitata [8]	1	Medium/air		18
	Mature	Medium/air		24
[9]	1	Medium		17
	4	Medium		20
	6	Medium		27
	Mature	Old medium	1400	26
	Mature	Medium + water		43
	Mature	Fresh medium		36
[12]	1	Medium	222	
	5	Medium	643	
	7	Medium	688	
	Prepupation			19
Dacus cucurbitae [9]	1	Medium		43
	5	Medium		44
	Mature	Old medium	1600	44
	Mature	Medium + water		80
	Mature	Fresh medium		68
Dacus dorsalis [9]	1	Medium	300	29
	4	Medium		36
	Mature	Old medium	1600	34
	Mature	Medium + water		64
	Mature	Fresh medium		63
Dacus oleae [8]	1	Medium/air	19	
	Mature	Medium/air	25	
[13]	Prepupation	Air		48
Dacus tryoni [10]	1	Medium	<400	<20
	3	Medium		<20
	5	Medium		<20
	7	Medium	<1600	<20
Dacus zonatus [14]	1	Medium		<20
	4	Medium		20

the pupa itself is formed), there was evidence in many instances that the larva was not able to form a pupa [15].

The dose required to reduce the emergence of adults from treated mature larvae by 95% ranged from < 15 Gy for the Mexican fruit fly, *Anastrepha ludens* (Loew) [7], to 44 Gy for the melon fly [9] and 48 Gy for the olive fly, *Dacus oleae* (Gmelin) [11]. The dose required to reduce adult emergence from the treated mature larvae was dependent on the substrate used during treatment. If the larvae were transferred to fresh rearing medium prior to treatment, or if the spent medium was covered with water to prevent the larvae from leaving the old medium, the dose required for a 95% reduction in adult emergence was 50–100% greater than the dose required to treat mature larvae in the old medium [9]. When Queensland fruit fly, *Dacus tryoni* (Froggatt), larvae were treated in nitrogen, the dose required to reduce adult emergence by 95% was about three times the dose required for treatment in air [16].

4.1.4. Infested fruits

4.1.4.1. Eggs

When infested fruits were treated to evaluate the effectiveness of irradiation as a quarantine treatment on fruit fly eggs, there was considerable variation in the dose required to reduce the hatch of the eggs (Table III) [9, 12, 17–19]. The age of the eggs, as well as the moisture content of the fruits and other factors, may have an influence on the effectiveness of irradiation on egg hatch. Higher doses were required to prevent the hatch of eggs in fruits with a high moisture content [17]. This was similar to findings that, when eggs were treated in nitrogen, carbon dioxide or water, an increase of at least 50% in the dose was required to prevent egg hatch [9]. When Queensland fruit fly eggs were treated in oranges, the dose required to prevent a 95% emergence of adults was 12.5 Gy, compared with 0.5 and 6 Gy when they were treated in apples and avocados, respectively [18].

4.1.4.2. Larvae

When fruits infested by larvae were irradiated, attempts were usually made to hold the fruits in order that older larvae would be present at the time of treatment. This was not always possible, especially when naturally infested fruits were being treated. Doses of 25–50 Gy prevented the emergence of adult Queensland fruit flies treated as young larvae in avocados, apples and oranges, while a dose of 75 Gy was required for older larvae in these fruits (Table IV) [15, 18, 19, 20–27]. Little difference was observed in the emergence between larvae that were reared on medium (Table II) [9] and those that were treated in papayas, mangoes, pumpkins or other fruits (Table IV) [24]. Several scientists have reported the emergence of

TABLE III. DOSE (Gy) REQUIRED TO REDUCE HATCH, PUPATION AND SUBSEQUENT ADULT EMERGENCE BY 95% FOLLOWING EXPOSURE OF FRUIT FLY EGGS IN FRUITS, WATER OR VARIOUS GASES TO GAMMA RADIATION [9, 12, 17–19]

Species/reference	Medium	Age of eggs (h)	Dose (Gy) for 95% reduction		
			Egg hatch	Pupation	Adult emergence
Ceratitis capitata [12]	Water	1–24	69		19
	Water	24–48	61		20
[17]	Peaches	24–48	< 10		
	Peaches	48–52	< 150		
	Peaches	68–72	< 350		
	Peaches	72–76	< 400		
	Plums	72–76	664		
	Nectarines	64–76	336		
Dacus dorsalis [9]	Air	2–3	20		
	Water	2–3	30		
	Air	2–3	12		
	3% O_2	2–3	14		
	CO_2	2–3	20		
	N_2	2–3	21		
Dacus tryoni [18]	Apples				0.5
	Avocados				6
	Oranges				12.5
Dacus zonatus [19]	Guavas	0–24		183	

occasional adults under conditions where such emergence was not expected. Once, in a test of Caribbean fruit fly, *Anastrepha suspensa* (Loew), larvae infesting grapefruits, we found the emergence of house flies, *Musca domestica* L. (unpublished data). In a test of melon flies infesting papayas in Hawaii, two Mediterranean fruit flies emerged [3]; these were obviously due to contamination of the fruits.

TABLE IV. DOSE (Gy) REQUIRED TO REDUCE PUPATION AND SUBSEQUENT ADULT EMERGENCE BY 95% FOLLOWING EXPOSURE OF FRUIT FLY LARVAE IN FRUITS TO GAMMA RADIATION [15, 18, 19, 20–27]

Species/reference	Age of larvae (d)	Host	Dose (Gy) for 95% reduction	
			Pupation	Adult emergence
Anastrepha ludens [20]	Mature	Grapefruits	2208	<50
Anastrepha suspensa [21]	Mixed	Grapefruits	246	
[22]	Mixed	Grapefruits	<600	<154
[23]	Early	Mangoes	12	
	Mid	Mangoes	12	
	4	Mangoes	37	
Ceratitis capitata [24]	Mixed	Papayas	<300	<25
Dacus cucurbitae [24]	Mixed	Fruits	<1400	<69
Dacus dorsalis [24]	Mixed	Papayas	<1200	<67
	Mixed	Avocados	<450	<70
	Mixed	Mixed	<400	<94
[25]	2	Mangoes	<500	<60
	6	Mangoes	<1000	<250
[26]	1–2	Bananas	>150	<50
	5–6	Bananas	>150	<50
Dacus tryoni [27]	Young	Apples		9
	Old	Apples		23
[18]	Young	Avocados		6
	Young	Oranges		13
	Old	Avocados		16
	Old	Oranges		14
Dacus zonatus [19]	1–2	Guavas	526	
	2–4		390	
	>4		848	
Rhagoletis indifferens [15]	Mixed	Cherries	>200	13

TABLE V. EFFECTS OF EXPOSURE OF FRUIT FLY LARVAE TO GAMMA RADIATION ON PREVENTION OF SUBSEQUENT ADULT EMERGENCE [7, 8, 10, 12–16, 18–24, 26–34]

Species/reference	Age of larvae (d)	Host	Dose (Gy)	
			No emergence	Probit 9 (99.9968%) mortality
Anastrepha ludens [7]	Mature	Air		22
[20]	Mature	Grapefruits	50	
Anastrepha suspensa [21]	Mixed	Grapefruits	25	
[22]	Mixed	Grapefruits	154	
[23]	Mixed	Florida Mangoes	17.5	
	Mixed	Mangoes	10–25	
	Mixed	Haiti Mangoes	80	
[28]	Mixed	Carambolas	50	
[29]	Mixed	Grapefruits	50 + cold	
Ceratitis capitata [8]	1	Medium		32
	Mature	Medium		37
[30]	1	Medium		44
	4	Medium		52
	6	Medium		56
[12]	Pre-pupation	Medium		26
[24]	Mixed	Papayas	25	
Dacus cucurbitae [30]	2	Medium		268
	3	Medium		145
[24]	Mixed	Fruits		156
[31]	3–4	Pumpkins		150
Dacus dorsalis [30]	2	Medium		66
	4	Medium		134
	6	Medium		63
[24]	Mixed	Papayas		206
	Mixed	Avocados		219
	Mixed	Mixed		280
[32]	Mixed	Mangoes	150	
[26]	1–2	Bananas	50	
	5–6	Bananas	50	
[33]	2	Mangoes	50	
	7	Mangoes	100	
[31]	3–4	Mangoes	150	

TABLE V. (cont.)

Species/reference	Age of larvae (d)	Host	Dose (Gy)	
			No emergence	Probit 9 (99.9968%) mortality
Dacus jarvisi[16]	5	Mangoes	75–90	
Dacus oleae [8]	1	Medium		37
	Mature	Medium		40
[13]	Pre-pupation	Medium		270
Dacus tryoni [10]	1	Medium	20	
	3	Medium	20	
	5	Medium	20	
	7	Medium	20	
[16]	5	Mangoes	75–90	
[34]		Mangoes	50	
[27]	Young	Apples	75	9
	Old	Apples	75	23
[18]	Young	Avocados	50	
	Young	Oranges	50	
	Old	Avocados	75	
	Old	Oranges	75	
Dacus zonatus [19]	1–2	Medium	50	
	2–4	Medium	50	
	>4	Medium	55	
[14]	1	Medium	20	
	4	Medium	50	
Rhagoletis indifferens [15]	Mixed	Cherries	97	18.6

4.2. Confirmatory studies

Applied research, in which infested fruits are treated in confirmatory tests, must be conducted in order to evaluate the effectiveness of a proposed dose of irradiation as a quarantine treatment. Theoretically, these tests are conducted at a dose that is calculated from the dose mortality curves based on data obtained in laboratory studies. However, frequently confirmatory tests are based on intuition or other factors instead of probit 9 estimates. Also, statisticians do not consider that it is proper to use probit 9, since it is such an extreme value. No adult emergence was observed

when infested fruits were treated at doses of 150 Gy, or less, for a number of species of fruit fly infesting several different species of fruit (Table V) [7, 8, 10, 12–16, 18–24, 26–34]. In several instances, the dose predicted for probit 9 quarantine security was less than the dose tested with no adult emergence. In some instances, the dose calculated for probit 9 was greater than 150 Gy, or the dose at which no adults emerged.

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REVIEW OF IRRADIATION AS A QUARANTINE TREATMENT FOR INSECTS OTHER THAN FRUIT FLIES

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Abstract

REVIEW OF IRRADIATION AS A QUARANTINE TREATMENT FOR INSECTS OTHER THAN FRUIT FLIES.

The status of gamma irradiation as a potential quarantine treatment for insects other than fruit flies was reviewed. Research has been reported on five orders of insects and four groups of mites. Recent research on several species of Coleoptera, one scale insect, three species of Lepidoptera, one leaf miner and two thrips has also been reported. Past research has emphasized the effects of irradiation on Coleoptera and Lepidoptera as pests of stored products. In general, a dose of 300 Gy was found to be effective as a quarantine treatment. Integration of irradiation with other treatments used for disease control or for enhancing the quality of commodities should be considered, along with alternatives to the probit 9 concept as applied to quarantine security.

1. INTRODUCTION

Irradiation has many advantages as a quarantine treatment for disinfestation when used on fresh, dried or processed fruits, grains and plant materials. Historically, most interest has been focused on its use against fruit flies [1]. In 1986, an International Consultative Group on Food Irradiation Task Force Meeting on Irradiation as a Quarantine Treatment was held at Chiang Mai, Thailand [2]. The Task Force Group considered that tephritid fruit flies had been researched so well that a dose of 150 Gy could be recommended for any species in the absence of specific data to support a lower dose. The Group also considered insects other than fruit flies, recommending for further consideration, inter alia, a generic disinfestation dose of 300 Gy which could be appropriate for all insects and allied forms in the absence of specific data to support a lower dose.

This review has been undertaken to establish whether the results of research carried out during the intervening time (since the last Task Force Meeting) were in

accord with the generic dose recommendation. The history of irradiation research on insects and allied forms has been reconsidered to some extent, largely to establish the identity of these species, together with the commodities at risk.

2. HISTORY

A Panel Meeting in Hawaii in 1970 identified the potential for irradiation as a quarantine disinfestation treatment for fruit, primarily against fruit flies [3]. It also identified more than 19 insects and other possible target pests as having significant destructive potential and, hence, quarantine status. This was followed in 1983 by a Consultants Group Meeting in Hawaii on the Use of Irradiation as a Quarantine Treatment of Agricultural Commodities [4].

The report of the above mentioned 1986 Task Force Group [2] listed 20 species of pests of major economic and quarantine importance other than fruit flies. These were from five orders of insects, the majority from Lepidoptera, Coleoptera and Hemiptera–Homoptera; others were from Diptera and Thysanoptera as well as from Acarina (mites).

A computer search of a database [5] located 39 papers reporting irradiation based disinfestation research on 25 species of insect and mite which could, or did, lead to the development of irradiation as a quarantine treatment. This was a relatively small proportion of the irradiation related research reported on insects. Several species warranted addition to the earlier list.

The 1986 Task Force Meeting focused on a range of related issues, including irradiation technology, irradiator design and operation, dosimetry, the effects of irradiation on fruit as related to increased shelf-life and phytotoxicity, and the cost–benefit considerations [2].

The imminence of cancellation of the registration of ethylene dibromide (EDB) for quarantine disinfestation purposes provided new impetus for research on irradiation as an alternative. This appears to have prompted the 1983 Meeting of a Consultants Group [4]. A further factor would have been the adoption in 1983 by the Codex Alimentarius Commission of the recommended international standard for irradiated foods and the recommended international Code of Practice for the operation of irradiation facilities used for the treatment of foods at an average dose of up to 10 kGy [6]. Also foreshadowed was the action in the United States of America by the Food and Drug Administration to approve irradiation as a food process up to a dose of 1 kGy [7]. Both these doses were considerably in excess of the insect disinfestation requirements for quarantine purposes.

The 1970 meeting had recognized that irradiation could meet the quarantine requirements by preventing development to the adult stage or by preventing reproduction in any fruit fly adults that might emerge [3]. Subsequent meetings explored this aspect more fully. The existing criteria for quarantine treatments were

mortality of the stage treated, although in fruit fly eggs and larvae this was demonstrated typically as mortality before the pupal stage. EDB normally achieved mortality within 2 days, followed by a period of morbidity during which no further development occurred. This was favoured by regulatory workers on procedural grounds, since it precluded detections of live treated larvae, pupae or sterile adults, even though these might not have constituted a quarantine threat.

A related meeting in Hawaii in 1983 [8] produced a number of technical papers relating to the use of irradiation for quarantine disinfestation and other purposes (control) on fresh and dried produce.

All these meetings set the scene for recommendations of major importance in the 1986 report [2], namely:

(1) "The criteria for efficacy of irradiation as a quarantine treatment should be based on the inability to perpetuate the species at a new location rather than in causing immediate mortality".

(2) A generic treatment schedule for fresh plant produce infested by insect eggs, larvae, pupae or adults of "exposure of any insect stage present to a minimum dose of 300 Gy in order to prevent emergence of normal adults from treated eggs, larvae or pupae or to sterilize any treated adults present or emerging from treated larvae or pupae". This would avoid any requirement for confirmatory data on the efficacy of the treatment against specific pests. Also recommended were specific doses for the mango seed weevil, *Sternochaetus mangiferae* (F.), and the codling moth, *Cydia pomonella* (L.), based on research data.

These recommendations [2], proposed for inclusion in a planned new edition of the International Plant Quarantine Manual [9], would in this way open the way for use of irradiation as a quarantine treatment on a wide range of insect pest species of importance. As of the end of 1990, the new revised edition of the manual had not been published. In the absence of acceptance of a generic treatment, only specific schedules are likely to be established. These will be restricted to pests for which culturing methods are available and laboratory infestation is possible, e.g. the codling moth, the light brown apple moth, *Epiphyas postvittana* (Walker), and mites.

The major trendsetter in internationally recognized quarantine standards has been the United States Department of Agriculture. Its scientists have assembled an array of treatments related to produce with an intended efficacy of probit 9, first proposed by Baker [10]. The current requirements to demonstrate probit 9 at the 95% probability level require cumulative tests against a total of 93 616 individuals [11, 12]. This approach has been adopted by Japan for gazetted species, except that it requires tests against a total of 30 000 individuals in three or four trials, without any survivors. The 30 000 standard in three trials is required by New Zealand, while Australia has historically accepted a cumulative total of 30 000 treated insects, without survivors. All are linked to the life stage most toler-

ant to the treatment. This approach more or less limits testing to insects which can be laboratory bred and their hosts infested, having been developed initially for fruit flies, which are relatively easily handled in this way. A proposal by Landolt et al. [13] for an approach to quarantine based on a minimum pest establishment risk has been developed by New Zealand researchers [14]. They have set a very stringent maximum pest limit (MPL) for fruit flies of five pests per million units based on treatment efficacy plus other factors. However, personnel from the Australian Plant Quarantine Inspection Service and the New Zealand Ministry of Agriculture and Fisheries have set much lower risk standards of 0.5% for other quarantine pests, such as for scale insects, red banded thrips, *Selenothrips rubrocinctus* (Giard), mango seed weevil and the mango planthopper, *Colgaroides acuminata* (Walker), as quarantine pests of mangoes [15].

For quarantined pests with an MPL of five per million units, mandatory treatments are required. However, pests with an MPL of 0.5%, injurious non-quarantine pests with an MPL of 5% and contaminants with an MPL of 10% are monitored with tolerances of 0, 21 or 48, respectively, in a sample of 600 fruits. This approach permits exporters to adopt the control measures most appropriate to their local situation in order to meet these tolerances and may be appropriate to commodities where the phytotoxicity restricts the maximum dose but where pests are low risk species.

3. CURRENT SITUATION

The current situation as regards the status of research on irradiation of pests other than fruit flies is summarized as follows (Table I).

3.1. Coleoptera

3.1.1. Asynonychus cervinus (Bohemann) (= *A. godmani* Crotch)

Research on the Fuller rose beetle by Johnson et al. [16] showed that hatch could be prevented for the most tolerant (older) eggs on lemons irradiated at 150 Gy. Because this weevil is not regarded as a critical quarantine pest, probit 9 efficacy of the disinfestation treatment is not demanded. However, Japan has a nil tolerance for the species at undefined pre- and post-export inspection sampling and more stringent requirements are foreshadowed. In this instance, the dose and method of irradiation would be at the discretion of the exporting country; a minimum dose of 150 Gy is indicated. The electron beam irradiation disinfestation method could be ideal for this insect.

3.1.2. *Callosobruchus* spp.

Legume weevils (bruchids) of this and related genera are not as widespread as other more cosmopolitan stored products pests. There is a move towards greater international trade in these high protein grains (pulses) and irradiation disinfestation could find a role, especially electron beam irradiation, which is ideally suited to grains. Doses as low as 70 Gy have been shown to be fully effective on members of this genus [17].

There is also a role for irradiation disinfestation in the control of stored products beetles in internationally traded shipments of grains and stored commodities. This is not strictly a quarantine problem where the insects are cosmopolitan species, although it is sometimes argued that insecticide resistant strains justify quarantine action. The validity of this argument is subject to some doubt. Where only control of populations is required, it may be argued that the slow death of the treated insects following irradiation makes the method unsuitable. However, much control of this nature is undertaken on relatively lightly infested commodities, because multiplication during shipment (around 50 times in each 6 week period) will result in rejections of outloading. Irradiation even at 200 Gy [17] prevents further multiplication and induces mortality within that period. On this basis, there is an apparently little exploited use for irradiation disinfestation, especially electron beam irradiation. Storage moths may prove to be more difficult to deal with, the adults seemingly requiring considerably higher doses than beetles.

3.1.3. *Helipus lauri* Bohemann

No recent research on this avocado seed weevil has been located. Reports of vascular browning [18] in avocados at doses of irradiation as low as 75 Gy indicate that irradiation may not be appropriate for this pest.

3.1.4. *Popillia japonica* Newman

No recent reports on irradiation disinfestation against the Japanese beetle has been found.

3.1.5. *Prostephanus truncatus* (Horn)

The spread of the larger grain borer from Central America into Africa has been one of the most devastating quarantine introductions of recent years. No recent research on irradiation disinfestation against this pest has been located, but it would appear to be a highly appropriate method for preventing further spread of this pest.

TABLE I. SOME PESTS OF MAJOR INTERNATIONAL ECONOMIC AND QUARANTINE IMPORTANCE OTHER THAN FRUIT FLY

Scientific name	Common name	Primary economic hosts	Distribution
		Coleoptera	
Asynonychus cervinus	Fuller rose beetle	Citrus	North America, Australia
Callosobruchus spp.	Legume weevils	Pulses	Absent from some growing areas
Helipus lauri	Avocado seed weevil	Avocado	Mexico, Central America
Popillia japonica	Japanese beetle	Ornamentals, lawns	North America, Asia, Azores
Prostephanus truncatus	Larger grain borer	Maize	Central America, Africa (parts)
Sternochaetus frigidus	Mango weevil	Mango	Thailand, various SE Asian countries
Sternochaetus mangiferae	Mango seed weevil	Mango	Asia, Africa, Australia, West Indies
Sternochaetus olivieri	Mango seed weevil	Mango	Thailand, various SE Asian countries
Tragoderma granarium	Khapra beetle	Grains	India, Africa (parts)
		Diptera	
Liriomyza trifolii	Serpentine leaf miner	Chrysanthenum, Gypsophilia, tomato	North America, South America, Europe, Africa
		Hemiptera–Homoptera	
Aleurocanthus woglumi	Citrus black fly	Citrus, ornamentals	Mexico, Florida, West Indies, Central America
Hemiberlesia lataniae	Latania scale	Avocado, other fruits	North/South America, Asia, Europe, Africa
Leptoglossus chilensis		Various deciduous fruits	Chile

TABLE I. (cont.)

Scientific name	Common name	Primary economic hosts	Distribution
Quadraspidiotus perniciosus	San José scale	Apple, other fruits	North/South America, Asia, Europe, Africa
Pseudococcus spp.	Mealybugs	Citrus, ornamentals	Various
		Lepidoptera	
Clepsis spectrana	Rose leaf roller	Rose	Europe
Cryptophlebia leucotreta	False codling moth	Cotton, deciduous fruits	South Africa
Cryptophlebia ombrodelta	Macadamia nut borer	Macadamia, lychee	Australia
Cydia molesta	Oriental fruit moth	Deciduous fruits	USA, Asia
Cydia pomonella	Codling moth	Deciduous fruits	North America
Epiphyas postvittana	Light brown apple moth	Deciduous fruits	Australia, Hawaii, New Zealand, UK
Lobesia botrana	Vine moth	Grapes	Europe
Prays citri	Citrus flower moth	Citrus	Europe, Asia
		Thysanoptera	
Caliothrips fasciatus	Bean thrips	Beans	North America, Europe
		Acarina	
Brevipalpus chilensis		Grapes	Chile
Halotydeus destructor	Red legged earth mite	Strawberries	Australia (parts)
Tetranychus mcdanieli	McDaniel mite	Deciduous fruits	North America
Tetranychus urticae	Two spotted mite	Deciduous fruits and flowers	Cosmopolitan

3.1.6. Sternochaetus spp.

3.1.6.1. *S. frigidus* F.

Larvae of this species, the mango (pulp) weevil, infest the flesh of mango fruit. It is a much more destructive pest than the mango seed weevil. It occurs widely throughout the South-East Asian mainland [19] and has recently been confirmed from one island of the Philippines [20]. No research work on irradiation disinfestation has been found, but it is a pest which is in need of consideration. It is an ideal target species for the 300 Gy generic dose concept, although subsequent research could well show a lower dose to be adequate.

3.1.6.2. *S. mangiferae* F.

Quarantine barriers against this insect, which causes little or no damage to commercial fruit, preclude trade in mangoes from many countries into mainland USA. Seo et al. [21] showed that a dose of 300 Gy prevented development in the immature stages and reduced adult longevity. Studies by Heather and Corcoran [22] corroborated these findings when observation periods on mortality were extended to match the long development times of this insect. Research in South Africa was directed towards obtaining a quicker kill, because in late season mangoes adult weevils occasionally emerge from the ripening fruit. In this study, Milne et al. [23] found that a dose of 500 Gy was required to prevent the emergence of adults from fruit, but this usage exceeded the requirements for quarantine security. The mango seed weevil is a very important example when irradiation disinfestation is being taken into consideration. Irradiation is currently the only disinfestation treatment capable of achieving quarantine security against this pest. It is of further importance in that no laboratory culture method is currently available. This virtually excluded probit 9 style disinfestation research against a stage identified as the most tolerant to irradiation. It is therefore an obvious application for the generic dose principle, supported by such disinfestation results as are available from naturally infested fruit.

3.1.6.3. *S. olivieri* Faust

In 1987, this seed weevil was identified as *the* pest species in commercial mangoes from Thailand [24], although it has been well documented taxonomically. A subsequent survey by Cunningham [25] identified it as the common seed weevil species in that country, although its distribution is likely to be wider and records will have been confounded by identification as the closely similar *S. mangiferae*. There is no reason to expect that this species will react any differently to irradiation, and the 300 Gy generic dose should be acceptable to any importing country accepting that dose against *S. mangiferae*.

3.1.7. Trogoderma granarium Everts

The Khapra beetle is probably the most serious quarantine species of all the stored products pests. It is restricted in its distribution to South-East Asia, the Indian subcontinent and parts of Africa. The Khapra beetle has a long tolerance to irradiation compared with other *Trogoderma* species and total sterilization of males has been achieved at 250–300 Gy [17]. Considerably lower doses were adequate to prevent development of a population through the sterility of adults of mixed sex — possibly 180 Gy on juvenile stages. It is probable that the mature pupal stage would be difficult to kill outright. Again, the electron beam method has excellent applicability when grains are being handled in bulk.

3.2. Diptera

3.2.1. Liriomyza trifolii (Burgess)

The serpentine leaf miner and other leaf miners are important pests of cut flowers and ornamental plants in international trade. They can, however, infest a range of other plants if they are introduced into a country from which they were previously absent. The study by Wit and de Vrie [26] showed that development to the adult in this species could be prevented by a dose of 80 Gy, which gave mortality of mature larvae, the most tolerant stage. Only relatively small numbers of insects were treated and the stages did not include pupae which might be present in commercial shipments. The species is very sensitive to irradiation and it should be noted how close the doses required are to those for the major Dipteran pest fruit flies. This is strong evidence of the value of the generic dose concept across pest groups.

3.3. Hemiptera–Homoptera

3.3.1. Aleurocanthus woglumi Ashby

No recent research has been located on this species, the citrus black fly. However, a paper on irradiation disinfestation of asparagus spears [27] determined that a dose of 100 Gy would achieve quarantine security against *Brachycorynella asparagi* (Mordvilko). On this basis, it is likely that similar homopterans would easily be killed, although in the absence of further data the generic dose of 300 Gy may be justified. This could cause phytotoxic effects on soft tissues such as asparagus shoots. Wit and de Vrie [26] reported that 100 Gy would sterilize the green peach aphid, *Myzus persicae* (Sulzer).

3.3.2. Hemiberlesia lataniae (Signoret)

No recent irradiation disinfestation research has been located on this pest, the latania scale.

3.3.3. Leptoglossus chilensis Spinota

No recent irradiation disinfestation research has been found for this pest. This type of insect occurs as a contaminant rather than as outright infestations of fruit in shipments.

3.3.4. Quadraspidiotus perniciosus (Comstock)

This pest, the San José scale, now occurs widely throughout the world, approaching cosmopolitan status. In preliminary work in Chile by Sánchez [28], all the stages were killed by a dose of 100–300 Gy. A study in Canada by Angerilli and Fitzgibbon [29] examined the combined effects of irradiation and cold storage on the survival of this species on apples, but recorded short term survival in cold storage at all doses up to 600 Gy. This need not indicate that 300 Gy would not provide quarantine security.

3.3.5. Pseudococcus spp.

Mealybugs infest a wide range of plant commodities, but are most important as quarantine pests on citrus and ornamentals. Because they occur on the exterior of fruits and plants, electron beam technology is highly appropriate. No recent quarantine disinfestation research on this group has been located.

3.4. Lepidoptera

3.4.1. Clepsis spectrana (Treitschke)

Wit and de Vrie [26] showed that for small numbers of larvae (n = 73) a dose of 200 Gy would prevent adult rose leaf rollers developing from fifth instars, the most tolerant stage.

3.4.2. Cryprophlebia spp.

3.4.2.1. *C. leucotreta* (Meyrick)

No recent reports have been found on the false codling moth.

3.4.2.2. *C. ombrodelta* (Lower)

No recent reports have been located on the macadamia nut borer.

3.4.3. Cydia spp.

3.4.3.1. *C. molesta* (Busck)

No recent reports have been found on the oriental fruit moth.

3.4.3.2. *C. pomonella* (L.)

A mean radiation dose of 139–177 Gy has been shown by Burditt and Hungate [30] to prevent development of the non-diapausing immature codling moth larvae to the adult in apples. Although the 79 540 larvae tested were less than the 93 616 required to demonstrate probit 9 efficacy, this is the most comprehensive quarantine testing programme involving a lepidopteran. They also predicted a number of other probable doses, e.g. to prevent pupation, and showed a difference in tolerance to irradiation between sexes. An earlier trial, involving the same species in walnuts [31], predicted that a dose of 230 Gy would be required to achieve an efficacy level of probit 9. Predictions such as these frequently overestimate probit 9 because of distortion of the real regression line. When 5954 larvae were treated in walnuts at a dose of 177 Gy, no normal adults developed. These results are an important source of support for the generic dose of 300 Gy and the Task Force Group recommended a dose of 250 Gy.

3.4.4. Epiphyas postvittana Walker

This species (the light brown apple moth) has been investigated by Batchelor et al. [32] and Dentener et al. [33]. They showed on small numbers that a dose of 199 Gy would prevent development of the larvae to the adult, although the LD_{99} for the penultimate and most tolerant larvae (fifth instar) was more than twice that dose. Again, this conforms with the expectations of quarantine security from a generic dose of 300 Gy implicit in the 1986 Task Force recommendations.

3.4.5. Lobesia botrana (Schiffermueller)

No recent reports have been located on the vine moth.

3.4.6. Prays citri Milliere

No recent reports have been found on the citrus flower moth.

3.5. Thysanoptera

3.5.1. Caliothrips fasciatus (Pergande)

No recent disinfestation research has been located on bean thrips. However, thrips as a group are a major quarantine problem in cut flowers and some fresh vegetables. They are not only a pest that debilitates and damages plants, but they can be the vectors of virus diseases which have devastating effects on plant production. Wit and de Vrie [26] showed that for two species of thrips on flowers, *Frankliniella pallida* Uzel and *Thrips simplex* (Morison), development to the prepupa was almost completely prevented by a treatment of 100 Gy and development to the adult was prevented. Thrips are close to Diptera as an insect group and again it is to be noted how close the dose required for disinfestation is to that for fruit flies. Reproduction in thrips is both parthenogenetic and sexual. A dose of 200 Gy was required to sterilize sexually reproducing females, but a lower dose achieved sterility where the reproduction was parthenogenetic. This is a group of insects where considerable research effort on irradiation disinfestation is warranted. At doses as low as 80 Gy little phytotoxic damage would be expected, but at 200 Gy cut flowers with soft tissues could be affected adversely.

3.6. Acarina

3.6.1. Brevipalpus destructor Baker

This species belongs to the Tenuipalpid group of mites which infest a wide range of fruit and ornamental crops. It is a pest of quarantine importance in produce exported from Chile, where Sánchez [28] reported complete mortality at 300 Gy, the generic dose proposed for insects. Although the numbers tested were small, these results support the adoption of the 300 Gy dose across all insect and mite species.

3.6.2. Halotydeus destructor (Tucker)

No recent irradiation disinfestation research has been located on this species, the red legged earth mite. However, in southern Australia its possible presence on strawberries is a major quarantine impediment to trade. Strawberries are a commodity highly tolerant of irradiation. Where doses of irradiation around 2 kGy are applied for shelf-life extension, quarantine disinfestation against the red legged earth mite should be acceptable without any requirement for mortality studies.

3.6.3. *Tetranychus* spp.

The tetranychid mites, the two spotted spider mite, *T. urticae* Koch, and the McDaniel spider mite, *T. mcdanieli* McGregor, are two examples of a ubiquitous group of mite pests of plants. These mites can cause severe production losses in fruit and flower crops and probably have largely achieved cosmopolitan distribution. No irradiation disinfestation research was located on the McDaniel mite, but Wit and de Vrie [26] reported that a dose of 350 Gy was necessary to sterilize both sexes of *T. urticae*, although egg and larval mortality was achieved at lower doses. However, Goodwin and Wellham [34] found that 300 Gy would satisfactorily disinfest cut flowers of all stages of *T. urticae* and that any adults developing from irradiated juveniles were sterile. These results, obtained on larger numbers of test insects, support the 300 Gy generic dose. Although it may be argued that the Dutch and Australian populations could have different tolerances to irradiation, it is more likely to be the result of differing experimental conditions.

3.6.4. *Other mite species*

From the responses to irradiation of mites as a group, it can be expected that 300 Gy will prove adequate. A comprehensive study by Ignatowicz and Brzostek [35] showed that for the mould mite, *Tyrophagus putrescentiae* (Schrank), and the bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin), mortality, or at least adult sterility, resulted from doses of 260 Gy or higher.

With the exception of the anomalous result on adult sterility from the work of Wit and de Vrie [26], mites as a group appear to have a physiological response to irradiation that is similar to insects. Mites as a group are cosmopolitan and are not generally placed in the highest quarantine category. This should avoid the necessity of having to prove treatments at the level of probit 9 efficacy, simply requiring a means by which exporting countries can avoid detection of live fertile insects at inspection. It does, however, require negotiation between exporting and importing countries to accept that any live irradiated insects detected will be infertile.

4. GENERAL COMMENT

The list of insects considered in this review is by no means exhaustive and, because the publication of results is fragmented and scattered, the literature search has also not been as extensive as possible. Nevertheless, the 300 Gy concept is comprehensively supported. A further consideration brought out is the need to apply the maximum pest level concept to quarantine pests. If this is linked to a realistic tolerance for each pest, considerable rationalization of quarantine security will

result, leading to a freeing of trade in commodities according to the risk and the efficacy of available treatments.

Low dose irradiation is the most widely applicable quarantine disinfestation measure available. It has fewer problems of phytotoxicity. Nevertheless, these, together with logistic, operational and public acceptance problems, mean that irradiation cannot be universally applicable across all pests in all crops in all quarantine situations.

Serious consideration needs to be given to integrating quarantine schedules using irradiation with those for other physical, residue free methods, particularly heat and cold [36]. This could lead to alternative schedules for greater operational flexibility and combined schedules in which the advantages of two or more methods are obtained and the disadvantages avoided.

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