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Irradiation as a quarantine treatment of arthropod pests

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FOREWORD

Fresh horticultural produce from tropical and sub-tropical areas often harbours insects and mites and are quarantined by importing countries. Such commodities cannot gain access to countries which have strict quarantine regulations such as Australia, Japan, New Zealand and the United States of America unless treated by an approved method/procedure to eliminate such pests. Current approved methods include fumigation by methyl bromide, hot water dip, vapour heat, dried heat and irradiation. Methyl bromide is being phased out globally under the Montreal Protocol in view of its strong ozone depleting properties. Countries such as the USA and those of the European Union are required to phase out the production of this chemical by the year 2005. Among other phytosanitary treatments, irradiation appears to have an edge as it is more versatile in controlling various pests and causes insignificant changes in quality of the treated products.

Based on data generated by a Co-ordinated Research Project (CRP) on Irradiation as a Quarantine Treatment of Fresh Fruits and Vegetables, in operation between 1986 and 1990, irradiation as a quarantine treatment of fresh fruits and vegetables against tephretid fruit fly is gaining wide acceptance by national and international authorities. It has been applied on a small commercial scale on fruits being exported from Hawaii for the US mainland market since 1995. The scope for expansion of this application is high as the USDA has established a policy in 1996 to accept irradiation as a quarantine treatment against major species of tephretid fruit flies regardless of commodities. In addition, the ASEAN countries have adopted a unified protocol on this subject.

The CRP on Irradiation as a Quarantine Treatment of Mites, Nematodes and Insects other than Fruit Flies, in operation between 1992 and 1997, attempted to fill the gap of information on the effectiveness of irradiation against other quarantine pests. Significant data were generated by this CRP to demonstrate that a minimum dose of between 200 and 400 Gy would render a number of non-fruit fly insects and mites sterile, thus meeting quarantine requirements. However, only a limited species of insects and mites were studied. Additional data are required to provide conclusive evidence that such a dose would render most, if not all, species of non-fruit fly insects and mites sterile. Plant parasitic nematodes appear to be resistant to irradiation as the dose required to render them sterile would cause damage to fresh horticultural commodities. Irradiation would be a useful quarantine treatment against this pest only for durable commodities such as pot soil, wood products, etc.

This publication presents the research results of the CRP presented at the final FAO/IAEA Research Co-ordination Meeting hosted by the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, and Department of Agriculture, State of Hawaii, held in Honolulu, Hawaii, from 3 to 7 November 1997.

The Scientific Secretary of this CRP was P. Loaharanu of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY OF THE CO-ORDINATED RESEARCH PROJECT

INTRODUCTION

The past few decades have seen increasing interest in the use of irradiation as a quarantine treatment of fresh horticultural commodities. Increasing acceptance and application of irradiation as a quarantine treatment against fruit flies which was the subject of a co-ordinated research programme sponsored by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture from 1986 to 1990. In particular, the following positive developments on this subject have occurred in recent years in the United States of America, especially in Hawaii:

(1) The issuing of the Notice of Policy by the USDA/APHIS in May 1996 which proposed regulation of irradiation as a quarantine treatment of fresh fruits and vegetables at specific minimum doses against major species of fruit flies regardless of commodities.

(2) The publication of the Final Rule by USDA/APHIS in July 1997 on Irradiation as a Phytosanitary Treatment of Carambola, Litchi, and Papaya from Hawaii at a minimum dose of 250 Gy against fruit flies in Hawaii.

(3) The market acceptance of and increasing demand for irradiated fruits from Hawaii, treated to meet quarantine requirements, in several States in the USA in the past few years.

In view of the global phasing out of methyl bromide, a major fumigant used for treating food and agricultural commodities to meet quarantine requirements, there is an urgent need to develop data on irradiation as a phytosanitary treatment of other arthropod pests of quarantine importance. Data generated in the past 5 years by the participants of the Co-ordinated Research Project (CRP) on Irradiation as a Quarantine Treatment of Mites, Nematodes and Insects other than Fruit Flies have shown promise on additional applications of irradiation as a quarantine treatment. Yet, much more work needs to be done to meet increasing national requirements with regard to global trade liberalization of food and agricultural commodities following the GATT Uruguay Round.

OVERALL OBJECTIVES OF THE CRP

The CRP puts emphasis on the following aspects of research in the past 5 years on irradiation as a quarantine treatment of agricultural commodities against mites, nematodes and insects other than fruit flies:

(1) Determine criteria, e.g. inability to reproduce, for accepting irradiation as a quarantine treatment against these pests.

(2) Determine the effect of irradiation on the most resistant stage of these quarantine pests at the time of treatment.

(3) Evaluate the quality of agricultural commodities irradiated at 2–3 times the dose(s) required to meet quarantine requirements.

(4) Develop method(s) for identifying surviving insects/other pests which were subjected to irradiation at a dose required for quarantine purposes.

On the basis of research work conducted by various investigators under the scope of this CRP, the following was achieved:

1. IRRADIATION AS QUARANTINE TREATMENT OF MITES (ACARINA) INFESTING HORTICULTURAL PRODUCE

Introduction:

Various spider mite species of the family *Tetranychidae* are notorious pests on several crops. The economic significance of spider mites as a pest species has increased considerably during recent decades because of their great ability to develop resistance to a wide variety of pesticides used in the field. Spider mites in various commodities have been the concern of several importing countries and exports require pre-shipment, phytosanitary fumigation to reduce or eliminate them. For example, the presence of diapausing females of the two-spotted spider mite (*Tetranychus urticae*) on New Zealand kiwifruit resulted in a methyl bromide fumigation in Japan of about 50% of the export. Interception of spider mites on nectarines exported to Australia also resulted in methyl bromide fumigation.

However, fumigation with methyl bromide is a technology that is being phased out globally. This effective fumigant will be phased out in all developed countries including the USA by 2005 and in developing countries by 2015 under the Montreal Protocol. Irradiation as a quarantine treatment could be an alternative to fumigation for the control of spider mites on cut flowers, vegetables and some fruits.

Radiation doses of 1–3 kGy resulting in the immediate mortality of mite pests are not recommended because they cause phytotoxicity to horticultural produce. Lower doses, less damaging to the plant/produce, but resulting in (a) loss of the ability of the treated mites to reproduce and/or (b) the inhibition of the mites' development should be studied.

Objectives:

Determine doses resulting in the inability of treated quarantine mites to reproduce and/or inhibit their development.

Criteria for quarantine treatment of mites:

(a) Inability of treated mites to reproduce (when all stages of mites occur on a horticultural commodity);

(b) Inhibition of development (when a particular immature stage is present within a commodity).

Achievements:

The following mites were studied under this CRP: *Tetranychus urticae*, *T. cinnabarinus*, *Panonychus citri*, *P. ulmi*, and *Oligonychus biharensis* (spider mites in the family

Tetranychidae). Some radiobiological data on effects of irradiation on the acarid mites (*Acaridae*) has been collected. It was found that various species of tetranychid mites responded to irradiation rather in a similar way.

Eggs of spider mites exposed to gamma irradiation early in embryonic development were considerably more susceptible to irradiation than older eggs. The tolerance of eggs to gamma radiation increased in 3–4 day old eggs when the eye-spot was formed.

Viable eggs of the European red mite, *Panonychus ulmi*, on apples have been the concern of several importing countries and exports require preshipment, phytosanitary treatment (fumigation with methyl bromide) to reduce or eliminate live eggs. Irradiation could be a new strategy method. The data obtained indicate that a low dose of gamma radiation equal to or higher than 0.15 kGy appears to be adequate to prevent post-diapause hatching of wintering eggs of the European red mite. Thus, this dose is suggested for quarantine treatment of apples infested with wintering eggs of the European red mite.

Nymphs were more resistant to radiation than eggs and larvae. Deteriorating effects of irradiation treatment were reflected in immatures by their mortality in subsequent developmental stages. A positive relationship between dosage and the percentage egg mortality or the mortality of subsequent stages were usually found when immature stages were irradiated. The sex ratio of the few adults that developed following irradiation of eggs, larvae, and nymphs was skewed towards males. Irradiation of females and males resulted in increased mortality, lowered fecundity, reduced egg viability, and sex ratio distortion in their progeny. The higher the dose of gamma irradiation applied to adults of spider mites, the higher the mortality of mites during their embryonic development. Complete sterility of both sexes was caused by a dose of 320 Gy. Ionizing irradiation needed for *T. urticae* female sterility has been reported to range from 200to 400 Gy, and for male sterility to range from 300 to 350 Gy. When both sexes were irradiated, the dose range 300 to 400 Gy caused sterility. Other spider mites also were susceptible. Doses of 300, 280, and 300 Gy were found to sterilize females, males, and both sexes of *T. arabicus*.

In spider mites, the progenies from fertilized eggs were all females and those of parthenogenetic ones were all males. In the parthenogenetic mode of reproduction, if the sex chromosome of the male is inactivated by a suitable dose (200 Gy for *O. biharensis*) of radiation that they retain their mating capability, their crossing with normal females will give rise to the production of unfertilized eggs resulting in a completely male population in the F_1 generation. Males and females of *O. biharensis* treated with 0.2 kGy and above made them sterile with subsequent production of male progenies in F_1 generation.

From the results obtained it is possible to determine the precise minimum dose of radiation required to cause sterility of treated mites. This dose was found to be 320 Gy, and spider mites irradiated with this dose fulfil the criterion for quarantine security, i.e., "inability to reproduce".

Because irradiation applied at doses ranging around 0.3 kGy does not cause immediate mortality of all mites, live pests could be present after treatment of horticultural produce intended for international trade. Thus, a simple method is needed for identification of irradiated mites.

2. IRRADIATION AS A QUARANTINE TREATMENT AGAINST INSECTS OTHER THAN FRUIT FLIES

Introduction:

Because of the multiplicity of insect pests of fresh fruits and vegetables which are traded internationally there are frequent instances where products are unacceptable without an approved prior disinfestation treatment. However the majority of these pests are regulated by quarantine inspections at the ports of exit and entry. Where there is a risk of rejection of product if pests are found at inspection it is frequently prudent to apply a disinfestation treatment. Irradiation can be highly appropriate for both of these uses with the second sometimes having a lower efficacy requirement than the first. Most if not all major orders of insects are represented but the most common are thrips, lepidopterans, coleopterans and dipterans in addition to fruit flies. In the absence of research data on a specific pest, quarantine disinfestation should be achievable at the recommended generic dose of 300 Gy. However there are obvious advantages of determining a specific dose for a pest, especially if it can be shown to be lower. For many of the pests other than fruit flies there are no laboratory rearing methods needed for large scale testing. Where these are not possible special consideration will need to be given to assessment of treatment efficacy.

Objective:

To determine irradiation doses for insects other than fruit flies that will meet quarantine security requirements of trade in fresh horticultural produce while allowing the produce to meet acceptable market quality and shelf life.

Criteria:

The criteria for assessment of a successful quarantine treatment are:

(1) It must prevent reproduction and establishment of the pest in the new environment, or through mortality of the stage treated or a subsequent stage; or through sterility of adult insects; or through physical injury which will prevent mating; or prevention of reproduction where the pest can reproduce parthenogenetically

(2) The level of efficacy required of the treatment:
e.g. (99.9968%): 100 000 of most tolerant stage (USA)
(99.99%): 3 × 10,000 of most tolerant stage (Japan, Australia)
(99.5%): 3 × 200 of most tolerant stage (low risk pests).

Achievements:

Litchi stem-end borer

Disinfestation of litchi stem-end borer *Conomorpha sinensis* Bradley for export of litchi fruits with irradiation. Results of 5 years' research have shown that:

(1) Complete kill of third instars occurred following irradiation at 0.25 kGy.

(2) Using prevention of adult emergence from fruit infested with the third instars as the criterion of effectiveness probit analysis showed that the irradiation dose (with 95% fiducial limits) for 99.5% mortality of third instars was 0.254 kGy (0.22–0.289 kGy) and for 99.9968% mortality was 0.267 kGy (0.184–0.351 kGy).

(3) When infested fruits were held at 7^0C after treatment there was complete kill of all larvae at 0.2 kGy after 12 days with death of initial survivors on commencement of pupation.

(4) Egg hatch was decreased to 61% at 0.25 kGy and 94% at 0.6 kGy.

(5) At 0.5 kGy there was complete kill of pre-pupae. For 4–5 day-old pupae , a dose >0.6 kGy was necessary, but preliminary testing indicated that all emerging adults from treatment at 0.3 kGy (possibly lower) would be sterile.

(6) An incidental effect of quarantine irradiation was to improve fruit quality by reduction of rotting. Parameters for fruit quality and wholesomeness were not changed at the irradiation doses required for disinfestation.

(7) A new laboratory rearing method was developed by the end of the project which should enable large scale confirmatory testing in subsequent research.

Thrips on cut flowers

Results of 5 years' research on post-harvest control of thrips on orchids and cut flowers by irradiation showed that:

(1) From an assessment of the occurrence of natural infestation, the impact of thrip infestation in cut flowers for export from Thailand between October 1992 and July 1997 that the incidence of infestation was 4.48–5.7% during 1992–1995, increasing to 8.44–12.91% in 1996–1997.

(2) For mass rearing of thrips using natural plants, of six different kinds of substrate used in this study, although thrips infested the plants and flowers there was insufficient increase of populations for required test methods; mass rearing using snap beans was then studied and preliminary results indicated that it would provide adequate populations of *Thrips palmi* for testing. However, further development and confirmation of the snap bean method are required.

(3) Effects of irradiation on cut flowers: for *Dendrobium* orchids the maximum dose tolerated at an ambient temperature of 28–30^0C was <0.5 kGy. However, if cut flowers were kept at a lower temperature of 15–18^0C the tolerance increased to 0.75–1 kGy before the vase life was affected.

(4) Results of *in vitro* trials using *T. palmi* on petals in test tubes directly exposed to irradiation were shown to correspond with results for natural infestation where thrips were released on whole inflorescences and were more practicable; the minimum effective dose using mortality as a criterion 7 days after treatment, was 0.75 kGy.

(5) Males and females of *R. syriacus* (1–2 day-old) when exposed to an irradiation dose of 0.15 kGy or greater laid similar numbers of eggs, 15–20, as untreated controls, 12–31, but they did not hatch. "Inability to reproduce" dose for eggs and larvae was 0.10 kGy compared to 0.15 kGy for pupae and adults.

Orchid weevils

Results of research carried out showed that:

(1) The life-cycle for orchid weevil (*Orchidophilus aterrimus)*, the most damaging pest of orchid, from egg to adult was found to be 70.15–12.04 days.

(2) The adult stage was the least sensitive and eggs the most sensitive to irradiation. Radiosensitivity also varied within a developmental stage. Irradiated larvae were smaller and weaker and did not feed as much as the control. About 7% of 30 day-old larvae treated with 150 Gy could pupate but none emerged into adult.

(3) Pairing of adults (11 to 30 day old) irradiated with 150 Gy with their non-irradiated counterparts showed that females could still lay eggs, and the eggs could still hatch. However, surviving larvae died 6 days after hatching.

(4) Melanization test for irradiated orchid weevil larvae produced inconsistent results.

(5) Irradiation with dose from 100 to 450 Gy affected the vase-life of Dendrobium and some cultivars of Heliconia adversely.

Mango seed weevils (*Sternochetus mangiferae, S. oliveri*)

Results of research carried out in the past five years showed that:

(1) A dose of 0.45 kGy prevented emergence of adult mango seed weevils from seeds of treated fruit. (Earlier work in South Africa had shown that 0.5 kGy would prevent emergence of adult weevils from fruits and research conducted in Hawaii (Seo et. al, 1974) had shown that adults irradiated at a minimum dose of 0.2 kGy did not produce eggs).

(2) A minimum dose of 0.3 kGy would cause mortality of all stages within 6 months instead of the normal life-span of adults of up to 2 years (Seo et. al., 1974; Heather et. al., 1990). This work was done on populations composed of all of the stages present within mature fruit at harvest.

(3) That anatomical changes such as the difference in development of the supra-esophageal gland in fruit flies or phenoloxidase assays were not practicable indicators of whether larvae, pupae or teneral adults had been irradiated, nor would electrophoresis based tests be sufficiently reliable.

The effect of irradiation as a quarantine treatment against mites and insects other than fruit flies conducted under this CRP is summarized below:

Irradiation as a quarantine treatment of mites and insects other than fruit flies (summary)

Scientific name	Common name	Major host(s)	Minimum dose required (Gy)
Coleoptera			
Sternochaetus mangiferae	mango seed weevil	mango	300 (no emergence)
Orchidophilus aterrimus	orchid weevil	orchid flowers	150 (no adult emergence; adult sterility)
Diptera			
Liriomyza trifolii	leafminer	leaf vegetables, cut flowers	100 (no emergence)
Homoptera			
Myzus persicae	green peach aphid	vegetables, ornamentals	200 (sterility)
Pseudococcus comstocki	mealybug	ornamentals, tree fruits	400 (sterility)
Coccus virdis	green scale	ornamentals, tree fruits	750–1,000 (partial adult mortality*)
Lepidoptera			
Conopomorpha sinensis	litchi stem-end borer	litchi, longan	250 (no emergence)
Spodoptera litura	cutworm	cut flowers	100 (no emergence)
Thysanoptera			
Frankliniella schutzei	blossom thrips	tree fruits	250–300 (sterility*)
Retithrips syriacus	slender thrips	common cut flowers	200 (sterility)
Thrips tabaci	onion thrips	common cut flowers	400 (sterility)
Thrips palmi	melon thrips	cut flowers, vegetables	1,000 (larval mortality) 400 (sterility)
Acarina			
Panonychus ulmi (eggs)	European red spider mite	fruits, fruit trees	150 (no emergence)
Tetranychus cinnabarinus	carmine mite	vegetables, cut flowers	320 (sterility)
Tetranychus urticae	two-spotted spider mite	vegetables, cut flowers	320 (sterility)
Oligonychus biharensis	red spider mite	ornamentals	200 (sterility)

*) Preliminary results.

3. IRRADIATION AS A QUARANTINE TREATMENT OF PLANT-PARASITIC NEMATODES

Introduction:

Plant-parasitic nematodes appear to vary in their sensitivity to irradiation. Effective mortality dosages have ranged from 2.0 up to 5.25 kGy. Based on these previous studies it appears that nematodes require relatively high dosages of irradiation in order to assure total kill. Plant-parasitic nematodes cause serious problems to plant materials that are important in

international trade. There are relatively few chemicals that can be used in plant protection because some leave harmful residues and other cause environmental problems. Heat and cold treatment cannot be used to disinfest many plant types because of low plant tolerance. Irradiation may be a viable alternative treatment. However, there is not much information available about irradiation effects on nematodes including those that cause quarantine problems. Also, there is a scarcity of data about the differential sensitivity of plant parasitic nematodes to irradiation, and there is not much data available on effects of irradiation on the different nematode developmental stages.

Criteria:

Investigate the ability of irradiated nematodes to infest and develop in plant hosts. Understand the differential effectiveness of irradiation within and among species and races of nematodes and between different genera of plant-parasitic nematodes.

Objectives:

Determine the minimum effective dosage to be recommended for quarantine plant-parasitic nematodes

Achievements:

The following nematodes were studied under the CRP: *Meloidogyne javanica*, *M. incognita*, *Ditylenchus dipsaci* (onion and garlic), *D. destructor* and *Globodera rostochiensis* on potato, and some *Meloidogyne* and *Ditylenchus* species on ginger.

The decrease of *M. javanica* egg hatch was observed when doses of 4 kGy and higher were applied. The percentage of the juveniles obtained from treated egg masses dropped from over 90% in the control to about 10 and 0% for doses 4 kGy and 6.5 kGy. The hatchability of eggs of *M. incognita* also was not affected when eggs masses were exposed to 1.5 kGy irradiation.

The 100% mortality of second stage juveniles of *M. javanica* was provided by doses higher than 7 kGy. All juveniles were killed by 7 kGy within 5 days and by 7.5 kGy and 8 kGy in 1 day. After application of 4 kGy or lower the majority of the J2 nematodes lived longer than 2 weeks. A similar experiment conducted in Thailand on *M. incognita* indicated that J2 may not be killed by 1.2 kGy within 14 days. These doses are phytotoxic to many fresh plant materials.

Bioassay appears to be a more sensitive indicator of the effectiveness of mortality or hatchability. The reduction of galling and the number of egg masses per gram of root decreased when a dose of 3.25 kGy was used. Exposure to 4.25 kGy resulted in no gall production and no reproduction. Also, J1 and J2 from irradiated eggs of *M. javanica* produced neither galling nor reproduction.

Irradiation treatments did not affect galling or numbers of egg per gram of wet root on tomato plants by J2 or eggs as compared to the unirradiated nematodes. Heat treatments, however, reduced galling and nematode reproduction on tomato plants. Combined treatments (heat and irradiation) did not show any advantages over a single treatment of either alone. Heat

treatment at 49°C for 10 or 20 minutes effectively controlled *M. incognita* and there was no significant improvement in control when irradiated with doses 5, 10, and 15 Gy.

Larval and adult nematodes emerged from onions and garlic samples on the 3rd week after irradiation with doses up to 0.5 kGy, and from potatoes treated with doses up to 2.0 kGy. However, irradiation of onions infested with *D. dipsaci* influenced the development and growth of larval nematodes to mature forms. Doses of gamma radiation ranging from 0.1 to 0.5 kGy had only a slight effect, if any, on the development and growth of *D. dipsaci* nematodes infesting garlic, but they increased larval mortality. Gamma radiation at doses up to 2.0 kGy induced the increased mortality of young nematode larvae of the potato tuber nematode, *D. destructor*, rather than inhibiting their development to mature forms. As a result doses up to 2 kGy influenced development and mortality of juvenile stages by decreasing the adult–juvenile ratio. This ratio also was changed by doses used on *D. dipsaci* on garlic.

Irradiation inhibited the development of *Globodera rostochiensis* juveniles when their cysts within soil were irradiated with a dose of 0.5 kGy or higher. A dose as low as 0.5 kGy reduced the infestation level and the density of females/cysts on the root of infested plants. However, a few cysts were often found on roots of plants grown in the pots with soil treated with a dose of 3.0 kGy. The ratio of the number of cysts to the number of females was about 1.0 in the control and in the 0.5 kGy-treatment, but it increased up to 2.0 with treatment at 1.0 kGy. At higher dosages of gamma radiation females were not found at the time of checking. These data indicate that cysts (= dead females) originating from irradiated juveniles were formed earlier than those from the untreated juveniles. The higher the dose of irradiation, the earlier the formation of such cysts.

The development of the second generation of the potato cyst nematode (= F_1 cysts, originated from irradiated cysts) was much weaker than of the parental generation. Females and/or cysts were found only in the control and in the 0.5 kGy treatment. The number of F_1 cysts found on potato roots was much lower than in the P generation.

Species of nematode genera *Meloidogyne* and *Ditylenchus* spp. were the most common along with other species that caused rotting, blackening at the budding zones and lesions. Data on mortality of second stage of juveniles (J_2) of *Meloidogyne* spp. in ginger at different intervals after exposure to various doses of gamma radiation, from 0.0 kGy to 4.0 kGy indicated that only 34.2% reduction of J_2 stages were obtained at 4.0 kGy after 14 days. Complete mortality of J_2 nematodes was obtained in distilled water at 3.0 kGy after 24 hours. It is apparent from the result that even at the highest dose (4.0 kGy) complete elimination of nematodes could not be achieved although the dose level affect the quality of ginger within this period.

Virtually no protein band and deformities were observed in both irradiated and unirradiated nematodes when samples were run on 5% PAGE in TBE and were observed under microscope.

Plant-infesting nematodes were found to be resistant to the irradiation treatment. Therefore, use of gamma irradiation for nematode disinfestation of agricultural products seems to be impractical, if the aim of the treatment is to kill these pests within a few weeks. The level of radiation required to kill nematodes in infected plants would damage plant tissues of tomatoes, onion, garlic, potatoes and ginger.

The doses needed to immobilize, kill and decrease hatch or development estimated so far cannot be applied on plant materials. For example, ginger irradiated at doses higher than 1 kGy would not be acceptable to the market. The high doses cause weight losses in a short period of time. Deterioration of color and texture also create additional problems. Doses reported for the control of nematodes in onion, garlic, or potato are too high result in plant tissues deterioration.

4 TOLERANCE AND QUALITY OF VARIOUS HORTICULTURAL COMMODITIES IRRADIATED FOR QUARANTINE PURPOSES

Introduction:

Many fresh herbs, ornamental plants, fruits, and some other produce in different parts of the world are infested with various pests such as insects, aphids, mites, thrips, and scales. Control of these pests in agricultural produce by fumigation is no longer desirable from the points of view of human health and global environment. Therefore, the move to find alternatives to chemical fumigation of fresh commodities for disinfection and disinfestation has been intensified in recent years. However, finding an efficacious quarantine treatment is often difficult because most fresh plant materials have limited tolerance to heat or cold. Irradiation could be a feasible and practical alternative with broader applicability to commodities and pests. Radiation tolerance is specific to individual commodities. Quality of irradiated fresh commodities should be studied to determine the applicability of quarantine treatment and the optimum irradiation condition for each commodity.

Objective:

To determine the radiation tolerance of various commodities, i.e. cut flowers, fresh herbs, and tropical fruits, and classify them according to the level of tolerance in relation to quarantine treatment.

Criteria:

To determine the following:

(1) Marketable life — The marketable life (either shelf life or vase life) of these irradiated produces should be approximately that of the control (10–14 days for cut flowers at 21–22^{o}C, 14–21 days for fresh herbs at 10^{o}C, 10–12 days for tropical fruits at 21–22^{o}C).

(2) Absence of negative quality changes in cut flowers, fresh herbs, or tropical fruits.

Examples of negative quality changes are:

(a) Wilting of petals or septals
(b) Bud not opening
(c) Yellowing, browning or wilting of leaves
(d) Necrosis of the core of flower
(e) Stem bending
(f) Dropping of petals or flowers
(g) Curling of leaves
(h) Discoloration of petals or flowers
(i) Browning of skin or pulp

(j) Softening of pulp
(k) Off-aroma and/or off-flavor
(l) Loss of nutrients (e.g. Vitamin C).

Achievements:

(1) Radiation tolerance of various cut flowers, fresh herbs, and tropical fruits:

Tolerance of various cut flowers, fresh herbs and tropical fruits to radiation is classified as follows:

a) Highly tolerant (up to 1,000 Gy):

Fruits:	papaya, rambutan, carambola, litchi, mango
Cut flowers:	ferns, phoenix leaf, narcissus, rhodante, limonium, helichrysum

b) Tolerant (up to 700 Gy)

Fresh herbs:	rosemary, thyme, oregano, parsley, chives
Cut flowers:	tulips, prairie gentian, carnation, gypsophila, bellflower, statice, triteleia, celosia gomphrena, red ginger, freesia

c) Moderately tolerant (up to 500 Gy)

Fresh herbs:	spearmint, taro leaf (food grade)
Cut flowers:	gladiolus, oncidium, alstromeria, stock, gloriosa, marigold, tube rose, callistephus

d) Low tolerance (below 200 Gy)

Fruits:	atemoya (var. Gesner), avocado (var. Sharwil)
Fresh herbs:	dill, basil (sweet, Thai, opal), arugula
Cut flowers:	chrysanthemum, rose, lily, calla, anthurium, sweetpea, strelitza, matthiola, aechmea, consolida, ranunculus, denphale, dendrobium, heliconia, gerbera

(2) Effects of treatment above tolerance doses:

a) Cut flowers and fresh herbs:

	Discoloration or wilting of petal or sepal	Dropping of petal or flower	Bud not opening	Necrosis of flower core	Bending of stem	Browning or wilting of leaves or stems	Curling of leaves
aechmea	x						
alstromeria	x						
anthurium	x						
arugula						x	
basil						x	
calla	x				x		
callistemon	x						
callistephus				x		x	
chrysanthemum	x		x	x		x	
consolida						x	
dendrobim	x	x					
denphale			x				
dill						x	
freesia	x						
gerbera	x				x		
gloriosa	x						
helianthus						x	x
heliconia	x				x		x
hemerocallis			x				
iris	x		x	x		x	
lily	x		x				
marigold	x						
matthiola			x				
oregano						x	
parsley						x	
prairiegentian			x				
ranunculus					x		
rose		x	x	x	x	x	
stock	x				x		
strelitzia	x						
sweet pea		x					
tube rose	x						
tulip	x						

b) Tropical fruits

	Skin browning	Pulp browning	Pulp softening/separation
atemoya	x	x	x
avocado		x	x

3) Major findings on phytotoxicity of irradiated cut flowers

a) The tolerance of fresh horticultural produce to radiation is proportional to their initial quality. Poor quality flowers are more sensitive to radiation than high quality ones.

b) High temperature before, during and after irradiation accelerates the senescence.

c) Certain preservation solutions showed promise in retaining the quality of some irradiated flowers such as chrysanthemum, gerbera, callistephus, and gladiolus.

5. PHYSIOLOGICAL MARKERS INDICATING EXPOSURE OF INSECTS TO IONIZING RADIATION

Introduction:

A potential problem in the use of irradiation as a quarantine treatment of fresh horticultural produce is the relatively high dosage necessary to kill insects. These high dosages may be well above the limits that can be applied to fresh fruits or vegetables, and some other commodities. Although low doses of irradiation in the range of 100 Gy to about 300 Gy suffice to sterilize insects and render them unable to reproduce, some may continue to live for some time following irradiation and may be found in irradiated produce at a port of entry. In such a case a marker of irradiation that is easily applied may be very useful in allaying concerns of some quarantine inspectors that inevitably occur when a living insect of quarantine importance is found in a shipment.

Among the reasons why high doses are needed to cause mortality of insects are the following:

(1) They do not utilize blood cells (hemocytes) to transport oxygen, as vertebrates do.

(2) The cellular defense system of insects is not so sensitive to irradiation as is the immune system of vertebrates.]

(3) In most cases insects have only a few cells that must divide, and cell division is periodic rather than constant. Insects are most sensitive to ionizing radiations during development of the embryo in the egg, at specific times when one instar is preparing to molt into the next stage, during the pupal stage when adult tissues are being formed as a result of rapid cell divisions, and when egg and sperm cells are being formed in the late pupal or early adult stage. These are times when significant cell divisions occur.

A database on irradiation dosages, abnormalities caused, and potential detection techniques of radiation exposure is needed for insects of quarantine importance. This report summarizes work sponsored by the International Atomic Energy Agency to develop such a database.

Objectives:

(1) to identify ways to determine that insects, mites, and nematodes of quarantine importance have been exposed to irradiation, and

(2) to provide scientific documentation to support the techniques and methods developed.

Criteria:

The criterion for quarantine security is that insects, mites, and nematodes should not be able to reproduce and start a new population . Nematodes should not be infective or capable of infecting a new plant.

Achievements:

Markers of irradiation have been developed or explored for the following insects of quarantine importance (or model insects representing a group of quarantine important insects).

Tephritid fruit flies:

Markers for irradiation of larval stages are needed; pupal and adult stages of fruit flies do not normally occur in fresh fruits and vegetables. Seven markers or tests that indicate that tephritid fruit flies have been irradiated have been developed. The Caribbean fruit fly, *Anastrepha suspensa* (Loew) , a quarantine pest in Florida, was used as the model insect for developing the tests. The tests consist of (1) failure of whole body melanization after death of an irradiated 3rd instar larva, (2) a spot test in which there is failure to get a color in the spot if irradiation equal to at least 50 Gy has occurred, (3) a quantitative measurement of the enzyme phenoloxidase, (4) counting the number of hemocytes (blood cells) in a 3rd instar larva, (5) larval weight, (6) measurement of the ratio between size of the supraesophageal ganglion and the proventriculus, and (7) abnormalities in the structure of the imaginal disks or complete failure of disk development for some structures to be made in the adult during pupation.

Tests 1 through 5 can be applied in the sequence 5, 4, 1, 2, and 3 to a single larva . Tests 1 and 2 are easiest to use and require no specific training. Application of test 2 requires the prior preparation or availability of a chemical substrate dried into a spot on transparent film. Prepared spots are stable for months, and perhaps for much longer, and could be available as prepackaged test film strips. Tests 1, 2, and 3 have been confirmed in the Mediterranean fruit fly, *Ceratitis capitata,* and in the Oriental and melon flies, *Bactrocera dorsalis* and *B. cucurbitae*, respectively. Quantitative measurements of phenoloxidase (test 3) and counts of hemocytes (test 4) have also been confirmed as indicators of irradiation in house flies, *Musca domestica*, which is not a quarantine insects but may serve as a model of other dipterans.

Electrophoretic profiles of proteins from control and irradiated larvae of *B. tryoni* (Froggatt) were similar, irrespective of the age at which irradiation occurred. Protein profiles

were also similar for control and irradiated pupae for the first 2 days of pupation, but a new protein band not present in irradiated insects was detected in control pupae 3 days after pupation began. However, it will likely prove difficult or impossible to utilize the new protein band as a reliable marker of irradiation because it is not possible to know the age of pupae that have not been carefully maintained under laboratory observation. The size of the supraesophageal ganglion in irradiated larvae of *B. tryoni*, corrected for age, was smaller than in control larvae. The reduction was about 64% in samples treated as eggs (26 h), first (72 h), and second (96 h) instars, and 34% smaller in third instars. Irradiated larvae had a significantly longer larval and pupation period, possibly because of the inability of irradiated larvae to feed and process food normally. There was also a significant increase in larval mortality with decreasing age at irradiation.

The reduction in growth of the supraesophageal ganglion in irradiated larvae may be a suitable marker in *B. tryoni*, as it is also in several other fruit flies, but application of the technique requires some ability in dissection of small insects. Although the whole-body melanization and phenoloxidase spot test apparently have not been tested for applicability to *B. tryoni*, it seems highly probable that they will work satisfactorily.

Simple and inexpensive tests are available to detect irradiated third instar fruit flies. Whole body melanization in control larvae or failure of it in irradiated larvae, and the phenoloxidase spot test are reliable indicators in tephritid fruit flies, and probably other dipterans, at radiation at doses that will be used to treat fresh fruits and vegetables for shipment.

Stored product pests:

(1) Models for lepidopterans

The melanization of the whole body was not uniform in control (unirradiated) insects, and after irradiation, the bodies of larvae did not always show failure of melanization. Thus, at present, the melanization reactions do not seem to be reliable indicators for lepidopterans. Disruption of the midgut, particularly the failure of the immature regenerative cells to successfully divide and grow into mature midgut cells after irradiation, seems to be a more reliable test, but more difficult to perform.

Plodia interpunctella, *Ephestia* sp., and *Cadra* sp. are model lepidopterans in which the disruption of the midgut has been tested and found to be reliable indicators of irradiation. Non-irradiated larvae of the Indian meal moth, *Plodia interpunctella* Hbn., the Mediterranean flour moth, *Ephestia (Anagasta) kuehniella* Zell., and the almond moth, *Cadra cautella* Wlk., showed strong melanization after killing by freezing. However, there were some insects which showed lack of melanization or melanized only partially, sometimes like those that were irradiated. Part of the larval body was dark black, while the rest of the body was of natural color or only slightly gray. Also, black and gray patches were observed in the larvae. After the irradiation treatment, the number of non-melanized larvae and larvae exhibiting a slight melanization usually increased. The degree of melanization in treated larvae was significantly different from untreated insects. Generally, it decreased with increasing dose and time elapsed after the treatment. The melanization test for detecting irradiated moth larvae may produce sometimes inconsistent results because (a) irradiation does not completely prevent melanization in mature moth larvae, and (b) the untreated larvae, killed by freezing and examined at room temperature, often show incomplete melanization.

(2) A model for Coleoptera (*Trogoderma granarium*)

The melanization reaction may be useful for younger stages, but there is also some ambiguity because of a lack of a completely uniform pattern of darkening in normal insects and similar ambiguity in irradiated insects. The midgut disruption reaction appears to be more reliable. After the irradiation treatment with doses ranging from 0.1 to 0.5 kGy, the melanization process was significantly inhibited in the 1st and 2nd instars of the khapra beetle, *Trogoderma granarium* Ev., killed by freezing in the 1st and 2nd week after irradiation. Also, irradiation significantly inhibited melanization of 4th instars after their death, but the relationships between the dose, time elapsed after irradiation, and the degree of melanization were not clear. A double freezing and thawing of control larvae increased the melanization index from 26.4 to 52%. Multiple freezing of irradiated larvae had no effect on the further increase of melanin formation. Phenoloxidase activity was very low in irradiated larvae, and only a few larvae produced the red/black color with 2-methyl-DOPA in the spot test. The effect of elapsed time after irradiation was evident. Almost all larvae irradiated with a 0.3 or 0.5 kGy dose failed to produce color with the substrate when they were assessed on the 2nd and 3rd week after the irradiation. However, these changes in melanization of the khapra beetle larvae cannot be used for indicating the previous exposure of these insects to irradiation, because of the great variability in response of the melanization process to the irradiation treatment.

(3) Mango seed weevil

The mango seed weevil is a quarantine pest. Protein separation by electrophoresis is not a reliable indicator of irradiation, and the melanization reaction was also ambiguous and inconclusive. At present there is no proven marker for the weevil, but it seems likely that disruption of the midgut may be reliable as an indicator of irradiation.

Conclusions:

The CRP on Use of Irradiation as a Quarantine Treatment of Mites, Nematodes and Insects other than Fruit Flies has generated much needed original data on the effect of irradiation of these important quarantine pests. Some of these data were difficult to develop as no information was available in literature about the life-cycle and laboratory rearing methods of a number of insects studied. The data from this CRP, although not complete, showed the promise of irradiation as a quarantine treatment of a number of fresh horticultural commodities against various pests. In view of the impending global phasing out of methyl bromide which is widely used to control pests of quarantine importance in the trade in fresh horticultural products, further efforts should be made by national and international organizations to generate data on irradiation as a phytosanitary treatment of these products.

The following conclusions were reached:

(1) Generic dose for sterilization of both males and females of spider mites was determined to be 320 Gy. Further studies should determine whether this dose would apply to all species of tetranychid family to meet quarantine requirements.

(2) With regard to insects other than fruit flies, it appears that a minimum dose of 300 Gy would cause either no adult emergence or sterility of most species of insects studied. Further studies are required, however, to confirm the most tolerant stage of various insects to irradiation and the minimum radiation dose required to meet quarantine requirements

on a pest by pest basis. Large scale confirmatory tests (95% confidence level) at higher efficacy levels, i.e. 30 000 and 100 000 insects, should be performed. For mango seed weevil, a comparative study should be made to determine the response to radiation between *S. mangiferae* and *S. oliveri.* In case of thrips, a minimum radiation dose required for inhibiting feeding which would prevent transmission of viruses of quarantine importance, should be determined.

(3) Little data are available on the effect of irradiation to various insect species belonging to the order Homoptera, including aphid, mealy bugs, and green scale which are important quarantine pests to fresh horticultural commodities. Further studies on these pests are urgently required.

(4) Radiation doses required to cause complete mortality to various infective stages of plant parasitic nematodes appeared to be higher than 6 kGy. In most cases, the minimum dose required to prevent gall development and reproduction of these nematodes is over 2 kGy which is too high for most fresh plant materials to tolerate. Thus, irradiation should be considered as an alternative to methyl bromide fumigation to control nematodes in non-perishable materials. Irradiation of materials such as soil and wood products and others infested by nematodes of quarantine/economic importance should be determined, and trials of the treatment should be performed at semi- and commercial scale levels.

(5) Although many fresh fruits and vegetables could tolerate radiation doses required for quarantine purposes, the response of various types of cut flowers to irradiation varied widely. Some cut flowers and ornamentals such as ferns, phoenix leaf, narcissus, tulips, prairie gentian, carnation, red ginger, etc. appeared to be tolerant to irradiation up to at least 700 Gy, others such as chrysanthemum, rose, lily, anthurium, dendrobium, heliconia, gerbera, etc. cannot tolerate radiation dose above 200 Gy. The tolerance of cut flowers to irradiation is proportional to their initial quality.

(6) Certain preservation solutions showed promise in retaining the quality of some irradiated flowers such as chrysanthemum, gerbera, callistephus and gladiolus. Further studies should be conducted on the combined use of irradiation with other environmental factors such as low temperature and different atmospheric conditions to increase the tolerance of certain cut flowers which are sensitive to irradiation alone.

(7) The melanization test appears to be a simple and reliable marker of irradiated larvae of various species of fruit flies subject to irradiation at the egg or early larval stages. This method, however, does not appear to be reliable as a marker for irradiated insects of other species. Other methods such as histological changes in the midgut and electrophoretic separation of proteins and other macromolecules should be explored as markers of other species of insects and mites. In the absence of a reliable marker for irradiated insects, quarantine inspectors are advised to follow strictly the information on irradiation treatment in the certificate of consignment which accompanies the shipment.

IRRADIATION AS AN ALTERNATIVE TO METHYL BROMIDE
The Australian Situation

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Abstract

International agreement to phase out the fumigant Methyl bromide (MeBr) will have serious implications for pest and disease control in Australia, particularly grain pest control, quarantine usage on fresh horticultural produce and control of soil pathogens or nematodes. Irradiation is a practical alternative but is not currently approved for use in Australia. Other options are available but none of the viable methods except irradiation have the short application time needed for treatment of grains found to be infested during loading at export. This usage is vital, as Australian grain is exported at very high standards of freedom from insects, assured by Government regulatory requirements. Irradiation is contrasted against other alternatives including heat and cold, especially for fresh horticultural produce.

1. INTRODUCTION

In Australia Methyl Bromide (MeBr) is widely used as a fumigant for the control of pests of stored grain, pests of timber, soil insects, soil nematodes, soil pathogens of plants, and as a quarantine treatment against pests of fresh horticultural produce and pests and diseases in propagation material. If the announced intention to phase out its use internationally, commencing from the year 2000 goes ahead [1], there will be no single replacement treatment appropriate for Australian requirements. However, MeBr was not at any time the only option for many uses and for some commodities it was not an appropriate or preferred treatment. A further disincentive for use of MeBr in Australia is the recent revision of the Maximum Residue Level (MRL) to 50 parts per billion (ppb), a 100 fold reduction. Irradiation will be a potential alternative for almost all purposes except field soil disinfestation and disinfection, and probably, logs for quarantine purposes and timber in service. Soil usage is responsible for more than half of the MeBr currently used in Australia.

Consumer preference in Australia as with most other countries in the world is for food free of pesticide residues regardless of their level of health hazard. Although fumigants tend to leave less residue than chemical dips, sprays, or dust applications, residues are detectable and this will increase with increasing sophistication of analytical technology. Therefore, future insect disinfestation, plant pathogen disinfection, and plant and seed devitalization need to use residue free technology based on physical rather than chemical options wherever possible. Irradiation is one option which needs to be considered seriously. At present in Australia, irradiation is not an approved treatment for food for human consumption, but a recent government decision approved its use on food for export when the buyer requests it. There is little reference material on this topic [1], [2], [3], [4] and considerable reliance has been placed on personal communication with sources given in Acknowledgments.

2. RELEVANT CURRENT USES OF MeBr IN AUSTRALIA

Grain of cereals, maize, sorghum, birdseeds, and legume seeds for human and animal consumption is protected against insect and mite pests in Australia by a range of pest management procedures. Options include insecticide additives, temperature reduction by natural or refrigerated

aeration, reduction of moisture content, hermetic sealing of storages after fumigation or atmosphere modification with carbon dioxide or nitrogen, insect proof packaging, heat disinfestation and manipulation of handling operations such as cleaning or grading procedures, or vertical impact when grain is relocated in vertical or horizontal storages involving high velocities and rapid decelerations. Products such as flour are sometimes disinfested mechanically by impact from a spinning bar known as an "entoleter" in the flow path.

Fumigation is done with MeBr or phosphine, each of which has preferred uses. The major advantage of MeBr is the short time needed to disinfest grain, 5 - 24 hours with MeBr compared to 5 - 12 days for phosphine. This makes it ideal for disinfestation of grain found to be infested at export or as a phytosanitary requirement of a contract of sale. It also has the advantage of not having temperature or relative humidity constraints at the time of fumigation such as apply to phosphine. Against these advantages are its legislative restriction to specially licensed operators and deleterious effects on germination, which cannot be alleviated entirely, of malting barley and seeds, and control of moisture content.

Millions of tonnes of stored products are fumigated with MeBr in Australia each year, mainly at export grain shipping terminals, but also to a lesser extent at up country storages, on farms, and in the storage facilities of produce merchants (Table I). Due to the high efficiency of fumigations in the Australian grain industry, usage is estimated at only 50 tonnes of MeBr to treat approximately 15 million tons of grain (Table II). Where closed system recirculatory fumigation is used, such as at shipping terminals, there is a belief that the used gas can be recovered without loss into the atmosphere, although the cost effectiveness of fumigation as a disinfestation treatment would then need to be reassessed.

Total grains production in Australia approximates 20 million tonnes annually, with considerable year to year variation due to seasonal conditions. Exports average 90% of production. In Queensland two thirds of the total is wheat and one quarter is sorghum, with the remainder maize, malting barley, rice, grain legumes, and other produce such as birdseed (panicums), linseed, and canola (rape). In the more temperate southern states the proportion of cereals is higher. Some commodities, such as malting barley, were never fumigated with MeBr because of its detrimental effect on germination. In other states the proportion of cereal grains, mainly wheat, is much higher. In warmer regions about three quarters of the export grains are fumigated with MeBr. The proportion is much higher than in the past due to the discontinuation of protection of grain after harvest with low toxicity residual insecticides such as fenitrothion, chlorpyrifos methyl, or synthetic pyrethroids and insect growth regulators.

The main reason for use of MeBr at export is to comply with Australia's clean (insect free) grains policy, which is enforced by regulatory orders administered by the Australian Quarantine and Inspection Service (AQIS). All regulated grains are sampled for insect infestation at the point of shipment at 6.75 L per 100 tonnes and loading is stopped whenever a live insect is found. Hence, there is "nil tolerance". Regulated commodities include the cereal grains wheat, oats, and barley as well as sorghum, but not maize, canola, or mung beans at the present time. In addition, some grain markets (USA, Fiji, and Papua New Guinea) require fumigation with MeBr as a condition of sale. This is a requirement before phytosanitary certification can be given. Tonnages are not large for any of these markets. All dedicated grain export terminals have recirculatory fumigation facilities mostly combined with high density concrete silos that have excellent gas retention and permit disinfestation in a time as short as 5 hours. The rate used is 150 mg/h/L or approximately 40-60 g/m^3. This enables export terminals to disinfest grain found to be infested at inspection and fully utilize ship loading rate capacities of from 1000-4000 tonnes/h. It is not clear what difficulties if any will be encountered in meeting the new lowered MRL before export. However from experience in meeting a similarly low MRL for EDB, purging of the treated commodities with an airflow would not be an unexpected requirement.

2.1. Fresh horticultural produce

Commodities involved are fresh fruit, vegetables [1] and cut flowers (Table I). The purpose of fumigation is to meet intrastate, interstate, and overseas phytosanitary requirements [3]. The most important pests of fruits are fruit flies and codling moth, but mites, scale insects, and thrips are also frequently a reason for fumigation. Fumigation with ethylene dibromide (EDB) is still approved for use against fruit flies in Australia, although it is no longer acceptable for most overseas export markets. MeBr was not as effective as EDB against eggs and larvae of fruit flies within fruit, because at the dosage necessary to meet quarantine efficacy requirements, fruit quality could be affected adversely. However, MeBr is very effective against surface pests such as mites, thrips, scale insects, and dried fruit beetle, all of which are subject to phytosanitary constraints in some markets. MeBr

Table I . COMMODITIES FREQUENTLY DISINFESTED WITH METHYL BROMIDE IN AUSTRALIAN COMMERCE

Commodities	Pests	Usage importance
Agricultural commodities		
Ginning cotton	Boll weevil	significant
Barley (not malting)	Storage pests	major
Oats	Storage pests	major
Wheat	Storage pests	major
Maize	Storage pests	major
Sorghum	Storage pests	major
Rice	Storage pests	major
Grain legumes	Bruchid weevils	significant
Baled hay	Mites	minor
Horticultural commodities		
Apples	Fruit fly, codling moth	significant
Pears	Fruit fly, codling moth	significant
Stone fruits	Fruit fly	minor
Oranges	Fruit fly, scales, thrips	significant
Mandarins	Fruit fly, scales, thrips	minor
Lychee	Fruit fly	minor
Mango	Fruit fly ,scales	minor
Melons	Fruit fly, thrips	significant
Broccoli	Lepidopterous larvae	minor
Cut flowers	Thrips, mites, others	minor
Nursery stock	Various	significant
Forest products		
Sawn timber Imports	Wood borers, termites	major
Logs	Wood borers, termites, and snails	major
Veneers	Lyctid borers	significant
Furniture	Wood borers	significant

Table II. QUANTITIES AND VALUES OF AGRICULTURAL AND HORTICULTURAL COMMODITIES COMMONLY DISINFESTED WITH METHYL BROMIDE IN AUSTRALIA [2].

Commodity	Production 1996 (ktonnes)	Estimated amount treated with Methyl Bromide		Value of Methyl Bromide treated component (AUD)
		Total (ktonnes)	Export (ktonnes)	
Wheat	16975	12731	9974	$2862M
Coarse grains	4114	3085	836	$567M
Raw Cotton	421	22	n/a	$16M
Pome fruits	48	0.5	0.3	$0.9M
Citrus	612	?56	50	$ n/a

became an option for fruits such as lychee and watermelon when acceptability of EDB on exports was lost. Recent research efforts in Australia for treatments of fresh horticultural produce have focused on circulated hot air, hot water, or cold treatments, and these are used commercially for major crops such as citrus (cold) and mangoes (hot air). The MRL for MeBr residues in fruit is the same as for grains (50 ppb) and could prove difficult to achieve.

The Australian usage of MeBr for fresh horticultural produce is fragmented and difficult to determine with accuracy. There was an upsurge in use recently as a result of intra- and interstate quarantine restrictions consequent to the discovery of the fruit fly, *Bactrocera papayae*, a form of *B. dorsalis* originally restricted to the Malayan peninsula, Malaysia, and the Indonesian islands. However, MeBr is only one of a number of options and the low cost one of dipping fruit in dimethoate or fenthion solutions has become the predominantly used treatment. An estimate of the commodities treated with MeBr is given in Table II.

2.2. Irradiation as an alternative to MeBr

2.2.1. Stored products

Irradiation will disinfest stored grains at relatively low treatment dosages. In Australia the annual production of human and animal food grains is subject to major seasonal variations, but exceeds 20 million tonnes in most years. Where storage time exceeds one stored grain pest lifecycle, which is about 6 weeks, pest suppression measures are necessary because of pests such as weevils, flour beetles, sawtooth grain beetle, other beetle pests, and moths such as the tropical warehouse moth. Other pests common in colder climates, such as mites and psocids, are of much less importance in Australia. All of these pests are cosmopolitan in distribution and their status as pests of quarantine importance is arguable, especially given the outcomes of the Uruguay round of the General Agreement on Tariffs and Trade (GATT). If non-quarantine status is accepted for these pests, less efficaceous treatments than those required for quarantine pests should be acceptable provided that produce quality and wholesomeness standards are met. For example USA typically requires an efficacy of 99.9968% ("probit 9") for a quarantine disinfestation schedule and many other

countries including Japan require 99.99%. By contrast, grain disinfestation treatment in Australia has a typical target efficacy of 99.9% which enables grain to meet the sampling rate of 6.75 L per 100 tonnes with a nil tolerance for live insects. Grain shipments treated to this standard have experienced less than 2% found to be infested at arrival after an average shipping time of 6 weeks. If Australian regulatory requirements can be modified to accept the slower mortality of irradiated insects with suppression of reproduction, irradiation has the basic capability to be an alternative to disinfestation with MeBr at the point of export. However, technology would need to be developed to meet grain flow rates involved in loading up to 4000 tonnes/h, and possibly more in the future as other alternative handling strategies developed. The grain stream involved in these loading rates is up to 30 cm deep.

Irradiation as a disinfestation method for grain has not been a serious component of Australian stored grains research. Justifications for its low priority have included political discouragement of research by way of a moratorium on government funding for this purpose, lobbying and other activities by anti-irradiation pressure groups, a belief by some researchers that delayed death of insect infestations subjected to low dose irradiation would lead to export rejections because of the presence of "live" insects at export, and concern about the effect of irradiation on the baking quality of flour. However, the major impediments to its consideration were the established grain export infrastructure designed around fumigation, and the inability of existing grain irradiation technology, whether Electron Beam or ^{60}Co, to cope with ship loading rates of some thousands of tonnes per hour.

The slow kill exhibited by grain insects treated at low disinfestation doses is not as disadvantageous as it might at first appear. Firstly, reproduction can be inhibited at least in the beetles, at doses around 250 Gy, and possibly at much lower dosages when adequate research data are collected. Secondly, mortality apparently results from damage to the midgut lining resulting in cessation of feeding and eventual starvation. Good sanitation practices in grain storages and physical control measures such as modified atmospheres can deliver grain to the point of export with levels of infestation below the level of detection at specified sampling levels. Irradiation can then ensure that these infestation levels do not increase during transit whereas infestations in untreated grain could be expected to increase by a factor of 50 times in the average 6-weeks transit time taken for Australian grain. In practice, infestations would decrease significantly as a result of irradiation induced mortality, some soon after treatment. Inhibition of reproduction in adult grain moths reputedly requires much higher doses than for beetles, possibly as high as 1000 Gy. Again, even if true, this is unlikely to be of as much significance as at first apparent because grain moths have a very short adult life, as little as 1-2 days so the majority of an infesting population will be present as eggs, larvae, and pupae which are likely to be much more susceptible. It will be necessary to treat grain as a moving stream and this in itself will have an adverse effect on adult survival and their ability to reinfest.

2.2.2. Fresh horticultural produce

Irradiation has been shown to be highly efficacious against fruit flies, codling moth, and lightbrown apple moth, the pests which are predominantly responsible for export constraints on Australian fruit. There is adequate Australian research data to show that a dose of 75 - 100 Gy will disinfest fruit against the main native fruit fly pest species, and there is international data which shows that this dose is more than adequate against the Mediterranean fruit fly, which in any case is restricted in its distribution to a region isolated from the main export-fruit production areas. However, MeBr is little used for fruit fly disinfestation in Australian export fruit, and is being restricted to watermelons for New Zealand and citrus, stone- and pome-fruits for Fiji, New Caledonia, and Papua New Guinea. MeBr combined with cold treatment against codling moth is under development for proposed exports of pome-fruits to Japan. An alternative for this can be expected to be needed and irradiation would be more than adequate as a replacement if Japan comes to accept irradiation as a quarantine treatment for fresh fruit imports.

Other pests for which MeBr is used as a disinfestation treatment in Australia include thrips, scale insects, and mites. Exported cut flowers are sometimes fumigated with MeBr as a way of ensuring that they will not be infested with mites or insects such as thrips, which, if found, result in fumigation on arrival in countries such as Japan and Korea. Again, the usage of MeBr for this purpose is low compared with alternatives. Irradiation is a suitable treatment for this purpose for many flowers, but as air transit times are short, acceptance of live irradiated insects needs to be negotiated if high and consequently injurious doses needed for immediate mortality are to be avoided.

3. OTHER RESIDUE FREE ALTERNATIVES TO MeBr

3.1. Heat

3.1.1. Fluidised Bed: For grain a heat disinfestation process known as "Fluidised Bed" has been developed to disinfest a moving stream of grain with a hot air stream. It uses a disinfestation temperature of 60^0 C. A semi-commercial unit was built and operated some years ago, but the method was not adopted widely presumably because of cost and logistical constraints. These constraints could change with the loss of MeBr.

3.1.2. Forced air heating: For fruit this method, variously termed Vapor Heat Treatment (VHT) or High Temperature Forced Air (HTFA), is used to disinfest fresh fruit to international standards. It is in use against fruit flies for mangoes exported to Japan and is being developed for other fruits and vegetables for internal trade and international exports. The capital and treatment costs far exceed fumigation and insecticide dips.

3.1.3. Hot water dips: Although this is the most economical way of applying heat as a disinfestation treatment for fresh fruit, it has not been adopted widely because of the likelihood of injury at exposure times of more than the 1-hour exposure typically required for fruits such as mango. However it is widely used for postharvest disease control which involves shorter exposure times, typically 10 minutes, because of the surface nature of diseases such as anthracnose.

3.1.4. Cooling and cold storage: Reduction of temperature is used in Australia for both grains and fruit as a means of disinfestation. For grain this is done by selective forced aeration with coolest available ambient air or with refrigerated air from mobile or *in situ* refrigeration systems. Temperatures of 8-9 ^{0} C will disinfest grain over a period of some weeks, and cooler temperatures act more quickly. No cooling method is suitable for disinfestation at the point of shipment because of the time needed at practicable temperatures.

For fresh fruit, cold storage at temperatures just above freezing will result in disinfestation to international quarantine standards. An example is orange exports to Japan and USA which are disinfested against fruit flies by storage at 1^0C for 16 days before shipment. Fresh and semiprocessed fruits which can be frozen to commercial frozen food temperatures, for example -18 °C, are accepted as insect free.

3.1.5. Other methodology: There are a number of other disinfestation strategies. For grain, hermetic storage with atmospheres modified by addition of carbon dioxide, nitrogen, or oxygen depletion with a gas burner are possible. Reduction of moisture content alone will reduce susceptibility of grain to infestation but is rarely practicable. For products such as seed, insect proof packaging is a widely used technique. Fresh fruit can be produced in ways that avoid the need for disinfestation [3]. None of these offer the immediate treatment facility of irradiation.

Table III - TIMBER, TIMBER STRUCTURES AND TIMBER PRODUCTS WHICH MAY BE DISINFESTED WITH METHYL BROMIDE IN AUSTRALIA

Production volume	Production value	Exports value	Imports volume
18.391 000m^3	$2119M AUD	$1017.8M AUD	3.67 000m^3

Table IV. AUSTRALIAN AGRICULTURAL AND HORTICULTURAL PRODUCE FOR WHICH IRRADIATION IS A POTENTIAL DISINFESTATION OPTION. DEVELOPMENTAL RESEARCH WILL BE NECESSARY FOR SOME PRODUCTS.

Commodity	Pest
Cereal grains	Stored grain insects
Coarse grains	Stored grain insects
Birdseed	Stored Grain insects
Rice	Stored grain insects
Grain legumes	Bruchid beetles
Baled hay	Mites
Apples	Fruit flies, Codling Moth
Avocados	Fruit flies
Banana	Fruit flies
Grapes	Fruit flies
Lemon	Fruit flies
Lychee	Fruit flies, *Cryptophlebia*
Mandarin	Fruit flies
Mangoes	Fruit flies, scale insects
Melons	Fruit flies, thrips, Fuller rose beetle, Mealy bug
Oranges	Fruit flies
Peaches	Fruit flies
Persimmons	Fruit flies
Pineapple	Dried fruit beetle, scale insects
Plum	Fruit flies
Strawberry	Fruit flies
Kohl species	Various Lepidoptera
Carrot	Nematodes
Capsicum (Bell Peppers)	Fruit flies
Tomato	Fruit flies
Potato	Nematodes
Strawberry	Redlegged earth mite, Queensland fruit fly
Sweet corn	White fringed Weevil
Onions	Fruit flies
Cucurbit vegetables	Fruit flies
Cut flowers	Thrips, mites, various Lepidoptera, Hymenoptera
Nursery stock soil	Nematodes, soil insects, other quarantine pests

Table V. SCIENTIFIC NAMES OF SIGNIFICANT PESTS OF AGRICULTURAL AND HORTICULTURAL COMMODITIES FOR WHICH IRRADIATION IS A POTENTIAL DISINFESTATION TREATMENT IN AUSTRALIA

Common Name	Scientific name and family
Fruit flies	
Cucumber (fruit) fly	*Bactrocera cucumis* (French) Tephritidae
Queensland fruit fly	*B. tryoni* (Froggatt) Tephritidae
Jarvis' fruit fly	*B. jarvisi* (Tryon) Tephritidae
Mediterranean fruit fly	*Ceratitis capitata* (Wiedemann) Tephritidae
Fruit moths	
Codling moth	*Cydia pomonella* (L.) Tortricidae
Lightbrown apple moth	*Epiphyas postvittana* (Walker) Tortricidae
Fruit and flower thrips	
Melon thrips	*Thrips palmi* Thripidae
Grain beetles	
Bean weevil	*Acanthoscelides obtectus* (Say) Bruchidae
Cowpea weevil	*Callosobruchus maculatus* (Fabricius) Bruchidae
Granary weevil	*Sitophilus granarius* (Linnaeus) Curculionidae
Maize weevil	*S. zeamais* (Motschulsky) Curculionidae
Rice weevil	*S. oryzae* (Linnaeus) Curculionidae
Rust red flour beetle	*Tribolium castaneum* (Herbst) Tenebrionidae
Sawtoothed grain beetle	*Oryzaephilus surinamensis* (Linnaeus) Silvanidae
Grain moths	
Angoumois grain moth	*Sitotroga cerealla* (Olivier) Gelechiidae
Indian meal moth	*Plodia interpunctella* Hubner Pyralidae
Tropical warehouse moth	*Ephestia cautella* (Walker) Pyralidae
Mites	
Straw itch mite	*Pyemotes herfsi* Oudemans Pygmephoridae
Two spotted mite	*Tetranychus urticae* Koch Tetranychidae
Exotic wood borers	
Powder post beetles	*Lyctus spp.* Lyctidae
Furniture beetle	*Anobium punctatum* De Geer Anobiidae
Wood wasps	*Sirex spp.* Siricidae
European house borer	*Hylotrupes bajulus* Cerambycidae
Exotic termites	
Drywood termite	*Cryptotermes brevis* (Walker) Kalotermitidae

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GAMMA IRRADIATION AS A QUARANTINE TREATMENT FOR SPIDER MITES (*Acarina: tetranychidae*) IN HORTICULTURAL PRODUCTS

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Abstract

The carmine spider mite, *Tetranychus cinnabarinus* (Boisd.), and the two-spotted spider mite, *Tetranychus urticae* Koch, are closely related species of tetranychid mites *(Acarina, Tetranychidae)* that respond to gamma irradiation in a similar way. Eggs of both species exposed to gamma radiation early in embryonic development were considerably more susceptible to irradiation than older eggs. The tolerance of eggs to gamma radiation increased in 3-4-day-old eggs, when eye-spots were formed. Nymphs were more resistant to gamma radiation than eggs and larvae. Deteriorative effects of irradiation treatment were reflected in the immatures by their mortality in subsequent developmental stages. A positive relationship between dosage and the percent egg mortality or the mortality of subsequent stages was usually found when the immature stages were irradiated. The sex ratio of adults developed from irradiated eggs, larvae, and nymphs was affected by the irradiation treatment; the ratio was usually skewed towards males. Irradiation of females resulted in increased mortality, lowered fecundity, reduced egg viability, and sex ratio distortion in their progeny. Two-day-old females of the carmine spider mite and the two-spotted spider mite irradiated with 200 or 300 Gy lived as long as the controls. Mortality occurred after 3 weeks. The number of eggs laid by irradiated females of spider mites was considerably lower than in the control, and it decreased as the absorbed dose increased. The higher the dose of gamma radiation applied to adults of the spider mites (the parental generation, *P*), the higher the mortality of the *F1* mites during their embryonic development. Viability of eggs laid by irradiated females of spider mites mated with irradiated males was significantly reduced. Young females treated with a dose of 0.2 kGy produced 40-50% nonviable eggs, while control mites produced only 6.0-6.6% nonviable eggs. A dose of 0.3 kGy caused high mortality of eggs; 88% and 97% nonviable eggs were found for the carmine spider mite and the two-spotted spider mite, respectively. In general, viability of eggs produced by mites irradiated as young females and old females was similar. The higher the dose of gamma radiation applied to adults of the spider mites, the higher the mortality of offspring during their embryonic development. However, a dose of 0.3 kGy did not cause complete sterility. To determine a sterilizing dose for both sexes, spider mites were irradiated with the following doses: 0.3, 0.31, 0.32, 0.33, 0.34, 0.35, and 0.4 kGy. When mites were treated with 0.30 and 0.31 kGy, a few eggs hatched. Data obtained indicate that a dose of 0.32 kGy is the lowest dose causing complete sterility in the carmine spider mite and the two-spotted spider mite, when both males and females were irradiated. This dosage could be used for irradiation as a quarantine treatment of horticultural products infested with spider mites.

1. INTRODUCTION

Various spider mite species of the family *Tetranychidae* are notorious pests on several crops. The economic significance of spider mites as pest species has increased considerably during recent decades because of their great ability to develop resistance to a wide variety of pesticides. Until recently, chemical control of spider mites has not been a problem since a few very effective acaricides are still applicable. However, irradiation as a quarantine treatment will be the next alternative to chemical control of spider mites on cut flowers, vegetables and some fruits.

High doses (1-3 kGy) of ionizing radiation resulting in the immediate mortality of mite pests are not recommended as they cause phytotoxicity to horticultural produce. Lower doses, friendly to the produce, but resulting in (a) the inability of treated mites to reproduce and/or (b) the inhibition of mite development should be used [1]. The effects of ionizing radiation on tetranychid mites belonging to the following species have been studied so far: *Tetranychus urticae* Koch [2-7], *T. arabicus* Attiah [8-10], *Panonychus citri* (McGregor) [11], *P. ulmi* Koch [12] and *Oligonychus biharensis* (Hirst.) [13]. Effects of ionizing radiation on the two-spotted spider mite have been investigated in details, but there is no information in the literature on the susceptibility to ionizing radiation of the carmine spider mite, *T. cinnabarinus* (Boid.), a closely related species.

The carmine spider mite is a cosmopolitan species with a range of hosts, including weeds, ornamental plants, fruit trees, vegetable crops, and greenhouse plants. Infested host plants are yellowish or brownish, and heavily covered with webs which affect their photosynthesis. Feeding of mites results in crumpling, curling, and twisting leaves, which eventually dry-up and fall-off. Growth, flowering and fruit setting of horticultural plants is always severely affected. Economic importance of these mites is especially significant in subtropical and tropical countries. The two-spotted spider mite is a typical polyphagous and cosmopolitan pest. It infests more than 300 plant species belonging to different families. Feeding symptoms and damage to the host plants of the two-spotted spider mite are similar to those caused by the carmine spider mite. In the present study, the effects of gamma radiation on development of immature stages, fecundity, and fertility of adults of the two-spotted spider mite and the carmine spider mite were investigated and compared to determine the doses required for a quarantine treatment.

2. MATERIAL AND METHODS

Spider mites were obtained from a greenhouse at the Warsaw Agricultural University, where they were reared on the common bean (*Phaseolus vulgaris* L.) at 20-25^{o}C, 70$\pm$5%R.H., and under a long day photoperiod (16:8 L:D). For experiments, mites were kept on detached leaf cultures. A culture consisted of glass dish (6 cm diam.) containing cotton wool saturated in water. A young bean leaf was pressed on top of a wad of cotton wool and this leaf rooted usually after some days. Such leaf cultures remained fresh for about 3-4 weeks under the rearing and experimental conditions. A number of leaf cultures were placed in a tray containing a little water. This water formed an isolating barrier between the various cultures and prevented desiccation of the leaves [14].

Young mites were obtained by separating them from the stock colony in the last quiescent stage before adulthood (teleiochrysalis), and they were placed on the leaf culture. Several young males and females of the spider mites were placed on the leaves, and females were allowed to produce eggs. Every other day, the mites were transferred to new leaf cultures, producing 'waves' of eggs left in the previously used leaf cultures. About 10 such transfers were made. The eggs were then incubated at 22$\pm$2^{o}C, 70-80% R.H., under a long day photoperiod. After the last transfer, mites at different ages and stages of development were obtained and then irradiated with gamma radiation.

Mites (eggs, immature stages of known age, and adult males and females) on detached leaf cultures were irradiated with a Co-60 source installed at the Faculty of Veterinary, Warsaw Agricultural University. The dose rate was 48.3 Gy/min. A Fricke dosimeter was used for calibration [15]. The irradiator was operated at ca. 20^{o}C and 50-60% R.H. Mites were treated with the following doses of gamma radiation: 0 (control), 50, 100, 200, 300, and 400 Gy. Spider mites belonging to both species were simultaneously treated with the same dose. After irradiation, mites were held under the rearing conditions described above. After irradiation, mites were reared until adult emergence. Development, number, and percent developing to the next stage of irradiated eggs, larvae, and

nymphs were recorded. Adults (F1) were sexed and the sex ratio of their progeny was calculated. Every 3 to 4 days, treated adult mites were transferred to fresh leaf cultures. The mortality of females was recorded for 3 weeks, and the number of eggs produced was noted. Emerged larvae were reared to obtain the F1 adults. Males and females of the F1 generation were paired to obtain adults of the F2 generation. Fecundity and fertility of irradiated females of the P generation, and the sex ratio of the F 1 and F2 progeny were recorded.

3. RESULTS

3.1. Effects of gamma radiation on development of immature stages of the carmine spider mite and the two-spotted spider mite

3.1.1. Irradiation of eggs

The data obtained indicate a positive relationship between dosage and percent egg mortality (Tables I and II). Mortality of 4-5-day-old eggs of the carmine spider mite was 36, 60, 81, and 95% after treatment with doses of 100, 200, 300, and 400 Gy, respectively. The respective mortalities of the oldest eggs of the two-spotted spider mite treated with the same dosages were 52, 66, 89, and 99%. Complete or almost 100% mortality of spider mite eggs was obtained when 0-2-day-old eggs received a dose of 100 Gy or higher. The oldest eggs were the most resistant to radiation, and only irradiation of them with a dose of 400 Gy resulted in almost complete mortality (94.8%).

TABLE I. DEVELOPMENT OF GAMMA-IRRADIATED EGGS OF THE CARMINE SPIDER MITE, *T. cinnabarinus*

Dose (Gy)	Age of eggs (days)	Number of eggs	Eggs to larvae (%)	Eggs to protonymphs (%)	Eggs to deutonymphs (%)	Eggs to adults (%)
100	0-1	173	1.7	0.6	0	0
	1-2	175	2.9	1.7	0	0
	2-3	164	18.9	12.2	11.0	10.4
	3-4	195	35.4	27.1	25.1	22.1
	4-5	132	63.6	52.3	40.2	37.9
200	0-1	166	0	0	0	0
	1-2	139	0	0	0	0
	2-3	163	0.6	0	0	0
	3-4	154	9.0	2.6	1.3	0.7
	4-5	204	40.2	12.3	6.9	5.4
300	0-1	199	0	0	0	0
	1-2	166	0	0	0	0
	2-3	211	0	0	0	0
	3-4	189	6.9	2.6	0.5	0
	4-5	214	19.2	3.7	0.9	0.5
400	0-1	135	0	0	0	0
	1-2	135	0	0	0	0
	2-3	141	0	0	0	0
	3-4	165	0	0	0	0
	4-5	172	5.2	2.9	0.6	0.6
Control	0-5	730	84.9	84.0	81.9	81.1

TABLE II. DEVELOPMENT OF GAMMA-IRRADIATED EGGS OF THE TWO-SPOTTED SPIDER MITE, *T.urticae*

Dose (Gy)	Age of eggs (days)	Number of eggs	Eggs to larvae (%)	Eggs to protonymphs (%)	Eggs to deutonymphs (%)	Eggs to adults (%)
100	0-1	141	2.8	0.7	0.7	0
	1-2	170	2.9	1.8	0.6	0.6
	2-3	160	8.1	8.1	7.5	7.5
	3-4	127	68.5	61.4	58.3	55.9
	4-5	184	47.8	45.1	42.3	40.2
200	0-1	200	0	0	0	0
	1-2	144	0	0	0	0
	2-3	158	1.3	0.6	0	0
	3-4	107	10.3	2.8	0	0
	4-5	116	33.6	28.4	22.4	19.8
300	0-1	149	0.7	0	0	0
	1-2	221	0	0	0	0
	2-3	213	3.8	2.8	1.4	1.4
	3-4	133	0.8	0	0	0
	4-5	130	11.5	8.5	3.4	3.1
400	0-1	148	0	0	0	0
	1-2	148	0	0	0	0
	2-3	123	0	0	0	0
	3-4	133	3.0	0	0	0
	4-5	151	0.7	0	0	0
Control	0-5	767	82.4	81.6	80.4	79.9

Eggs of both species exposed to gamma radiation early in embryonic development were considerably more susceptible to irradiation than older eggs. Exposure of 0-1-day-old eggs of *T. cinnabarinus* to 100 Gy caused 98.3% mortality, whereas only 36.4% of the 4-5-day-old eggs died after the treatment with the same dose. The tolerance of eggs to gamma radiation increased in 3-4-day-old eggs, when eye-spots were formed.

At low doses, a significant number of eggs hatched, but deleterious effects of irradiation were reflected by mortality in subsequent stages. Tables I and II summarize the effects of egg irradiation on survival of subsequent developmental stages. A gradual increase of mortality of immature stages developing from irradiated eggs was usually observed in both spider mite species.

No adults of the carmine spider mite developed from (a) 0-2-day-old eggs exposed to a dose of 100 Gy or higher, (b) 2-3-day-old eggs exposed to a dose of 200 Gy or higher, and (c) 3-4-day-old eggs exposed to a dose of 300 or 400 Gy. When the oldest eggs were exposed to 100, 200, 300, and 400 Gy, adult survivors were 37.9, 5.4, 0.5, and 0.6%, respectively. More than 80% of eggs developed to adult stage in the control (Table I). Similar results were recorded for the two-spotted spider mite (Table II). A small percent of adults emerging from irradiated old eggs of both spider mite species exhibited malformations of the hind pair of legs, or their whole body was malformed. The sex ratio of adults developing from irradiated old eggs was affected by the treatment. The sex ratio of the carmine spider mite was 2.46 (no. of females : no. of males) in the control, but it was reduced to 1.97 in adults developing from eggs treated with a dose of 100 Gy (Table III). A more pronounced effect was

observed in the two-spotted spider mite, in which the sex ratio was 3.79 in the control, and only 1.8 in adults from irradiated eggs (Table IV).

TABLE III. SEX RATIO OF CARMINE SPIDER MITES, *T. cinnabarinus*, THAT DEVELOPED FROM IRRADIATED IMMATURE STAGES

Dose (Gy)	Developmental stage treated	Sex ratio		
		Number of females	Number of males	Females:males
100	eggs*	73	37	1.97
	larvae	46	26	1.77
	nymphs	60	43	1.40
200	eggs*	8	4	-
	larvae	16	23	0.70
	nymphs	41	42	0.98
300	eggs*	0	1	-
	larvae	9	9	-
	nymphs	33	31	1.06
400	eggs*	0	1	-
	larvae	0	0	-
	nymphs	1	3	-
Control	-	421	171	2.46

* 4-5-day-old eggs were irradiated.

TABLE IV. SEX RATIO OF TWO-SPOTTED SPIDER MITES, *T. urticae*, THAT DEVELOPED FROM IRRADIATED IMMATURE STAGES

Dose (Gy)	Developmental stage treated	Sex ratio		
		Number of females	Number of males	Females:males
100	eggs*	99	59	1.8
	larvae	38	14	2.71
	nymphs	49	50	0.98
200	eggs*	20	3	-
	larvae	21	24	0.88
	nymphs	60	37	1.62
300	eggs*	5	2	-
	larvae	8	17	-
	nymphs	61	62	0.98
400	eggs*	0	0	-
	larvae	1	3	-
	nymphs	0	1	-
Control	-	485	128	3.79

* 4-5-day-old eggs were irradiated.

3.1.2. Irradiation of larvae

At low doses (100 or 200 Gy), a significant number of larvae developed to the protonymph or deutonymph stage, and more than 60% of them reached the adult stage. Deleterious effects of irradiation of larvae with a higher doses were reflected in mortality of subsequent stages. Tables V and VI summarize the effects of irradiation of larvae on survival of subsequent developmental stages. A gradual increase of mortality of immature stages that developed from irradiated larvae was usually observed in both spider mite species.

TABLE V. DEVELOPMENT OF GAMMA-IRRADIATED LARVAE OF THE CARMINE SPIDER MITE, *T. cinnabarinus*

Dose (Gy)	Number of larvae	Larvae to protonymphs (%)	Larvae to deutonymphs (%)	Larvae to adults (%)
100	72	86.1	75.0	63.9
Control	61	95.1	90.2	86.9
200	63	87.3	79.4	61.9
Control	85	96.5	95.3	95.3
300	81	66.7	50.6	22.2
Control	113	95.6	88.5	88.5
400	104	5.8	3.8	0.0
Control	78	96.2	96.2	94.9

TABLE VI. DEVELOPMENT OF GAMMA-IRRADIATED LARVAE OF THE TWO-SPOTTED SPIDER MITE, *T. urticae*

Dose (Gy)	Number of larvae	Larvae to protonymphs (%)	Larvae to deutonymphs (%)	Larvae to adults (%)
100	82	84.1	69.6	63.4
Control	103	99.0	99.0	96.1
200	73	89.0	76.7	61.6
Control	52	100.0	96.2	90.4
300	81	72.8	59.3	30.9
Control	49	98.0	98.0	98.0
400	99	26.3	14.1	4.0
Control	87	97.7	90.8	90.8

No adults of the carmine spider mite developed from larvae exposed to 400 Gy, and only 4% adults of the two-spotted spider mites developed from larvae treated with the same dose of gamma radiation. These data indicate that larvae of the spider mites are less susceptible to irradiation than eggs. The sex ratio of adults was affected by radiation treatment of larvae. The sex ratio of the carmine spider mite was 2.46 (no. of females : no. of males) in the control, whereas it was lowered to 1.77 and 0.7 in adults that developed from larvae treated with doses of 100 and 200 Gy, respectively (Table III). A more pronounced effect was observed in the two-spotted spider mite, in which the sex ratio was 3.79 in the control, and 2.71 and 0.88 in adults that originated from larvae irradiated with 100 and 200 Gy, respectively (Table IV).

3.1.3. Irradiation of nymphs

Tables VII and VIII summarize the effects of irradiation of protonymphs on survival of subsequent developmental stages. Mortality of spider mites that developed from irradiated protonymphs increased with increase of absorbed dose of gamma radiation. A few adults of the carmine spider mite and the two-spotted spider mite developed from protonymphs irradiated with a dose of 400 Gy. Protonymphs of the spider mites are more resistant to gamma radiation than eggs and larvae.

TABLE VII. DEVELOPMENT OF GAMMA-IRRADIATED PROTONYMPHS OF THE CARMINE SPIDER MITE, *T. cinnabarinus*

Dose (Gy)	Number of protonymphs treated	Protonymphs to deutonymphs (%)	Protonymphs to adults (%)
100	125	86.4	82.4
Control	136	91.2	86.0
200	119	85.7	69.7
Control	150	86.0	84.0
300	148	75.7	43.2
Control	161	96.9	95.0
400	121	7.4	3.3
Control	157	92.9	90.4

TABLE VIII. DEVELOPMENT OF GAMMA-IRRADIATED PROTONYMPHS OF THE TWO-SPOTTED SPIDER MITE, *T. urticae*

Dose (Gy)	Number of protonymphs treated	Protonymphs to deutonymphs (%)	Protonymphs to adults (%)
100	126	88.9	78.6
Control	158	93.7	81.6
200	154	82.5	43.0
Control	154	90.3	85.1
300	199	81.9	61.8
Control	150	89.3	84.0
400	147	17.0	0.7
Control	203	89.2	86.2

The sex ratio of adults that developed from irradiated nymphs was affected by the treatment. The sex ratio of the carmine spider mite was 2.46 in the control, whereas it was 1.4, 0.98, and 1.06 in adults that developed from nymphs treated with a dose of 100, 200, and 300 Gy, respectively (Table III). More pronounced effect was observed in the two-spotted spider mite: the sex ratio was 3.79 in the control, and 0.98, 1.62, 0.98 in adults that originated from nymphs irradiated with 100, 200 and 300 Gy, respectively (Table IV).

3.2. Effects of gamma radiation on fecundity and fertility of the carmine spider mite and the two-spotted spider mite

Two-day-old females of the carmine spider mite and the two-spotted spider mite irradiated with 200 or 300 Gy lived as long as the controls. Mortality occurred after 3 weeks (Tables IX and X). Data on mortality of gamma irradiated females of the carmine spider mite and the two-spotted spider mite irradiated as 7-10 day-old adults are summarized in Tables XI and XII. Control females of the carmine spider and females irradiated with 50 Gy dose lived longer than 21 days, whereas females irradiated with 100, 200, and 300 Gy were all dead after 21, 21, and 15 days, respectively (Table XI). Similar results were recorded for the two-spotted spider mite (Table XII).

Irradiated females of the carmine spider mite produced eggs only during the first two weeks after treatment, but the controls laid eggs during the total observation period. The number of eggs laid by irradiated females was considerably lower than in the control. A dose of 300 Gy reduced fecundity by about 61% (Table XIII). Females of the two-spotted spider mite treated with 300 Gy produced eggs only during the first two weeks after the treatment, but those irradiated with 200 Gy and the controls laid eggs during the total observation period. Number of eggs laid by irradiated females was considerably lower than in the control, and it decreased as the absorbed dose increased. A dose of 300 Gy did not inhibit completely production of eggs by treated females, but it reduced fecundity by about 70% (Table XIV). The effect of irradiation on spider mite fecundity was more pronounced, when 7-10 day old females were treated. However, the highest dose tested did not inhibit the production of eggs by aged females (Tables XV and XVI).

TABLE IX. MORTALITY (%) OF GAMMA-IRRADIATED FEMALES OF THE CARMINE SPIDER MITE, *T. cinnabarinus*, IRRADIATED AS 2-DAY- OLD FEMALES

Dose (Gy)	200	300	Control
Number of females	35	17	29
Days after irradiation			
0-3	8.6	23.5	0.0
3-7	11.4	47.0	13.8
7-10	34.3	52.9	27.6
10-14	48.6	52.9	37.9
14-17	57.1	76.5	58.6
17-21	62.8	82.3	86.2
>21	100.0	100.0	100.0

TABLE X. MORTALITY (%) OF GAMMA-IRRADIATED FEMALES OF THE TWO-SPOTTED SPIDER MITE, *T. urticae*, IRRADIATED AS 2-DAY- OLD FEMALES

Dose (Gy)	200	300	Control
Number of females	26	28	14
Days after irradiation			
0-3	11.5	10.7	0.0
3-7	19.2	21.4	0.0
7-10	26.9	32.1	0.0
10-14	26.9	46.4	21.4
14-17	42.3	46.4	50.0
17-21	50.0	50.0	57.1
>21	100.0	100.0	100.0

TABLE XI. MORTALITY (%) OF GAMMA-IRRADIATED FEMALES OF THE CARMINE SPIDER MITE, *T. cinnabarinus*, IRRADIATED AS 7-10- DAY-OLD ADULTS

Dose (Gy)	50	100	200	300	Control
Total number of females	69	58	61	73	76
Days after irradiation					
0-3	7.2	19.0	13.1	30.1	3.9
3-6	18.8	72.4	62.3	80.8	6.6
6-9	27.5	74.1	62.3	90.4	14.5
9-12	31.9	75.9	65.6	98.6	18.4
12-15	40.6	93.1	67.2	100.0	22.4
15-18	50.7	98.3	88.5		36.8
18-21	65.2	100.0	100.0		51.3
>21	100.0				100.0

TABLE XII. MORTALITY (%) OF GAMMA-IRRADIATED FEMALES OF THE TWO SPOTTED SPIDER MITE, *T. urticae*, IRRADIATED AS 7-10- DAY-OLD ADULTS

Dose (Gy)	50	100	200	300	Control
Total number of females	65	58	63	71	72
Days after irradiation					
0-3	7.7	12.1	30.2	81.7	4.2
3-6	41.5	13.8	34.9	88.7	4.2
6-9	52.3	13.8	49.2	91.5	5.6
9-12	69.2	17.2	58.2	97.2	9.7
12-15	72.3	37.9	65.1	98.6	16.7
15-18	75.3	56.9	77.8	100.0	18.1
18-21	93.8	98.3	100.0		30.6
>21	100.0	100.0			100.0

TABLE XIII. FECUNDITY AND FERTILITY OF FEMALES OF THE CARMINE SPIDER MITE, *T. cinnabarinus*, IRRADIATED AS 2-3- DAY- OLD FEMALES

Dose (Gy)	Number of females observed	Number of eggs laid (number of eggs per female*)	Viability of eggs (%)
200	17	125 (7.4)	58.4
300	35	537 (15.3)	11.7
Control	29	1374 (47.4)	93.4

*Number of eggs per female laid during a week period.

TABLE XIV. FECUNDITY AND FERTILITY OF FEMALES OF THE TWO-SPOTTED SPIDER MITE, *T. urticae*, IRRADIATED AS 2-3-DAY-OLD FEMALES

Dose (Gy)	Number of females observed	Number of eggs laid (number of eggs per female*)	Viability of eggs (%)
200	29	515 (17.8)	49.5
300	28	374 (13.4)	3.2
Control	14	1250 (89.3)	94.0

*Number of eggs per female laid during a week period.

TABLE XV. FECUNDITY AND FERTILITY OF FEMALES OF THE CARMINE SPIDER MITE, *T. cinnabarinus*, IRRADIATED AS 7-10-DAY-OLD FEMALES

Dose (Gy)	Number of females observed	Number of eggs laid (number of eggs per female*)	Viability of eggs (%)
50	69	919 (13.3)	77.3
100	58	257 (4.4)	61.5
200	61	206 (3.4)	49.7
300	73	120 (1.6)	4.8
Control	76	1788 (23.5)	77.4

*Number of eggs per female laid during a 3 day period

TABLE XVI. FECUNDITY AND FERTILITY OF FEMALES OF THE TWO-SPOTTED SPIDER MITE, *T. urticae*, IRRADIATED AS 7-10-DAY-OLD FEMALES

Dose (Gy)	Number of females observed	Number of eggs laid (number of eggs per female*)	Viability of eggs (%)
50	65	614 (9.4)	74.5
100	58	223 (3.8)	68.4
200	63	248 (3.9)	41.7
300	71	52 (0.7)	11.1
Control	72	1161 (16.1)	81.3

*Number of eggs per female laid during a 3 day period.

Viability of eggs laid by irradiated females of spider mites kept with irradiated males was significantly reduced. Young (2-3-day-old) females treated with a dose of 200 Gy produced 40-50% nonviable eggs, while the control mites produced only 6.0-6.6% nonviable eggs. A dose of 300 Gy caused high mortality of eggs; 88% and 97% nonviable eggs were found for the carmine spider mite and the two-spotted spider mite, respectively. In general, viability of eggs produced by mites irradiated as young females (Tables XIII and XIV) and old females (Tables XV and XVI) was similar. The higher the dose of gamma radiation applied to adults of the spider mites, the higher the mortality of offspring during their embryonic development. However, a dose of 300 Gy did not cause sterility. To determine a sterilizing dose for both sexes, spider mites were irradiated with 300, 320, 340, 360, 380, and 400 Gy. Hatchability of eggs produced by treated mites is presented in Table XVII. Data obtained indicate that a dose of 320 Gy is the lowest dose causing sterility in the carmine spider mite and the two-spotted spider mite, when both males and females were irradiated.

Mites emerged from eggs laid by irradiated females were reared on the bean leaves to the adult stage. Their mortality during the development was recorded (Tables XVIII-XX). Mortality of control mites during the embryonic and post-embryonic development was similar and it ranged from 4.3 to 6.6%, whereas in the treatments most mites died during embryonic development. Mortality of treated mites during the post-embryonic development was low (<6%) and similar to the control. Only in immatures that developed from eggs laid by *T. cinnabarinus* females irradiated with a 200 Gy dose did post-embryonic mortality reach the level of 14.4% (Table XVIII). However, when the percent of mortality of immatures during the post-embryonic development is referred to the total number of emerged larvae, then it may be stated that the highest mortality during the post-embryonic development occurred in progeny originated from eggs laid by 300 Gy-treated females than in mites originated from 200 Gy-treated females and in the control (Table XX).

TABLE XVII. DETERMINATION OF A DOSE OF GAMMA RADIATION CAUSING STERILITY OF BOTH SEXES OF THE CARMINE SPIDER MITE AND THE TWO-SPOTTED SPIDER MITE

Spider mite species	Dose (Gy)	Number of eggs observed	Viability of eggs (%)
T. cinnabarinus	Control	1374	93.4
	300	537	11.7
	310	421	6.5
	320	370	0.0
	330	367	0.0
	340	314	0.0
	350	298	0.0
	360	280	0.0
	380	293	0.0
	400	178	0.0
T. urticae	Control	1250	94.0
	300	374	3.2
	310	453	2.5
	320	389	0.0
	330	290	0.0
	340	267	0.0
	350	250	0.0
	360	185	0.0
	380	243	0.0
	400	107	0.0

TABLE XVIII. MORTALITY OF PROGENY OF IRRADIATED FEMALES (2-3-DAY-OLD) OF SPIDER MITES DURING THE EMBRYONIC AND POST-EMBRYONIC DEVELOPMENT

Mite species	Dose (Gy)	Number of eggs observed	Mortality (%)		
			Embryonic development	Post-embryonic development	Total
T. cinnabarinus	200	125	41.6	14.4	56.0
	300	537	88.3	5.8	94.1
	control	1374	6.6	5.6	12.2
T. urticae	200	515	50.5	4.3	54.8
	300	374	96.8	2.7	99.5
	control	1250	6.0	4.3	10.3

The F1 generation was obtained in treatments involving irradiation of adults with 200 and 300 Gy. The sex ratio of the F1 progeny developed from irradiated females was affected by the irradiation, and it was very low. There were more males than females in the treatments than in the control. The sex ratio of the carmine spider mite was 2.3 (number of females : number of males) in the control, whereas it was reduced to about 0.6 in the F1 adults that developed from females treated with a dose of 200 and 300 Gy. Similar effects were found for the two-spotted spider mite. The sex ratio was 2.3 and 0.3 in the control and in progeny from females that were irradiated with 200 Gy, respectively (Table XXI). In the next generation (F2) the value of sex ratio increased and was similar or only slightly lower than in the control (Table XXII).

TABLE XIX. DEVELOPMENT OF EGGS LAID BY GAMMA-IRRADIATED FEMALES OF SPIDER MITES

Mite species	Dose (Gy)	Number of eggs observed	Eggs to larvae (%)	Eggs to protonymphs (%)	Eggs to deutonymphs (%)	Eggs to adult stage (%)
T. cinnabarinus	300	537	11.7	10.6	7.2	5.9
	200	125	58.4	55.2	49.6	44.0
	control	1374	93.4	92.2	89.4	87.8
T. urticae	300	374	3.2	1.6	0.8	0.5
	200	515	49.5	48.1	46.6	45.2
	control	1250	94.0	92.3	90.6	89.7

TABLE XX. MORTALITY OF IMMATURES ORIGINATED FROM IRRADIATED FEMALES OF SPIDER MITES DURING THE POST-EMBRYONIC DEVELOPMENT

Mite species	Dose (Gy)	Number of larvae	Mortality of immatures at the indicated developmental stages: number (%) dead mites			
			Larva	Protonymph	Deutonymph	Total
T.cinnabarinus	300	63	6 (9.5)	18 (28.6)	7 (11.1)	31 (49.2)
	200	73	4 (5.5)	7 (9.6)	7 (9.6)	18 (24.7)
	control	1283	16 (1.2)	39 (3.0)	22 (1.7)	77 (6.0)
T. urticae	300	12	6 (50.0)	3 (25.0)	1 (8.3)	10 (83.3)
	200	255	7 (2.7)	8 (3.1)	7 (2.7)	22 (8.6)
	control	1175	21 (1.8)	21 (1.8)	12 (1.0)	54 (4.6)

TABLE XXI. SEX RATIO OF THE F_1 PROGENY DEVELOPED FROM EGGS LAID BY IRRADIATED FEMALES OF SPIDER MITES

Mite species	Dose (Gy)	Number of eggs observed	Number (%) of F_1 adults developed	Sex ratio		
				Number of females	Number of males	Females: males
T. cinnabarinus	300	537	32 (6.0)	12	20	0.6
	200	125	55 (44.0)	22	33	0.6
	0	1374	1206 (87.8)	843	363	2.32
T. urticae	300	374	3 (0.8)	0	3	-
	200	515	233 (45.2)	57	176	0.32
	0	1250	1121 (89.7)	780	341	2.29

TABLE XXII. SEX RATIO OF THE F2 PROGENY DEVELOPED FROM EGGS LAID BY THE F1 FEMALES ORIGINATED FROM IRRADIATED FEMALES OF SPIDER MITES

Mite species	Dose (Gy)	Number of eggs observed	Number (%) of F1 adults developed	Sex ratio		
				Number of females	Number of males	Females: males
T. cinnabarinus	300	928	791 (85.2)	511	280	1.82
	200	2225	1677 (75.4)	1133	544	2.08
	0	1374	1206 (87.8)	843	363	2.32
T. urticae	200	1991	1284 (64.5)	818	466	1.75
	0	1250	1121 (89.7)	780	341	2.29

4. DISCUSSION

Several papers on the effects of ionizing radiation on the spider mites *(Tetranychidae)* have been published, and general information on these effects has been amassed. According to these data, the deteriorative effects of radiation on spider mites include lethality, reduced longevity, delayed molting, ceasing of oviposition, reduced fecundity (ovipositional rate), egg sterility, reduction of egg hatch, delayed embryonic and postembryonic development, and disturbance in the sex ratio of progeny. These effects occur at certain dose levels. Application of ionizing radiation to mite control problems appears promising in two ways: radiation can be applied in doses lethal to the population, or in doses which sterilize the pests.

Lethal effects of high doses of ionizing radiation on adults of the spider mites have not been studied. However, from studies on other mite pests it appears that the mites are very resistant to irradiation, and doses higher than 2 kGy are required for immediate mortality of the acarid mites *(Acaridae)* [1]. High doses of ionizing radiation resulting in accelerated mortality of mites are not recommended as they may cause phytotoxicity to horticultural produce. Lower doses, friendly to the produce, but resulting in (a) the inability of treated mites to reproduce, and/or (b) the inhibition of the development should be suggested [15].

4.1. Effects of gamma radiation on development of immature stages of the carmine spider mite and the two-spotted spider mite

The effects of ionizing radiation on developmental stages of the spider mites previously have been studied only in the two-spotted spider mite and *T. arabicus* [2, 8, 9, 16]. In general, these results are in agreement with those obtained in the present study. Tolerance of spider mite eggs to ionizing radiation (gamma rays, electron beam) increases with increased age. Eggs irradiated at an early stage of embryonic development are considerably more susceptible than those exposed at later stages. A large shift in susceptibility of eggs occurs in 3-day-old eggs. Irradiation of eggs results usually in retardation of embryonic and postembryonic development. It results in high mortality of larvae and nymphs that hatched from treated eggs. Fecundity and fertility of adults that emerged from irradiated eggs is also much affected. Goodwin and Wellham [5] observed that survivors of eggs treated with 150 Gy and 300 Gy completed their development at a slower rate. At 150 Gy, females laid 0.09 eggs per female per day. At 300 Gy, females failed to develop to maturity, and no eggs were laid. We found that a few adult mites (up to 3%) developed from old (4-5-day-old) eggs treated with a dose of 300 Gy, but none at a dose of 400 Gy (Tables I and II).

Some authors [2, 8] have reported that haploid (unfertilized) eggs are more susceptible to irradiation than diploid (fertilized) eggs fated to be female. However, data from this study (Tables III

and IV) do not support these findings, but indicate the effect of irradiation on the sex ratio in mites that developed from irradiated eggs is strongly biased towards males.

Larvae of spider mites were not killed immediately by treatment with a dose ≤400 Gy, and some of them completed their development. No adults of the carmine spider mite developed from larvae exposed to a 400 Gy dose, and only 4% adults developed from larvae treated with this dose of gamma radiation (Tables V and VI). Goodwin and Wellham [5] reported that no larvae treated at 600 Gy or higher completed their development. Also Wakid *et al.* [16] noted that larvae of *T. arabicus* suffered high mortality at 500 Gy, and at 600 Gy adult emergence from treated larvae was completely inhibited. These data indicate that larvae of spider mites are less susceptible to irradiation than eggs.

Wakid et al. [16] found that *T. arabicus* males emerging from irradiated larvae were more susceptible to the treatment than females. At 200 Gy, a few males were able to mature, while at 250 Gy all emerged adults were females. We observed that the sex ratio of adults that originated from treated larvae was shifted towards males in both mite species (Tables III and IV).

Irradiation of larvae results in reduced number of adults developing from them, and in delayed and incomplete development. Females that emerged from irradiated larvae produce fewer eggs than the controls, fewer progeny, and more non-viable eggs. At 300 Gy, females of *T. arabicus* were sterile [16]. Irradiation of spider mite nymphs results in reduction of adult emergence and/or delayed adult emergence. Mites at this stage are more resistant to irradiation than eggs and larvae. Elbadry et al. [9] noted that treated deutonymphs were able to develop to the adult stage at 2,100 Gy. However, sublethal doses of gamma radiation (80-300 Gy) applied to nymphs significantly reduced longevity, fecundity, and fertility of adults. We found that a few adults emerged from protonymphs treated with 400 Gy, but at lower doses a significant number of nymphs completed their development. The sex ratio was biased towards males.

4.2. Effects of gamma radiation on fecundity and fertility of the carmine spider mite and the two-spotted spider mite.

The effects of low doses of ionizing radiation on longevity of spider mite adults were considered only by Goodwin and Wellham [5], Dohino and Tanabe[2], and Bhuiya *et al.* [13]. Goodwin and Wellham [5] observed no mortality over periods of 0-4 days and 5-12 days after gamma irradiation in two-spotted spider mite females irradiated with doses as high as 1,200 Gy. However, Dohino and Tanabe [2] found that the survival rate of irradiated *T. urticae* females slowly decreased 5 days after irradiation with electron beams applied in doses up to 800 Gy, while that of non-irradiated females rapidly decreased on the 7th day after irradiation and was lower than that of irradiated females on the 9-10th day after irradiation. The inequality of survival rate in irradiated females was found to reverse on the 12th day after irradiation. A clear relationship between dose and survival rate of the two-spotted spider mite was not found by these authors. Bhuiya *et al.* [13] observed gradual decrease of longevity in *O. biharensis* adults treated with gamma radiation. Control males died after 20 days but males irradiated with 100, 200, 300, 400, and 500 Gy died after 13, 15, 13, 9, and 8 days, respectively. In the present study, we found that the survival rates of control females of the carmine spider mite and the two-spotted spider mite and females irradiated with 200 or 300 Gy of gamma radiation were similar. Complete mortality of both control and treated mites occurred after 3 weeks (Tables IX and X). Aged females (7-10 day old) of the carmine spider mite irradiated with 100, 200, and 300 Gy were all dead after 21, 21, and 15 days, respectively; whereas control females irradiated with 50 Gy lived longer than 21 days (Table XI). Similar results were recorded for the two-spotted spider mite (Table XII).

Dohino and Tanabe [2] reported that ovipositional rate (eggs/female/day) of irradiated females rapidly declined in the first 7 days and oviposition stopped on the 17th, 10th, 7th, and 7th day after irradiation with 200, 400, 600, and 800 Gy of electron beams, respectively. Similar results are

reported in the present study with the carmine spider mite and the two-spotted spider mite. Irradiated females of the carmine spider mite produced eggs only during the first two weeks after the treatment, but controls laid eggs during the total observation period. As a consequence, the fecundity (number of eggs laid per female) of irradiated females was greatly affected (Tables XIII-XVI). Somewhat different results were reported by Goodwin and Wellham [5]. They noted that ovipositional rate was similar during both the 0-4 day and 5-12 day periods after the treatment in young (1 day old) and old (5 day old) *T. urticae* females treated with the same dose of gamma radiation. However, they found that ovipositional rate decreased as the dose of irradiation increased.

Viability of eggs produced by adults of the spider mites irradiated with low doses is usually significantly reduced. We noted that irradiation with 300 Gy caused high mortality of eggs; 88% and 97% nonviable eggs were found in the carmine spider mite and the two-spotted spider mite, respectively. The higher the dose of gamma radiation applied to adults of the spider mites, the higher the mortality of mites during their embryonic development (Tables XIII-XVI). Complete sterility of both sexes was produced by a dose of 320 Gy (Table XVII). Table XXIII summarizes data of different authors on the doses of ionizing radiation causing the sterility in females, males or both sexes of the spider mites. Doses needed for *T. urticae* female sterility range from 200 Gy to 400 Gy, and for male sterility from 300 to 350 Gy. When both sexes were irradiated, doses in the range of 300 to 400 Gy caused sterility. Doses of 300, 280, and 300 Gy sterilized females, males, and both sexes of *T. arabicus*, respectively [10].

Dohino and Tanabe [2] observed at 200 kGy dose that hatchability of *T. urticae* eggs recovered from 0.6-2.1% in the first 7 days up to 28.5-46.2% in the next 7 days. Also, Goodwin and Wellham [5] noted that at 150 Gy more eggs laid in the first 4 days were sterile (99%) than eggs laid 5-12 days after irradiation (87-91%). Recovery of egg viability was also observed in acarid mites. Almost all the eggs laid during the first 2 weeks after treatment of *T. putrescentiae* adults with 90 Gy were dead. Later, viability of eggs produced by these mites gradually increased to 74% [1]. Eggs of mites before and after oviposition differ in their susceptibility to irradiation. Szlendak *et al.* [19] found that spermatogonia and spermatozoa are more tolerant than spermatocytes and spermatids to electron beams. They suggested that primary gonial cells repopulate testes after irradiation at 300 Gy and less. It may be that egg cells and oogonia of mites are more tolerant to ionizing radiation than oocytes. Electron beam irradiation is similar to gamma irradiation in sterility effect for spider mites [2] and for acarid mites [20].

TABLE XXIII. DOSES OF IONIZING RADIATION CAUSING STERILITY IN FEMALES, MALES, OR BOTH SEXES OF SPIDER MITES

Spider mite species	Sterilizing dose (Gy) for			Reference
	Females	Males	Both sexes	
Oligonychus biharensis	-	-	200	[13]
Panonychus citri	320	240	-	[11]
Tetranychus arabicus	300	280	300	[9, 10]
T. cinnabarinus	-	-	320	this paper
T. urticae	400	-	-	[2]
T. urticae (= T. telarius L.*)*	320	320	-	[6]
T. urticae	-	-	300	[5]
T. urticae	200	300	-	[7]
T. urticae	-	-	320	this paper
T. urticae	-	350	-	[17]
T. urticae	400	-	-	[18]
T. urticae	80	240	-	[4]

Henneberry [6] reported that untreated females of the two-spotted spider mite mated with males exposed to a dose in the 80-240 Gy range produced fewer females and more nonviable eggs than females of untreated pairs. Untreated females mated to males exposed to 320 Gy produced only males and nonviable eggs. Females exposed to 80 Gy and mated to untreated males produced fewer female progeny than untreated pairs. Females irradiated with 160 Gy produced fewer females and males that the untreated controls. However, the sex ratio (no. of females : no. of males) was 2.5, as was the ratio in the control. Females exposed to 240 Gy and 320 Gy and mated to untreated males produced a few progeny or only nonviable eggs.

At doses 1,120 Gy and 1,280 Gy, more males and fewer nonviable eggs were produced. Henneberry [6] interpreted the effect produced by these doses as sperm injury or sperm activation. Fertilization of eggs did not occur to produce diploid females and the mated females responded as if unmated. In *T. arabicus* and *P. citri*, at doses >800 Gy, untreated females mated to treated males produced more males and fewer nonviable eggs, indicating the induction of sperm injury [9-11]. Sperm inactivation was also observed in the acarid mite, *Tyrophagus putrescentiae* (Schrank), by Ignatowicz *et al.* [21] after irradiation of adult mites with a dose of or higher.

The sex ratio of the citrus red mite was close to 1:1 in the control, and it was variably affected by irradiation. Treated females mated to untreated males produced less viable eggs and variable percent of females as the dose increased. At a dose 160 Gy, mites produced 43.4% viable eggs, and about 14 % females were found among progeny that developed from these eggs. The corresponding data for 240 Gy were 21% viable eggs and 41.2% females. At 320 Gy, treated females were sterile and they produced only nonviable eggs [11].

When both sex of *T. arabicus* were treated with gamma radiation, the sex ratio in their progeny decreased as the dose increased. The control sex ratio was 1.77, and it decreased to 0.65 at a dose of 80 Gy. A few progeny was obtained from mite pairs irradiated with 160 Gy, and their sex ratio was 0.29 (2 females and 7 males). When treated females were mated to untreated males, the sex ratio in progeny of the parent generation increased with the dose, and at 240 kGy mite pairs produced only females [10].

Somewhat different results are reported from our studies with irradiated females and males of the two-spotted spider mite and the carmine spider mite. We found that the sex ratio in the control spider mites was about 2.3, and it was significantly biased in progeny of irradiated parents. Usually, F1 males developed from eggs produced by treated females, and males were more numerous than females in both spider mite species studied (Table XXI). In the F2 generation, the sex ratio increased and approached the control value, indicating post radiation recovery (Table XXII).

The effects of irradiation on the sex ratio in progeny of treated mites is evident. We found that the sex ratio is usually skewed towards sons after the treatment. Factors such as irradiation which influence sex-ratio are critical to mite population growth rates. Because daughters determine growth, the proportion of offspring that are female affects the rate of population increase. Furthermore, as adult females are the dispersing stage [22], sex ratio is important to population expansion.

Data on premature mortality of mites that emerged from eggs produced by irradiated adults of *T. arabicus* were provided only by Wakid *et al.* [9, 10]. They noted that mortality of mites during post-embryonic development (larvae, nymphs) was very low, not exceeding 8.0%. In the present study, we found that mortality of progeny from irradiated adults of spider mites during the post-embryonic development was also low, similar to the control, and in the most cases not exceeding 6.0%. One exception was that progeny of 200 Gy-treated adults of the carmine spider mite was 14.4%) (Table XVIII). The highest mortality of mites during post-embryonic development occurred at the protonymphal stage (Table XX).

5. CONCLUSIONS

Mortality of 4-5-day-old eggs of the carmine spider mite was 81 and 95% after treatment with 300 or 400 Gy, respectively. The respective mortalities of the oldest eggs of the two-spotted spider mite treated with the same dosages were 89 and 99%. Complete or almost 100% mortality of spider mite eggs was obtained when 0-2-day-old eggs received a dose of 100 Gy or higher. The oldest eggs were the most resistant to radiation, and only irradiation of them with a dose of 400 Gy resulted in almost complete mortality (94.8%). No adults of the carmine spider mite developed from 3-4-day-old eggs exposed to 300 or 400 Gy. When the oldest eggs of the two-spotted spider mite were exposed to 300 Gy and 400 Gy, adult survivors were 0.5% and 0.6%, respectively. A small percent of adults that emerged from irradiated old eggs of both spider mite species exhibited malformations of the hind pair of legs, or their whole body was malformed. The sex ratio of adults that developed from irradiated old eggs was affected by the treatment.

Deteriorative effects of irradiation of larvae with a higher doses were reflected by mortality in subsequent stages. A gradual increase of mortality of immature stages developed from irradiated larvae was usually observed in both spider mite species. No adults of the carmine spider mite developed from larvae exposed to a 400 Gy dose, and only 4% adults of the two-spotted spider mites originated from larvae treated with the same dose of gamma radiation. Sex ratio of adults developed from irradiated larvae was affected by the treatment.

Gamma irradiation of protonymphs affected the survival of their subsequent developmental stages. Mortality of subsequent stages of spider mites originated from irradiated protonymphs increased with the increase of the absorbed dose of gamma radiation. A few adults of the carmine spider mite and the two-spotted spider mite developed from protonymphs irradiated with a 400 Gy dose. Protonymphs of the spider mites were more resistant to gamma radiation than eggs and larvae. Sex ratio of adults developed from irradiated nymphs was affected by the treatment. Sex ratio of the carmine spider mite was 2.46 in the control, whereas it was lowered to 1.06 in adults developed from nymphs treated with a dose of 300 Gy. More pronounced effect was observed in the two-spotted spider mite: the sex ratio was 3.79 in the control, and 0.98 in adults originated from nymphs irradiated with 300 Gy.

Two-day-old females of the carmine spider mite and the two-spotted spider mite irradiated with 300 Gy lived as long as the controls. Their complete mortality occurred after 3 weeks. Control females of the carmine spider mite lived longer than 21 days, whereas females irradiated with 300 Gy were all dead after 15 days. The similar results were recorded for the two-spotted spider mite. Irradiated females of the carmine spider mite produced eggs only during the first two weeks after the treatment, but the controls laid eggs during the total observation period. Number of eggs laid by irradiated females was considerably lower than in the control. A dose of 300 Gy reduced the fecundity by about 61%. Females of the two-spotted spider mite treated with 300 Gy produced eggs only during the first two weeks after the treatment. Number of eggs laid by irradiated females was considerably lower than in the control, and it decreased as the absorbed dose increased. Viability of eggs laid by irradiated females of spider mites kept with irradiated males was significantly reduced. A dose of 300 Gy caused the high mortality of eggs; 88% and 97% nonviable eggs were found for the carmine spider mite and the two-spotted spider mite, respectively. Viability of eggs produced by mites irradiated as young females and old females was similar. The higher the dose of gamma radiation applied to adults of the spider mites, the higher the mortality of offspring during their embryonic development. A dose of 320 Gy was found to be the lowest dose causing sterility in the carmine spider mite and the two-spotted spider mite, when both males and females were irradiated.

Higher mortality during the post-embryonic development occurred in progeny originated from eggs laid by females treated with 300 Gy than in mites that originated from females treated with 200 Gy and in the control. The F1 generation was obtained in treatments involving irradiation of adults

with 200 and 300 Gy. The sex ratio of the F1 progeny that developed from irradiated females was affected by the irradiation, and there were more males than females in the treatments. In the next generation (F2) the value of sex ratio increased and was similar or only slightly lower than in the control.

According to the above data, gamma irradiation as a quarantine treatment for spider mites *(Tetranychus urticae, T. cinnabarinus)* in cut flowers, vegetables, some fruits, and other agricultural commodities may be applied at doses higher than 320 Gy, which is friendly to the produce, but results in the inability of treated mites to reproduce and/or the inhibition of their development.

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EFFECTIVENESS OF ELECTRON IRRADIATION AS A QUARANTINE TREATMENT OF CUT FLOWERS

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Abstract

The effects of electron beams on spider mite (*Tetranychus urticae*) and flour beetle (*Tribolium freemani*) were slightly smaller than those of gamma-rays. "Soft-electrons" (low-energy electrons) at an energy of 170 keV inactivated eggs, larvae, pupae, and adults of the flour beetle. Electron beams at doses up to 400 Gy killed or sterilized all the pests for cut flowers tested; spider mite (*Tetranychus urticae*), mealybug (*Pseudococcus comstocki*), leaf miner (*Liriomyza trifolii*), thrips (*Thrips palmi*, *Thrips tabaci*), cutworm (*Spodoptera litura*), and aphid (*Myzus persicae*). Carnation, alstromeria, gladiolus, tulip, statice, stock, dendrobium, prairie gentian, oncidium, campanula, gloriosa, fern, gypsophila, freesia, lobelia, triteleia, and gerbera were resistant to radiation, while chrysanthemum, rose, lily, calla, antherium, sweet pea, and iris were sensitive. Radiation-induced deterioration of chrysanthemum could be prevented by post-irradiation treatment with commercial preservative solutions or sugar solutions.

1. INTRODUCTION

The number of cut flowers imported to Japan has been rapidly increasing, and currently more than 800 million stalks of cut flowers are imported. Around 100 million stalks do not pass quarantine inspection and are subjected to quarantine treatments, generally fumigation with methyl bromide or cyanide, in methods that cannot treat a large quantity of flowers at the same time. Fumigation with methyl bromide is not desirable from the viewpoints of both human health and environment, and the development of alternative methods is required. One such method is radiation treatment, and the Japanese Government is interested in the irradiation of cut flowers with electron beams. However, not enough data are available on the effects of radiation on pests and cut flowers to prove the efficacy of irradiation as a quarantine treatment. We examined the effects of electron beams on various pests and cut flowers and evaluated a method to prevent radiation-induced damages of host commodities.

2. MATERIALS AND METHODS

2.1. Cut flowers

Cut flowers were purchased at a flower market 2 days before irradiation. Irradiated flowers were soaked in tap water without preservatives and stored at ambient temperature.

2.2 Test insects

2.2.1. Spider mites

Two spotted spider mites, *Tetranychus urticae*, obtained from the Faculty of Horticulture of Chiba University were tested. Mites were reared on kidney bean leaves at 22°C and 70% RH under an illumination of 16L:8D. The duration of egg, larval and nymphal stages was 4-7 days and 7-9 days, respectively. Adult females were obtained about 9 days after hatching [1].

2.2.2. Leafminers

Twenty females of the leafminer, *Liriomyza trifolii*, were released in a cage which contained 3 kidney bean seedlings. Each seedling had only the first two true leaves, and the cut end of the stem was soaked in water. The flies were allowed to lay eggs in leaves freely for hours. After eggs were deposited, the leaves were cut at the petioles and moved to petri-dishes with agar. The cut end was put into agar to prevent the leaf from drying [2].

2.2.3. Mealybugs

Comstock mealybugs, *Pseudococcus comstocki*, were reared on pumpkin at 23°C and 70% RH in the dark. The developmental duration of eggs was 12 days. Adult males emerged in about 30 days, and adult females laid eggs about 50 days after hatching [3].

2.2.4. Thrips

Thrips palmi individuals were obtained from the National Research Institute of Vegetable, Ornament Plants and Tea in Ano, Mie. *Thrips tabaci* were field-collected. The insects were reared with cucumber leaves in a cage that was covered with Bemberg nets (Asahi Chemical Industry Co.Ltd.) at 25°C and 60-80% RH, under an illumination of 16L:8D. Under these conditions, the duration of eggs, larval stages, and pupal stage were 4-5 days, 4-6 days, and 4-5 days, respectively. Adults were obtained about 10 days after hatching [4].

Ten adult females were allowed to oviposit into a cucumber leaf which was put on a filter paper in a polyethylene bag. Collections of eggs of *T. palmi* and *T. tabaci* were performed for 3 days and for 1 day, respectively. The eggs laid in the cucumber leaves were held under the rearing conditions above until irradiation. Second instars and adults for irradiation were collected from the colonies in a rearing cage and placed on a cucumber leaf in a polyethylene bag.

2.2.5. Cutworms

Spodoptera litura was obtained from Yokohama Research Center, Mitsubishi Chemical Corporation. Seven or eight adult pairs were allowed to oviposit on the inside of a tracing paper bag at 25°C and 60-80% RH with illumination of 16L:8D. The eggs were put in a petri dish and held under the above conditions for preparing 1-, 2-, 3-, and 4-day-old eggs for irradiation. Larvae were given an artificial diet (Insecta LF, Nihon Nosan Kogyo K.K.) in a plastic container (21x29x10cm). Third instars were obtained 6-7 days after hatching and fifth instars were obtained 12-13 days after hatching, and prepared for irradiation. Newspaper straps were spread in the container for their pupation. Pupae were obtained about 20 days after hatching [5].

2.2.6.Aphids

Myzus persicae was obtained from the Yokohama Research Center, Mitsubishi Chemical Corporation. Aphids were reared on leaves of potted Chinese cabbage at 25°C and 60-80% RH with

illumination of 16L:8D. Two adult females were allowed to deposit nymphs on an uninfested Chinese cabbage leaf, the base of which was covered with moistened cotton and aluminum foil in a Petri-dish. The adult females were removed after 24 hours. The nymphs on the leaf were kept at 25°C under an illumination of 16L:8D for 5 days to obtain 3rd and 4th instars and adults for irradiation.

2.3. Irradiation

One gamma-ray irradiator, the Gammacell 220 from Nordion International Inc., installed at National Food Research Institute, and two electron accelerators, the Van de Graaff electron accelerator from Nissin High Voltage Engineering Co., LTD, installed at the National Food Research Institute and the Dynamitron from Radiation Dynamics Inc., installed at Tsukuba Electron Irradiation Center, Sumitomo Heavy Industries, LTD were used to irradiate the biological samples. Insects were irradiated with the Van de Graaf electron accelerator and the gammacell, and cut flowers were irradiated with the Dynamitron and the gammacell.

3. RESULTS AND DISCUSSION

3.1. Comparative effects of gamma-rays and electron beams on insects

A smaller number of adults from gamma-irradiated pupae of the flour beetle, *Tribolium freemani*, laid eggs as compared with adults from electron-irradiated pupae (Table I). The hatchability of eggs laid by the adults from gamma-irradiated pupae was slightly lower than that from electron-irradiated pupae (Table I). Younger eggs of the two spotted spider mite, *Tetranychus urticae*, were more sensitive to both gamma-rays and electron beams (Table II) [6]. The effect of gamma-rays on the hatchability of the eggs was slightly larger than that of electron beams, although the difference was insignificant (Table II).

These results (Tables I and II) indicate that the insecticidal effects of gamma-rays are the same as, or slightly greater, than those of electron beams, which agrees with results reported previously [7].

3.2. Effects of "Soft-electrons" on flour beetle

Soft-electrons or low-energy electrons at 170 keV and 4 μA for 15 min (ca.7.5 kGy) inactivated eggs, larvae, pupae, and adults of *Tribolium freemani* immediately after irradiation. Larvae and adults were alive without moving for 1 week and then died. Treated eggs did not hatch and the pupae did not develop into adults. All stages of the flour beetle exposed to 170 keV-electrons for 45 min or 200-keV electrons for 15 min were dead immediately after the treatment. Such electrons penetrate only 50-150 μm of materials and enables decontamination of dry food ingredients with little quality

TABLE I. STERILITY OF *Tribolium freemani* FEMALE ADULTS FROM IRRADIATED PUPAE

	Eggs laid (%)		Eggs hatched (%)	
Dose	Gamma-ray	Electron beam	Gamma-ray	Electron beam
100Gy	0	0	---	---
50Gy	0	13.3	---	0
25Gy	40.0	88.2	16.1	44.6
10Gy	93.3	93.3	55.8	73.9
0Gy	85.0	82.7		

TABLE II. PERCENT HATCHABILITY OF *Tetranychus urticae* EGGS IRRADIATED WITH GAMMA-RAYS OR ELECTRON BEAMS

Age of egg	Dose (Gy)	Electron beam	Gamma-ray
3-days-old	50	90.4 ± 6.3	88.3 ± 11.2
	100	89.4 ± 6.8	88.6 ± 6.7
	150	84.1 ± 11.8	82.8 ± 7.8
	200	69.6 ± 12.8	65.4 ± 7.8
4-days-old	200	97.7 ± 3.8	95.7 ± 4.8
	400	96.0 ± 4.3	84.7 ± 7.8
	600	77.5 ± 9.4	76.8 ± 13.3
	800	52.6 ± 16.7	50.7 ± 13.9
	1000	29.7 ± 20.2	23.4 ± 22.2

change[8,9]. Soft-electrons can reduce both microorganisms and insects contaminating dry food ingredients such as grains, pulses and spices with little quality deterioration.

3.3. Effects of electron beams on insects

Six different insects subjected to the experiment showed different sensitivities to electron beams (Table III) [1-5]. Leafminer and cutworm were highly sensitive to radiation, and spider mite

Table III. - EFFECTS OF ELECTRON BEAMS ON INSECTS

Species	Stage	100Gy	200Gy	400Gy	600Gy
Spider mite	egg			O	O
(Tetranychus urticae)	larva & nymph			O	O
	adult		X	O	O
Mealybug	egg		O	Z	Z
(Pseudococcus comstocki)	larva		O	O	O
	adult		X	O	O
Leafminer	egg	Z	Z		
(Liriomyza trifolii)	larva	Z	Z		
	pupa	Z	Z		
Thrips	egg	Z	Z	Z	
(Thrips palmi)	larva	O	O	O	
	adult	X	X	O	
Thrips	egg	Z	Z	Z	
(Thrips tabaci)	larva	O	O	O	
	female adult	X	X	O	
Cutworm	egg	Z	Z	Z	
(Spodoptera litura)	larva	Z	Z	Z	
Aphidnymph & adult		O	O	O	
(Myzus persicae)					

Z = dead, inhibition of adult emergence; O = sterilized; X = no effect

and mealybug were less sensitive. Electron-irradiation at 400 Gy inhibited the hatching, larval growth, pupation, adult-emergence, and/or oviposition and sterilized the adults, irrespective of the species and stage of insect, which suggests that 400 Gy is the optimum dose for inactivation of pests for cut flowers.

3.4. Effects of electron beams on cut flowers

Carnation (5 cultivars), Alstromeria (2 cultivars), Gladiolus (3 cultivars), Tulip (5 cultivars), Statice (2 cultivars), Stock (2 cultivars), Dendrobium, Prairie gentian, Oncidium, Campanula, Gloriosa, Fern, Gypsophila, Freesia, Lobelia, Triteleia, and Gerbera were tolerant to 400 Gy of electron beams. Some of these cut flowers showed slight deterioration caused by irradiation at 400 Gy, but not to the degree that they are not marketable. Chrysanthemum, rose, lily, calla, antherium, sweet pea and iris were intolerant (Table IV) [10-12]. The detrimental effects of irradiation were delay/inhibition of flowering, withering/browning of flowers and leaves and bending of petioles.

3.5. Prevention of radiation-induced deterioration of chrysanthemum

Commercial floral preservative solutions and aqueous solutions (2%) of sucrose, glucose, fructose, and maltose delayed bloom wilting and foliage yellowing of cut chrysanthemums caused by irradiation at 750 Gy ; irradiated chrysanthemum stems placed in such sugar solutions showed almost the same vase-life as unirradiated stems placed in water (Table V) [13-16]. Solutions of 8-hydroxyquinoline sulfate (HQS), silver thiosulfate (STS), sodium dodecylbenzenesulfonate (DBS), polyoxyethylene lauryl ether (PEL), potassium sorbate, mannitol, sorbitol, glycerol, 6-benzylamino purine (BA), and gibberelline (GA) did not reduce radiation -induced deterioration. Placing chrysanthemum stems in 2% sucrose prior to and during the time of irradiation did not influence the vase-life (Table VI), but placing chrysanthemum stems in 2% sucrose following irradiation prolonged vase-life. These results indicate that only sugars influence post-irradiation metabolism responsible for radiation-induced deterioration of chrysanthemum cut flowers. Sugar solution also prevented foliage yellowing of roses caused by irradiation.

Table IV. CUT FLOWERS INTOLERANT TO ELECTRON BEAMS

Species	Adverse effects
Chrysanthemum	flower wilting, browning of inflorescence core, foliage yellowing
Rose	delay and inhibition of flowering, foliage yellowing
Lily	delay and inhibition of flowering, withering and browning of petals
Calla	browning of bract leaf, petiole bending
Antherium	withering of bract leaf
Sweet pea	abscission of flower
Iris	foliage yellowing, necrosis of bud edge

4. CONCLUSIONS

The effects of electron beams on insects were slightly smaller than the effects from gamma rays, so doses necessary to disinfest cut flowers with electron beams will be slightly higher than with gamma rays. All the pests for cut flowers can be inactivated by electron beams at 400 Gy. Some cut flowers are tolerant to electron beans at 400 Gy and others are intolerant. Electron-irradiation is practical for cut flowers that are resistant to radiation. Radiation-induced deterioration of chrysanthemum can be prevented by sugar solutions.

TABLE V. QUALITY OF IRRADIATED CHRYSANTHEMUMS HELD IN VARIOUS VASE SOLUTIONS FOR 28 DAYS AT 25°C.

Vase solution	Onset of flower wilting (days)[z]	Onset of leaf yellowing (days)[y]	Flowers fresh weight (g)[x]	No. of yellowed leaves[w]
water	8.3 ± 2.2a[u]	5.9 ±0.9a	3.17 ±0.51a	25.0 ±0.0a
0.02% HQS	8.5 ±2.5a	5.5 ±1.8a	3.56 ±0.48a	25.0 ±0.0a
0.1% sorbate	7.9 ±1.8a	6.1 ±1.5a	3.33 ±0.79a	25.0 ±0.0a
0.03% DBS	8.0 ±2.2a	6.2 ±1.1a	3.75 ±0.81a	25.0 ±0.0a
0.01% PEL	8.3 ±2.5a	6.3 ±1.6a	3.55 ±0.76a	25.0 ±0.0a
0.024% STS	7.8 ±2.6a	5.9 ±1.8a	3.78 ±0.79a	25.0 ±0.0a
0.01% BA	8.2 ±2.7a	5.5 ±1.8a	3.88 ±0.68a	25.0 ±0.0a
0.001% GA	8.6 ±2.4a	5.9 ±1.8a	3.68 ±0.88a	25.0 ±0.0a
2% glycerol	7.9 ±2.5a	5.7 ±1.8a	3.21 ±0.92a	25.0 ±0.0a
2% mannitol	7.8 ± 2.8a	5.8 ±1.6a	3.59 ±0.78a	25.0 ±0.0a
2% sorbitol	8.3 ±2.9a	5.8 ±1.7a	3.03 ±0.87a	25.0 ±0.0a
2% sucrose	22.0 ±1.8b	19.9 ±2.6b	13.56 ±0.84b	2.0 ±1.2b
2% glucose	21.5 ±1.4b	19.2 ±2.1b	13.25 ±0.78b	2.2 ±0.9b
2% fructose	22.6 ±1.5b	19.1 ±1.9b	12.98 ±0.91b	2.0 ±0.8b
2% maltose	22.6 ±1.4b	18.9 ±2.3b	13.32 ±0.89b	2.2 ±1.0b
sucrose+HQS	22.0 ±1.7b	18.2 ±2.2b	12.58 ±1.09b	2.5 ±0.9b
sucrose+sorbate	22.5 ±1.6b	19.6 ±1.9b	13.35 ±0.85b	2.6 ±1.1b
Control[v]	22.3 ±1.7b	19.4 ±2.0b	7.93±0.83c	2.4 ±1.3b

[x]Days after irradiation when the first wilted flower was observed, [y]Days after irradiation when the first yellowed leaf was observed, [x]Flower fresh weight 21 days after irradiation, [w]Number of yellowed leaves out of 25 on five stems 21 days after irradiation, [v]Unirradiated chrysanthemums held in water, [u]Means in a column followed by the same letter are not significantly different (Duncan's multiple range test, $P = 0.05$).

TABLE VI. QUALITY OF IRRADIATED CHRYSANTHEMUMS TREATED WITH SUCROSE BEFORE, DURING, AND/OR AFTER IRRADIATION, AND HELD FOR 28 DAYS AT 25°C

Treatment	Onset of flower wilting (days)[z]	Onset of leaf yellowing (days)[y]	Flower fresh weight (g)[x]	Number of yellowed leaves[w]
water-water[v]	8.2 ± 2.3a[t]	5.7± 1.7a	3.09 ± 0.57a	25.0± 0.0a
2% sucrose-water	7.9 ± 2.1a	6.2 ± 1.3a	3.25 ± 0.59a	25.0 ± 0.0a
2% sucrose-2% sucrose	22.5 ± 1.8b	18.2 ± 2.3b	13.56 ± 1.05b	2.2 ± 1.2b
water-2% sucrose	21.6 ± 1.5b	19.1 ± 2.1b	12.85 ± 0.97b	2.9 ± 1.3b
Control[u]	22.9 ± 1.8b	19.8 ± 1.9b	8.25 ± 0.87c	2.8 ± 1.5b

[z]Days after irradiation, [y]Days after irradiation, [x]Flower fresh weight 21 days after irradiation, [w]Number of yellowed leaves out of 25 on five stems 21 days after irradiation, [v]Stems were held in the first solution before and during irradiation, and in the second solution after irradiation, [u]Unirradiated chrysanthemum held in water, Means separated by Duncan's multiple range test. Values in the same column separated by different letters are significantly different ($P = 0.05$).

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IRRADIATION AS A QUARANTINE TREATMENT OF CUT FLOWERS, GINGER AND TURMERIC AGAINST MITES, THRIPS AND NEMATODES

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Abstract

Effect of radiation on different developmental stages of mites, thrips, and nematodes were observed to determine their sterility doses and to develop a method for detection of irradiated and unirradiated specimens. A brief survey on cut - flower and tuber associated pests, and their biological study along with the tolerance level of host products were conducted. Mites *Oligonychus biharensis* (Hirst) and *Tetranychus* sp., as well as four species of thrips viz. *Retithrips syriacus* (Mayet), *Haplothrips gowdeyi* Franklin, *Frankliniella intonsa* Tribom, and *Microcephalothrips abdominalis* Crowford were recognized as common pests damaging plants and cut-flowers. Common species of nematodes infesting ginger and turmeric were *Meloidogyne* spp. and *Ditylenchus* spp. Results indicated that a dose 0.2 kGy and above caused complete sterility of male and female mites and insects. Various preadult developmental stages required less irradiation dose (0.05 - .1 kGy) for sterilization. Variation of melanization in treated and untreated life stages of mites and thrips could not be observed even at 0.2 kGy with the 2 - methyl DOPA spot test. Inhibition of melanization in irradiated pupal stages of thrips were observed at doses above 0.4 kGy. Both irradiated and unirradiated thrips were identical in their protein banding pattern. Virtually no protein bands were observed in irradiated and unirradiated nematodes when samples were run on 5% PAGE in TBE. Tube rose and marigold treated with higher dose (0.3 to 0.5 kGy) caused no remarkable morphological degradation for 7-8 days after irradiation. Nematodes were resistant to radiation. Complete elimination and abnormalities of J_2 stages of *Meloidogyne* spp. and *Ditylenchus* spp. were not observed even at 4.0 kGy although significant weight loss and spoilage of tubers were recorded after 14 days of radiation exposure.

1. INTRODUCTION

Different varieties of cut-flowers grown in Bangladesh have promising economic potential and prospects in international trade. The plants and flowere may be infested with various pests, including mites, thrips, aphids and moths inflicting considerable damage to cash earning crops. Control of these pests are essential for boosting production as well as satisfying quarantine requirements. At present strict and effective quarantine measures are applied to prevent the establishment of pests into areas where they do not occur. Moreover, use of pesticides and fumigants are likely to be restricted in near future because of their residual and hazardous problems. Among the various methods of quarantine requirements, radiation treatment emerged as one of the most suitable and practical alternatives for fresh commodities including cut-flowers. Two species of mites, *Oligonychus biharensis* (Hirst) and *Tetranychus* sp., are most abundant and found throughout the year. Four species of thrips collected from cut - flowers and flowering plants were identified in co-operation with "The Identification Services" of the International Institute of Entomology, London, UK. These species are *Retithrips syriacus* (Mayet), *Microcephalothrips abdominalis* Crowford, *Frankliniella intonsa* Trybom, and *Haplothrips gowdeyi* Franklin. Ginger and turmeric mainly used as spices are infested by various nematodes species causing damage in the field and in post harvest storage. Among various species of nematodes, *Meloidogyne* spp. and *Ditylenchus* spp. are common and widely spread

throughout the country [1] [2]. Before advocating irradiation as a suitable alternative method for cut-flowers, ginger, and turmeric, emphasis should be given on standardization of effective radiation dose and to identify the irradiated insects and mites present in the samples. Several studies have been made to distinguish irradiated insects from unirradiated ones. Rahman *et al.* [3] [4] and Nation *et al.* [5] studied the effects of radiation on the size of supraoesophageal ganglion in relation to the proventriculas in several tephritid fruit flies. Failure of melanization and a spot test for phenoloxidase activity with 2-methyl DOPA was developed by Nation *et al.* [6] for detection of irradiated tephritid fruit flies. Reduced melanization of the confused flour beetle, *Tribolium confusum* following radiation treatment has been observed by Ignatowicz [7]. Radiation sensitive protein markers have been detected in gamma irradiated larvae and pupae of oriental fruit flies by using SDS polyacrylamide gel electrophoresis [8].

Keeping the above views in mind the following investigations have been carried out during the past five years under this project:
(a) Biology and radiosensitivity of mites and thrips infesting cut-flowers.
(b) Determination of tolerance level and vase-life extension of cut-flowers applying radiation doses and low-temperature treatment.
(c) Isolation and culture of nematodes infesting ginger and radiation disinfestation of nematodes along with tolerance level of the plant materials.
(d) Methods for identification of treated insects, mites, and nematodes to determine ways to detect when any stage of these pests found in a particular treated produce have been irradiated.

2. MATERIALS AND METHODS

2.1. Rearing of mites and thrips

The biology and behavior of mites and thrips were studied both in the laboratory and under natural conditions. Two species of mites, *O. biharensis* and *Tetranychus* sp., as well as four species of thrips, *R . syriacus*, *H. gowdeyi*, *F. intonsa*, *M. abdominalis*, were frequently obtained from the experimental garden throughout the year . In case of mites, the biology and radiosensitivity of *O. biharensis* were considered for this study. Among the thrips, *R . syriacus* was the most common species causing maximum damage to rose gardens. These were used as experimental mites and insects and an attempt was made to determine the doses at their various life cycle stages to cause inability to reproduce. Rose leaves were chosen for laboratory rearing of the experimental mites and thrips [9] [10]. The eggs of both species were collected either from infested host leaves or from laboratory reared females. The egg laden host leaves were placed over water soaked cotton in petri dishes. All the life stages of mites were reared in the laboratory on rose leaves placed over water soaked cotton. The eggs of thrips were reared similarly but larvae, pupae, and adult thrips were cultured on rose leaves whose main stalks were usually inserted in wet cotton stubs placed in small plastic vials. The whole sets were kept in a glass beaker covered with fine meshed nylon net. Laboratory rearing were maintained at ambient temperature (25 $\pm$ 5°C, 60 $\pm$ 10% R.H.). The mites and thrips were transferred to fresh leaves from time to time to ensure food supply. The different life stages of the mites and thrips were observed under a binocular dissecting microscope.

2.1.1. Irradiation of mites and thrips

Radiosensitivity and reproductive efficiency of mites and thrips were determined after irradiating different developmental stages of *O. biharensis* and *R . syriacus* with doses ranging from 0.01 to 0.5 kGy for eggs, larvae, pupae, and adults. The crosses between irradiated female deutonymphs and normal males, and irradiated males with normal female deutonymphs were conducted to determine doses to cause inability of reproduction in *O. biharensis*. Sterility doses for males and females *R.. syriacus* were determined from the success of the F_1 generation after crossing

between treated and untreated adults. Sterilizing doses of developmental stages were determined from the unsuccess of the treated life stages up to adult transformation. Irradiation was from a Co^{60} source at a dose rate of 0.33 kGy / hr.

2.2. Detection of irradiated mites / insects.

2.2.1. Melanization spot test

Phenoloxidase activities in larvae, nymphs / pupae, and adults of *O. biharensis* and *R. syriacus* were determined with the help of the 2 - methyl DOPA test. Twenty mg of 2-methyl-DOPA was dissolved in 10 ml of sodium phosphate buffer (pH 7.2) and 50 µl of this solution containing 100 µg of the substrate was air dried on a non-absorbent transparent sheet. Samples of 50 irradiated and unirradiated larvae, nymphs / pupae and adults were crushed separately in one drop of tap water and the crushed sample was placed by micropipette on each of the dried spots of 2-methyl DOPA in order to observe any change of color. Radiation doses ranging from 0.01 to 0.2 kGy were applied to the biological organisms for this purpose. Morphological deformation and color change, if any, were observed after radiation exposure (0.01 - 0. 5 kGy) to detect the radiation treated species.

2.2.2. Electrophoresis

Electrophoresis was conducted on 5% PAGE in TBE buffers. Test samples were different stages of the life cycle of *R. syriacus*. The samples were irradiated at 20, 40, and 50 Gy. Four to five specimens from each stage were squashed in TBE and bromphenol blue 24 hr. after irradiation. These were run on a vertical slab gel apparatus at 240 V and stained with 0.5% Coomassie Blue. Samples of fruit fly pupae irradiated (50 Gy) at larval stage were run with them for comparison.

2.3. Disinfestation and vase life extension of cut flowers by irradiation and low temperature

Freshly cut marigold and tube rose were collected from the experimental garden and twelve bunches containing 10 flowers of each type were taken for studies. Each bunch of flowers was put inside a perforated polythene pouch and with a cotton plug. From time to time water was sprinkled on the cotton plug with a syringe. Two sets containing six bunches of each type of flower were prepared and irradiated at doses of 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 kGy. One set of irradiated flowers was kept at fixed temperature (15°C and 40 $\pm$ 5% R.H.) in an incubator, and the second set at ambient temperature (25 $\pm$ 5°C, 60 $\pm$ 10% R.H.) inside a glass box with a netted door. Observations were made regularly at 24 hr. intervals regarding pest incidence and storage ability of flowers.

2.4. Rearing of nematodes

Nematodes were maintained on fresh and uninfested ginger collected from the experimental garden. The uninfested ginger was innoculated with nematodes in the laboratory either by placing small amount of infested ginger or by few drops of water containing nematodes on the fresh ginger. After three days of intermixing, the gingers were stored in moist condition in petri dishes at the ambient temperature (25 $\pm$ 5° C and 60 $\pm$ 10% R.H.) for a period of 3 months.

2.5. Effect of radiation on ginger

Infested and uninfested ginger packed in 200g polythene bags were used to determine radiation sensitivity. Observations on the extent of damage, occurrence of nematodes, weight loss, and physical appearance of the experimental items were made at different intervals after treatment. A small piece of ginger was placed into 10 ml of water and shaken well for nematode collection. The collected nematodes were kept in distilled water for radiation treatment.

2.6. Irradiation of nematodes

Nematode infested and uninfested ginger was irradiated at doses ranging from 0.0 to 2.0 kGy. The effects of radiation on the J_2 stage of *Meloidogyne* spp. was determined from exposure of infested ginger with doses ranging from 0.0 to 4.0 kGy at a dose rate of 1.2 kGy / hr. Nematodes were collected and kept in distilled water prior to irradiation at the same dose to observe abnormalities and damage.

2.7. Detection of irradiated nematodes

Electrophoresis procedures for nematodes (*Meloidogyne* spp.) were similar to those used with thrips and fruit flies. Five specimens (J_2 stage) from irradiated and unirradiated samples were collected from the laboratory culture and squashed for this study. The sample was placed on the gel and electrophoresed.

3. RESULTS AND DISCUSSION

The life-cycle of the mites, *O. biharensis* and *R . syriacus,* was completed within 20 - 22 days from egg to adult at 25 $\pm$ 5° C and 60 $\pm$ 10% R.H. *O. biharensis* has five distinct stages in their life-cycle, the egg, larva, protonymph, deutonymph, and adult. *R . syriacus* completed its life-cycle in four stages, the egg, larva, pupa, and adult. There was only one larval instar in *O. biharensis, but* two larval instars were present in *R . syriacus*. The pupal stage in *R . syriacus* could be divided into two sub stages, pupa - I of one day duration and pupa - II with two days duration prior to adult emergence. *O. biharensis* laid eggs on both surfaces of leaves, and the thrips *R. syriacus* laid eggs inside the host tissue below the epidermal layer. The incubation period of both mite and thrips was about 8 days and the larval period / instars continued for 3 - 7 days. The nymphal and pupal period was completed in 4 - 6 days. Adult longevity varied from 20 - 30 days in the laboratory. *O. biharensis* and *R . syriacus* were capable of reproducing both sexually and parthenogenetically. The progenies from fertilized eggs were all females and males were produced parthenogenetically. Based on the mode of reproduction, it is believed that if the sex chromosome of the male is inactivated by a suitable dose of radiation, males that mate with normal females will give rise to unfertilized eggs resulting in only males in the F_1 generation. On the other hand, a female deutonymph treated with a sub lethal dose and crossed with normal males will either produce both males and females or will not lay any viable eggs if exposed to a sterilizing dose. Deutonymphs were utilized in this experiment only to ensure their virginity prior to desired pairings.

3.1. Male mite sterility

The irradiated males were apparently sexually active even after treatment with 0.5 kGy. They guarded the female deutonymph and mated with them immediately after adult emergence. An average of four eggs were laid by control females per day, but egg laying totally ceased during the last 2-3 days of adult life. From this cross an average of 21 females and two males were obtained in F_1 generation, giving a sex ratio of about 9:1 in the adult population. The average number of eggs laid by the normal female paired with treated males ranged from 12 - 31 (Table I). Longevity of males was about 13, 15, 13, 9, and 8 days, and hatchability of eggs was 72%, 48%, 33%, 29%, and 15% in the crosses of normal females with males treated with 0.1, 0.2 , 0.3, 0.4, and 0.5 kGy, respectively. The sex ratio started to become male biased from the normal 9:1 female to male ratio to 1:3 when 0.1 kGy treated males were crossed with normal females. Total cessation of production of F_1 females resulting from pairing of normal females with males irradiated with 0.2, 0.3, 0.4, and 0.5 kGy, producing only 8.6%, 16.6%, 14.3%, and 15.1% male progeny, respectively. It is apparent from the results that treating males with 0.2 kGy and above made them sterile, resulting in subsequent production of male progeny in the F_1 generation.

TABLE I. EFFECT OF RADIATION ON CHANGING SEX RATIO AND INDUCED STERILITY IN THE F_1 GENERATION IN *O. BIHARENSIS* WHEN CROSSES WERE MADE BETWEEN TREATED MALES AND UNTREATED FEMALES, AND UNTREATED MALES AND TREATED FEMALES.

Dose (kGy)	Untreated female x treated male				Treated female x untreated male			
	Mean ± (S.D.) eggs laid	Mean ± (S.D.) adult emergence		Male sterility	Mean ± (S.D.) eggs laid	Mean ± (S.D.) adults produced		Female sterility
		Females (F_1)	Males (F_1)			Females (F_1)	Males (F_1)	
0.0	25 (7.9)	21.0 (1.6)	2.0 1.16	None	16 (3.7)	9 (1.6)	1 (0.7)	None
0.1	25 (7.6)	1.5 (0.9)	4.5 (0.8)	Almost all	18 (4.5)	5 (1.6)	1 (1.22)	Some
0.2	31 (4.4)	Nil	2.5 (1.2)	All	6 (1.6)	1 (1.22)	Nil	Almost all
0.3	12 (3.2)	Nil	2 (1.0)	All	4.5 (1.9)	Nil	Nil	All
0.4	14 (2.92)	Nil	2 (1.0)	All	3.0 (1.6)	Nil	Nil	All
0.5	16.5 (5.7)	Nil	2.5 (1.5)	All	5 (2.7)	Nil	Nil	All

Female mite sterility : No effect on adult emergence from the deutonymphal stage to adult has been observed up to 0.3 kGy. About 50% eggs hatched in 0.1 kGy treated females compared to 100% egg hatch in control pairs. The average number of eggs laid by females treated with 0.2, 0.3, 0.4, and 0.5 kGy when crossed with normal males were 6.0, 4.5, 3.0, and 5.0 eggs, respectively. All eggs laid by females treated with 0.3 kGy failed to hatch. It appeared that the sterility dose for adult females lies within 0.2 kGy. This result also agrees with the sterility dose of 0.25 kGy for male and female mites as reported by Ignatowicz [11].

Mite egg sterility : All control eggs hatched and passed through the subsequent developmental stages to become adults, except 5.9% due to factors other than radiation. Egg hatchability gradually decreased from 90.5 - 76.4%, resulting in still lower percent of adults (79.7 - 38.0%) when treated with 10.0 - 50.0 Gy. Only a few percent (6.0 - 3.4%) of eggs hatched after exposure to $\geq$ 60.0 Gy, and all of them failed to reach the adult stage.

3.2. Radiosensitivity of thrips

Table II indicates the effects of radiation on eggs, larvae, pupae, and adults of *R . syriacus*. Eggs (5 - 6 days) treated with 0.1 kGy showed some sort of development initially, but failed to be transformed into the adult stage. The early eggs (0 - 24 hr.) of *R . syriacus* irradiated with 0.1 kGy were damaged, and without further development. Larvae (I and II) treated with 0.1 kGy and above also did not to develop to the following life stage. A few adults were obtained from late eggs and larvae exposed to radiation dose of 0.05 kGy, whereas most of the unirradiated eggs and larvae successfully developed into adults in the laboratory. Pupae (I and II) exposed to a dose of 0.01 - 0.25 kGy showed 86 to 99% adult emergence. Less than 50% adult emergence of the above two stages were observed when treated with $\geq$ 0.3 kGy, and adult emergence totally stopped after exposure of $\geq$ 0.5 kGy. Eggs laid by emerging adults that were treated with $\geq$ 0.15 kGy failed to develop into adults. When adult males and females (1 - 2 days age) were exposed to an irradiation

TABLE II. ADULT *R. SYRIACUS* EMERGENCE AND STERILITY OF ADULTS AFTER VARIOUS DEVELOPMENTAL STAGES WERE TREATED WITH DIFFERENT DOSES OF GAMMA RADIATION.

Dose (kGy)	Egg (5-6 days)		Larva I + II		Pupa I + II		Adult (1-2 days)	
	% adults emerging Mean ± (S.E.)	% Sterility Mean ± (S.E.)	% adults emerging Mean ± (S.E.)	% Sterility Mean ± (S.E.)	% adults emerging Mean ± (S.E.)	% Sterility Mean ± (S.E.)	% Sterility ± S.E (Male)	% Sterility ± S.E (Female)
0.0	86.0 (1.4)	0.0	94.0 (2.5)	0.0	100	0.0	0.0	0.0
0.01	81.0 (1.87)	6.3 (1.08)	85.3 (1.78)	4.3 (1.08)	99.3 (2.12)	2.6 (1.08)	7.0 (1.41)	2.6 (0.81)
0.025	65.0 (1.9)	44.3 (2.16)	68.6 (2.95)	26.3 (2.55)	96.6 (1.47)	13.6 (2.49)	27.0 (3.59)	22.0 (2.27)
0.05	24.0 (1.87)	88.6 (2.40)	40.0 (2.16)	693 (2.95)	98.6 (1.08)	66.6 (4.3)	68.6 (3.56)	64.0 (2.1)
0.10	0.0	100	0.0	100	97.3 (1.5)	83.6 (3.9)	90.0 (4.96)	85.0 (3.25)
0.15					98 (1.41)	100	100	100
0.20					94.0 (2.55)			
0.25					86.0 (1.41)			
0.30					49.0 (2.12)			
0.35					31.3 (2.95)			
0.40					17.3 (2.95)			
0.45					4.3 (1.78)			
0.50					0.0			

dose of 0.15 kGy and paired, the females laid 15-20 eggs but they did not hatch. In controls the total number of eggs laid by a pair within the life span varied from 18 - 25. A dose for eggs and larvae that results in inability to reproduce was 0.10 kGy, whereas a higher dose of 0.15 kGy was required for pupae and adults.

3.3. Identification of irradiated insects / mites

Pupae I and II of *R. syriacus* are reddish in color and the adults are black in general appearance. The enzyme responsible for blackening the body of the adults apparently starts in the early pupa II stage. None of the pupa I attained adulthood, although some of them were transformed to pupa II stage even after exposure of 0.5 kGy. Pupae II that developed from pupae I treated with 0.5 kGy were always reddish in color. However, pupae II that transformed from pupae I treated with 0.4 kGy showed blackening in about 50% of the individuals, while the rest appeared reddish without showing any sign of melanization. Thus, in *R. Syriacus* thrips, the occurrence of reddish pupae I and II in radiation treated floricultural produce is an indicator of their exposure at least to a dose of 0.5 kGy.

The 2-Methyl DOPA test for larvae (I and II), pupae (I and II), and adults of *R . syriacus* was negative, and did not produce the red color turning to black color that indicates phenoloxidase. Third instars of the tephritid fruit fly, *Bactrocera cucurbitae,* did give a positive test, turning the 2-methyl DOPA spot black. Second instars of *B. Cucurbitae* irradiated with 0.04 kGy transformed into third instars, the spot test with such a third instar was negative indicating the lack of phenoloxidase after the radiation treatment. These results agree with those found in other tephritid fruit flies [6] [12]. The 2-methyl DOPA test for larvae, protonymphs, deutonymphs, and adults of *O. biharensis* was negative for both control and irradiated samples. All the stages of irradiated and unirradiated *R.*

syriacus were identical in their protein banding pattern, whereas in the fruit flies the banding patterns in the irradiated samples were different from those of the controls as indicated by the absence of a protein band in pupae.

3.4. Vase life of flower

No difference could be detected between the control and irradiated samples of marigold and tube rose after irradiation with respect of physical condition, color, and odor. All flowers treated up to a dose of 0.5 kGy were acceptable for keeping in the vase for 7 - 8 days under laboratory conditions, but flower bunches kept at low temperature (15° C, 40 ± 5% R.H.) were in good condition for about 12 - 14 days. About 8 days after radiation treatment (0.5 kGy), flowers became shrunken with curling of petals, and black spots started to appear on the sepals. A large number of mites and thrips, along with other pests, were observed in the polythene bag containing control flowers at ambient temperature. Thus, irradiation and low temperature treatment seemed to be effective for disinfestation and shelf-life extension of marigold and tube rose.

3.5. Nematodes infesting ginger

The contaminated ginger showed heavy infestation of nematodes after 2 - 3 weeks. Both irradiated and unirradiated ginger contained various species of nematodes. Among the ematodes, *Meloidogyne* spp. and *Ditylenchus* spp. were most common, but there also were other species. Rotting, blackening at the budding zones, and lesions were observed. Table III represents the mortality of second stage of Juveniles (J_2) of *Meloidogyne* spp. in ginger at different intervals after exposure to 0.0 kGy to 4.0 kGy gamma radiation. Results after 14 days indicated that only 34.2% reduction of J_2 stages was obtained at 4.0 kGy. Significant deterioration in color and texture was observed at 1.0 kGy and above. Most of the sample irradiated above though the dose level affects the quality of ginger within this period. Virtually no protein band and deformities were observed in irradiated and unirradiated nematodes when samples were run on 5% PAGE in TBE and were observed under the microscope. Table IV represents loss of weight of control and irradiated gingers in ambient temperature at different intervals. In controls the average weight loss was 67% after 90 days. In contrast the weight loss in irradiated samples was quicker and higher. Significant deterioration in color and texture was observed at 1.0 kGy and above. Most of the samples irradiated above 1.0 kGy became brittle after 45 days of observations. After 30 days, these became unacceptable for marketing and human consumption. Because of deterioration of the qualities of the product, the highest dose (4.0 kGy) was not applied for storage studies.

TABLE III. MORTALITY OF J_2 STAGES OF *MELOIDOGYNE* SPECIES OF NEMATODES IN GINGER AT DIFFERENT DOSES OF RADIATION.

Dose (kGy)	Mean percentage of mortalities on different days ± (S.E.)							
	0	2	4	6	8	10	12	14
0.0	-	0	1.26 (0.10)	2.5 (0.18)	3.3 (0.21)	3.6 (0.14)	5.13 (0.21)	6.5 (0.69)
1.0	-	0	1.86 (0.21)	2.6 (0.12)	4.96 (0.42)	6.8 (0.42)	6.13 (0.49)	9.13 (0.55)
2.0	-	1.12 (.11)	1.93 (0.17)	2.6 (0.12)	6.26 (0.29)	8.53 (0.43)	12.2 (0.35)	16.33 (0.91)
3.0	-	1.60 (0.14)	2.23 (0.22)	5.73 (0.49)	7.5 (0.21)	14.93 (0.29)	17.73 (0.80)	29.4 (0.65)
4.0	-	2.0 (0.24)	2.63 (0.17)	6.06 (0.29)	11.86 0.67	16.53 0.85	18.26 (1.56)	34.2 (1.43)

TABLE IV. PERCENTAGE OF WEIGHT LOSS OF IRRADIATED AND UNIRRADIATED GINGER AFTER THREE MONTHS OF STORAGE AT AMBIENT TEMPERATURE.

Doses (kGy)	Mean percentage of weight loss after varying storage time in days ± (S. E.)						
	0	15	30	45	60	75	90
0.0	-	21.6	29.9	41.5	56.8	62.4	67.6
		(5.8)	(3.6)	(6.3)	(7.4)	(3.8)	(4.4)
0.50	-	29.4	45.8	62.4	60.2	74.4	77.3
		(4.7)	(6.4)	(5.8)	(2.8)	(2.2)	(2.2)
1.00	-	35.6	52.5	70.4	74.6	89.4	90.4
		(4.9)	(6.6)	(5.4)	(4.2)	(2.0)	(1.2)
1.50	-	37.4	60.4	71.3	80.2	89.4	91.3
		(5.5)	(6.7)	(6.6)	(4.4)	(1.2)	(1.2)
2.00	-	31.6	63.3	87.5	91.2	91.8	92.0
		(4.7)	(4.1)	(4.6)	(2.4)	(1.2)	(0.88)

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POST HARVEST CONTROLLING OF ORCHID THRIPS ON CUT FLOWERS BY IRRADIATION

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Abstract

Post-harvest controlling of orchid thrips, *Thrips palmi* Karny on cut flowers by irradiation was conducted during October 1992 to September 1997 at the Thai Irradiation Centre (TIC) and Division of Entomology and Zoology, Department of Agriculture, Thailand. The studies were carried out by conducting experiments on irradiation of cut flowers for controlling thrips with doses ranging from 0.1 to 1 kGy. The vaselife of radiated cut flowers was evaluated. Colonies of thrips were established in the laboratory in order to determine radiation sensitivity of various development stages of thrips and also to assess the occurrence of natural infestations by examining commercial market quality flowers from growers where management practices can be identified. Results from five years of research on post harvest control of thrips on orchids and cut flowers by irradiation showed that despite intensive investigation, difficulty in permanent establishment of a laboratory colony of *Thrips palmi* Karny for bioassays continued. The snap bean rearing method for rearing large number of thrips has bean developed, although it is less satisfactory than desirable. It has given sufficient numbers for testing in the 6th experiment. The maximum dose tolerated by *Dendrobium* orchid flowers at ambient temperature (25 - 30 °C) was below 0.5 kGy, but at a pre-and post irradiation temperature 15 - 18 C, the maximum dose tolerated approached 0.75 - 0.8 kGy. The effective dose for control *Thrips palmi* Karny, however, was higher than 0.75 kGy.

1. INTRODUCTION

Thrips are important agricultural pests not only because of the mechanical injury caused by their feeding, but they also are vectors for bacterial, fungal and viral disease organisms. They are abandant in vegetation and live mainly in flowers without any noticeable damage. Among the pests of ornamental crops in Thailand, the orchid thrips *Thrips palmi* Karny and *Dichromothrips corbetti* Prisner are considered to be the most destructive sucking insect in both the high land and low land areas. Severe damage causes silvery or whitish patches that gradually become light brown to brown, and finally the flowers fall off. The few research reports on thrips in Thailand deal mainly with which chemical control method can be used, but sensitivity to a number of commercially available insecticides is low. Eggs are deposited in plant tissues where the larvae feed. They pupate in the soil. These properties make it difficult to control thrips with chemicals unless they are sprayed many times. The major problems from orchid thrips in Thailand is not only the severe damage on cut flowers, especially orchid and chrysanthemum, but also the presence of thrips found in export products, and then necessitating a quarantine treatment. In order to avoid or reduce the use of pesticides, radiation of orchid cut flowers is an alternative that needs intensive study. The orchid flowers irradiated and studied in this report were obtained and packed both from growers and exporting companies in Bangkok, Thailand.

2. MATERIALS AND METHODS

2.1. Assessing the occurrence of natural infestation

Monthly investigations, explorations, and recordings were made from 1992-1997 in five locations of commercial and local market quality from growers. The result showed that the infestation of thrips in cut flowers for exportation from October 1992 to December 1995 was low and averaged 4.48% - 5.7% (Tables I, II). Infestation increased to 8.44 - 12.91 % in 1996-1997 (Table III).

Possibly the attractive cost of cut flowers during 1992 to 1997 may have induced growers to use more pesticide to control thrips. Without control, thrips caused 100% infestation of plants and damaged plants in many ways, such as producing galls, causing patching or white spot on flowers, and feeding on the sap of living plant cells. If the flower surface is fed on extensively, silvery, whitish, or colorless patches are formed. These patches or spots gradually become light brown to brown, and finally the flowers fall off.

2.2. Mass rearing of thrips

Mass rearing of thrips is very difficult, because of the lack of proven mass rearing techniques. Thrips were collected from areas where high usage of pesticides has occurred, and lack of a susceptible strain made evaluation of resistant and susceptible tests difficult. Rearing studies were divided into two parts: first, the use of natural diets, and second, the use of artificial diets.

2.2.1. The use of natural substrate

Live thrips were collected from orchid growing areas and reared on 6 different plant flowers (as a natural diet) including orchid (*Vanda spp.),* okra (*Abelmoschus esculentus*), chrysanthemum (*Chrysanthemum hortorum),* egg plant (*Solanum melongena*), cucumber (*Cucumis spp.*), and watermelon *(Citrullus lanatus).* The result showed that thrips appeared to only survive tested crop flowers, and populations of *Thrips palmi* Karny did not increase enough for testing.

TABLE I. ORCHIDS INFESTED BY THE THRIPS *Thrips palmi* Karny IN 1992-1993.

Month 1992	No. of orchid flowers Examined	Infested	% infestation	Month 1993	No. of orchid flowers Examined	Infested	% infestation
JAN	200	10	5	JAN	250	11	4.4
FEB	250	14	5.6	FEB	200	13	6.5
MAR	370	29	7.83	MAR	380	39	10.26
APR	150	13	8.66	APR	200	26	13
MAY	100	8	8	MAY	150	14	9.33
JUN	250	11	4.4	JUN	250	11	4.4
JUL	200	3	1.5	JUL	300	8	2.66
AUG	170	6	3.52	AUG	200	9	4.5
SEP	350	8	2.28	SEP	400	15	3.75
OCT	400	5	1.25	OCT	320	9	2.81
NOV	300	7	2.33	NOV	300	7	2.33
DEC	350	12	3.42	DEC	190	4	2.1
Total	3,090	126	53.79	Total	3,140	166	66.04
Average	2,575	105	4.48	Average	261.66	13.83	5.5
Max	400	29	8.66	Max	400	39	13
Min	100	3	1.25	Min	150	4	2.1

TABLE II. ORCHIDS INFESTED BY THE THRIPS *Thrips palmi* Karny IN 1994-1995.

Month 1994	No. of orchid flowers Examined	Infested	% infestation	Month 1995	No. of orchid flowers Examined	Infested	% infestation
JAN	180	9	5	JAN	190	9	4.73
FEB	220	16	7.27	FEB	210	9	4.28
MAR	300	22	7.33	MAR	350	39	11.14
APR	160	14	8.75	APR	180	21	11.66
MAY	140	9	6.42	MAY	160	17	10.62
JUN	180	7	3.88	JUN	220	13	5.9
JUL	190	6	3.15	JUL	200	17	8.5
AUG	150	7	4.66	AUG	170	9	5.29
SEP	230	5	2.17	SEP	-	-	-
OCT	300	7	2.33	OCT	210	7	3.33
NOV	280	3	1.07	NOV	300	4	1.33
DEC	340	11	3.23	DEC	290	5	1.72
Total	2,670	116	55.26	Total	2,480	150	68.5
Average	223	9.66	4.6	Average	206.66	12.5	5.7
Max	340	11	8.75	Max	350	39	11.66
Min	140	3	1.07	Min	150	4	1.72

2.2.2. Mass rearing by the snap bean method

This thrips rearing method was learned from Dr. Ron Mau, University of Hawaii, and Dr. Diane Ullman and Dr. Wayne Hunter, University of Davis. Young to mature pods of snap bean, *Phaseolus vulgaris,* were selected as the test plant part. The studies were conducted at the Entomology and Zoology laboratory, Bangkok, under temperature and humidity controlled conditions. Beans were covered completely with 2% Clorox bleach for 10 minutes, then given a quick water rinse and allowed to drain. The bean pods were placed on tissue papers to dry, and when dry, the ends were trimmed off in order to reduce mold. The beans were then ready to use for mass rearing of the thrips in plastic cups.

At least 15-20 cups and about 5 beans per cup were used, and live adult thrips (collected from farmer's plots) were released into each cup. For adult egg laying, a small amount of diluted honey was placed in the groove of a bean, but the entire bean was not covered. Tapping on the top of containers knocked adults to the bottom of the cup. Also opening the lid slightly and blowing one's breath on the thrips caused them to jump to the bottom of the cup. A small brush was used to remove any adults on beans that were to be moved to new cups. Moisture was provided and controlled by placing pieces of filter paper in the bottom of cup to help absorb excess moisture. This rearing procedure provided eggs, larvae, pupae, and adults.

TABLE III. ORCHIDS INFESTED BY THE THRIPS *Thrips palmi* Karny IN 1996-1997.

Month 1996	No. of orchid flowers Examined	Infested	% infestation	Month 1997	No. of orchid flowers Examined	Infested	% infestation
JAN	214	5	2.42	JAN	236	16	6.77
FEB	231	20	9.65	FEB	224	19	8.48
MAR	210	20	9.52	MAR	228	26	11.40
APR	172	18	10.46	APR	218	41	18.80
MAY	224	34	15.17	MAY	176	19	10.79
JUN	204	25	12.26	JUN	178	32	17.97
JUL	241	32	13.27	JUL	126	23	18.25
AUG	141	13	9.21	AUG	176	27	15.34
SEP	270	19	7.03	SEP	201	17	8.45
OCT	269	21	7.80	OCT	-	-	-
NOV	272	8	2.94	NOV	-	-	-
DEC	201	3	1.56	DEC	-	-	-
Total	2649	218	101.29	Total	1,763	220	116.25
Average	220.75	18.16	8.44	Average	195.88	24.44	12.91
Max	272	34	15.17	Max	236	41	18.80
Min	141	3	1.56	Min	126	16	6.77

3. RESULTS AND DISCUSSION

3.1. The egg stage

Eggs were collected from adults at two-day intervals; for more precise staging, eggs should be collected at 24-hr intervals. In practice, eggs cups were made every two days and about 15-20 cups for egg collection were maintained. It was not possible to synchronize development of the thrips and to have all individuals in the same stage at one time.

3.2. The larval stage

The first instars of thrips prefer the youngest beans. After beans with eggs are removed from cups, they should be put in a clean cup, with a filter paper in the bottom. It was also practical to combine different cups together (usually 2 egg cup beans into one cup). Fresh beans were added to the previous egg cups for collection of more eggs. Larvae can hide in the wrinkles of older beans, so they must be inspected carefully. After first instars are observed on fresh beans, the old beans can be removed. Additional beans were added as needed on the second day, and again 2 days later. Only 2-3 beans were needed to maintain a food source. It is important to observe the quality of the beans in the cups, and touching them aids in determining if they are too dry, and need replacing.

3.3. The adult stage

The emergence of new adults should be recorded to determine the developmental time; adults were produced in 10-15 days at 28 °C, but the time can be as long as 18-24 days at 20 °C. The first

addition of beans to new adults was plain beans. Streaked beans can be used, but it takes a few days for females to mature and then lay more eggs on the 2nd changing of beans. If only larvae are needed and adults of a specific age are not needed, then one can put two cups of adults into one cup of beans for higher egg production. Developing and learning to use the snap bean rearing method was started in 1996-1997, and larval thrips obtained increased enough for testing. However, it was not possible to get large numbers of the same instar of larval thrips at the same time. Therefore, development of and confirmatory rearing studies of thrips on snap beans or other substrates, and improved alternative rearing techniques under laboratory conditions needs continued study.

3.4. Experiments on irradiation of cut flowers for controlling thrips

Six experiments were performed. The first experiment was conducted at ambient room temperature 27 - 30 °C and 70 - 75% RH (no control of temperature and moisture), but experiments 2-6 were conducted under temperature and humidity controlled conditions in which the orchid flowers were transported by an air conditioned bus from growers to the irradiation center and stored in the laboratory at 12 - 18 °C and 70 - 75% RH. Doses of 0, 0.1, 0.3, 0.5, 0.8, and 1.0 kGy (also 1.5 kGy in experiments 5 and 6) from a cobalt-60 source were used to treat cut flowers of *Dendrobium* Pompadour. Before irradiation, 625 inflorescences of orchid flowers were prepared and packed in 25 cardboard boxes (size 24 x 63.5 x 6.6 cm). A Randomized Complete Block design was used with 5 replications and 6 treatments. After irradiation the treated flowers were kept under controlled conditions at 20+2 °C and 80-90% RH, and were put in vases that contained 100 ml of distilled water.

Observations were made on vase life and damage symptoms 24 hours after treatment and for 12 days post treatment. Observations of the number of thrips (live and dead) and % mortality was done under a microscope 24 hours after irradiation, and continued for 12 days post treatment. For evaluation, five inflorescences were randomly removed from 25 of each treatment. In general, shelf life or vase life was determined both in opened and unopened flowers. Export standards require acceptable vase life for 7 days or more.

Vase life effects of irradiation were divided into 6 grades as follows:
1 = Fresh, no damage (no difference in quality between treated and untreated).
2 = Slight damage symptoms, 5-10% of treated flowers (opened and unopened) show symptoms of wilting and beginning to yellow. This is considered to be commercially acceptable damage that will be acceptable to consumers.
3 = Moderate damage symptoms with 10 - 15% wilted; this is considered acceptable to consumers.
4-6 = Severe damage, more than 25 -50% wilted, flowers or buds completely dropped over, and unacceptable to consumers.

4. RESULTS AND DISCUSSION

4.1. Effects on cut flowers

Effect of irradiation varied according to flower quality and cultivar. In many cases the vase life and quality of treated flowers were comparable to the untreated. A longer vase life occurred after treatment with Co-60 at the rate of 0.3 and 0.5 kGy. In a number of cases the effects were partly influenced by physiological state of the flowers. Indications were found in 3 reactions, as follows:

(1) When there was no temperature control during transportation before and after irradiated, damage was more severe and vase life was shorter.

(2) Damage in low quality (local grade) of orchid flowers was more severe than in exporting grade (high quality).

TABLE IV. EFFECT OF IRRADIATION ON OPENED ORCHID FLOWERS AND VASE LIFE IN EXPERIMENT 1, 19 JAN.-2 FEB. 1994 AT 27-30 °C, 70-75% RH. MEAN RATING GIVEN TO OPENED FLOWERS AT INDICATED DOSE AND DAYS AFTER TREATMENT (DAT).

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	9 DAT	11 DAT
Untreated	1.00	1.75 b	2.16 d	2.79 d	2.99 d	3.54 c
0.1	1.00	1.66 b	2.37 c	2.58 d	2.93 d	3.43 c
0.3	1.00	1.77 b	2.83 b	3.22 c	3.41 c	3.83 bc
0.5	1.00	1.95 a	2.92 b	3.57 b	3.77 b	4.10 ab
0.8	1.00	1.97 a	2.49 a	4.05 a	4.47 a	4.40 a
CV%	NS	7.20	5.42	7.56	4.52	7.52

TABLE V. EFFECT OF IRRADIATION ON UNOPENED ORCHID FLOWERS AND VASE LIFE IN EXPERIMENT 1, 19 JAN.-2 FEB. 1994 AT 27-30 °C, 70-75% RH. MEAN RATING GIVEN TO UNOPENED FLOWERS AT INDICATED DOSE AND DAYS AFTER TREATMENT (DAT).

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	9 DAT	11 DAT
Untreated	1.00	1.03	1.11 c	1.28 c	1.56 c	1.99 c
0.1	1.00	1.04	1.14 bc	1.24 c	1.53 c	1.87 c
0.3	1.00	1.04	1.18 bc	1.27 c	1.51 c	1.84 c
0.5	1.00	1.04	1.27 b	1.53 b	2.04 b	2.56 b
0.8	1.00	1.06	1.46 a	2.07 a	2.98 a	3.42 a
CV%	NS	NS	7.53	12.13	15.52	13.17

Note : Level 1 - 3 is acceptable to consumers.
Level 4 - 6 is unacceptable.

TABLE VI. EFFECT OF IRRADIATION TREATMENT ON OPENED ORCHID FLOWERS, EXPERIMENT 2, 7-18 JUNE, 1995 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 18 ° C, 70-75% RH. MEAN RATING GIVEN TO OPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	9 DAT	11 DAT
Untreated	1.00	1.11 b	2.00	2.87 b	3.32 b	3.49 c
0.1	1.00	1.10 b	2.25	3.16 b	3.75 b	4.1 bc
0.3	1.00	1.10 b	2.16	3.24 b	3.75 b	3.93 bc
0.5	1.00	1.45 a	3.26	3.81 a	4.58 a	4.81 a
0.8	1.00	1.2 b	2.39	3.34 ab	3.90 b	4.24 ab
CV%	NS	12.82	NS	11.29	12.42	11.81

TABLE VII. EFFECT OF IRRADIATION TREATMENT ON UNOPENED ORCHID FLOWERS, EXPERIMENT 2, 7-18 JUNE, 1995 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 18 ° C, 70-75% RH. MEAN RATING GIVEN TO UNOPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	9 DAT	11 DAT
Untreated	1.00	1.00	1.03 c	1.05 b	1.10 c	1.29 c
0.1	1.00	1.00	1.07 bc	1.22 b	1.55 bc	1.71 bc
0.3	1.00	1.05	1.25 abc	1.59 ab	1.95 ab	1.93 b
0.5	1.00	1.00	1.40 ab	2.01 a	2.39 a	2.58 a
0.8	1.00	1.01	1.44 a	1.79 a	2.17 a	2.30 ab
CV%	NS	NS	19.22	25.42	20.51	22.44

Note : Level 1 - 3 is acceptable to consumers.
Level 4 - 6 is unacceptable.

(3) On average, the lower flowers of each inflorescence had a shorter vase life, more severe damage, turned yellow, and dropped down within 5-7 day after being irradiated at 0.5, 0.8, and 1 kGy when there was no temperature control. With temperature controlled conditions, similar damage occurred only at 0.8 and 1 kGy.

Therefore, the effects of irradiation on cut flowers trend to depend on flowers quality and cultivar. Negative effects of higher doses of radiation occurred in a number of cut flowers. For the five doses studied, the percentages of flowers that showing no damage or acceptable damage were 87.85% at 0.1 kGy, 86.80% at 0.5 kGy, and 86.10% at 0.8 kGy 7 days after treatment (DAT).

5. THRIPS CONTROL

5.1. Mortality of orchid thrips caused by Co-60 radiation

The effect of irradiation on the second instar (larvae stage) was studied in six experiments with Co-60 radiation at doses of 0, 0.1, 0.3, 0.5, 0.75, 1.0, and 1.5 kGy. Experiments 1-4 were done with an unknown number of thrips, but in experiments 5 and 6, the original number of thrips 2nd instars was known. A Complete Randomized Block (CRD) was designed with six treatments and five replications. Observations were made at 1, 3, 5, 7, 10, and 12 days after treatment by recording the number of live and dead thrips in each replications in order to calculate percent mortality from irradiation with Co-60. For evaluation, five inflorescences were taken randomly from 25 flowers in each treatment. Results are tabulated separately for experiments in which the original number of thrips was unknown and for those experiments in which the original number was known. In the tables that follow values in a column followed by the same letter are not significantly different.

6. RESULTS AND DISCUSSION

6.1. Unknown number of thrips in the test

Experiments 2-4 (when the mass rearing method is not applicable) were done with an unknown number of thrips. In experiments 3-4, when immediate death was the criterion evaluated, the results presented in detain in Tables XIV-XVI, showed that 0.5 kGy gave 50% mortality at 3 DAT, 0.8 kGy gave 85.71% in experiment 3, and 85% and 90% mortality in experiment 4.

TABLE VIII. EFFECT OF IRRADIATION TREATMENT ON OPENED ORCHID FLOWERS, EXPERIMENT 4, 25 APR.- 4 MAY, 1996 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 12 - 15 ° C, 70-75% RH. MEAN RATING GIVEN TO OPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	1.00	1.00	1.99	2.48 b	2.93 cd	3.14 c
0.1	1.00	1.00	2.03	2.43 b	2.79 d	3.66 b
0.3	1.00	1.00	2.00	2.70 b	3.17 bc	3.96 d
0.5	1.00	1.06	2.05	2.64 d	3.19 bc	3.80 d
0.8	1.00	1.00	2.00	2.78 ab	3.40 b	3.89 b
1.0	1.00	1.00	2.00	3.07 a	4.00 a	4.78 a
CV%	NS	NS	NS	8.19	5.67	4.83

TABLE IX. EFFECT OF IRRADIATION TREATMENT ON UNOPENED ORCHID FLOWERS, EXPERIMENT 4, 25 APR.- 4 MAY, 1996 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 12 - 15 ° C, 70-75% RH. MEAN RATING GIVEN TO UNOPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	1.00	1.00	1.58 b	1.42 d	1.85 ab	2.05 b
0.1	1.00	1.00	1.58 b	1.52 cd	1.75 b	2.11 b
0.3	1.00	1.00	1.57 b	1.60 bcd	2.11 a	2.14 ab
0.5	1.00	1.16	1.67 ab	1.63 bc	1.88 ab	2.11 b
0.8	1.00	1.00	1.77 a	1.75 ab	2.09 a	2.27 a
1.0	1.00	1.00	1.75 a	1.88 a	2.07 a	2.28 a
CV%	NS	NS	4.51	7.68	8.82	4.43

TABLE X. EFFECT OF IRRADIATION TREATMENT ON OPENED ORCHID FLOWERS, EXPERIMENT 5, 12 - 24 JUNE 1997 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 18 - 26 ° C, 72-76% RH. MEAN RATING GIVEN TO OPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	1.59 b	2.22 c	2.49	2.95 bc	3.32 ab	3.65 ab
0.3	1.08 a	1.33 a	2.28	2.67 ab	3.34 ab	3.68 ab
0.5	1.04 a	1.62 ab	2.28	2.39 a	2.97 a	3.32 a
0.75	1.26 ab	1.83 bc	2.69	3.36 c	4.10 c	4.76 c
1.0	1.10 a	1.69 ab	2.52	2.96 bc	3.62 bc	3.91 b
1.5	1.22 a	1.92 bc	2.54	3.93 d	5.51 d	5.90 d
CV%	15.9	18.1	NS	11.8	9.6	8.2

TABLE XI. EFFECT OF IRRADIATION TREATMENT ON UNOPENED ORCHID FLOWERS, EXPERIMENT 5, 12 - 24 JUNE 1997 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 18 - 26 ° C, 72-76% RH. MEAN RATING GIVEN TO UNOPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	1.02	1.07	1.34	1.67 ab	1.84 ab	2.03 b
0.3	1.00	1.08	1.28	1.42 a	1.52 a	1.56 a
0.5	1.00	1.07	1.29	1.47 ab	1.86 ab	2.07 b
0.75	1.00	1.12	1.54	1.79 b	2.11 b	2.38 b
1.0	1.00	1.13	1.54	1.69 b	2.09 b	2.18 b
1.5	1.00	1.01	1.43	2.12 c	2.59 c	2.78 c
CV%	NS	NS	NS	13.3	14.9	12.4

TABLE XII. EFFECT OF IRRADIATION TREATMENT ON OPENED ORCHID FLOWERS, EXPERIMENT 6, 17 - 30 JULY 1997 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 20 - 21 ° C, 70-82% RH. MEAN RATING GIVEN TO OPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	1.00	1.05	1.47 a	3.18 a	3.31 a	3.75 a
0.3	1.00	1.07	2.53 de	3.12 a	3.60 a	3.87 a
0.5	1.00	1.07	2.12 c	3.21 a	4.18 b	4.37 b
0.75	1.02	1.06	2.34 cd	3.55 b	4.48 b	4.91 c
1.0	1.00	1.03	1.79 b	3.22 ab	4.61 b	5.23 c
1.5	1.00	1.03	2.71 e	4.73 c	5.85 c	5.96 d
CV%	NS	NS	10.8	6.3	6.0	6.2

TABLE XIII. EFFECT OF IRRADIATION TREATMENT ON UNOPENED ORCHID FLOWERS, EXPERIMENT 5, 17 - 30 JULY 1997 WITH TEMPERATURE AND RELATIVE HUMIDITY CONTROLLED AT 20 - 21 ° C, 70-82% RH. MEAN RATING GIVEN TO UNOPENED ORCHIDS TREATED WITH THE DOSE INDICATED AND AT DAYS AFTER TREATMENT (DAT)

Dose (kGy)	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	1.00	1.04	1.18 a	1.41 a	1.51 a	1.62 a
0.3	1.00	1.03	1.48 bc	1.63 ab	1.82 bc	1.90 ab
0.5	1.00	1.10	1.55 bc	1.81 b	1.99 bc	2.00 b
0.75	1.00	1.02	1.38 ab	1.85 b	2.10 c	2.13 b
1.0	1.00	1.04	1.16 a	1.60 ab	1.80 b	1.83 ab
1.5	1.00	1.03	1.66 c	2.13 c	2.63 d	2.85 c
CV%	NS	NS	10.7	10.3	10.5	10.7

Note : Level 1-3 is acceptable to consumers.
Level 4-6 is unacceptable.

TABLE XIV. PERCENTAGE MORTALITY OF THRIPS AFTER IRRADIATION IN EXPERIMENTS 3 AND 4 WHEN THE ORIGINAL INFESTATION NUMBER OF THRIPS WAS NOT KNOWN

Dose (kGy)	EXP 3 MARCH 1996				EXP 4 APRIL-MAY 1996			
	1 DAT	3 DAT	5 DAT	7 DAT	1 DAT	3 DAT	5 DAT	7 DAT
Untreated	0	0	0	0	0	0	0	0
0.1	10.00	12.50	0	0	81.42	20.00	0	0
0.3	0	0	0	0	87.49	66.66	0	0
0.5	0	50.00	0	0	85.00	42.85	0	0
0.8	82.60	85.71	0	0	90.00	54.54	0	0
1.0	-	-	-	-	100	90.00	0	0

TABLE XV. EFFECT OF COBALT -60 IRRADIATION ON *Thrips palmi* Karny DAYS AFTER TREATMENT IN EXPERIMENTS 3 AND 4

Dose	EXP 3 MAR 1996						EXP 4 MAY 1996					
(kGy)	0	0.1	0.3	0.5	0.8	1.0	0	0.1	0.3	0.5	0.8	1.0
Number of thrips studied	7	8	4	4	7	-	11	10	9	7	11	10
No. live thrips after treatment	7	7	0	2	1	-	11	8	3	4	5	1
No. dead thrips after treatment	0	1	0	2	6	-	0	2	6	3	6	9
% mortality	0	12.5	0	60	85.71	-	0	20	66.66	42.25	54.54	90

TABLE XVI. EFFECT OF COBALT - 60 IRRADIATION ON *Thrips palmi* Karny SEVEN DAYS AFTER TREATMENT IN EXPERIMENTS 3 AND 4

Dose	EXP 3 MAR 1996						EXP 4 MAY 1996					
(kGy)	0	0.1	0.3	0.5	0.8	1.0	0	0.1	0.3	0.5	0.8	1.0
Number of thrips studied	7	3	2	2	2	-	3	2	5	1	8	8
No. live thrips after treatment	0	0	0	0	0	-	3	2	5	1	8	1
No. dead thrips after treatment	0	0	0	0	0	-	0	0	0	0	0	1
% mortality	0	0	0	0	0	-	0	0	0	0	0	12.5

TABLE XVII. PERCENTAGE MORTALITY OF THRIPS AFTER IRRADIATION WHEN A KNOWN NUMBER OF THRIPS WAS RELEASED ON ORCHID FLOWERS IN EXPERIMENTS 5 AND 6

Dose (kGy)	EXPERIMENT 5 JUNE 1997						EXPERIMENT 6 JULY 1997					
	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	0	0	0	0	0	0	0	0	0	0	0	0
0.3	-	-	-	-	-	-	13.79	18.49	21.21	41.58	64.70	100
0.5	11.42	15.15	21.21	39.13	46.15	76.47	11.66	13.95	37.25	57.57	87.50	100
0.75	11.76	13.15	20.00	46.15	62.50	71.42	26.00	34.64	47.05	61.38	100	-
1.0	25.00	40.54	62.00	71.42	100	-	28.94	33.33	78.78	95.95	100	-
1.5	33.33	81.69	100	-	-	-	81.69	92.94	100	-	-	-

TABLE XVIII. EFFECT OF COBALT- 60 IRRADIATION ON *Thrips palmi* Karny FIVE DAYS AFTER TREATMENT IN EXPERIMENT 5 ON 17 JUNE 1997 AND EXPERIMENT ON 22 JULY 1997

Dose	EXP 5						EXP 6					
(kGy)	0	0.3	0.5	0.75	1.0	1.5	0	0.3	0.5	0.75	1.0	1.5
Number of thrips studied (released)	100	-	100	100	100	100	100	100	100	100	100	100
No. live thrips after treatment	133	-	78	80	38	0	105	78	64	54	20	0
No. dead thrips after treatment	0	-	21	20	62	99	0	21	38	48	79	99
% mortality	0	-	21.21	20	62	100	0	21.21	37.25	47.05	78.78	100

TABLE XIX. EFFECT OF COBALT -60 IRRADIATION ON *Thrips palmi* Karny SEVEN DAYS AFTER TREATMENT IN EXPERIMENT 5 ON 19 JUNE 1997 AND IN EXPERIMENT 6 ON 24 JULY 1997

Dose	EXP 5						EXP 6					
(kGy)	0	0.3	0.5	0.75	1.0	1.5	0	0.3	0.5	0.75	1.0	1.5
Number of thrips studied (released)	100	-	100	100	100	100	100	100	100	100	100	100
No. live thrips after treatment	135	-	70	70	28	0	103	59	42	39	4	0
No. dead thrips after treatment	0	-	45	60	70	126	0	42	57	62	95	95
% mortality	0	-	39.13	46.15	71.42	100	0	41.58	57.57	61.38	95.95	100

TABLE XX. PERCENTAGE MORTALITY OF THRIPS IRRADIATED AND HELD IN TEST TUBES AFTER IRRADIATION WHEN THE NUMBER OF THRIPS IN THE TEST WAS KNOWN

Dose (kGy)	EXPERIMENT 5 JUNE 1997						EXPERIMENT 6 JULY 1997					
	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Untreated	0	0	0	0	0	0	0	0	0	0	0	0
0.3	-	-	-	-	-	-	2	19	51	90	100	-
0.5	9	16	42	68	-	-	8	22	53	91	100	-
0.75	12	18	58	98	-	-	16	41	75	100	-	-
1.0	15	25	96	-	-	-	10	53	90	100	-	-
1.5	12	49	100	-	-	-	26	87	100	-	-	-

TABLE XXI. EFFECT OF COBALT - 60 IRRADIATION ON *Thrips palmi* Karny FIVE DAYS AFTER TREATMENT IN EXPERIMENTS 5 AND 6

Dose	EXP 5 17 JUNE 1997						EXP 6 22 JULY 1997					
(kGy)	0	0.3	0.5	0.75	1.0	1.5	0	0.3	0.5	0.75	1.0	1.5
Number of thrips studied (released)	100	-	100	100	100	100	100	100	100	100	100	100
No. live thrips after treatment	114	-	58	42	4	0	131	49	47	25	10	0
No. dead thrips after treatment	0	-	42	58	96	118	0	51	53	75	90	131
% mortality	0	-	42	58	96	100	0	51	53	75	90	100

TABLE XXII. EFFECT OF COBALT - 60 IRRADIATION ON *Thrips palmi* Karny SEVEN DAYS AFTER TREATMENT IN EXPERIMENTS 5 AND 6

Dose	EXP 5 19 JUNE 1997						EXP 6 24 JULY 1997					
(kGy)	0	0.3	0.5	0.75	1.0	1.5	0	0.3	0.5	0.75	1.0	1.5
Number of thrips studied (released)	100	-	100	100	100	100	100	100	100	100	100	100
No. live thrips after treatment	136	-	32	2	0	0	135	10	9	0	0	0
No. dead thrips after treatment	0	-	68	98	100	118	0	90	91	114	131	131
% mortality	0	-	68	98	100	100	0	90	91	100	100	100

TABLE XXIII. WORKING PROGRAM AND ENVIRONMENTAL CONDITIONS FOR THE VARIOUS EXPERIMENTS

		1st Experiment	2nd Experiment	3rd Experiment	4th Experiment	5th, 6th Experiment
1.Duration of studies		19-29JAN94	7-18JUN95	25MAR-4APR96	22APR-1MAY96	12JUN-SEP97
2.Vaselife studied		_/	_/	_/	_/	_/
3.Mortality studied		X	_/	_/	_/	_/
4.Harvesting and	Temp.(°C)	27-35	27-35	27-30	28-35	28-35
packaging	%RH	80	80	80	80	80
5.Transportation	Temp.(°C)	23-25	15	15	15	20±2
	%RH	70-75	90	90	90	72-75
6.At TIC* or before	Temp.(°C)	27-35	12-18	12-18	12-18	27±2
irradiation	%RH	93-95	90-95	90-95	90-95	90
7.After irradiation	Temp.(°C)	23-25	15	15	15	25
	%RH	70-75	90	90	90	70
8.Laboratory or	Temp.(°C)	19-27	20-35	19-22	19-22	20±2
stored room	%RH	70-75	70-75	80-90	80-90	80-90

* TIC = Thai Irradiation Center

6.2. Known original number of thrips (Tables XVII-XXIII)

When the snap bean rearing method had been developed, it was possible to release 100 thrips 2nd instars in each replication of experiments 5 and 6. The experiments were separated according to studies in test tube with thrips on tested plants (flowers).

In the test tube method, thrips were released in test tubes and directly exposed to Co-60 at the tested doses. The results noted 5 DAT, shown in Table 20, are that in experiment 5 0.5 kGy gave 42% mortality, 0.75 kGy gave 58%, 1.0 kGy gave 96%, and 1.5 kGy gave 100% mortality. At 7 DAT 0.75 kGy gave 98% mortality. In experiment 6, 5 days after treatment, 0.5 kGy gave 53% mortality, 0.75 kGy gave 75%, 1.0 kGy gave 90%, and 1.5 kGy gave 100% mortality. At 7 DAT 0.3 kGy gave more than 90% mortality.

In the tests with thrips on the tested plants (flowers), with 100 2nd instars on orchid inflorescences in each replications, the results are shown in Table I7. In experiment 5 at 5 DAT, 1.0 kGy gave 62%, and 1.5 kGy gave 100% mortality, and 1.0 kGy gave 71.42% mortality at 7 DAT.
In experiment 6 at 5 DAT, 1.0 kGy gave 78.78% while 1.5 kGy gave 100% mortality. At 7 DAT 0.75 kGy gave 61.38%, and 1.0 kGy gave 95.95% mortality.

In conclusion, results from tests with thrips in test tubes and on tested orchid flowers showed that the effective dose for death of the thrips should be higher than 0.75 kGy at 7 DAT. The use of Co-60 radiation to control thrips in cut flowers depends on acceptation of irradiation as a quarantine treatment. When applied at the rate of 0.1, 0.3, 0.5, 0.8, and 1 kGY (also 1.5 kGY) with unknown and known of original number of thrips in each treatment, live larvae of thrips were found in each treatment, and the population gradually increased starting from two days after radiated. Because Co - 60 radiation does not directly kill all thrips at the doses used, other criteria than insect mortality need to be established to describe treatment efficacy. When the rearing technique for thrips is more successfully established, and large numbers of the same instar can be obtained, it should be possible

to determine radiation sensitivity and to compare tolerance of life stages of *Thrips palmi* Karny. This should allow determination of the most tolerant and sensitive stages, and enable prediction of dose needed to meet quarantine requirements of markets.

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IRRADIATION AS A QUARANTINE TREATMENT FOR ORNAMENTALS

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Abstract

The orchid weevil, *Orchidophilus aterrimus* (Waterhouse), was the most damaging and most difficult to control among the insect pests surveyed. The duration of development of the different stages of orchid weevil were as follows: egg incubation was 7.20 ± 1.47 days, larval period was 58.70 ± 11.24, and the pupal period was 10.83 ± 1.54 days. The total developmental period from egg to adult was 70.15 ± 12.04 days. The pre-oviposition period of the adult female was 44.27 ± 12.18 days and the mean number of eggs laid by a female per week was 3.95 ± 1.36 eggs. Radiosensitivity, in general, decreased with the age of the orchid weevil; the adult was the least sensitive and the eggs the most sensitive to radiation. However, radiosensitivity also varied within a developmental stage. Pairing studies on orchid weevils showed that older adults (11-to-30-days-old) irradiated with 150 Gy and paired as I x U and U x I laid eggs, but surviving larvae died 6 days after egg hatch. The melanization test for irradiated orchid weevil larvae produced inconsistent results. A shorter vaselife was found on *Dendrobium* cutflowers irradiated with 100 to 450 Gy. Among the different varieties of *Heliconia* , the variety Parrot was the most tolerant tolerant to radiation. Irradiation affected the growth of the seedlings and ready-to-bloom *Dendrobium* plants. It also affected the formation of spikes on the latter. The percentage of dropped/wilted flowers in flowering *Dendrobium* plants was higher on irradiated plants as compared with the control.

1. INTRODUCTION

The potential of the Philippines as a major trader in the world's ornamental industry is indicated by its exports of fresh blossoms, decorative cut foliage, and nonflowering or green polished plants. From US $405, 319 in 1982, the value of ornamentals exported was US $727,354 in 1987, and more than doubled to US $963,305 in 1990. However, this is a very insignificant amount compared to the estimated world import market of US $1.3 billion.

In the past, quarantine treatment for ornamentals involved the use of chemical control such as fumigation with methyl bromide or spraying with pesticides. However, with the adverse effects of pesticides to human and environment, the use of gamma radiation as an alternative quarantine treatment for ornamental is deemed necessary to realize the growth potential of the ornamental industry and to produce quality ornamental as source of foreign exchange earnings for the country. This study was conducted to establish an alternative quarantine treatment for ornamentals intended for export.

2. METHODOLOGY

2.1. Survey and identification of pests

Surveys of orchid pests, particularly pests of *Dendrobium*, were conducted in three gardens in Laguna Province about 60 km. south of PNRI and about 10 km. north of the University of the Philippines at Los Baños (UPLB). The gardens include Varunee's garden and Nene's garden in Calamba, Laguna, and Economic Garden of the Bureau of Plant Industry in Los Baños. The survey was conducted at bimonthly interval from January to June 1992. The *Dendrobium* plants were examined for the presence of pests and the degree of damage caused by the pests was observed in the

field. Immature stages of the insect pests were also observed in the field. These stages of the insect pests were brought to the laboratory and reared until they attained the adult stage for identification. In the survey, the owners were interviewed on their crop protection practices in their respective gardens and their observations solicited on serious pest problems encountered in their plants.

2.2. Survey on orchid weevil infestation

Among the pests surveyed, rearing and life history studies were conducted on the orchid weevil since this pest was found the to be the most persistent and the most difficult to control. Both the larval and pupal stages are spent inside the pseudobulb or stem of the plant. During the months of March and April 1992, a survey on the degree of orchid weevil infestation was conducted in the three gardens. Twenty plants were selected at random and labeled. Each plant was examined for the presence of exit holes in the pseudobulb, feeding punctures in the leaves, or presence of orchid weevil adults.

2.3. Rearing of orchid weevil, *Orchidophilus sp.*

Field-collected adults were reared in the laboratory at 25.06 ± 1.17 °C and 72.88 ± 5.78% RH in glass jars covered with nylon-organdy cloth. Fresh *Dendrobium* leaves were provided two to three times a week for them to feed on and for oviposition of eggs. Sexing of adult weevils followed the procedure of Buchanan (1935) as cited by Mau [1].

Orchid leaves were exposed to about 50 orchid weevil adults (25 : 25) in nylon-organdy covered jars for oviposition. Eggs dissected from infested leaves were transferred on wet blotting papers to Petri dishes. Soon after hatching, the larvae were individually transferred on cut stems provided with artificial tunnels made by puncturing the stem with a nail. The infested stems were placed inside test tubes (2.5 cm. diam. x 20 cm.) with nylon-organdy covers and held in trays. Emerged adults were transferred to clean vials provided with fresh *Dendrobium* leaves and wet cotton rope.

Orchid plants in cages were infested with adult weevils to augment the stock culture in the laboratory. This was done by enclosing a succulent pseudobulb with the weevils in a nylon-organdy bag tied at the base with a string or rubber band. The adults were transferred to uninfested orchids after three to four days.

2.4. Life cycle studies

Fresh leaves of *Dendrobium* were placed in an oviposition jar. Two to three days after infestation, the leaves were soaked in water for five minutes to soften the epidermis at the sites of oviposition. Entrance holes were made in the leaves with the use of forceps or nail to enable young larvae to enter the leaves. After five to 10 days, the larvae were removed from the leaves by dissection and transferred to small puncture tunnels made with a nail in cut stems. The infested stems were placed inside test tubes (2.5 cm. diam. x 20 cm ht.) with nylon-organdy cloth and held in trays for about a month. Afterwards, the stems were dissected and checked daily to determine the stage of development of the insect. The larval period was calculated by subtracting the length of the incubation period from the egg to pupal stage.

The pre-oviposition period, fecundity, and longevity were determined by two methods. In the first method, each pair of adult weevils (1 male: 1 female) was contained in a glass vial and fed with fresh *Dendrobium* leaves. Eggs were collected at weekly interval until death of the female, and the number of dead adults was also determined. In the second method, field-collected adult weevils (8 males: 11 females with unknown date of emergence) were confined in a glass jar and provided with *Dendrobium* leaves. Mortality was taken at weekly interval. The number of eggs laid per female per week was calculated by the number of females alive at the end of the week.

2.5. Irradiation of different stages of the orchid weevil

Studies on the effect of irradiation on different stages of the orchid weevil, including eggs (1 to 3 days old and 4 to 7 days old), larvae (2 to 4 days old and 30 days old), early pupae not showing browning, late pupae, and adults (pre-ovipositing female x less than one-month-old male and ovipositing female x more than one-month-old male), were undertaken with the Gamma Cell 220 Irradiator at PNRI. The different developmental stages were exposed to 150, 300, and 450 Gy radiation doses. Unirradiated lots served as controls. For each developmental stage, mortality was recorded daily. Larvae and pupae were reared to the adult stage. Adults were confined in glass vials and provided with fresh *Dendrobium* leaves. Dissection of infested leaves was done at weekly interval to collect any eggs laid by the adult female.

2.6. Pairing studies on irradiated and unirradiated adults

One- to 10-day-old adults and 11- to 30-day-old adults were irradiated with 150 Gy in the Gamma Cell Irradiator. Adults were paired as follows: Untreated (U) x U , Irradiated (I) x U , U x I, and I x I . The presence or absence of mating, the number of eggs laid, if any, and percentage egg hatchability were recorded for each pairing combination.

2.7. Melanization studies

Newly-hatched larvae were placed in orchid leaves punctured with forceps to serve as entrance holes. The larvae were irradiated with 5, 10, 20, and 50 Gy doses. When the larvae reached the last instar, they were placed in a freezer (-20°C) for 24 hours to kill them. The larvae were removed from the freezer, laid on white background at room temperature, and observed for melanization.

2.8. Vaselife determination of cutflowers following irradiation treatment

2.8.1. Dendrobium. Dendrobium cutflowers, var. Norashikin Blue Fairy, were packed in boxes and irradiated with 100, 150, 300, and 450 Gy in the Multipurpose Gamma Irradiator. Unirradiated lots served as controls. After irradiation, the cutflowers were individually placed in test tubes containing distilled water and kept at 25°C and 70-80% RH. The number of dropped and wilted flowers was recorded daily. The data were analyzed by a Completely Randomized Design (CRD) with five replications.

2.8.2. Heliconia. Heliconia cutflowers, var. Parrot, Parakeet and Sase, were exposed to doses ranging from 100 to 600 Gy in the Multipurpose Gamma Irradiator of the PNRI to determine the effect of irradiation on their vaselife. Unirradiated cutflowers served as the control lot. Following irradiation, the cutflowers were individually placed in test tubes containing distilled water, held in test tube racks, and observed daily for browning of bracts and browning/falling of petals.

The variety of *Heliconia* was based on the description of Vergara [2]. The number of dropped flower was counted and the degree of browning of flowers and bracts was recorded based upon the following rating: 0 = fresh, 1 = 5 to 10%, 3 = 11 to 25%, 5 = 26 to 50%, and 7 = 51 to 100% browning.

2.9. Irradiation of different growth stages of Dendrobium plants

Different growth stages of *Dendrobium* including seedlings, ready-to-bloom, and flowering were exposed to 150, 300, and 450 Gy radiation doses to determine if irradiation affects the growth and/or flowering of *Dendrobium*. The data collected include plant height, number of leaves, and number of stems/shoots for seedlings; plant height, number of leaves, number of stems and formation of spikes for ready-to-bloom; and number of dropped and wilted flowers for flowering Dendrobium plants. Five plants were used for each dose and treatments were replicated three times. The data were analyzed as a Randomized Complete Block Design (RCBD).

3. RESULTS

3.1. Survey and identification of pests

Based on the survey, the commonly encountered orchid pests include snails, millepede, mites, and insects such as the orchid weevil and thrips. Other pests such as scale insects, mealybugs, grasshoppers, katydids, and some caterpillars of lepidopterous pests have been reported by the owners or managers of the gardens surveyed. However, these pests were not considered of significant importance since they are mostly general feeders.

Among the insect pests, the orchid weevil was present in the three gardens surveyed and was most prevalent at the Economic Garden, especially in the dry months of March and April. Adults are dark brown to almost dull black in color with distinct longitudinal, depressed lines or furrows extending from the base to the apex of the elytra. They are characterized by the projection of the head into a snout used by the newly-emerged adults to get out of the pseudobulb after emergence. Adult weevils feed on leaves, flowers, pseudobulb, and exposed roots of *Dendrobium*. Damage is usually in the form of holes or cavities made during adult feeding. Based on the description of Buchanan given by Mau [1], the orchid weevil was identified as *Orchidophilus aterrimus* (Waterhouse). The identification was confirmed by the International Institute of Entomology in London.

Crop protection measures which include weekly or bimonthly spraying of insecticides, fungicides, and foliar fertilizers is the usual practice at the Varunee's and Nene's Garden. However, at the Economic Garden pesticide spraying was not done regularly but only on a case to case basis, although fertilizer was applied at monthly intervals.

3.2. Survey of weevil infestation

Results of the survey on orchid weevil infestation are shown in Table I. The data show higher weevil infestation observed at the Economic Garden as compared to the other two gardens. The high infestation may be due to erratic application of pesticide resulting in the rapid multiplication of the pest, or the insecticide being used may not have been adequate to control the weevils.

3.3. Life cycle studies

3 3 1 Sexing and measurement of adult weevils

Sexing of field-collected orchid weevils showed a low female to male ratio (1 : 1.6) from a total of 152 adults examined. The phenomenon cannot be explained, and our results are contrary to those reported in Hawaii where a ratio of 1.2 : 1 was found [1]. Body measurements of the different life stages of the weevil are shown in Table II.

3.3.2. Oviposition and incubation period

The eggs of the orchid weevil are laid singly in cavities caused by adult feeding. The egg is oval and white when newly-laid, later becoming yellowish within a few days before hatching. Prior to hatching the mandibles of the developing larva become visible as brown spots and later the head capsule can also be observed about one day prior to hatching. The Incubation period varied from 4 to 13 days with a mean of 7.20 ± 1.47 days at ambient conditions as determined from a total of 25 eggs.

3.3.3. Larval development

The newly-hatched larva is yellowish with a light brown head. It feeds readily into leaf tissues after hatching. Dissection of infested plant showed that larval development occurred within the pseudobulbs. The larva produces a gallery that is oriented longitudinally and downwards from the site of oviposition. The gallery is usually filled with solid larval excrement or frass up to where the grub is

TABLE I. MEAN % INFESTATION OF ORCHID WEEVILS IN THREE GARDENS

Gardens	Percent infestation	
	March 1992	April 1992
Economic Garden	57.5	85.0
Varunee's Garden	30.0	47.5
Nene's Garden	40.0	52.5

TABLE II. MEAN BODY MEASUREMENTS OF THE DIFFERENT DEVELOPMENTAL STAGES OF THE ORCHID WEEVIL

Stage	Body width (mm)	Body length (mm)	No. of Individuals
Egg	0.51±0.07	0.81±0.07	34
Mature larva	2.66±0.35	8.75±1.25	16
Pupa	2.10±0.26	5.51±0.68	20
Adult	2.18±0.16	4.47±0.31	30
	2.36±0.27	4.85±0.52	30

found. Mature larvae were 6 to 10 mm long and 2 to 3 mm wide. The duration of the larval stage, calculated by subtracting the length of the incubation period from the egg to pupa, ranged from 39 to 80 days with a mean of 58.70 ± 11.24 days (N=23).

3.3.4. Pupation

Pupation occurred within the gallery made by the larva. The newly-developed pupa was creamy white in color. The eyes darken and become visible 2 to 3 days after pupation. Browning of the eyes and rostrum and blackening of the wing pads were evident on the 6th to 9th day. Prior to adult emergence, the color of the wing pads and rostrum deepened to black and dark brown, respectively. Antennal segmentation and browning of femora-tibial joints, pretarsus and part of the abdomen were also indicative that the pupa is about to emerge. The pupa measured 5.51 ± 0.68 mm long and 2.10 ± 0.26 mm wide. Pupal period ranged from 9 to 15 days with a mean of 10.83 ± 1.54 days.

3.3.5. Adult emergence

The newly-formed adult was light to dark brown in color and became totally black in 2 to 3 days. The adult stayed inside the pupal cell for sometime and started feeding only 6 to 10 days. After emergence, sex ratio was determined to be 1: 1.14 (male: female) (N = 22). Total developmental period from egg to adult ranged from 54 to 103 days with a mean of 70.15 ± 12.04 days.

3.3.6. Pre-oviposition period, fecundity and longevity

The pre-oviposition period of the female adult weevil ranged from 29 to 66 days with a mean of 44.27 ± 12.18 days. The mean number of eggs laid by a female per week ranged from 2.0 to 5.33 with a mean of 3.95 ± 1.36 eggs. Maximun adult longevity was observed at 310 days (ca. 10 months). The number of eggs collected weekly from field-collected adults were also taken. The mean number of eggs laid per female per week based on the number of females alive at the start of the week ranged from 0.2 to 4.0 with a mean of 1.69. The adults inside the oviposition jar lived for approximately 20 weeks. It was also observed that the females laid very few eggs during the first month of confinement in the oviposition jar.

3.4. Irradiation of different developmental stages of orchid weevil

Results of the irradiation studies of the different ages of orchid weevil eggs were summarized in Table III. Data shows that younger eggs (1- to 3-day-old) were more sensitive than older ones (4- to 7-day old). Generally, insect eggs have been found to be most sensitive to radiation [3] and this could be due to a high degree of mitotic activity and lack of differentiation which are correlated with sensitivity to radiation [4]. Irradiation of young eggs resulted to 100% mortality while for older eggs mortality increased with an increase in the dose applied. Significant differences in egg mortality was observed among the doses used. Our results indicate that orchid weevil eggs are more radiosensitive than eggs of other species of beetles like Fuller rose beetle [5] and other species of Coleoptera [6] [7] [8]. The larvae that hatched from irradiated eggs lived for only five to 10 days.

When 2- to 4-day old larvae were irradiated, larval longevity was significantly shorter compared with the control (Table IV). Treated larvae fed very little to none at all especially those receiving higher doses. In effect, treated larvae were smaller and weaker compared with the control. In the irradiation of 30-day-old larvae, all larvae treated with 150 and 300 Gy survived five days after irradiation while only 8% of the larvae treated with 450 Gy survived. However, no significant difference was observed among the doses used (Table V). About 7% of the larvae treated with 150 Gy pupated but no adults emerged.

The data presented in Table VI show pupal mortality of early pupae (pupae not showing browning) treated with 450 Gy. Also, none of the surviving pupae emerged into adult. In contrast,

TABLE III. PERCENT MORTALITY OF YOUNG AND OLD ORCHID WEEVIL EGGS EXPOSED TO DIFFERENT DOSES OF RADIATION

Age of eggs	Dose (Gy)	Corrected egg mortality * (%)
1 - 3 a	450	100.00 b
	300	100.00 b
	150	100.00 b
	0	0.00 a
4 - 7 b	450	74.89 d
	300	28.87 c
	150	8.78 b
	0	0.00 a

* Data are the means of 5 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).

TABLE IV. PERCENT MORTALITY OF 2-4-DAY-OLD ORCHID WEEVIL LARVAE EXPOSED TO DIFFERENT DOSES OF RADIATION AND LONGEVITY OF SURVIVING LARVAE

Dose (Gy)	Longevity * (days)	Pupation	Adult Emergence
450	11.82 c	0.00	0.00
300	11.83 c	0.00	0.00
150	17.33 b	0.00	0.00
0	99.90a	85.00	81.00

* Data are the means of 3 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).

irradiation of late pupa resulted in zero mortality and 100% adult emergence with all doses used (Table VII). On the other hand, the longevity of emerged adults decreased with an increase in dose. Significant differences were observed among the doses used.

Table VIII shows that irradiated pre-ovipositing females paired with less than one-month old males survived treatment five days after irradiation. None of the adult females laid eggs throughout the entire life span. The longevity of the adults was also affected by exposure to radiation. Similarly, all ovipositing females paired with more than one-month old males survived irradiation treatment five days after irradiation (Table IX). However, only adult females treated with 150 Gy laid eggs but none of the eggs hatched. Irradiated adults had a much shorter life-span compared with the controls.

In general, the radiosensitivity of the orchid weevil decreased with the age of the insect based on the mortality obtained for each stage five days after irradiation with dose ranging from 150 to 450 Gy. The adult was the least sensitive and the eggs the most sensitive to irradiation. However, within a developmental stage the sensitivity of the insect also varied with younger age being more sensitive than older ones.

TABLE V. PERCENT MORTALITY, PUPATION, AND ADULT EMERGENCE OF 30-DAY-OLD ORCHID WEEVIL LARVAE EXPOSED TO DIFFERENT DOSES OF RADIATION

Dose (Gy)	Larval mortality at 5DAI (%)1	Pupation (%)	Adult Emergence (%)	Longevity (days)
450	8.00 a	0.00 d	0.00 b	19.25 d *
300	0.00 a	0.00 c	0.00 b	26.25 c *
150	0.00 a	6.60 b	0.00 b	42.75 b **
0	0.00 a	86.67 a	83.33 a	83.38 a **

* Data are the means of 6 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).
* Longevity of surviving larvae.
** Longevity of surviving larvae and pupae.

TABLE VI. PERCENT MORTALITY OF, AND ADULT EMERGENCE, FROM EARLY PUPAE OF THE ORCHID WEEVIL EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION AND LONGEVITY OF SURVIVING PUPAE AND ADULTS

Dose (Gy)	Pupal mortality at 5DAI (%)	Adult emergence (%)	Longevity (days)
450	28.57 a	0.00 d	9.56 *
300	0.00 b	38.09 c	12.62 **
150	0.00 b	76.19 b	21.50 **
0	0.00 b	100.00 a	54.69 **

Data are the means of 7 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).
* Longevity of surviving pupae.
** Longevity of surviving pupae and emerged adults.

TABLE VII.PERCENT MORTALITY OF, AND EMERGENCE FROM, LATE PUPA OF THE ORCHID WEEVIL EXPOSED TO DIFFERENT DOSES OF RADIATION AND LONGEVITY OF EMERGED ADULTS

Dose (Gy)	Pupal mortality at 5DAI (%)	Adult emergence (%)	Longevity of emerged adult * (days)
450	0.00	100	13.75 d
300	0.00	100	21.00 c
150	0.00	100	29.00 b
0	0.00	100	88.50 a

* Data are the means of 5 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).

TABLE VIII. ADULT MORTALITY AND LONGEVITY OF PRE-OVIPOSITING FEMALES AND LESS THAN 1-MONTH OLD MALES EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION

Dose (Gy)	Adult mortality at 5 DAI		Adult Longevity	Mean no. of eggs laid
	Female (%)	Male (%)		
450	0.00	22.16 d	18.67 d	0
300	0.00	25.16 c	20.83 c	0
150	0.00	28.00 b	20.00 b	0
0	0.00	64.00 a	49.40 a	1.00*

*Data are the means of 5 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).

TABLE IX. ADULT MORTALITY, LONGEVITY, AND FECUNDITY OF MATURE ADULTS (MORE THAN 1-MONTH-OLD) EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION

Dose (Gy)	Adult mortality at 5DAI		Adult Longevity *	Mean no. Eggs laid	Egg hatchability (%)
	Female (%)	Male (%)			
450	0.00 a	14.77 a	12.77 d	0.00	-
300	0.00 a	15.53 a	14.80 c	0.00	-
150	0.00 a	23.49 b	20.56 b	0.83	0.00
0	0.00 a	60.48 c	57.45 a	5.00	93.00

*Data are the means of 5 replicates. Means followed by the same letter in each column are not significantly different ($P = 0.05$, DMRT).

3.5. Pairing studies on irradiated and unirradiated adults

Results of the different mating combinations involving irradiated and unirradiated adults (less than 10-day-old and 11- to 30-day old adults) in general showed shorter longevity of irradiated ones compared to untreated ones.

TABLE Xa. LONGEVITY, OVIPOSITION, AND EGG HATCHABILITY OF THE DIFFERENT MATING COMBINATIONS INVOLVING IRRADIATED (I) AND UNTREATED (U) LESS THAN 10-DAY-OLD ORCHID WEEVILS IRRADIATED WITH 150 GY

Adult Pair	No. of pairs	Mean Longevity (days)		No. of Eggs Laid	Egg (%)	Hatch
		Male	Female			
U X U	3	55	41	4	100	
U X I	3	63.3	20	0	-	
I X U	3	19	49.7	0	-	
I X I	3	22.7	19	0	-	

TABLE Xb. LONGEVITY, OVIPOSITION AND EGG HATCHABILITY OF THE DIFFERENT MATING COMBINATIONS INVOLVING IRRADIATED (I) AND UNTREATED (U) 11- TO 30-DAY-OLD ORCHID WEEVILS IRRADIATED WITH 150 GY

Adult Pair	No. of pairs	Longevity (days) Male	Longevity (days) Female	No. of Eggs Laid	Egg (%)	Hatch
U X U	2	100	83	4	100	
U X I	2	62	43	1	100	
I X U	2	27	140	1	100	
I X I	2	16	13.25	0	-	

Tables Xa and Xb show that only mating combinations involving both untreated male and female orchid weevil were able to lay eggs. Mating was observed on all mating combinations. However, only mating combinations involving less than 10-day old untreated males and females were able to lay eggs. In contrast with 11- to 30-day old adults, eggs were also collected on pairs I x U and U x I . The eggs were able to hatch into larvae. However, death of larvae followed after 5 to 6 days.

3.6. Melanization studies

Based on visual observations, last instar orchid weevil larvae irradiated as first instars with 5, 10, 20, and 50 Gy and unirradiated controls did not show any significant differences in the degree of melanization. From a total of 11 trials, none of the larvae turned completely brown or dark brown. Melanization test for irradiated orchid weevil larvae produced inconsistent results. Incomplete melanization or browning as shown by brown or dark brown patches in the larvae was observed on both treated and control even after 24 hours. The results obtained were not comparable to the ones obtained with the Caribbean fruit fly [9].

3.7. Vaselife determination of cutflowers following irradiation treatment

3.7.1. Dendrobium

Studies on the tolerance of *Dendrobium* cutflowers (var. Norashiking Blue Fairy) to varying doses of radiation showed a shorter vaselife on cutflowers irradiated with doses ranging from 100 to 450 Gy. This was shown by a significantly higher percentage of wilted and dropped flowers on irradiated compared with untreated flowers at two days after irradiation (DAI) onwards. More than 50% dropped and wilted flowers occurred at 6, 10 and 14 DAI. There were no significant difference observed between 100 and 150 Gy at 4 DAI onwards although still significantly different from the control.

3.7.2. Heliconia

Based on the vaselife studies using different varieties of *Heliconia* viz. Parrot, Parakeet, and Sase the Parrot variety had the longest vaselife of 8 to 9 days. Parakeet and Sase easily showed symptoms of browning of tips and dropping of flowers. Most of the flowers dropped on the third day. Among the varieties used, the Parrot variety was also the most tolerant to radiation. It could tolerate even a dose as high as 600 Gy.

3.8. Irradiation of the different growth stages of *Dendrobium*

Increment in plant height and leaves of irradiated seedlings were significantly lower compared with the control. The effect of irradiation on seedlings was more evident on the occurrence of change in leaf color from green to somewhat rusty red.

In contrast, on ready-to-bloom *Dendrobium* plants, irradiation has no significant effect on plant height but on the number of leaves and shoots. Significantly fewer shoots were formed, especially at 300 and 450 Gy, than in those treated with lower doses. In addition, only the unirradiated plants produced flowers or spikes (53%) during the seven-month observation period. The formation of spikes in ready-to-bloom *Dendrobium* plants was either delayed or inhibited. Apparently, irradiated plants have a greater percentage of dropped and wilted flowers compared with unirradiated ones.

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the data obtained, 300 Gy could be used as the disinfestation dose for orchid weevil. Studies on the effects of irradiation on the vaselife of other cutflowers, e.g., *Heliconia*, *Oncidium*, *Anthurium*, etc., and other insects and mites of quarantine importance should be conducted. In addition, studies on irradiation effects on other foliage plants for export particularly to Japan, e.g., *Dracaenia*, leathery ferns, etc., should also be included.

Melanization could not be used as an indicator of irradiated orchid weevil since incomplete melanization or browning was observed in both irradiated and unirradiated larvae.

Studies to determine the lowest sterilizing dose in orchid weevil and on the reproductive system of adult male and female weevil following exposure to gamma radiation should be pursued.

5. PROBLEMS ENCOUNTERED

1. The additional tests on the vaselife of irradiated *Dendrobium* cutflowers in controlled temperature from harvest to irradiation have not been pursued due to the unavailability of the cutflowers. The garden where we obtain cutflowers became heavily infested with the weevil. As a result, they stopped growing *Dendrobium* to eliminate the source of infestation.

2. The long life cycle of the orchid weevil would not allow us to complete some of our biological and experimental tests.

3. In the dissection of the adult weevil for reproductive system study, difficulty was encountered in the separation of the reproductive organs. Therefore, techniques are being looked into to eliminate this problem.

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TOLERANCE OF CUT FLOWERS TO GAMMA-RADIATION

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Abstract

Cut flowers were gamma-irradiated with doses of 0, 200, 400, 600, and 1000 Gy. *Dianthuscaryophyllus* (Caryophyllaceae), *Gypsophila paniculata* (Caryophyllaceae), *Freesia* sp (Iridaceae), *Limonium sinuatum* Mill. (Plumbaginaceae), *L. latifolium* Kuntze (Plumbaginaceae), *Narcissus tazetta* L. (Amaryllidaceae), *Helichrysum bracteatum* Andr. (Compositae) and *Rhodanthe manglesii* Lindl (Compositae) were tolerant up to 1000 Gy, without visible negative changes after irradiation and during the vase-life. *Callistephus chinensis* (Compositae) and *Lilium longiflorum* Thunb. (Liliaceae) were moderately tolerant, but were modified by high doses. *Anthurium* sp (Araceae), *Strelitzia* sp (Musaceae), *Matthiola incana* R. Br. (Cruciferae), *Aechmea distichanta* (Bromeliaceae), *Consolida ajacis* Niew (Ranunculaceae), *Ranunculus* sp (Ranunculaceae), *Dendrobium phalenopsis* (Orchidaceae) and *Gerbera* sp (Compositae) were not tolerant to a dose of 200 Gy. The most adequate flowers to be submitted to irradiation treatment for disinfestation purpose were those of the Caryophillaceae family and those which can be used as dried flowers, such as members of the *Rhodanthe*, *Helichrysum* and *Limmonium* genera.

1. INTRODUCTION

Brazil has a huge diversity of exotic flora that includes flowers and other ornamental plants of commercial value. Some of these plants have great potential to be explored and expanded to the international market. However, fresh products have to be submitted to a phytosanitary inspection when exported to other countries. The tropical climate of Brazil favors infestation by many pest organisms that do not exist in non-tropical regions. Radiation could be an effective treatment against insects that will prevent their reproduction in the host country. The radiation treatment could be also an alternative to substitute for chemical fumigation with methyl bromide, which destroys the ozone layer of the Earth.

The cut flower is alive and in some cases it continues developing during its vase-life. It can be composed of one or many flowers, and may include leaves and stem, or only flower and stem. Some are harvested as bud and others after the blossom. The vase-life varies with the species or cultivar. Handling and environmental conditions during the vase-life can also affect flower vigor. Infestation by pests can damage drastically the final product, so that it can be considered commercially unsuitable. Flowers do not differ from other biological organisms in that they can be damaged by radiation, depending on the dose. Some authors have irradiated cut flowers with gamma-radiation [1-5], while others have used electron beams [6, 7] for disinfestation.

To avoid the deleterious effects of radiation, cut flowers can be supplied with preservative or holding solutions containing sugar [1, 4, 5]. Unfortunately, this procedure also must be adopted by the florist and the final consumer to assure continued flower quality during the vase-life.

The problem of insects or other arthropods infesting live plants, such as cut flowers, foliage, or cacti is perhaps the most limiting factor to increased international trade. Currently disinfestation by insecticides that are sprayed or dipped is a necessity. However, due to the architecture of flowers, there are many parts of the flower on which an application of the correct amount of pesticide is almost impossible or very difficult. Besides, because misapplication and other biological factors occur, there may be a significant increase of resistance by the pests rendering pesticide application more and more inefficient, if not useless. Moreover, handling or maintaining plants with a potentially toxic residue in their homes could be a nightmare for housewives [2, 8-15].

This paper presents the tolerance and the sensitivity of some cut-flowers to gamma-radiation, and indicates those more suitable for disinfestation by radiation.

2. MATERIALS AND METHODS

2.1. Plant materials

Cut flowers were obtained in the São Paulo city flower market, about 4 km from the laboratory. The stems of the flowers were cut and soaked in tap water for about 15 hours before irradiation in order for the flowers to recover their turgidity.

The following cut flowers were irradiated: *Dianthus caryophyllus* (Caryophyllaceae), *Gypsophila paniculata* (Caryophyllaceae), *Freesia* sp (Iridaceae), *Rhodanthe manglesii* Lindl (Compositae), *Limonium sinuatum* Mill. (Plumbaginaceae), *L. latifolium* Kuntze (Plumbaginaceae), *Narcissus tazetta* L. (Amaryllidaceae), *Helichrysum bracteatum* Andr. (Compositae), *Callistephus chinensis* (Compositae), *Lilium longiflorum* Thunb. (Liliaceae), *Anthurium* sp (Araceae), *Strelitzia* sp (Musaceae), *Matthiola incana* R. Br. (Cruciferae), *Aechmea distichanta* (Bromeliaceae), *Consolida ajacis* Niew (Ranunculaceae), *Ranunculus* sp (Ranunculaceae), *Dendrobium phalenopsis* (Orchidaceae) and *Gerbera* sp (Compositae).

2.2. Irradiation of flowers

Two sources of irradiation were used as follows: a Panoramic cobalt-60 source (Yoshizawa Kiko Co. Ltd.) (205-493Gy/h) or a Gammacell 220 (Nordion International Inc.) (338.0-418.1Gy/h). The flowers were irradiated with the stems soaked in water.

2.3. Maintenance of flowers

After irradiation, the flowers were maintained in holding solutions at room temperature of 24-30°C in summer (December to March), 15-24°C in winter (June to September), 19-27°C in spring (September to December) and autumn (March to June), and light exposure for 9 or 10 hours.

The compositions of the holding solutions were:
- Solution A: 0.02% 8-hydroxyquinoline sulfate, 0.01% citric acid, 25ppm silver nitrate;
- Solution B: 0.02% 8-hydroxyquinoline sulfate, 0.001% ampicillin, 0.001% streptomycin.

3. RESULTS

3.1. Tolerant flowers (tolerant up to 700Gy or more)

3.1.1. Plant material

Dianthus caryophyllus (Caryophyllaceae)

Irradiation source: Gammacell 220
Doses: 0, 300, 600, and 900 Gy (418.1 Gy/h)
Holding solution: A

Carnations were not modified by gamma-radiation if they were irradiated in the developed flowering stage, and they toleratied doses up to 900 Gy. In the irradiated samples there was no new shoot formation as in the control ones. However, this fact did not compromise the commercial value of the irradiated carnations.

Gypsophila paniculata (Caryophyllaceae)

Irradiation source: Gammacell 220
Doses: 0, 200, 400, 600, 800, and 1000 Gy (342 Gy/h)
Holding solution: A

The leaves and opened flowers were not affected by radiation. Bud opening was inhibited, but it did not affect the product value because the majority of the flowers are normally commercialized as semi or totally opened flowers.

Freesia sp (Iridaceae)

Irradiation source: Gammacell 220
Doses: 0, 200, 400, 600, and 800 Gy (342 Gy/h)
Holding solution: A

Bud opening was not inhibited by radiation. The number of opened flowers reached the maximum between 2 - 5 days after irradiation in all samples (Figure 1A) and the number of buds decreased similarly in all doses (Figure 1B), indicating that the inflorescence continued developing even after irradiation. However, flowers originating from buds irradiated with 800 Gy had pale coloration.

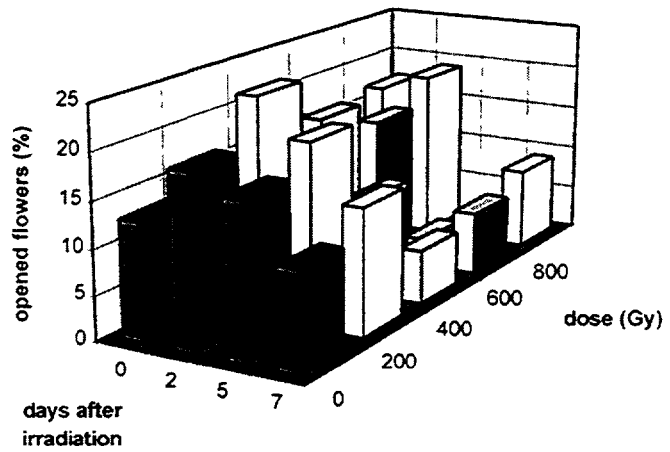

FIG. 1A. Percentage of Freesia opened flowers after gamma-irradiation.

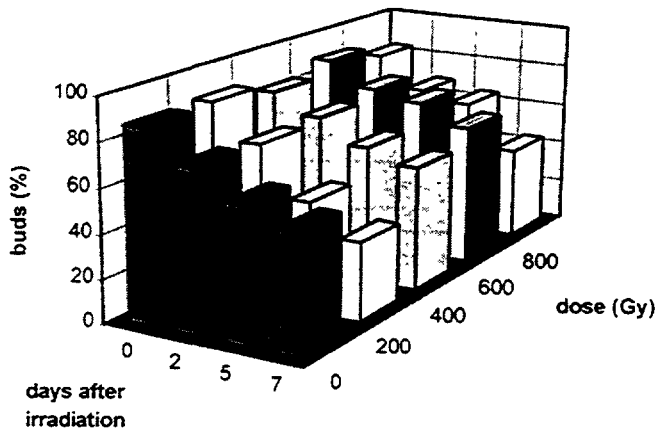

FIG. 1B. Percentage of Freesia *buds after gamma-irradiation.*

Rhodanthe manglesii Lindl (Compositae)
Irradiation source: Gammacell 220
Doses: 0, 200 400, 600, 800, and 1000 Gy (338 Gy/h)
Holding solution: A

Rhodanthe was very resistant to irradiation, without visible alterations. The flower coloration was not affected and the leaves also continued green as did the control ones.

Limonium latifolium Kuntze *and L. sinuatum* Mill. (Plumbaginaceae)
Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800 and 1000 Gy (206 Gy/h)
Holding solution: B

Bud opening of *Limonium* was inhibited by radiation. Thus, only the control samples presented new flowers during 4 or 5 days. However, the main visual aspect of *L. sinuatum* is the colored bracts, which are dry, and these were not modified by radiation. *L. latifolium* also did not lose their commercial value, because they are used as a complement to a flower arrangement and bud color was not affected by the treatment, in spite of the opening inhibition. The leaves of both cultivars were also tolerant to radiation.

Narcissus tazetta L. (Amaryllidaceae)
Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (206 Gy/h)
Holding solution: B

Gamma-radiation up to 1000 Gy did not inhibit bud opening (Figure 2A) and the percentage of opened flowers increased similarly in all samples (Figure 2B). However, there was a delay in the opening process of irradiated buds, which consequently slowed the wilting process of the flowers, and the decrease in fresh weight of irradiated flowers during the vase-life was lower than values for control flowers (Table I).

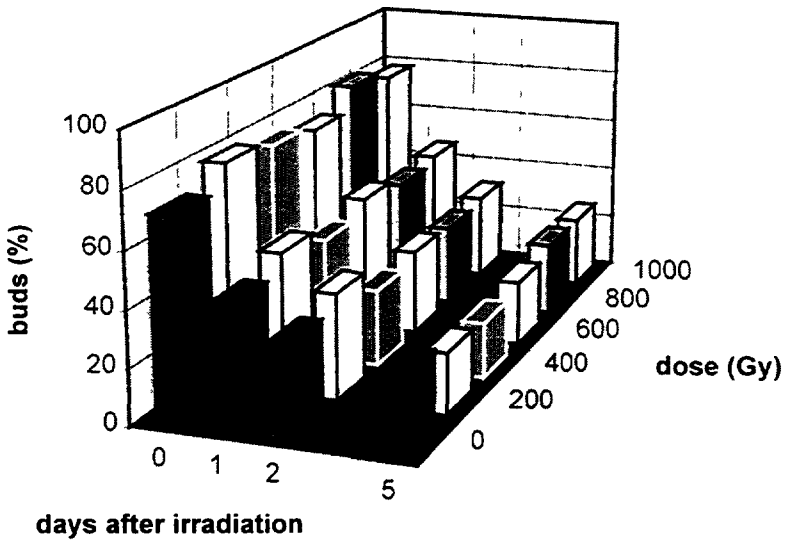

FIG 2A. Percentage of Narcissus *buds after gamma-irradiation.*

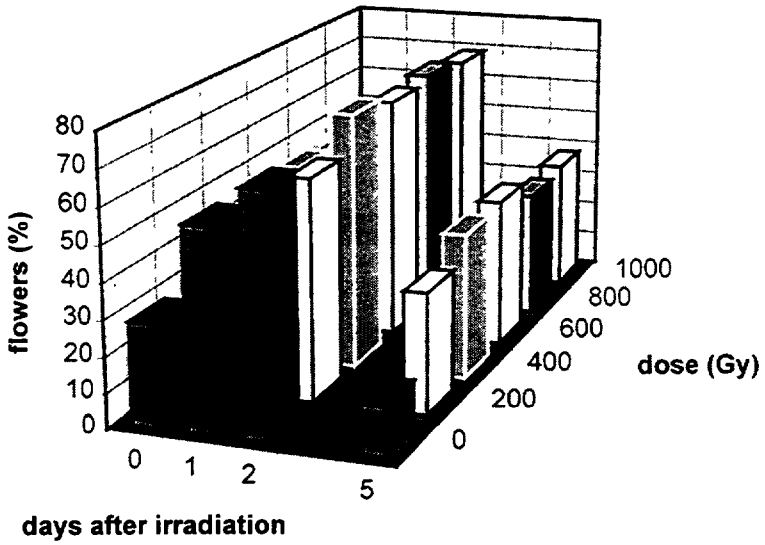

FIG. 2B. Percentage of Narcissus *flowers after gamma-irradiation.*

TABLE I. *Narcissus tazetta* FRESH WEIGHT (%) AFTER GAMMA-IRRADIATION. NUMBER OF STEMS BY DOSE, N=9.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	92.36±4.63	95.38±8.93	94.12±5.24	96.13±7.35	100.85±3.60	95.45±1.48
5 days	80.64±3.00	87.23±13.28	88.90±4.54	92.64±9.71	95.64±5.31	92.66±2.53

Helichrysum bracteatum Andr. (Compositae)
Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (205 Gy/h)
Holding solution: B

In spite of the fresh weight decrease with doses above 400 Gy (Table II), the irradiated samples were not visually modified comparing to the control ones. The development of the inflorescence was not inhibited by any of the doses used.

TABLE II. *Helichrysum bracteatum* FRESH WEIGHT (%) AFTER GAMMA-IRRADIATION. NUMBER OF STEMS BY DOSE, N=9.

Dose (Gy)	0	200	400	600	800	1000*
0 day	100	100	100	100	100	100
2 days	98.90±0.73	99.49±0.72	97.99±3.81	97.81±1.38	96.45±1.15	93.83±1.45
5 days	93.52±4.28	94.97±1.56	85.68±5.90	87.25±0.30	82.75±4.61	82.21±5.22
7 days	87.32±9.78	88.91±4.67	76.93±2.22	80.74±1.96	72.99±6.89	73.88±5.35
9 days	78.88±17.6	81.16±6.84	67.25±0.62	73.19±0.30	63.50±8.28	65.93±4.44
12 days	57.88±21.77	63.11±17.82	49.00±1.80	54.58±4.94	46.84±4.21	47.56±5.71

*N=8

3.2. Moderately tolerant flowers (tolerant up to 500 Gy)

3.2.1. Plant material

Callistephus chinensis (Compositae)
Irradiation source: Gammacell 220
Doses: 0, 200, 400, 600, 800, and 1000 Gy (234 Gy/h)
Holding solution: A

Doses over 400 Gy caused wilting of leaves. Doses of 600, 800, and 1000 Gy also were deleterious, damaging the inflorescence and decreasing vase-life. The fresh weight of irradiated samples decreased significantly 5 days after irradiation (Table III).

TABLE III. FRESH WEIGHT (%) OF *Callistephus chinensis*, AFTER GAMMA-IRRADIATION. NUMBER OF INFLORESCENCE BY DOSE, N=5.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	103.09±1.42	102.40±0.12	99.81±1.11	101.47±0.96	100.10±0,15	101.08±0.48
5 days	101.44±2.59	95.47±2.19	93.51±1.32	90.30±1.74	93.54±3.49	94.06±0.98

Lilium longiflorum Thunb. (Liliaceae)
Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (208 Gy/h)
Holding solution: B

The *Lilium* samples were irradiated in the bud or in semi-opened flower development stages. The buds opened normally up to 400 Gy. With higher doses at the bud stage, flower development reached only the semi-opened stage. When the semi-opened flower was irradiated, however, the opened stage was reached.

3.3. Flowers with low tolerance (below 250 Gy)

3.3.1. Plant material

Anthurium sp (Araceae)
Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, and 600 Gy (236.6 Gy/h)
Holding solution: A

Anthurium was very radiosensitive, presenting discolored spots in the spathe (colored bract) 2 days after irradiation. After 5 days, black dots also appeared in those spots. Fresh weight decreased similarly in all samples up to 5 days after the treatment (Table IV).

TABLE IV. *Anthurium* sp FRESH WEIGHT (%) AFTER IRRADIATION. NUMBER OF FLOWERS BY DOSE, N=5.

Dose (Gy)	0	200	400	600
0 day	100	100	100	100
2 days	100±0.00	99.93±0.10	99.69±0.27	99.31±0.15
5 days	98.28±2.22	99.37±0.51	98.28±1.74	94.73±2.34

Strelitzia sp (Musaceae)

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200,400, 600, and 800 Gy (236.6 Gy/h)
Holding solution: A

Strelitzia was not tolerant to gamma-radiation. The vase-life was shortened by radiation (Table V) and processes of discoloration and wilting were observed. The fresh weight did not modify notably due to the treatment (Table V).

TABLE V. *Strelitzia* FRESH WEIGHT (%) AFTER GAMMA-IRRADIATION AND VASE-LIFE. NUMBER OF FLOWERS BY DOSE, N=5.

Dose (Gy)	0	200	400	600	800
0 day	100	100	100	100	100
2 days	101.27±0.28	98.32±0.90	98.83±0.50	97.76±1.01	98.48±0.49
5 days	96.70±1.98	94.64±2.45	96.65±0.41	95.39±2.60	95.02±0.42
7 days	91.97±3.43	91.06±3.28	-	-	-
vase-life (days)	8	6	5	5	5

Matthiola incana R. Br. (Cruciferae), white and wine color cultivars.

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (234 Gy/h)
Holding solution: A

Matthiola flowers were radiosensitive even at a dose of 200 Gy, presenting bud opening inhibition. Development of the inflorescence was stopped in irradiated samples, while control buds continued generating new flowers. Vase-life was shortened by only one day compared to the controls (Table VI).

TABLE VI. *Matthiola incana* VASE-LIFE (DAYS) AFTER GAMMA-IRRADIATION. NUMBER OF INFLORESCENCE BY DOSE, N=5.

Dose (Gy)	0	200	400	600	800	1000
wine color	6	5	5	5	5	5
white color	7	6	6	6	6	6

Aechmea distichanta (Bromeliaceae)

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (234 Gy/h)
Holding solution: A

Doses of 800 and 1000 Gy caused discoloration of the inflorescence 2 days after irradiation. This was observed later with 200, 400, and 600 Gy, also. The inflorescence discoloration was followed by a decrease of the fresh weight with doses of 600, 800, and 1000 Gy (Table VIII).

TABLE VII. *Aechmea distichanta* FRESH WEIGHT (%), AFTER GAMMA-IRRADIATION. NUMBER OF INFLORESCENCE BY DOSE, N=5.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	99.46±0.38	100.63±1.47	99.11±3.05	96.65±0.11	98.39±4.34	96.31±0.04
5 days	90.92±1.30	95.85±3.79	90.67±4.96	86.51±3.34	87.76±11.77	81.37±6.74

Consolida ajacis Niew (Ranunculaceae)

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (234 Gy/h)
Holding solution: A

Coloration of *Consolida* flowers was not altered by irradiation. However, the leaves were not tolerant even to 200 Gy, presenting an accelerated chlorosis and wilting. Wilting of the flowers was evident at a dose of 400 Gy 5 days after irradiation.

Ranunculus sp (Ranunculaceae)

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (206 Gy/h)
Holding solution: B

The main negative quality change of irradiated *Ranunculus* flowers was the stem bending (Table VIII). All samples were not viable 5 days after irradiation, including the control ones, due to the wilting of the petals. The fresh weight decrease was more accentuated on irradiated samples (Table IX).

TABLE VIII. NUMBER OF STEM BENDING OF *Ranunculus* FLOWERS, 5 DAYS AFTER GAMMA-IRRADIATION. NUMBER OF FLOWERS BY DOSE, N=10.

Dose (Gy)	0	200	400	600	800	1000
No. of flowers	2	5	5	7	7	10

TABLE IX. *Ranunculus* FLOWERS FRESH WEIGHT (%) AFTER GAMMA-IRRADIATION. NUMBER OF FLOWERS BY DOSE, N=10.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	94.18±6.20	90.30±2.43	89.67±2.59	92.59±5.39	88.30±1.18	91.79±8.86
5 days	80.03±9.60	77.10±5.30	73.29±6.67	76.17±4.33	66.48±5.32	71.14±5.37

Dendrobium phalenopsis (Orchidaceae)

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (234 Gy/h)
Holding solution: A

There was bud opening inhibition with all doses, but 200 Gy did not cause visible damages when the irradiation was done on opened flowers. Doses of 800 and 1000 Gy were definitely damaging because they caused flowers to drop.

Gerbera sp (Compositae), small size cultivar - light rose and wine colors; normal size cultivar - light rose color.

Irradiation source: Panoramic cobalt-60 source
Doses: 0, 200, 400, 600, 800, and 1000 Gy (208 Gy/h)
Holding solution: B

The irradiated *Gerbera* negative quality changes included stem bending, flower discoloration, curling of petal, and wilting of the flower. Fresh weight decrease after irradiation was a good parameter of measurement related with the visual quality loss of the flowers (Tables X-XII).

TABLE X. *Gerbera* FRESH WEIGHT (%), AFTER GAMMA-IRRADIATION. SMALL SIZE, LIGHT ROSE COLOR CULTIVAR. NUMBER OF INFLORESCENCE BY DOSE, N=4.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	101.57±0.06	99.79±2.11	100.02±1.15	101.36±0.61	101.50±1.34	95.54±2.41
5 days	98.75±0.38	96.00±1.57	86.33±12.81	95.48±1.00	93.86±3.75	85.47±3.53
7 days	95.20±1.54	90.66±0.32	74.11±21.07*	83.04±3.76	85.20±4.48	76.28±2.87
9 days	90.71±4.51	87.58±1.09	83.04±3.76*	80.87±5.95	79.82±4.64	66.90±4.26
12 days	85.76±4.58	83.17±3.18	75.45±2.62*	-	-	-

*N=3

TABLE XI. *Gerbera* FRESH WEIGHT (%), AFTER GAMMA-IRRADIATION. SMALL SIZE, WINE COLOR CULTIVAR. NUMBER OF INFLORESCENCE BY DOSE, N=4.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	101.81±0.40	100.24±0.48	100.62±0.01	98.21±0.65	99.27±0.36	98.45±3.42
5 days	93.93±1.25	89.46±4.45	86.80±2.58	81.16±1.41	81.14±0.41	79.38±2.74
7 days	87.13±1.14	82.66±4.43	78.87±5.30	73.03±0.84	70.94±2.31	69.45±0.66
9 days	82.59±1.22	78.83±3.32	75.25±6.63	68.73±1.53	67.46±3.65	65.89±0.37
12 days	78.67±0.15	74.64±3.53	-	-	-	-

TABLE XII. *Gerbera* FRESH WEIGHT (%), AFTER GAMMA-IRRADIATION. NORMAL SIZE, LIGHT ROSE COLOR CULTIVAR. NUMBER OF INFLORESCENCE BY DOSE, N=6.

Dose (Gy)	0	200	400	600	800	1000
0 day	100	100	100	100	100	100
2 days	100.77±1.60	94.05±8.69	100.30±3.94	99.24±2.00	95.14±0.08	100.08±0.66
5days	92.56±3.30	79.16±11.22	88.96±6.38	83.65±4.10	78.83±0.96	86.30±4.93

4. CONCLUSIONS AND DISCUSSION

Radiation could be an effective treatment against insects in some cut-flowers such as carnations (*Dianthus*) and *Gypsophila*, (Caryophillaceae), *Limonium* (Plumbaginaceae) and *Narcissus* (Amaryllidaceae). These flowers were tolerant to doses up to 900 or 1000 Gy, without negative alterations that could compromise the commercial quality of the flowers. *Dianthus* and *Gypsophila* also were reported as tolerant to a gamma-radiation dose of 500 Gy by Wit and van de Vrie [3]. Tanabe and Dohino [7] reported that *Gypsophila* was tolerant to 400 Gy electron beam irradiation, but had slightly withered petals with 600 Gy. *Limonium sinuatum* cv.'Sophia Soft Pink' was tolerant to 600 Gy electron beam radiation, but *L. sinuatum* cv. 'New Blue' had slight chlorosis of leaves and stems [7]. The *Limonium sinuatum* samples we irradiated were constituted by rose and blue colors, both tolerant to gamma-radiation.

The response to gamma-radiation varied by genus in the Compositae family. *Rhodanthe* and *Helichrysum* were tolerant and *Callistephus* was moderately tolerant. On the other hand, *Gerbera* was not tolerant, and neither was *Chrysanthemum* [4, 5]. *Callisthephus* was reported as tolerant to 500 Gy by Wit and van de Vrie [3] and *Gerbera* was tolerant to 400 Gy electron beam radiation [7], but not tolerant to 100 Gy gamma-radiation [3]. Because the Compositae is the most abundant family of the Angiospermae division, comprising about 19,000 species of plants [16], it could justify the variable response observed in this work. It would be interesting to examine why some genus/species are tolerant to radiation and others are sensitive.

Rhodanthe (Compositae), *Helichrysum* (Compositae) and *Limonium* (Plumbaginaceae) are used as dried flowers and have naturally low water content. Possibly low water content could be one of the reasons for tolerance to radiation.

The tropical plants, *Strelitza* (Musaceae), *Dendrobium phalenopsis* (Orchidaceae) and *Aechmea distichanta* (Bromeliaceae), were sensitive to gamma-radiation. *Strelitza* and *Aechmea* have a high level of water content, which may promote radiosensitivity.

Anthurium was very sensitive to gamma [3] and electron beam [7] radiation, and cannot be irradiated even with low doses, as observed in this work.

Fresh weight was a good parameter of measurement to evaluate the radiation effect in some cases, as with *Narcissus tazetta, Callistephus chinensis, Aechmea distichanta, Ranunculus sp* and *Gerbera sp.* However, it is necessary to develop predictive tests and quantitative measures of internal quality of the cut-flowers to evaluate the product [17].

Intercontinental trade of flowers and other ornamental plants is increasing significantly. Tropical exotic plants are very coveted by North American, Japanese, and European markets, the three major world flower markets. Quality improvement of flowers and quality control are being considered as important goals to the floricultural products world trade [17]. The improvement of the quality of some radiosensitive flowers can be a step to increase the tolerance to the radiation and to other kinds of stress that shorten the vase-life of the cut-flowers.

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RADIATION DISINFECTION OR DISINFESTATION OF NEMATODES, APHIDS, MITES, THRIPS, AND OTHER PESTS ON FOOD PLANT MATERIALS: EVALUATION FOR EFFECTIVENESS AND PRODUCT QUALITY[1]

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Abstract

Many fresh herbs, ornamental plants, and several varieties of taro grown in Hawaii are infested with various pests such as aphids, mites, thrips, and nematodes. Finding an efficacious quarantine treatment for these commodities is difficult because most cannot tolerate heat or cold, and a suitable chemical treatment is lacking. Irradiation could be a feasible, practical alternative. Quality of these irradiated materials should be studied to help determine if irradiation is a suitable quarantine treatment. Of the ten fresh herbs irradiated with up to 0.70 kGy, five (rosemary, thyme, oregano, parsley, chives) are very tolerant, and show no difference from the controls after two to three weeks at 7 °C. Red ginger and four cultivars of heliconia, very attractive ornamental plants, can be irradiated at 0.75 and 0.50 kGy, respectively, and have a vase life of 10 days or more at 21 °C. Leafminer in bean plants cannot emerge when irradiated at 0.15 kGy. The nematode, *Meloidogyne javanica,* which infects taro and ginger, is prevalent in Hawaii. To cause mortality in second stage juveniles (J2), a gamma-radiation dose higher than 4.0 kGy is necessary. Suppression of hatching of egg masses requires doses of 2.0 kGy and above. Galling of tomato plants inoculated with J2 and egg masses decreases when J2 and egg masses were irradiated at 3.25 kGy and above. Heating J2 at 43 °C for 10 min before inoculating them into the plants effectively reduces root galling. Synergism was not found between heat treatment (49 °C for 10 or 20 min) and irradiation with up to 0.015 kGy, the dose above which sprouting of ginger rhizomes and taro cormels is inhibited. The results suggest that irradiation is promising as a quarantine treatment for selected fresh herbs and ornamental plants, but not for control of nematodes in root crops.

1. INTRODUCTION

In the past few years, the move to find alternatives to chemical fumigation of fresh commodities for disinfection or disinfestation has intensified. Participating member states at the Montreal Protocol have agreed that methyl bromide would be phased out for all uses by January, 2001. Thermal and cold treatments have been used on limited number of commodities as a quarantine treatment, but these treatments are commodity-specific in terms of the treatment regime. Treatment time are usually quite long, and product quality has been affected due to some biochemical changes in the commodity resulting from an overexposure to the applied time-temperature regime (heat or cold) [1]. Among its various potential applications, irradiation as a quarantine treatment is perhaps one of the most feasible and practical alternatives to chemical, heat, or cold treatment for fresh commodities [2]. Quarantine treatment with irradiation is simple because the dose applied to control a certain group of pests such as fruit flies is becoming generic; it is efficacious because it is both effective in meeting quarantine requirements totally and efficiently in terms of a short treatment time.

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Irradiation is versatile because its application is broader than any other quarantine treatment method in terms of its applicability to many commodities and different species of pests.

Food plant growers and the floriculture industries in tropical and subtropical regions such as the Hawaiian islands are confronted with quarantine restrictions because their plant materials are often infested with melon thrips, aphids, mites, mealybugs, cockerell scale, other pests, or nematodes. Plant foods, cut flowers, and foliage plants shipped to the United States mainland or international destinations sometimes are not properly treated. Since 1988, over 4,000 floral plant shipments from Hawaii have been rejected at ports of entry in California alone when insects were found on the plant materials. Examples are dendrobium and red ginger. Elimination of these infestations by chemical or thermal means is difficult, and often with mixed results due to limited effectiveness in inactivating the pests and unwanted phytotoxicity to the plants.

When irradiation is applied as a quarantine treatment for fruit fly control, the concepts vary from causing sterility of the treated pest, to preventing adult emergence, to blocking the eggs from hatching. In the most recent publication of USDA-APHIS policy statements, the agency's expectation is that ". . . In those instances where pest organisms survive treatment, it is essential for quarantine purposes that the organism is unable to reproduce, and it is desirable for the organism to be unable to emerge from the commodity unless it can be easily distinguished from a non-irradiated pest of the same species." [3] A generic, minimum absorbed dose of 250 Gy is being proposed for treating papayas, lychees, and carambolas grown in Hawaii to prevent fruit fly spread. This is a modification of an earlier quarantine regulation on irradiation as a quarantine treatment of Hawaii grown papayas.

On pests other than fruit flies, the minimum effective dose for non-reproduction and non-emergence have been studied at various laboratories including those participating in this FAO/IAEA Research Coordinating Program. But the necessary minimum doses have not been defined or confirmed. At the same time, quality of commodities irradiated as a quarantine treatment to control those pests other than fruit flies need to be studied in order that their shelf or vase life, and marketability, are preserved. Determination of the upper dose for each commodity or a group of commodities, which can be called tolerance dose, is necessary to make the irradiation treatment practical. It has been observed that the tolerance dose of a given commodity should probably be 2 to 3 times higher than the minimum quarantine dose due to variations in dose rates in any given irradiator, package configuration, and product density.

The objective of our project was to explore the technical feasibility of using gamma irradiation alone or in combination with other means such as thermal treatment to disinfect or disinfest selected food and ornamental plant materials with pest problems that result in their being restricted in interstate and international shipments due to quarantine regulations.

Fresh herbs and a number of ornamentals grown in Hawaii have very good economic potential but are in need of a suitable quarantine treatment. Irradiation may just fit that need. Nematodes are another pest problem on root crops. With restrictions or unavailability of chemicals for nematode control, the possibility might exist for irradiation as an alternative quarantine treatment of plant materials for nematode control. The radiosensitivity of root-knot nematodes, *Meloidogyne* spp., has not been thoroughly documented. Irradiation doses required to kill or sterilize nematodes in all stages are unknown. Preliminary studies were carried out in Thailand of effects on second stage juveniles (J2) and egg masses of *M. incognita* exposed to gamma-radiation at 1.2 and 1.5 kGy, respectively. J2 stages were not killed in 14 days, and eggs were able to hatch (Chinnasri, unpubl.). Bioassays also showed that irradiated juveniles and juveniles from irradiated eggs induced galling and reproduced on tomato plants (Chinnasri, unpubl.). To understand the infection of plant materials by *Meloidogyne javanica,* which is prevalent in Hawaii, and whether or not irradiation is a feasible process, we need to know the effect of irradiation on J2 juveniles and eggs of *M. javanica*, and also the possibility of combining irradiation and heat treatment.

Our efforts have been mainly in these areas. We have intended to study the control of mites, thrips, aphids and other pests by irradiation. However, due to resource constraints, and lacking interested colleagues experienced in the rearing of these pests, this part of the study may be carried out at a later date.

2. MATERIALS AND METHODS

2.1. Irradiation of fresh herbs

Nine (9) herbs and taro leaves popularly grown in Hawaii were used as experimental materials. These included:

Thyme (*Thymus vulgaris*)
Rosemary (*Rosmarinus offinalis*)
Oregano (*Origanum vulgare*)
Parsley (*Petroselinum crispum*)
Spearmint (*Mentha spicata*)
Chives (*Allium schoenoprasum*)
Arugula (*Eruca vesicaria ssp. sativa*)
Dill (*Anethum graveolens*)
Basil, Sweet, Thai and Opal (*Ocimum basilicum*)
Taro leaves (*Colocasia esculenta cv. Bunlong*)

These fresh herbs and taro leaves were harvested from commercial farms on the island of Oahu, brought to the Food Technology Building, and irradiated at the Hawaii Research Irradiator either on the day of harvest or the next morning. The plant samples were irradiated with 0.25 to 0.70 kGy in replicates. After irradiation, they were kept in the refrigerators at 7 °C, except basil which was kept at ambient temperature (25-28 °C).

Evaluations of these plants were by visual inspections every two to three days up to three weeks. The irradiated samples were compared with controls in terms of color changes (discoloration), dehydration, wilting or curling of the leaves, and presence of burned or brown spots.

2.2. Irradiation of ornamentals

Popularly grown in Hawaii are two sizes of red ginger plants and four cultivars of Heliconia used in this study:

Red ginger (*Alpinia purpurata*)
Heliconia (*Heliconia caribaea*)
Heliconia (*Heliconia orthotrica*)
Heliconia (*Heliconia spathocircinata x psittacorum*)

Replicate irradiation experiments were conducted with doses varying from 0.25 to 0.75 kGy. After irradiation, the flower samples were kept in water in containers at 21 °C. Evaluations were also by visual inspections every two to three days up to two weeks. Irradiated samples were compared with controls in quality factors somewhat similar to fresh herbs indicated above. Any appearance of wilting/curling, softening (loss of turgidity) or burnt/brown spots would be on petals and small stems.

2.3. Irradiation of leafminer (*Liriomyza trifolii*) in bean plants

Beans plants infested with leafminer were irradiated up to 0.75 kGy to determine the minimum dose needed for non-emergence. Leafminers also infest many of the fresh herbs mentioned above.

2.4. Irradiation of root-knot nematode (*Meloidogyne javanica*)

2.4.1. Propagation of Meloidogyne javanica

M. Javanica populations were propagated on Rutgers tomato plants (*Lycopersicon esculentum*) in a greenhouse at 30 °C. Egg masses or juveniles were collected from 15-day-old cultures. Two experiments were conducted in the laboratory and the greenhouse.

2.4.2. Irradiation of M. javanica

For mortality tests, approximately 100 one-day-old J2s were placed in vials containing sterile water and irradiated at 2.0, 4.0, 6.0, 7.0, 7.5, and 8.0 kGy. Controls were not irradiated. Dose rate for these experiments was 9.93 Gy/min. Triplicate runs were made. J2s were characterized as dead when they were observed to lay motionless, their bodies swelled, or they turned brown. Juveniles were observed daily, and the number of dead J2s were counted and recorded.

To determine the effect of irradiation on hatch, egg masses collected from females on 45-day-old tomatoes plants were placed individually into vials containing sterile water and irradiated at 2.0, 3.0, 4.0, 5.0, 6.0, 6.25, and 6.5 kGy besides the control. Irradiated egg masses were transferred from vials into 5-cm-diam. Petri dishes filled with sterile water and incubated at 22-25 °C. These egg masses in the Petri dishes were examined daily and the number of J2 counted. At day-15, the gelatinous matrix covering the eggs was dissolved with sodium hypochlorite [4], and the unhatched eggs were counted. The total number of eggs per egg mass was determined by combining the total number of J2 with the number of unhatched eggs. This number was used to compare the percentage of hatch among treatments. There were three replicates per irradiation dose.

2.4.3. Bioassay

For bioassay, approximately 5,000 one-day-old J2s or 20 egg masses were placed in vials containing sterile water and irradiated at 1.0, 2.0, 3.0, 3.25, 3.75, 4.0, 4.25, and 4.5 kGy. Controls were not irradiated. The dose rate for these experiments was 9.71 Gy/min. Irradiated juveniles were then inoculated onto 5-cm-tall Rutgers tomato seedlings in 10-cm-diam. clay pots. Irradiated egg masses were observed under a microscope to insure that there was no contamination by any newly-hatched juveniles. The egg masses were then transferred into another vial containing sterile water and inoculated onto a 5-cm-tall Rutgers tomato seedling transferred in a 10-cm-diam. clay pot. Inoculated tomato plants were arranged in a randomized complete block (RCB) with five replicates per treatment, two plants per replicate, and maintained in the greenhouse. Forty-five days after inoculation, the tomato roots were rated for galling where 0 = no galling, 1 = trace infections with a few small galls, 2 = < 25%, 3 = 25-50%, 4 = 50-75%, and 5 and above = > 75% of roots galled [5]. Eggs were extracted from tomato roots with a sodium hypochlorite technique [4]. and the number of eggs per gram of wet root weight was calculated.

2.5. Combined heat treatment and gamma-radiation of root-knot nematode

Two 4 x 4 factorial experiments were conducted: one on J2s and the other on egg masses. Approximately 5,000 one-day-old J2s or 20 egg masses were placed in 10-ml vials containing sterile water. The vials were immersed in a water bath at either 43 °C for 10 min, 49 °C for 10 min, 49 °C for 20 min, or maintained at room temperature (25 °C) as control. Immediately after the hot water treatment, the J2s or egg masses were exposed to gamma-radiation with 0.005, 0.01, or 0.015 kGy. Controls were not irradiated. The hot water-treated and irradiated juveniles and egg masses were then inoculated onto 5-cm-tall Rutgers tomato seedlings grown in 1-cm-diam. clay pots and transferred into the greenhouse. The treatments were triplicated, with two plants per replicate. All tomato plants were harvested 45 days after inoculation. Roots were rated for galling as indicated above, and eggs were extracted from roots with sodium hypochlorite. The number of eggs per gram of wet root weight was calculated.

2.6. Radiation source

The Hawaii Research Irradiator with 100 ^{60}Co capsules of the U.S. National Brookhaven Laboratory design, located in the Food Technology Building, University of Hawaii at Manoa, was used as the gamma radiation source for all the experiments. During the experimental period, the

activity of the ^{60}Co source was 4,943 to 4,832 Ci (1.83×10^{14} to 1.79×10^{14} Bq). The dose rates were 9.93 to 9.71 Gy/min, and the maximum to minimum dose rates was 1.09.

3. RESULTS AND DISCUSSION

3.1. Shelf/Vase life as indicator of tolerance of fresh herbs and ornamental plants to irradiation

3.1.1. Fresh herbs

Evaluations of fresh herbs and taro leaves after irradiation were carried out mainly by R. Hamasaki who is very knowledgeable and experienced in the cultivation and quality of herbs. From replicate runs, the observed results are expressed in Table I. These nine herbs and taro leaves seem to fall into three categories in terms of their tolerance to gamma-radiation: quite tolerant (at 0.60 to 0.70 kGy); moderately tolerant (about 0.45 to 0.50 kGy); and not tolerant (0.25 kGy and below).

TABLE I. TOLERANCE OF VARIOUS FRESH HERBS AND TARO LEAVES TO GAMMA-RADIATION

Days quality was retained	Quite Tolerant @ 0.60 - 0.70 kGy	Moderately Tolerant @ 0.45 - 0.50 kGy	Not Tolerant @ 0.15 - 0.25 kGy
21	Rosemary		
14	Thyme		
14	Oregano		
14	Parsley		
14	Chives		
10		Spearmint	
10		Taro leaves	
7			Dill
3			Basil (Sweet, Thai, Opal)
2			Arugula

Observations of tolerance to gamma-radiation in terms of quality retention were as follows:

(1) Rosemary (*Rosmarinus offinalis*) - Tolerates irradiation very well. At 21 days, irradiated samples (0.25 to 0.70 kGy) were as good as the control.
(2) Thyme (*Thymus vulgaris*) - Tolerates irradiation to 0.60 kGy up to 14 days without any difference from the control.
(3) Oregano (*Origanum vulgare*) - Can tolerate irradiation up to 0.70 kGy quite well. At 14 days, most samples were as good as the control, except one or two showing necrosis in young leaves, and a few older leaves turning black. At 21 days, most samples (0.25 - 0.70 kGy) were in fair condition.
(4) Parsley (*Petroselinum crispum*) - Tolerates irradiation up to 0.60 kGy up to two weeks. The leaves begin to turn yellow after two weeks.
(5) Chives (*Allium schoenoprasum*) - Tolerates irradiation to 0.60 kGy up to 14 days, and shows no difference from the control.
(6) Spearmint (*Mentha spicata*) - Those treated up to 0.50 kGy appear to tolerate irradiation

quite well up to about 10 days. At 14 days, all samples including the control have their lower leaves turning brown or black.

(7) Taro leaves (*Colocasia esculenta cv. Bunlong*) - Used as one type of Hawaiian foods, taro leaves can tolerate irradiation to 0.45 kGy up to 10 days.

(8) Dill (*Anethum graveolens*) - Sensitive to irradiation. Can tolerate 0.25 kGy for one week with acceptable quality. Irradiation at 0.50-0.60 kGy causes yellowing of stems and leaves.

(9) Basil, Sweet, Thai and Opal (*Ocimum basilicum*) - Probably the most sensitive to irradiation among the group of fresh herbs tested. Judged acceptable three days after irradiation at 0.25-0.60 kGy, the stems and leaflets begin to show yellowing at 25 °C. By the seventh day, they become unmarketable. In general, the shelf life of untreated basil is rather limited. Lowering the storage temperatures, and keeping the plants in water may help prolong the life of cut basil.

(10) Arugula (*Eruca versicaria spp. sativa*) - This herb appears very sensitive to irradiation, even at the low dose of 0.15 kGy. Irradiation causes arugula to turn yellow much faster than the control.

3.1.2. Red ginger and Heliconia

(1) Red Ginger (*Alpinia purpurata*) - These are medium to large red ginger plants, which are very attractive ornamentals. The cut plants used in the experiments are mostly about 50 to 55 cm tall. Samples were irradiated at 0.25, 0.50, and 0.75 kGy and kept in water at 21° C after irradiation. There appears to be some differences between those grown in the summer months and those grown in the cooler months (spring and fall). Experimental samples collected in June had a vase life of abut 10 - 12 days with no observed differences between the controls and those irradiated with up to 0.75 kGy. Those sampled in cooler months (March and October) had 4 to 5 days of additional vase life.

(2) Heliconia - Several cultivars of Heliconia were collected and their tolerance to gamma-radiation evaluated. The names of the cultivars were given above. They were irradiated with 0.25, 0.50, and 0.75 kGy. It was observed that some sensitivities were exhibited by these plants at various doses, such as softening, bending, and some browning or burning of the tips. Overall results show that heliconias irradiated up to 0.50 kGy were comparable to the control for up to 7 - 10 days when keep in water at 21°C.

3.2. Irradiation to cause non-emergence of leafminer

Six replicate experiments were conducted to determine the minimum dose for non-emergence of the leafminers which infest many plants in Hawaii. The highest dose used was 0.75 kGy. It was found that 0.15 kGy was sufficient to cause non-emergence of the leafminers.

3.3. Effects of irradiation on root-knot nematode, *Meloidogyne javanica*

3.3.1. Mortality of J2 and hatch of egg masses

There was minimum effect on the J2s up to 15 days with treatment of up to 4.0 kGy. There was no significant difference in mortality between the control and those irradiated with up to 4.0 kGy ($P = 0.01$). After irradiated with 6.0 kGy, mortality of J2s began to increase after 5 days, reaching 80% at 15 days. With a dose of 7.0 kGy, death of J2s was observed by the second day and increased rapidly until all were dead by day 5 after exposure. Mortality was 100% on the day following exposure to 7.5 and 8.0 kGy.

Overall, hatching decreased with increasing radiation doses. Irradiation at doses of 2.0 and 3.0 kGy affected hatching. The number of J2 hatching from egg masses irradiated at 4.0 to 6.0 kGy was very low throughout 15 days of the experiment. Doses of 6.25 and 6.5 kGy completely inhibited egg hatch.

3.3.2. Bioassay

Bioassay proved to be a more sensitive indicator of the effect of irradiation on *M. javanica* juveniles and egg masses than the observations of mortality or hatch. A dose as low as 2.0 kGy resulted in a reduction of root galling by J2s and in the number of eggs per gram of root (Table II). Egg masses were less sensitive to irradiation than the J2s. A reduction in galling and in the numbers of eggs/g root did not occur below doses of 3.25 kGy. Exposure of J2s and egg masses to 4.25 kGy or greater resulted in no galling and no reproduction as measured by eggs/g root (Table II).

3.4. Effects of combined heat and irradiation on root-knot nematode, *M. javanica*

Irradiation treatments did not affect galling or numbers of egg/g root on tomato plants by J2s or eggs as compared to the nonirradiated nematodes ($P > 0.05$). Heat treatments, however, reduced galling and nematode reproduction on tomato plants (Table III). Additionally, there was no interaction between irradiation and heat treatment ($P > 0.05$). Reproduction was completely inhibited by heat treatment of 49 °C for 10 or 20 min (Table III). The J2s were adversely affected by heat treatment at 43 ° C for 10 min as measured by subsequent reduction in root gall index (Table III). In contrast, heat treatment at 43 °C for 10 min was insufficient to inactivate eggs, resulting in a greater number of egg production than the eggs that were not exposed to heat (Table III).

TABLE II. ROOT GALL INDEX AND NUMBER OF EGGS/ g ROOT OF *MELOIDOGYNE JAVANICA* ON TOMATO AFTER IRRADIATION OF J2 AND EGGS OF THE NEMATODE.

	Root gall index		Eggs/g root	
(kGy) Irradiation dose	J2	Eggs	J2	Eggs
0	5.0 a	4.4 a	5.3 a	5.3 a
1	5.0 a	4.8 a	5.2 a	4.9 ab
2	4.0 b	4.5 a	5.0 b	5.0 ab
3	3.4 c	4.6 a	4.4 c	4.5 b
3.25	2.7 d	2.3 b	3.0 d	3.9 c
3.5	2.6 d	2.3 b	2.6 e	2.7 d
3.75	1.8 e	1.4 c	2.7 e	2.3 d
4	1.0 f	0.5 d	2.2 f	1.5 e
4.25	0 g	0 d	0 f	0 f
4.50	0 g	0 d	0 f	0 f

Means in columns followed by the same letter are not different ($P<0.05$), according to Duncan's multiple-range test performed on $\log_{10}$-transformed data.

4. DISCUSSION

It is interesting and encouraging to note that five out of ten fresh herbs including taro leaves were found to be quite tolerant to gamma-radiation up to 0.70 kGy. The rest are moderately tolerant to radiation (around 0.50 kGy), or not tolerant to radiation (below 0.25 kGy). Those that are very tolerant to radiation would be good candidates to be irradiated as a quarantine treatment to control pests other than fruit flies, though the minimum dose required to control these various pests remains to be defined.

The two ornamental plants, red ginger and heliconia, also appear to be good candidates for irradiation as a quarantine treatment for export markets. Growers spend considerable time and efforts in washing to get rid of pests that might be on the plants before packing them for shipments. Yet it is

TABLE III. EFFECT OF HOT WATER TREATMENT ON REPRODUCTIVE CAPABILITY OF J2s AND EGGS OF *MELOIDOGYNE JAVANICA* AS MEASURED BY ROOT GALL INDEX AND NUMBER OF EGGS/g WET ROOT WEIGHT

	Root gall index		Eggs/g root	
Hot water treatment	J2	Eggs	J2	Eggs
Control	5 a	5 a	24,815 a	27,835 b
43°C for 10 min.	3.9 b	4.9 b	18,920 a	33,670 a
49°C for 10 min.	0 c	0 c	0 b	0 c
49°C for 20 min.	0 c	0 c	0 b	0 c

Means in columns followed by the same letter are not different ($P<0.05$), according to Duncan's multiple range test.

not a complete assurance that all pests are washed off the plants. That is the reason for rejection of many shipments from Hawaii of cut flowers at the port of entry in California. If irradiation is used, the floriculture industry should see an improvement in their postharvest handling of cut flowers and having a suitable quarantine treatment which should be readily acceptable to the public.

Plant-parasitic nematodes are often quarantine pest subject to importation bans. The infestations are particularly difficult to eliminate when the infested material is vegetative propagation materials, such as seed potatoes, ginger rhizomes, taro cormel, or bulbous crops. Currently, disinfestation procedures rely upon hot water treatments, chemical dips or combinations of the two [6]. Irradiation has proven to be an effective method to disinfect many processed and perishable foodstuffs of bacteria, fungi, and insects [7,8]. It may also have applicability for disinfection and disinfestation of nematodes.

One of the most important considerations for the control of nematodes with irradiation is the effect of irradiation on plants [9]. The treatment must not adversely affect the growth of the plant, and should be based on the inability of nematode to reproduce rather than its immediate death. Applicability of irradiation on crops such as ginger rhizomes or taro cormels for disinfestation of *M. Javanica* is dependent upon the quality of the rhizomes and the cormels after treatment. The sprouting of ginger rhizomes and taro cormels is inhibited by radiation doses of 0.025 kGy and below [10 and Chinnasri, unpubl.]. Higher doses would cause adverse effects on the rhizomes and cormels.

Disinfestation does not require the elimination of the nematode from the plant material, but only prevention of subsequent reproduction. Sublethal doses of irradiation did affect the viability of *M. javanica*. To enhance the efficacy of sublethal exposures, the nematodes were stressed with heat prior to irradiation. However, the heat stress was sufficient in itself to kill the nematodes and did not bring about any synergistic effect with irradiation.

5. CONCLUSIONS

(1) Results from this study of applying gamma-radiation to a number of fresh herbs and cut flowers show that some are quite tolerant to radiation up to about 0.75 kGy which is an encouraging sign that irradiation could be used as a quarantine treatment for these plant materials for controlling pests other than fruit flies.

(2) A low dose (0.15 kGy or less) required to prevent the emergence of leafminers is another encouraging sign that this pest, which invades a number of herbs and ornamental plants, could be controlled by irradiation. Between 0.15 kGy and the tolerance dose of the plants indicated above (0.50 to 0.75 kGy), a factor of 3 to 5 in the minimum required vs the maximum tolerable should be very feasible for the industry to adopt.

(3) Nematodes appear to be a very radiation-resistant pest. The doses required for non-hatching of the egg masses and mortality of the second stage juveniles are much too high for any plant material to tolerate. Since nematodes seem to be quite heat sensitive, further research to find a suitable combination treatment using heat, irradiation, and/or other means to achieve a synergistic effect of causing non-reproduction of nematodes in plant materials would be very useful.

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DISINFESTATION OF LITCHI STEM-END BORER *Conopomorpha sinensis* BRADLEY WITH IRRADIATION FOR EXPORT OF LITCHI FRUITS

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Abstract

Larvae of the litchi stem-end borer, *Conopomorpha sinesis* Bradley, in litchi fruits were exposed to ^{60}Co gamma irradiation doses ranging from 0-400 Gy as a quarantine treatment. Criterion of effectiveness of the irradiation dose was based on preventing adults emerging from treated fruits infested with the larvae. Probit analysis showed that the irradiation dose that caused 99.5% mortality of larvae was 254 Gy (220-289 Gy) and 99.9968% mortality of the 3rd instars was 267 Gy (184-351 Gy) with 95% fiducial limits. The mortality of litchi stem-end borer increased when the irradiation treatment was coupled with extended cool storage (76°C). Hatching of eggs was decreased with increasing dosages to the eggs of 250-600 Gy. Prepupae treated at 300-600 Gy were more radiosensitive than 4-5 days old pupae. The results of visual quality observation indicated that the rotten fruit rate of litchi fruits was reduced by 250-350 Gy treatment.

1. INTRODUCTION

The litchi fruit tree has been grown in China , the site of the first plantings in the world, for a long time. China has the largest area planted to litchi in the world [1]. The litchi stem-end borer, *Conopomorpha sinesis* Bradley [2], is a major insect pest on litchi fruits, shoots, and leaves [3], [4]. Presently the litchi stem-borer is reported from South China, Taiwan, Thailand, Nepal, and India. This insect pest not only causes economic loss of fruit in the orchard, but quality of fresh fruit is reduced because of its presence.

here has been a long history of the exportation of litchi fruit products from China to the Philippines, Singapore, and other countries in Southeast Asia. Exportation of the fruits now or will in the future extend to Europe and America. In order to prevent the dispersal of the litchi stem-end borer to other countries, it is necessary to disinfest the fruits. Thus, it is necessary to develop methods for quarantine treatment of this pest.

Revocation of the registration of ethylene dibromide (EDB) by the United States [5], as well as the intention of other countries to phase out its use, has necessitated development of alternative treatments. In addition, the impending ban on methyl bromide because of its potential to deplete stratospheric ozone is likely to strengthen further the acceptance and application of irradiation as a quarantine treatment. Gamma irradiation has been proposed as a possible quarantine treatment for commodities subject to infestation by fruit flies [6] and codling moth [7], [8]. Research is needed to determine whether irradiation can be used as an effective quarantine treatment for disinfestation of litchi stem-end borer.

Our project, partly funded by IAEA, aims at developing methods for disinfestation of this insect pest with gamma irradiation. This report is based on the results of our studies conducted from 1992-1997.

2. MATERIALS AND METHODS

2.1. Radiation Source

The irradiator used in the research is a cobalt-60 unit from the Nordion Company (Canada designed irradiator) located in the Biophysics Building, South China Agricultural University. The source during the period of the experiments from 1992-1997 was 14.8×10^{14} Bq , 0.954×10^{14} Bq, 0.838×10^{15} Bq , 3.7×10^{15} Bq, 3.19×10^{15} Bq and 2.76×10^{15} Bq, respectively, with a dose rate of 6.45 Gy/min, 6.04 Gy/min, 6.18 Gy/min, 6.69 Gy/min, 6.47 Gy/min and 4.99 Gy/min, respectively.

2.2. Source of infested litchi fruits and irradiation of larvae

The litchi variety used in our experiments was Wai Chee. Fruits containing larvae were picked up from orchards of Huadong Country, Guangzhou. Fruits intended for infestation were free of insecticides. In 1992-1996, the fruits were collected from the orchard, infested fruits with larvae were selected carefully in the laboratory, and then put into a mesh cloth bag to be treated at 4-7 doses with ^{60}Co gamma radiation from 0-400 Gy. Each dose was replicated three or five times. In 1994 and 1996, the infested fruits were divided into two groups; one group was stored at room temperature and another group was coupled with extended cool storage (7°C) after treatment. In 1997, fruits infested by the third instars of the stem-end borer were obtained from a colony maintained at a litchi orchard on fresh litchi fruit. The litchi trees with fruits in the pre-ripe period were covered with a nylon mesh cage 4 m in length, breadth, and height. Field-collected pupae were kept in the laboratory at room temperature for adult emergence. The emerging adults were then transferred to field cages and allowed to oviposit on the fruit surface. Hatching larvae bored into the fruits and developed. Fruits were held for 13 days to obtained third instars for the irradiation test. The infested fruits with third instars were divided into seven groups, and immediately treated with ^{60}Co gamma radiation at the dosage of 0, 100, 150, 200, 250, 300, and 350 Gy. There were five replicates in each treatment. After treatment the fruits were put into mesh cloth bags and fresh leaves were placed inside the bag. When the mature larvae came out from the fruits, they settled in the bags or leaves to spin cocoons. The number of cocoon and adult emergence was counted daily. Treated fruits were dissected and examined for larvae on the 7th day after treatment. Total mortality of test larvae was calculated on the 15th day after treatment.

2.3. Irradiation of the cocooned prepupae and cocooned 4-5-day-old pupae

Cocooned prepupae and pupae were collected from among the leaves of orchards of Huadu, Guangzhhou. Both the precocooned pupae and 4-5-day-old pupae were divided into five groups, and immediately treated with gamma radiation at dosages of 0, 300, 400, 500, and 600 Gy. Each dose was replicated three times. Mortality of cocooned pupae was counted seven days after treatment.

2.4. Irradiation of eggs

Eggs were selected from the litchi fruits. The female lays eggs singly, mostly placing them in grooves on the fruit surface. It is necessary to observe the fruit carefully in the laboratory for eggs, and make a round mark around the egg for further examination. Fruits with eggs were divided into five groups, and immediately treated with radiation at 0, 300, 350, 400, and 600 Gy, with three replications. Hatched and unhatched eggs and larvae were calculated 5 days later.

2.5. Visual quality observation

Fruits collected from the fields were treated immediately with gamma radiation. Five treatments were given at 0, 250, 300, 350, and 400 Gy. Each treatment was replicated three times, and in each replication there were about 110 fruits. The treated fruits were stored under laboratory

conditions (temperature about 27 °C). On the 7th day after treatment, the rotten fruits were collected and the percent rotten fruit was calculated.

2.6. Statistical Analysis

Percentages were transformed to arcsine values and analyzed by analysis of variance (ANOVA). Table data are reconverted to the actual percentages. Means were separated by Duncan's Multiple Range Test (P=0.05). Dose-mortality response lines were estimated by probit analysis (Minitab Software, 1985), and 99.95% (1996) or 99.9968% (1997) mortality and 95% fiducial limits (FL) were calculated.

3. RESULTS AND DISCUSSION

3.1. Effects of gamma radiation on larvae stored at room temperatures

Results in 1992 showed that none of the caterpillars of the litchi stem-end borer survived the 250 and 300 Gy treatments beyond 5 days. At 200 Gy, mortality of larvae was 69.8%, whereas in the

TABLE I. EFFECTS OF GAMMA IRRADIATION ON LITCHI STEM-END BORER LARVAE AT VARIOUS DOSAGES, 1992-1995

Dose (Gy)	No. fruits with Larvae	Mortality of larvae And pupae (%)	No. Fruits with larvae	Mortality of Larvae and pupae (%)
	1992		1993	
CK	51	0	115	1.70
200	51	69.8	--	--
250	49	100	117	100
300	51	100	113	100
350	--	--	109	100
400	--	--	115	100
	1994		1995	
	(Mean ± SEM)		(Mean ± SEM)	
CK	138	2.17 ± 0.1 c	268	9.34 ± 1.43 c
200	89	68.76± 5.6 b	264	68.56 ± 1.45 b
250	137	* 99.2 ± 1.4 a	263	100 ± 0.0 a
300	125	100 ± 0.0 a	251	100 ± 0.0 a
350	136	100 ± 0.0 a	249	100 ± 0.0 a
400	--	--	--	--

Means (±SEM) of three replicates. Means in a column with the same letter are not significantly different at P=0.05, Duncan's Multiple Range Test (DMRT). *The survivors made cocoons but died.

control (untreated group) none of the larvae died. Results from 1993 and 1994 showed that none of the larvae survived at the 7th day after treatment with 300, 350, and 400 Gy dosages, and at 250 Gy, only one larva survived and was able to pupate, but it could not emerge as an adult and it finally died. Larval mortality in the controls was only 1.7% and 2.17%, respectively, and survivors were able to pupate in a cocoon and to emerge as adults. In the experiments of 1995, none of the larvae survived on the 7th day after 300 and 350 Gy treatments, and at 250 Gy mortality of the larvae was 92.78%. Surviving larvae were able to pupate, but died in the pupal stage. Mortality of larvae and pupae treated at 200 Gy was 68.56%. Mortality of control larvae and pupae was 9.34%, and survivors pupated and emerged as adults. A dose of 250 Gy was effective for disinfesting fruits at room temperature that contained larvae. In all treatments, except the controls, of our experiments from 1992-1995, some of the larvae became brown or darkly spotted and finally died inside the fruits. Some were able to leave the fruits and make membranous silk cocoons, but they could not pupate and finally died inside their cocoons. Based on the results of our tests from 1992-1995, we are certain that the larvae of the litchi stem-end borer can be killed with gamma radiation at the dosage of 250 Gy (Table I). We conclude that 250 Gy is the lethal dosage of gamma irradiation to the larval stage of the litchi stem-end borer.

3.1.1. Dose-mortality irradiation test

In 1996, third instars of the litchi stem-end borer in fruits totaling 927, 311, 475, 831, 815, 865, and 807 were irradiated at 0, 100, 150, 200, 250, 300, and 350 Gy, respectively. The results of a dose-mortality of the litchi stem-end borer in fruits indicated that no adults were recovered when third instars were irradiated at 250 Gy. With the 100-Gy treatment, the mortality was only 27%. The control (untreated group) had 8.52% mortality.

TABLE II. EFFECTS OF GAMMA IRRADIATION ON LITCHI STEM-END BORER LARVAE AT VARIOUS DOSAGES AT GUANGZHOU, 1996-1997

Dose (Gy)	No. fruits with larvae	No. dead larvae	No. larvae surviving	% Total mortality
		1996		
CK	927	79	848	8.52 ± 1.68 e
100	311	84	227	27.01 ± 2.12 d
150	475	234	241	49.26 ± 1.38 c
200	831	564	267	67.87 ± 1.98 b
250	815	815	0	100.0 ± 0.0 a
300	865	865	0	100.0 ± 0.0 a
350	807	807	0	100.0 ± 0.0 a
		1997		
CK	1198	111	1087	9.27 ± 1.83 e
100	565	144	421	25.49 ± 1.31 d
150	705	297	408	42.12 ± 0.98 c
200	1099	783	316	71.25 ± 1.39 b
250	1063	1063	0	100.0 ± 0.0 a
300	1112	1112	0	100.0 ± 0.0 a
350	1059	1059	0	100.0 ± 0.0 a

Means (±SEM) of five replicates. Column means followed by the same letter are not significantly different at P=0.05, based on Duncan's Multiple Range Test (DMRT).

Increasing the dose to 150 Gy resulted in 49.26% mortality. A further increase to 200 Gy resulted in 67.87% mortality. Probit analysis gave a reasonable estimate of 254 Gy (220-289 Gy) needed for 99.5% mortality of third instars with 95% fiducial limits (Table II). In 1997, third instars in fruits collected from artificial rearing, totaling 1198, 565, 705, 1099, 1063, 1112, and 1059 were irradiated at 0, 100, 150, 200, 250, 300, and 350 Gy, respectively. The criterion of effectiveness of the irradiation dose was based on preventing adults emerging from treated fruits. The results indicated that no adults were recovered when the third instars were irradiated at 250 Gy. With 100 Gy the mortality was only 25.49 %. Control (untreated group) mortality was 9.27%. Increasing the dose to 150 Gy resulted in 42.12% mortality, and 200 Gy resulted in 71.25% mortality. Probit analysis (Minitab software, 1985) gave a reasonable estimate of 267 Gy (184-351 Gy) needed for 99.9968% mortality of third instars at 95% fiducial limits (Table II).

3.2. Effects of Irradiation coupled with extended cool storage (7°C) of litchi fruits infested with larvae

The results of our tests in 1994 and 1996 showed that when treated at 200 Gy, mortality of larvae was 93.7%-96.51% on the 7th day after treatment (Table III). Three surviving larvae also died on the 12th day after treatment. Our preliminary experiments showed that irradiation at 200 Gy coupled with extended cool storage at 7°C was effective for disinfesting fruit of the litchi stem-end borer. The effect of irradiation coupled with extended cool storage was greater than irradiation and storage at room temperature.

TABLE III. EFFECTS OF GAMMA IRRADIATION ON LITCHI STEM-END BORER LARVAE AT VARIOUS DOSAGES COUPLED WITH EXTENDED STORAGE, [1] GUANGZHOU, 1994-1996

	1994				1996			
Dose (Gy)	Fruits with larvae	No. dead larvae	No. surviving larvae	Percent total mortality[1]	Fruits with larvae	No. dead larvae	No. surviving larvae	Percent total mortality[1]
CK	90	14	76	15.56	339	59	280	17.4 ± 2.3 a
200	86	83	3[2]	96.51	320	300	20[2]	93.7 ± 3.8 b
250	70	70	0	100	312	312	0	100.0 ± 0.0 c
300	72	72	0	100	279	279	0	100.0 ± 0.0 c
350	88	88	0	100	351	351	0	100.0 ± 0.0 c

Means (±SEM) of five replicates. Column means followed by the same letter are not significantly different at P=0.05, based on Duncan's Multiple Range Test (DMRT). The mortality of larvae was calculated on the 7th day after treatment. The surviving larvae made cocoons, but could not pupate, and five days later they died.

3.3. Irradiation for eggs at various dosages

Exposure of eggs to 0, 250, 300, 350, 400, and 600 Gy of gamma irradiation showed that hatching of eggs was decreased with increasing dosages (Table IV). At 250 Gy the percentage of unhatched eggs and dead larvae was 60.90%, but at 600 Gy it was 93.64%. Some eggs died in the preblackhead and some in the blackhead stage. Some neonate larvae could not completely leave the

egg shell. Some larvae hatched normally but they did not develop beyond the larval stage and finally died.

3.4. Irradiation of prepupae and 4-5-day-old pupae at various dosages

Results of exposing prepupae and 4-5-day-old pupae to 300, 400, 500, and 600 Gy gamma irradiation showed that prepupae were more radiosensitive than 4-5-day-old pupae (Tables V and VI). No adults emerged when prepupae were treated at 500 and 600 Gy, and at 300 and 400 Gy only 6.36% adults emerged. The percentage of adults emerging from pupae treated at 4-5 days with 300, 400, 500, and 600 Gy was 81.84%, 76.49%, 74.93%, and 67.68%, respectively. Emergence from the control was 89.53%. Part of the emerged adults were malformed, or could not emerge from the cocoon. Most of the adults appeared to be normal, but they were sterile. We dissected the normal-appearing female adults that had been treated with 300, 350, and 400 Gy on the fourth day after emergence, and found the ovaries undeveloped. Ovaries developed normally in the controls.

TABLE IV. EFFECT OF GAMMA IRRADIATION ON LITCHI STEM-END BORER EGGS AT VARIOUS DOSAGES, JULY 1995

Dose (Gy)	No. eggs	No. hatched eggs	No. unhatched eggs	No. dead larvae	% Unhatched eggs and dead larvae (Mean ± SEM)
CK	81	69	10	2	14.80 ± 3.70 e
250	74	29	39	6	60.90 ± 11.71 d
300	65	18	38	9	72.37 ± 4.02 cd
350	82	18	57	7	78.07 ± 0.46 bc
400	79	10	56	13	87.37 ± 7.92 ab
600	79	5	63	11	93.64 ± 4.48 a

Means (SEM) of three replicates. Column means followed by the same letter are not significantly different at P=0.05, based on Duncan's Multiple Range Test (DMRT).

TABLE V. EFFECT OF GAMMA IRRADIATION ON COCOONED PREPUPAE AT VARIOUS DOSAGES, JULY 1995

Dose (Gy)	No. Cocooned prepupae	No. dead cocooned prepupae	Mortality cocooned prepupae (%) (mean ± SEM)	No. adults emerged	(%) Adults emerged (mean ± SEM)
CK	99	12	12.12 ± 6.06 c	87	87.88 ± 6.06 a
300	99	93	93.64 ± 3.49 b	6	6.36 ± 3.49 b
400	99	93	93.64 ± 3.49 b	6	6.36 ± 3.49 b
500	102	102	100.00 ± 0.00 a	0	0.00 ± 0.00 c
600	96	96	100.00 ± 0.00 a	0	0.00 ± 0.00 c

Means (SEM) of three replicates. Column means followed by the same letter are not significantly different at P=0.05, based on DMRT.

TABLE VI. EFFECT OF GAMMA IRRADIATION ON COCOONED PUPAE (4-5 DAYS OLD) AT VARIOUS DOSAGES, JULY 1995

Dose (Gy)	No. cocooned pupae	No. dead cocooned pupae	Mortality of cocooned pupae (%) (mean ± SEM)	Adults emerged	(%) Adults emerged (mean ± SEM)
CK	38	4	10.47 ± 4.26 c	34	89.5 ± 34.26 a
300	38	7	18.16 ± 8.52 bc	31	81.84 ± 8.52 ab
400	38	9	23.51 ± 7.06 ab	29	76.49 ± 7.06 bc
500	38	10	26.07 ± 11.20 ab	28	74.93 ± 11.20 bc
600	34	11	32.32 ± 4.63 a	23	67.68 ± 4.63 c

Means (SEM) of three replicates. Column means followed by the same letter are not significantly different at P=0.05, based on DMRT.

3.5. Visual quality observation

The results (Table VII) showed that with irradiation at 250, 300, 350, and 400 Gy the percent of rotten fruit was reduced by all irradiation treatments and most remarkable at the dosage of 350 Gy. These doses suggest the possibility of the extension of the shelf-life quality of stored litchi fruits.

TABLE VII. PERCENT OF ROTTEN LITCHI FRUIT ON THE 7TH DAY AFTER IRRADIATION HELD UNDER LABORATORY CONDITIONS AND IRRADIATED AT VARIOUS DOSAGES, JULY 1995

Dose (Gy)	CK	250	300	350	400
No. fruit used	115	117	113	109	115
Rotten fruits	88	48	47	31	50
% Rotten fruit	76.5 a	41.0 b	41.6 b	28.4 c	43.5 b

Means of three replicates in each column followed by the same letter are not significantly different at P=0.05, based on DMRT.

Gamma irradiation has been proposed for the disinfestation treatment of fruits and vegetables [9]. The United States Food and Drug Administration (FDA) published a final rule in 1986 regarding food irradiation and stated that food for human consumption is safe if irradiated with lower than 1000 Gy [10]. Based on our experiments from 1992-1997 on disinfestation of litchi containing the stem-end borer from 1992-1997, larvae can be killed with gamma radiation at a dosage of 250 Gy, with treated fruit stored at room temperature (about 29 °C). Thus, it might be concluded that exposure of larval stage of litchi stem-end borer at 250 Gy would prevent the emergence of the normal adults. No adults emerged from fruit given an irradiation treatment at 200 Gy coupled with extended cool storage (7 °C). Irradiation coupled with extended cool storage was significantly more effective than when the the infested fruit was irradiated and stored at room temperature. This dose is not only far less than the allowable dose, but also has no adverse effect on litchi fruits.

Our experiments in 1995 showed that hatch of eggs exposed to doses 250, 300, 350, 400, and 600 Gy was decreased with increasing dosages. In all treatments from 250-600 Gy, larvae hatching from eggs did not survive to pupate. Prepupae treated at 300-600 Gy were more radiation sensitive

than 4-5-day-old pupae. The percentage of emerged adults was higher even when 4-5-day-old pupae were treated at higher dosage (500-600 Gy) than from prepupae treated at the lower dosages of 300-400 Gy. However, the emerged adults were sterile. The results of visual quality observations indicated that the percent of rotten litchi fruits was reduced by 250-350 Gy treatment.

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DEVELOPMENT OF THE YELLOW POTATO CYST NEMATODE *Globodera rostochieitsis* (Woll.) ON POTATOES AFTER GAMMA IRRADIATION OF CYSTS

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Abstract

Gamma irradiation inhibited the development of the yellow potato cyst nematode, *Globodera rostochiensis* (Woll.) Behrens when cysts containing juveniles in anabiosis were irradiated with a dose of 0.5 kGy or higher. A dose of 0.5 kGy reduced the infestation level and the density of females/cysts on root of infested plants. However, a few cysts were found on roots of plants grown in pots with soil treated with a dose of 3.0 kGy. Development of the second generation of the potato cyst nematode (= F1 cysts that originated from irradiated cysts) was much weaker than that of the parental generation, The F1 females and/or cysts were found only in the control and in the 0.5 kGy treatment in low numbers.

1. INTRODUCTION

Potatoes are the main host of the yellow potato cyst nematode, *Globodera (=Heterodera) rostochiensis* (Woll.) Behrens, but this nematode can also occasionally attack tomatoes, aubergines, and some wild solanaceous plants [1 -3]. Second-stage juveniles of the nematode invade the roots of host plants, where they feed on a group of cells in the pericycle, cortex, or endodermis and transform the cells into a syncytium or transfer cell. During development fernale nematodes enlarge, thus disrupting the root surface. Finally, an enlarged, mature fernale dies and its cuticle hardens, forming the cyst around the eggs within. Cysts occur on roots and tubers, and during the harvest of potatoes they may be dislodged from the surface of the root and drop into the soil where they can remain infective for many years in the absence of solanaceous hosts [3]. The cysts also remain attached to plant materials, such as seed potatoes.

The symptoms of attack by *G. rostochiensis* are not specific. Patches of poor growth occur generally in the crop, sometimes with yellowing, wilting, or death of the foliage. Even with minor symptoms on the foliage, the size of the tubers can be reduced. Because the potato cyst nematode causes serious economic losses, and because it may be introduced easily with contaminated plant material into the pest-free areas, this nematode is listed in the EPPO A2 List [3], and in the quarantine lists of many countries.

Potatoes are by far the most important crop in the world, and are subject to intense international trade. Tubers infested with nematodes are not accepted by importing countries. Consequently, potato crops are usually treated with nematocides or soil fumigants to produce nematode-free materials for export [4, 5]. Irradiation of agricultural commodities may be an effective quarantine measure and disinfestation method against quarantined pests. Unlike chemical techniques, irradiation has the advantages of not leaving toxic residues, is technically efficacious, economically feasible, and can be safely used for disinfesting a wide variety of agricultural products and materials. A number of papers have been published on the effects of irradiation on potato cyst nematodes [6-12].

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We studied the development of juveniles of the yellow potato cyst nematode irradiated within cysts in soil to obtain more data on their ability to invade host plants, to develop, and to produce succeeding generation.

2. MATERIALS AND METHODS

Samples of about 1.0 kg of soil contaminated with cysts of *G. rostochiensis* containing juveniles in anabiosis were irradiated with doses 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. The treatments were repeated three times for dosages 0.5-2.5 kGy, and one time for 3.0 kGy. The average number of cysts of *G. rostochiensis* Ro_1 pathotype in the soil irradiated with 0.5-2.5 kGy and used in particular replications were 206, 125, and 125 per 100 g of soil, respectively. Soil irradiated with 3.0 kGy dose contained about 125 cysts per 100 g.

A few days after the irradiation treatment, soil samples were mixed thorougly with horticultural (untreated, nematode-free) peat. Peat mixed with irradiated soil was placed into pots, and potatoes of the susceptible variety "Bryza" were planted. Pots containing soil irradiated with 0.5-2.5 kGy were replicated 25 times, and pots with soil irradiated with 3.0 kGy dose of gamma radiation were replicated 20 times.

After potatoes bloomed, we checked for female nematodes and/or cysts on their roots. From each plant 20 roots were collected, i.e., 250 roots for each treatment dosage (except for the treatment with 3.0 kGy dose). The number of females and/or cysts found on roots were counted per 1 m of the root length, and the mean numbers of females and/or cysts per m of the root length are reported in the TABLES I and III.

After removing potato plants, we planted the tubers produced by these plants back into the same growing medium in which they had grown. When the plants matured, potatoes were checked for female nematodes and/or cysts on their roots as described above.

3. RESULTS

Irradiation inhibited the development of *G. rostochiensis* juveniles when their cysts within soil were irradiated with a dose of 0.5 kGy or higher (Tables I and II). A dose as low as 0.5 kGy reduced the infestation level and the density of females/cysts on roots of infested plants. However, a few cysts were found on roots of plants grown in the pots with soil treated with a dose of 3.0 kGy.

The ratio of the number of cyst to the number of females was about 1.0 in the control and the 0.5 kGy treatment, but the ratio increased to 2.0 in the treatment with 1.0 kGy dose. At higher dosages of gamma radiation females were not found at the time of checking. These data indicate that cysts (= dead females) originating from irradiated juveniles were formed earlier than those from untreated juveniles. The higher the dose of irradiation, the earlier the formation of such cysts.

Development of the second generation of potato cyst nematodes (= F2 cysts, originating from irradiated cysts) was much smaller than that of the parental generation (Tables III and IV). Females and/or cysts were found only in the control and in the 0.5 kGy treatment. The number of F1 cysts found on potato roots was much lower than in the parental generation.

Potato plants grown in the control soil lodged before lodging of plants grown in growing media with irradiated cysts. However, no significant dying and death of control plants was observed. These results could be caused by differences between conditions in pot cultures and the field, where significant dying of plants infested by the potato cyst nematode is often observed.

TABLE I. MEAN NUMBER OF FEMALES AND/OR CYSTS OF *Globodera rostochiensis* PER 1 M OF ROOTS OF POTATOES GROWN IN SOIL WITH CYSTS IRRADIATED WITH GAMMA RADIATION.

Dose (kGy)	Mean number of females per 1 rn root	Mean number of cysts per 1 m root length	Mean number of females and cysts per 1 m root length
Control	19.4	19.2	38.6
0.5	1.7	1.6	3.3
1.0	0.1	0.2	0.3
1.5	0.2	0.2	0.4
2.0	0	0.04	0.04
2.5	0	0.1	0.1
3.0	0	0.2	0.2

TABLE II. PERCENTAGE OF POTATO ROOTS INFESTED WITH FEMALES AND/OR CYSTS OF *Globodera rostocheinsis* WHEN POTATOES WERE GROWN IN THE SOIL WITH THE SECOND GENERATION CYSTS ORIGINATING FROM THOSE IRRADIATED WITH GAMMA RADIATION.

Dose (kGy)	Percentage of roots infested with females	Percentage of roots infested with cysts	Percentage of roots infested with females and/or cysts
Control	32.0	47.6	55.6
0.5	10.0	8.0	15.2
1.0	1.0	1.0	2.0
1.5	1.75	1.32	3.07
2.0	0	0.43	0.43
2.5	0	1.2	1.2
3.0	0	1.0	1.0

TABLE III. MEAN NUMBER OF FEMALES AND/OR CYSTS OF *Globodera rostochiensis* PER 1 M OF ROOTS OF POTATOES GROWN IN SOIL WITH SECOND GENERATION CYSTS ORIGINATING FROM THOSE IRRADIATED WITH GAMMA RADIATION

Dose (kGy)	Mean females per m root	Mean cysts per m root	Mean females and cysts per m root
Control	6.5	0.4	6.9
0.5	0	0.1	0.1
1.0	0	0	0
1.5	0	0	0
2.0	0	0	0
2.5	0	0	0
3.0	0	0	0

TABLE IV. PERCENTAGE OF POTATO ROOTS INFESTED WITH FEMALES AND/OR CYSTS OF *Globodera rostochiensis* WHEN POTATOES WERE GROWN IN THE SOIL WITH THE SECOND GENERATION CYSTS ORIGINATING FROM THOSE IRRADIATED WITH GAMMA RADIATION

Dose (kGY)	Percentage of roots infested with females	Percentage of roots infested with cysts	Percentage of roots infested females and/or cysts
Control	7.6	2.4	8.8
0.5	0	1.2	1.2
1.0	0	0	0
1.5	0	0	0
2.0	0	0	0
2.5	0	0	0
3.0	0	0	0

4. DISCUSSION

Results indicate that potato cyst nematodes are remarkably resistant to ionizing radiation. We found a few cysts on roots of plants grown in soil treated with 3.0 kGy. Our data are in agreement with results of other authors. Fassuliotis [7] irradiated cysts of *G. rostochiensis* and showed that no hatch occurred after a dose of 6.4 kGy. Dropkin (cited by Myers [9]) reported that eggs of *Meloidogyne arenaria* ssp. *thaniesi* and M. *incognita v. acrila* hatched in reduced numbers following irradiation with 3.6 kGy. No further hatch occurred after a dose of 7.2 kGy. Townshend [10] found that a few juveniles were recovered from soil exposed to 5.12 kGy dose of gamma radiation.

We found that irradiation of soil containing cysts of the the yellow potato cyst nematode with doses lower than 3.0 kGy does not inhibit hatching of the second-stage juveniles. Weischer [11] reported that irradiation with 0.288 kGy from a radium source had no apparent effect on the development of second stage juveniles irradiated within the cysts. He found that many more juveniles hatched from irradiated cysts of the yellow potato cyst nematode than from untreated ones. Similar results were reported by Townshend [10], who recovered more *Helerodera schachiii* juveniles from soil exposed to 0.08, 0.32, and 1.28 kGy than from non-irradiated soil.

We found that gamma radiation retarded or inhibited the development of *G. rostochiensis* juveniles when their cysts within soil were irradiated with a dose of 0.5 kGy or higher. Consequently, a low dose of 0.5 kGy reduced the infestation level and the density of females/cysts on roots of attacked plants. At higher doses of ionizing radiation (up to 0.784 kGy) development of juveniles of both *G. rostochiensis* and *Helerodera schachtii* was retarded and the rate of reproduction was reduced. Fewer juveniles invaded roots and those that did developed much more slowly [11]. Susceptibility of nematode juveniles to irradiation depends on the depth of their dormancy. Weischer [12] reported that *G. roslochiensis* juveniles from one-year-old cysts were less sensitive than juveniles from 3-month-old cysts, although both contained juveniles in the same molting stage. The degree of sensitivity to low doses (96-192 Gy) of radiation depends not only on the age of the cysts, but also on the depth of their dormancy. The gonads of juveniles activated by root diffusate were much more sensitive to irradiation than those of juveniles irradiated in anabiosis.

The ratio of the number of cysts to the number of females was about 1.0 in the control and in the 0.5 kGy-treatment, but it increased to 2.0 in the treatment with 1.0 kGy dose. At higher dosages of gamma radiation females were not found at the time of checking. These data indicate that cysts (= dead females) originating from irradiated juveniles were formed earlier than those from untreated

juveniles. Therefore, doses of gamma radiation higher than 1.0 kGy caused accelerated mortality of mature females, resulting in earlier formation of the cysts in treatments than in the control.

Development of the second generation of the potato cyst nematode (= F1 cysts originating from irradiated cysts) was possible only in the control and in the 0.5 kGy treatment. The number of F1 cysts found on potato roots was much lower than in the parental generation. These results indicate that doses higher than 0.5 kGy are sterilizing doses for *G. rostochiensis* when applied to the juveniles within the cysts. Similar results were obtained by Fassuliotis [7]. He found great variation in the proportion of viable second-generation eggs in cysts of *G. rostochiensis* after irradiation with 200 Gy, and sterilization occurred at doses of 400 to 800 Gy. Delayed emergence, reduced infectivity, altered motility, and chromosome damage were also noted. Myers [9] found that less than 1% reproduction occurred in *G. roslochiensis* after 0.2 kGy. Evans [6] noted that irradiation of second stage juveniles with doses up to 0.64 kGy had no effect on their activity, but irradiation of either cysts or juveniles impaired development of the F1 generation and fewer cysts with fewer eggs were formed than in unirradiated controls. Eggs that were present often failed to produce normal juveniles. Although juveniles from irradiated cysts compete poorly with those from unirradiated cysts when potato plants are inoculated with a mixture of the two, irradiation may be impractical for nematode disinfestation of plant materials or infested soil.

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PHYSIOLOGICAL MARKERS IN INSECTS INDICATING TREATMENT WITH IONIZING RADIATION

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Abstract

Seven markers or tests that can be applied to 3rd instars of the Caribbean fruit fly as indicators of exposure to ionizing radiation are described, including (1) whole body melanization, (2) phenoloxidase spot test, (3) quantitative phenoloxidase measurement, (4) measurement of the ratio between size of the supraesophageal ganglion and the proventriculus, (5) development of imaginal discs, (6) number of hemocytes in one µl of hemolymph, and (7) larval weight. The markers work best and are most definitive when larvae are exposed to at least 50 Gy no later than the first day of the 3rd instar. All of the tests are sensitive enough to be applied to a single 3rd instar larva. Combinations of some of the tests could be used on a single larva. Tests (1) and (2) are easiest to use and require no specific technical training, and seem to have the most potential for practical use in quarantine.

1. INTRODUCTION

The safety and efficacy of irradiation as a treatment of fruits, vegetables, and other food products is well established [1]. In addition irradiation of commodities is an effective way to control insects of quarantine importance, and can help prevent the spread of economically important insects to new areas with produce shipments [1], [2], [3], [4]. Moreover, irradiation is cost effective and environmentally sound.

One factor that creates some problems in irradiation of food products to control insects is that it takes extremely high dosages of radiation to immediately kill insects. These high dosages are well above the limits that can be applied to fresh fruits or vegetables, and most other food products. The reasons that insects are so hard to kill with radiation are at least three-fold. First, insects do not depend upon cellular transport of oxygen, such as in the case with vertebrate red blood cells, a system in vertebrates that is extremely sensitive to radiation. Relatively high dosages of irradiation do not alter the ability of insects to supply oxygen through the tracheal system to their tissues. Second, although insects do have cellular defense mechanisms against invading bacteria and other foreign agents similar to the vertebrate immune system, those defense mechanisms are not so easily damaged by radiation as is the immune system of vertebrates. Third, in the larval and adult stages, insects have only a few cells that have to undergo division, a process known to be highly susceptible to damage by exposure to irradiation. The main times of rapid and extensive cell division in most insects are during embryonic development in the egg, reorganization during pupation, and formation of eggs and sperm. At these particular times, insects are very sensitive to irradiation. In addition cell division occurs in the midgut of most insects as new cells grow in to replace old worn-out cells, and in the epidermal cell layer just beneath the cuticle, in which new cells are produced at each molt; these processes can be damaged by irradiation also. In a natural environment, insects are likely to occur simultaneously in all stages - as eggs, larvae, pupae, and adults. Thus, a portion of almost any population is likely to be sensitive to irradiation while another portion of the population is relatively resistant.

1.1. Imaginal discs are sensitive to irradiation.

Insects that pass through a complete metamorphosis, i.e., egg, larva, pupa, and adult, have within the larval body small clusters of undifferentiated embryonic cells called imaginal discs. There are independent groups of these discs for the various tissues that must be constructed in the adult,

including such structures as wings, legs, compound eyes, gonads, and numerous other adult structures. The imaginal disc cells divide to provide enough cells for an adult structure. Divisions take place slowly during larval life, but occur rapidly late in the last larval stage and early in the pupal stage. At these times an individual is very sensitive to damage by irradiation. Insects with complete metamorphosis include some of the insects of greatest concern in quarantine, such as tephritid fruit flies, moths, and beetles.

Insects with incomplete metamorphosis do not have a pupal stage, but the larval (nymphal) insect gradually transforms into an adult. Although the immature forms in these insects already have many adult features, such as compound eyes and general body shape, they still have to have cell division to develop wings (if the adult is winged) and internal reproductive tissues. Thus, they also have sensitive times to irradiation.

There is interest within the food industry and scientific community in easy to apply techniques to verify whether living insects found in a shipment have or have not been irradiated. Such methods would serve to back-up certificates of irradiation that every commodity will carry from the irradiation facility, and can quickly allay fears that inevitably occur when a living insect of quarantine importance is found. Thus, a data-bank of insect species, treatment dosages, abnormalities caused, and potential detection techniques is needed. The objective of our work has been to seek markers in insects exposed to irradiation. This report describes markers in larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) and another dipteran, *Musca domestica* L.

2. MATERIALS AND METHODS

2.1. Insects.

Eggs and larvae of the Caribbean fruit fly, *A. suspensa*, were provided by the Florida Department of Agriculture and Consumer Services (FDACS) mass-rearing laboratory. Pharate first instars within 5-6 hours of hatching, and instars 1, 2, and 3 were irradiated on the first day of the instar with varying dosages of gamma rays from a ^{137}Cs source maintained by the USDA Medical and Veterinary Laboratory and by FDACS in Gainesville, FL. Some larvae were also irradiated with a linear accelerator operated by the FDACS in Gainesville. Details of the measurement of phenoloxidase activity with 2-methyl-3(3,4-dihydroxyphenyl)-DL-alanine (2-methyl-DOPA) as a substrate have been published [5]. Procedures for dissection and measurement of the supraesophageal ganglion and proventriculus have been published [6].

2.2. Hemocyte counts.

Hemolymph (1 µl) from a small puncture in the body wall of a larva was collected in a 1-µl micropipet and added to 49 µl of SBP saline-stain solution [7] for a 50-fold dilution of the hemolymph. The hemolymph sample was mixed thoroughly with the SBP saline-stain and a drop added to the counting area of an AO Spencer Bright-Line Hemacytometer chamber. The ruled squares for counting human white cells were used, and hemocytes in ten squares, each 1 mm^2 x 0.1 mm deep, were counted. The number of hemocytes per cubic millimeter (= 1 µl volume) of hemolymph was calculated as follows:

hemocytes/µl = sum of hemocytes in 10 squares x dilution factor

3. RESULTS

Seven markers in larvae of the Caribbean fruit fly exposed to irradiation are listed in Table I. The results from various experiments designed to validate the markers are presented in Tables II-VI, and in Figures 1 and 2.

TABLE I. MARKERS OF IRRADIATION IN CARIBBEAN FRUIT FLY LARVAE

Marker	Nonirradiated	Irradiated
1. Whole body melanization	melanization	no melanization
2. Phenoloxidase spot test	red color	no color
3. Phenoloxidase measurement	high activity	low activity
4. Supraesophageal/proventriculus ratio	1.9	1.0
5. Imaginal disc development	large	small or not developed
6. Hemocytes/µl hemolymph	>29000	<1000
7. Larval weight	14 mg/larva	2-7 mg/larva

4. DISCUSSION

4.1. Whole body melanization.

This is the easiest and least difficult test to perform. Late 3rd instars that have been irradiated prior to the molt into 3rd instars do not melanize when exposed to room temperature after having been frozen for a few minutes to kill them. The irradiated larvae remain cream-colored to slightly gray, while control larvae treated similarly turn black in 5 to 10 minutes [5]. A simple application of this test is to freeze any "wiggler" or living larva of a fruit fly found in an irradiated commodity for a period of 15 minutes (or longer) to kill it and disrupt normal physiological control of enzyme activity in the body of the larva. Upon removal of the (normally white to cream colored) larva from the freezer, it can be placed on a table top or other observation surface and allowed to warm to room temperature. Almost immediately a non-irradiated larva begins to darken due to oxidation of phenolic compounds in the body, and within a few minutes becomes dark brown to black. Darkening is caused by action of the enzyme phenoloxidase, which normally exists in an inactive form in living larvae. Irradiation of Caribbean fruit fly larvae in the 1st or 2nd instar with at least 20 Gy [5] prevents the development of cells that synthesize phenoloxidase, and an irradiated larva does not darken.

Irradiation does not prevent the enzyme from functioning; it prevents the synthesis of the enzyme. The dosage that prevents the synthesis of phenoloxidase may vary with different insect species. Phenoloxidase is rapidly synthesized by Caribbean fruit fly larvae late in the 3rd instar (see section below), and irradiation after this time is too late to stop phenoloxidase synthesis.

Whole body melanization does not occur in 1st and 2nd instars of the Caribbean fruit fly regardless of irradiation or nonirradiation status because these stages have too little phenoloxidase (synthesis not yet started) for significant darkening to occur [5].

4.2. Spot test for phenoloxidase activity (qualitative test).

This test is similar to the whole body melanization test [5] in larvae. A single late 3rd instar that has been irradiated in an earlier instar can be tested for phenoloxidase activity by crushing the larva in a drop of water and adding 1 drop of the crushed larva to a test paper (transparency film or similar nonabsorbent paper or film) containing a dried spot of 100 µg of 2-methyl dihydroxyphenyl alanine (2-Methyl DOPA in phosphate buffer, pH 6.5). Lack of red color development indicates lack of phenoloxidase and exposure to ionizing radiation. Control, nonirradiated larva readily produce the red color that later turns to black. Test papers for the spot test can be prepared and stored for future use. The substrate, 2-Methyl DOPA, is stable for months in the spot on the transparent film. Dried

spots from testing an irradiated larva (no color or slightly yellow due to tissue color of the insect) and from a control (nonirradiated) larva (red turning to black spot) can be preserved as a permanent record for comparison with new tests or for later documentation of test results.

TABLE II. PHENOLOXIDASE ACTIVITY IN SUPERNATANTS FROM WHOLE BODY HOMOGENATES OF MATURE 3rd INSTARS IRRADIATED AT STAGES INDICATED

Units* of phenoloxidase / mg protein (± SD)

STAGE IRRADIATED**	IRRADIATION DOSE 0 GY	50 GY	150 GY	250 GY
1ST INSTAR	514.5a*** ± 107.4	22.0b ± 10.5	25.2b ± 8.4	54.3b ± 12.7
2ND INSTAR-EXP.1	686.3a ± 152.2	8.8b ± 2.5	11.1b ± 3.8	58.2b ± 8.3
EXP.2	450.9a ± 12.0	0.6b ± 0.4	1.9b ± 0.4	8.6b ± 7.0
3RD INSTAR	318.2a ± 80.9	35.1b ± 9.7	34.8b ± 12.6	35.0b ± 8.9
MATURE 3RD-EXP.1	645.4a ± 172.0	616.2a ± 45.1	573.3a ± 92.5	372.9b ± 44.0
EXP.2	397.5a ± 7.9	325.3b ± 35.8	356.5b ± 14.1	271.6c ± 5.3

*A unit of Phenoloxidase is the amount of enzyme producing a change in absorbance of 0.01/min at 475 nm, pH 6.5, at 30 C. **Each instar was irradiated on the first day of the instar, except mature 3rd instars, which were irradiated as mature larvae emerging from the food. Mean values in a row followed by the same letter are not significantly different ($P > 0.05$).

4.3. Quantitative measurement of phenoloxidase activity.

Phenoloxidase can be quantitatively measured in one larva in the 3rd instar. Table II shows that late 3rd instars that were irradiated prior to molting into the 3rd instar had approximately 10% or less of the normal level of phenoloxidase. Enzyme activity that is 10% of normal does not produce a red or black color in the spot test described above.

Although phenoloxidase activity was reduced at the 250-Gy dose when mature 3rd instars were irradiated (Table II), much of the enzyme had already been formed before irradiation, as shown in Table III. The whole body content of phenoloxidase is very low in Caribbean fruit fly larvae until late in the 1st day of the 3rd instar, but then synthesis of phenoloxidase begins to occur rapidly. By the 2nd day of the 3rd instar, specific phenoloxidase activity (activity per unit of body protein) is high and continues to rise during the 3rd day of the instar (Table III). Following pupariation, the phenoloxidase level gradually falls.

TABLE III. PHENOLOXIDASE ACTIVITY IN SUPERNATANTS FROM WHOLE BODY HOMOGENATES OF VARIOUS STAGES OF THE CARIBBEAN FRUIT FLY

Units* of phenoloxidase / mg protein (± SD)

	AGE WITHIN INSTAR			
	< 1 Day	1 Day	2 Days	3 Days
2nd INSTAR	ND[1]	53.3 ± 20.9	69.2 ± 48.8	
Transition to 3rd INSTAR[2]	24.2 ± 14.0			
3rd INSTAR	49.7[3] ± 15.8	41.1 ± 20.6	325.5 ± 85.6	432.5 ± 35.3
Pupariation	373.5[4] ± 98.6	340.3 ± 29.1	47.6 ± 16.6	114.8 ± 40.1

*A unit of Phenoloxidase is defined as the amount of enzyme producing a change in absorbance of 0.01/min at 475 nm, pH 6.5, at 30 C.

[1] ND = not determined

[2] The 2nd instar molts into the 3rd instar late on the 2nd day of the instar. Individuals were selected that showed visible mouthhooks for both 2nd and 3rd instars; i.e., the 2nd instar cuticle was not yet ecdysed.

[3] 3rd instar, 2-3 hr old

[4] 1 hour after pupariation

4.4. Growth of the supraesophageal ganglion.

The supraesophageal ganglion in the medfly [8] and several *Bactrocera* spp. of fruit flies [9], [10] failed to grow normally in insects irradiated as young larvae, and a ratio of the size of the ganglion divided by size of the proventriculus was indicative of irradiation. Using similar procedures, we found the same general results in Caribbean fruit fly larvae [6]. The cross sectional area of a plane through the two supraesophageal ganglionic hemispheres, or width across the top of the two supraesophageal ganglionic hemispheres, divided by the cross sectional area or width of the proventriculus, respectively, gave values < 1 if larvae were irradiated with 20 Gy or greater dosages, as compared to normal control ratios of >1.9.

These dissections and measurements must be made with 20 - 30 X magnification. Dissection takes some practice and skill, and if only a few larvae were found in a commodity, there will be little margin for error or poor dissection. Consequently, it does not seem likely that this marker will be a useful one in routine quarantine applications.

4.5. Growth of imaginal discs

The imaginal discs are small groups of undifferentiated cells in the larvae of those insects that undergo a complete metamorphosis, i.e., that have a pupal stage. The imaginal discs contain cells that will grow into characteristic adult structures during pupal transformation. The discs grow little in the

early instars of Caribbean fruit fly larvae, but they grow extremely rapidly by mitotic cell divisions in the 3rd instar, probably as a result of hormonal stimulation. Radiation damage that prevents normal cell division means that not enough cells will be available at pupation to form the adult structures. An imperfectly formed adult may develop, or one that is sterile, or possesses other defects, but usually development is aborted and the fly dies in the pupal stage. Relatively low dosages of radiation are sufficient to damage the imaginal discs. The imaginal discs of Caribbean fruit fly larvae irradiated with at least 50 Gy do not develop normally [6]. In addition to interfering with cell division, irradiation probably introduces many genetic and biochemical defects in the DNA of the cells. Thus, adult structures cannot develop or develop imperfectly because there are not enough cells, and possibly because of numerous biochemical and genetic defects induced by radiation.

The imaginal discs for the compound eyes, legs, and some other structures can be observed and abnormalities noted in the same dissections prepared to observe the supraesophageal ganglion and proventriculus. However, because of the difficulties and constraints imposed by dissection of small larvae, observation of the imaginal discs will not be a satisfactory marker of radiation exposure in quarantine administration.

4.6. Lack of hemocytes (blood cells) in irradiated larvae.

Larvae irradiated with at least 50 Gy at hatching have only 1 to 2% as many hemocytes as non-irradiated larvae (Table IV). To observe and count hemocytes requires the collection of 1 µl of hemolymph from a puncture in the body cuticle of a larva. Care must be taken not to puncture the gut or to get gut contents in the sample. The hemolymph sample is added to 49 µl of a dye/fixative solution [8], and a drop is placed on a hemacytometer counting slide used in the clinical measurement of red and white blood cells in humans. Hemocytes are counted in 1 square mm X 0.1 mm deep marked areas (the white cell counting area). Counting 10 such areas allows one to express the total hemocyte count as hemocytes per cubic mm. Late 3rd instar Caribbean fruit fly larvae have a total of about 30,000 cells per cubic mm of hemolymph (1 cubic mm = 1 µl volume). Student's unpaired *t*-test for data in Table IV is highly significant (t=15.2, P= < 0.0001). It is clear, even without statistics, that larvae irradiated with at least 50 Gy have extremely few hemocytes, while normal control 3rd instars have >29,000 cells per µl hemolymph. Hemocytes are produced primarily from the division of small hemocytes called prohemocytes [11], and a rapid increase in the number of circulating hemocytes occurs late in the 2nd day of the 3rd instar as shown in Figure 1.

4.7. Reduction in size of irradiated larvae.

Irradiated larvae are substantially smaller than nonirradiated larvae at every stage, but the size difference is particularly striking in late 3rd instars seeking a pupariation site (Table V).

TABLE IV. NUMBERS OF HEMOCYTES PER µl HEMOLYMPH IN CONTROL AND IRRADIATED (50 Gy AT HATCHING) 3rd INSTARS OF *Anastrepha suspensa*. MEAN ± SD OF 3 DETERMINATIONS

Replicate	Control	Irradiated
1	27833 ± 3912	467 ± 462
2	30967 ± 3875	150 ± 132
3	28717 ± 9624	33 ± 29

TABLE V. WEIGHT OF CONTROL AND IRRADIATED LATE 3rd INSTARS

Irradiation dose (Gy)	Weight/larva mg
0 (control)	14.2
5	12.4
10	10.4
20	10.8
50	7.3
75	4.7
100	5.1
150	2.6

Table VI - PHENOLOXIDASE ACTIVITY IN 4-DAY-OLD THIRD INSTARS OF THE CARIBBEAN FRUIT FLY PARASITIZED WITH THE PARASITOID *Diachasmimorpha longicaudata*

Stage/treatment	Units* phenoloxidase per mg protein (± SD)	Percent of control value
Control larvae	351.2 ± 119.2^{a}	100
Parasitized on 1st day of 3rd instar	129.2 ± 76.3^{bc}	37
Parasitized on 2nd day of 3rd instar	40.6 ± 9.2^{b}	12
Parasitized on 3rd day of 3rd instar	265.2 ± 60.3^{ac}	76

*A unit of phenoloxidase is defined as the amount of enzyme producing a change in absorbance of 0.01/min at 475 nm, pH 6.5, at 30 C. Means followed by the same superscript are not significantly different ($P > 0.05$).

Larvae were irradiated at hatching with the doses indicated. Groups of 10 larvae were weighed, and the weight recorded is the average weight per larva. Weight and size alone may not be definitive indicators of irradiation because inadequate nutrition can be a factor in final weight obtained in insects. However, in conjunction with several of the other markers noted in Table I, weight can be a useful part of the decision making process relative to determining if irradiation has occurred. Larvae may be smaller and weigh less because irradiation probably damages midgut cells which produce the digestive enzymes and which also absorb the products of digestion. In addition, irradiation probably damages the epidermal cells beneath the cuticle that are responsible for the synthesis of new cuticle at each molt. These cells must divide in order to provide a larger body size with each molt.

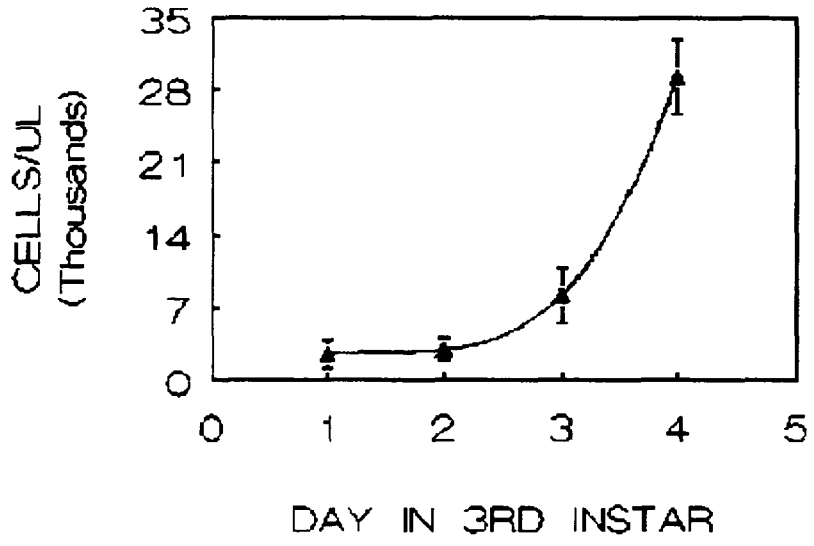

Figure 1 - *Temporal increase in hemocytes in nonirradiated 3rd instar Caribbean fruit fly*

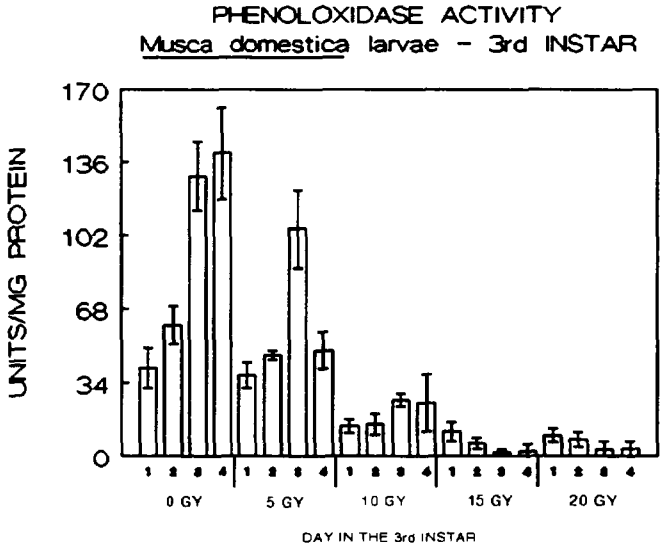

Figure 2 - Reduction in phenoloxidase activity during days 1-4 in 3rd instars of the housefly irradiated as 1st instars

4.8. Parasitization can influence phenoloxidase in Caribbean fruit fly larvae

The phenoloxidase system and encapsulation of foreign objects by hemocytes are two of the recognized defense systems used by insects against attack by foreign invaders. These defense mechanisms may be compromised by irradiation and by successful parasitism. We have conducted one brief experiment which does indeed show that phenoloxidase activity is lowered in Caribbean fruit fly larvae parasitized by *Diachasmimorpha longicaudata*. Larvae were parasitized by allowing female parasitoids to lay eggs in 3rd instar Caribbean fruit fly larvae on the 1st, 2nd, or 3rd day that larvae were in the 3rd instar. Measurements of phenoloxidase activity [5] were made on the 4th day when larvae started to crawl out of the food seeking a pupariation site. The results, presented in Table IV, confirm that parasitism lowers the specific activity of phenoloxidase, presumably by destroying tissues (cells) that synthesis the enzyme. When parasitoids of this species are successful in establishing themselves in larvae, they eventually kill their host.

4.9. Parallel results with *Musca domestica*, another dipteran

Irradiation with dosages as low as 10 - 20 Gy caused significant reduction in phenoloxidase activity and in numbers of hemocytes in mature 3rd instar larvae of the housefly, *Musca domestica*, when newly hatched 1st instars were irradiated with ^{137}Cs. Phenoloxidase activity in the 3rd instars that were irradiated as 1st instars is shown in Figure 2. Hemocytes in the hemolymph of control, nonirradiated larvae appeared only very late in 3rd instars. Less than 100 hemocytes per µl of hemolymph could be found on day 2 of the 3rd instar, but by day 3 the value was 13,770/µl, with the number rising to 45,460/µl on day 4. Late on day 4 and on day 5 larvae left the food seeking a pupariation site.

5. CONCLUSIONS

We have identified seven markers that can be used to determine if a 3rd instar Caribbean fruit fly has been irradiated with at least 50 Gy in an early instar. The tests can be applied to a single larva. The whole-body melanization and the phenoloxidase spot tests are the easiest tests to perform, and require no special skills or training. Several of the tests can be used in combination; for example, a 3rd instar can be weighed, a sample of hemolymph taken for a count of the hemocytes, and the larva can be crushed for the phenoloxidase spot test. The tests are not valid if radiation occurs later than the 1st day of the 3rd instar. By this time in the life of a Caribbean fruit fly larva, synthesis of phenoloxidase, growth of the supraesophageal ganglion, production of hemocytes, and overall growth of the larva are well advanced. It is inhibition of these physiological events by irradiation in an early instar that is the basis for the markers. The tests are not adequate to identify with certainty the dosage that larvae have been exposed to, but they are valid indicators of irradiation at well below the currently approved dosage of 250 Gy for quarantine control of fruit flies.

Mansour and Franz [12] have shown low activity of phenoloxidase and prevention of melanization in Mediterranean fruit fly larvae that were irradiated at or near hatching. We have shown reduced phenoloxidase, lack of melanization, and lack of hemocytes after irradiation of 1st instars of houseflies, *Musca domestica*. Thus, it seems probable that the methods described here will be generally applicable to dipterans although the exact dosage of radiation that causes the physiological changes will probably vary with species. For example, very dramatic reductions of phenoloxidase activity in 3rd instars can be achieved by irradiating housefly 1st instars with as little as 10-15 Gy, and few if any survive to the 3rd instar after irradiation with 25 GY, but as shown in the data of this paper, somewhat greater dosages are required to achieve the same reduction in phenoloxidase activity in Caribbean fruit flies.

Caribbean fruit fly larvae that are parasitized by the parasitoid *Diachasmimorpha longicaudata* have lower levels of whole-body phenoloxidase, and it is possible that a nonirradiated, but parasitized 3rd instar larva could be mistaken for one that had been irradiated because of the lower activity of phenoloxidase. This should not be of any consequence to quarantine officials, however, because fruit fly larvae parasitized with this parasitoid do not successfully emerge as adult fruit flies. The parasitoid is used as a biological control of the Caribbean fruit fly in some citrus production area of Florida.

First and second instars of tephritid fruit flies are extremely small and hard to detect in cut fruit or produce. Nevertheless, if these small stages should be found, it is unfortunate that the tests described do not work on the 1st or 2nd instar. The activity of phenoloxidase cannot be measured in just one 1st or 2nd instar larva because activity is too low. Moreover, the two early instars have not synthesized hemocytes, so they cannot be counted. Radiation damage to the supraesophageal ganglion and to imaginal discs is not detectable with reliability in the 1st and 2nd instars.

Some of these tests may be applicable to other groups of insects of quarantine importance, such as beetles and moths. However, case by case study is necessary to determine if these tests, or some

other tests that can be developed, actually work. It is important to recognize that the greatest damage caused by the dosages of irradiation used in fruit and vegetable treatments occurs in cells that are in an embryonic state, such as the imaginal disc cells, or in the process of division. Damaged cells may not be able to synthesize some of their normal products, such as the enzyme phenoloxidase. If phenoloxidase or some cellular product already has been synthesized prior to irradiation exposure, then it is unlikely that irradiation will be reflected in any measurable decrease or change in the enzyme or product. Thus, it is important to determine when in the various life stages of an insect it is sensitive to irradiation. Greater problems in detection are presented by insects with long larval development through several instars, such as many beetles and moths, as opposed to the short larval developmental time and few instars of fruit flies. Detailed study will be required to look for changes that irradiation treatment might influence.

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DETECTION METHODS FOR IRRADIATED MITES AND INSECTS

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Abstract

Results of the study on the following tests for separation of irradiated pests from untreated ones are reported: (a) test for identification of irradiated mites *(Acaridae)* based on lack of fecundity of treated females; (b) test for identification of irradiated beetles based on their locomotor activity; (c) test for identification of irradiated pests based on electron spin resonance (ESR) signal derived from treated insects; (d) test for identification of irradiated pests based on changes in the midgut induced by gamma radiation; and (e) test for identification of irradiated pests based on the alterations in total proteins of treated adults. Of these detection methods, only the test based on the pathological changes induced by irradiation in the insect midgut may identify consistently either irradiated larvae or adults. This test is simple and convenient when a rapid processing technique for dehydrating and embedding the midgut is used.

1. INTRODUCTION

Irradiation of agricultural commodities is an effective quarantine measure and disinfestation method against stored product pests [1]. Unlike chemical techniques, irradiation has the advantage of not leaving toxic residues, is technically efficacious, economically feasible and can be safely used for disinfesting a wide variety of agricultural products and materials [2].

In general, a dose of 0.3 kGy is recommended for quarantine treatment, and doses up to 1 kGy are suggested for radiation disinfestation of agricultural products [3]. These effective doses have no detrimental effects on most commodities being treated [4], but do not produce immediate mortality of stored product pests [5, 6]. This can be disadvantageous if the product is for immediate export and there is a zero tolerance for insects. Live mites, for example, present in an agricultural product will be of concern to quarantine and/or storage personnel. Therefore, to use irradiation as an effective quarantine control, a simple and quick method is needed to establish if live insects found within commodities at the point of import have been irradiated [1, 7]. Studies on this subject have been limited, and there are few appropriate practical techniques for the identification of irradiated pests [8, 9].

We studied the following detection methods (tests) for separation of irradiated pests from untreated ones: (a) a test for identification of irradiated mites based on lack of fecundity of treated females; (b) a test for identification of irradiated beetles based on their locomotor activity; (c) a test for identification of irradiated pests based on electron spin resonance (ESR) signals derived from treated insects; (d) a test for identification of irradiated pests based on changes in the midgut induced by gamma radiation; and (e) a test for identification of irradiated pests based on the alterations in total proteins of treated adults. The results obtained are reported in the present paper.

2. MATERIAL AND METHODS

2.1. Identification of irradiated mites

The bulb mite, *Rhizoglyphus echinopus* (F. et R.), was isolated from rotting onions in January 1987. These mites have been kept in a laboratory on yeast at 89% R.H., and 25±1°C. The mold mite,

Tyrophagus putrescentiae (Schrank), has been cultured at this laboratory for several years on wheat germ at 85±5% R.H. and 25±1°C. Inert deutonymphs were isolated from the stock culture, and on the following day all adults emerged. After 2-3 days, females and males were irradiated with 0.0 (control), 0.3, 0.6, 0.9, and 1.2 kGy of gamma radiation. After the treatment, mites were paired and transferred into rearing cages with food (yeast or wheat germ). The rearing cages were stored at 25°C and 85% R.H. The number of eggs laid was recorded at 2-3-day intervals.

Cages containing food infested with acarid mites were irradiated with the following doses of gamma radiation: 0.0 (control), 0.2, 0.3, and 0.6 kGy. Treated cages were stored for one, two, three or more weeks in darkness at 25±1°C and 85±5% R.H. After these periods, live adult mites were isolated and placed into separate rearing cages supplied with food. Usually, 20 females per treatment were prepared for microscopic study.

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

2.2. Locomotor activity of irradiated adults of the grain weevil and the rice weevil

Adults of the grain weevil, *Sitophilus granarius* (L.), and the rice weevil, *S. oryzae* (L.), were studied. The insects were from laboratory cultures on wheat grain at ca. 70% R.H. and 25°C in darkness. Beetles isolated from the cultures were irradiated with the following doses of gamma radiation: 0.0 (control), 0.25, 0.5, 0.75, and 1.0 kGy (dose rate - ca. 40 Gy/min.). Treated insects were stored for several days at ca. 70% R.H. and 25°C in darkness. Every day, irradiated beetles were used for determination of their locomotor activity.

Locomotor activity of irradiated insects was studied with the method of Wiygul and Haynes [10]. For each test, a piece of paper (ca. 25 x 35 cm) was prepared with 2.5 cm and 20 cm diameter circles drawn in the center. Then a small plastic funnel was placed in the 2.5 cm diameter circle, and ten beetles were placed in the funnel. Two minutes later (period of orientation) the funnel was removed, and the beetles were allowed to crawl for 1-5 minutes. A count was then taken of the insects that had crawled out of the 20 cm diameter circle. The procedure was replicated three times for a complete test. The score was computed as the percentage of beetles that moved out of the 20 cm diameter circle during the test period.

Additional tests were conducted to compare the effects of gamma radiation with the effects of some adverse environmental conditions on the locomotor activity of storage beetles. Groups of beetles were kept in glass jars without food for 3, 5, and 7 days. After these periods of time, insects were tested by the method outlined above. Another group of weevils was placed in a refrigerator (+3°C) for 24 hr, then removed, kept at a room temperatures, and tested for their locomotor activity.

2.3. Electron spin resonance (ESR) signal derived from irradiated insects

Adults of the confused flour beetle, *Tribolium confusum* (DuVal.), were irradiated with 0 (control), 0.75, and 1.0 kGy of gamma radiation, whereas adults of the rice weevil, *S. oryzae*, were given 0 (control), 0.25, 0.5, 0.75, and 1.0 kGy (dose rate = ca. 64 Gy/min.). Two weeks after irradiation, insects were killed by a temperature of 65°C, slowly dried, and used for ESR spectroscopic studies.

Additional experiments have been performed in order to show the effects of high (>1 kGy) doses of gamma radiation on the ESR peak height (= spin concentration) in adults of the rice weevil irradiated with the following doses of gamma radiation: 0 (control), 1, 3, 5, and 7 kGy. Three groups of weevils were studied: Group I insects were irradiated 4 weeks before the ESR recording; Group II insects were irradiated 2 weeks before the ESR recording, and Group III insects were irradiated a few days before the ESR recording.

Between 17.7 and 33.9 mg of dead insects and their fragments were weighed and transferred into a quartz ESR tube, with 5 mm outside diameter. The ESR spectra were recorded at ambient temperature with a Bruker ESP 300E spectrometer in X-band by staff of the Institute for Nuclear Chemistry and Technology, Warszawa-Zeran, Poland. Peak heights of ESR signals were measured with ESR software, and normalized to the material weight. Generally, the methods used by Stachowicz *et al.* [11] were followed.

2.4. Changes in the midgut of the confused flour beetle induced by gamma radiation

Adults of *T. confusum*, of different ages, from a laboratory culture maintained at 27±1°C and 60+5% R.H., were irradiated in a ^{60}Co irradiator with the following doses of gamma radiation: 0 (control), 0.1, 0.3, and 0.5 kGy. The treated and untreated (control) insects were kept in separate jars with wheat flour and powdered yeast (5%) for 2 or 3 weeks. After this period, beetles were fixed with 2.5% buffered glutaraldehyde for 3 hr., using 0.1 M cacodylate buffer, pH 7.2. Insects were then washed several times with the same buffer, dehydrated in a standard alcohol series and acetone, and embedded in Spurr's resin. Transverse semi-thin sections were cut on the LKB-8800 microtome, mounted on slides, stained with toluidine blue, and observed under a light microscope.

2.5. A rapid method of detection of irradiated insects: Changes in the midgut of larvae of the Indian meal moth induced by irradiation

Fifth instars of the Indian meal moth, *Plodia interpunctella*, were selected from a laboratory culture maintained at 27±1°C and 60±5% R.H. on crushed peanuts. Larvae were irradiated in a ^{60}Co irradiator with 0.3 kGy dose of gamma radiation (dose rate = ca. 30 Gy/min.). The treated and untreated (control) larvae were kept in separate jars with food for 2, 7, and 14 days. After these periods, larvae were prepared for histological analysis.

Buffer and fixative were prepared according to the formulas described by Hayat [12]. Plastic embedding mixture was prepared from components purchased from Polysciences Inc. (Warrington, PA) according to the schedule for embedding suggested by Shearer and Hunsicker [13]. A small part of a larva in which the midgut is located was cut and fixed for 30 min in Karnovsky's fixative at room temperature, and then rinsed three times for 2 min each step in 0.1 M cacodylate buffer (pH=7.4). Next, tissues were quickly dehydrated in 70% ethanol and in 1,4-dioxane, infiltrated with the resin/dioxan mixture and pure resin. After infiltration, the material was transferred to capsules containing embedding mixture and polymerized at 80°C for 3 hr. Transverse semi-thin sections were cut on the LKB-8800 microtome, mounted on slides, stained with toluidine blue, and observed under a light microscope.

2.6. Alterations in total proteins of irradiated adults of the confused flour beetle

2.6.1. Insects

Adults of the confused flour beetle, *T. confusum*, reared at 25°C and 60+5% R.H. on a medium consisting of bleached enriched wheat flour and brewers yeast (19:1) were used. The insects were taken, without determining their sex, from mass cultures which had been maintained at the Warsaw

Agricultural University. Groups of beetles were irradiated within the medium in a ^{60}Co irradiator (dose rate = ca. 35 Gy/min.). The following doses were given: 0.1, 0.3, or 0.5 kGy. A Fricke dosimeter was used for dosimetry [14]. A control group of beetles was not irradiated.

After the treatment, beetles were kept in Petri dishes with rearing medium. Dishes were stored in thermostatically-controlled cabinets at 25±1°C and 60±5% R.H. On the 5th, 8th, 11th, 14th, 17th, 20th, 23rd, and 26th day after the treatment, 10 insects in each group were selected for preparation of homogenate samples. Samples were pooled to avoid variability in electrophoretic patterns noted in individual insects [15].

2.6.2. Preparation of homogenate samples

Selected adults were washed free of the rearing medium with a buffer containing 10 mM sodium phosphate buffer, 100 mM KCl and 1 mM EDTA, pH 7.5. The cleaned insects were crushed in a mortar with 250 µL of the sample buffer to which 0.1 mM serine protein inhibitor, PMSF, was added. The resulting suspension was then transferred to the Potter homogenizer. Samples were packed in ice during the homogenization process to avoid melanization. Each homogenate was centrifuged for 6 min at 15,000*g*. The supernatant (100 µL) was pipetted into Eppendorf vials containing 10 µL of sample buffer (0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.001% bromophenol blue), mixed and stored in a freezer at -70°C.

2.6.3. Electrophoresis procedures

The Laemmli method for slab gel electrophoresis was followed [16]. Seven percent and 25% separation gels were used. The sample mixture (8 µL) and the mixture of known molecular weight markers (14.3, 18.0, 24.0, 45.0, and 66.0 kDa; Sigma-Aldrich Chemie GmbH) was loaded into each electrophoresis well. The running buffer was 1% SDS, 0.192 M glycine, 0.025 M Tris, pH 8.3. Samples were run at 150 V until the dye front moved to the end of the gel. Gels were removed from the plate and stained for 30 min with 2 g/L Coomassie R-250 blue in 10% acetic acid and 20% methanol. Gels were destained in a solution of 7.5% acetic acid and 5% methanol in water.

2.6.4. Densitometric analyses

Destained gels were scanned with a laser Personal Densitometer (Molecular Dynamics) at the following parameters: pixel size - 50 µm, 12 bytes per pixel. Each SDS-PAGE electrophoretic pattern of protein fractions was analyzed with a PC 468 DX2 computer and the Image Quant program (Molecular Dynamics), setting the option for automatic detection of electropherogram peaks and for integration of the area under the curves. Data on the protein concentration relative to stain intensity were thus obtained.

2.6.5. Statistical procedures

Data were studied as averages wherever possible. The statistical differences among the groups were evaluated with Student's *t*-test for significant differences of means with a probability of 5% or less [17].

3. RESULTS

3.1. Identification of irradiated mites

Results of previous studies [18, 19] indicated that the number of infecund pairs of acarid mites *(Acaridae)* increases with the increase of the irradiation dose. We observed also that females of the bulb mite, *Rhizoglyphus echinopus* (F. et R.), treated with a dose of 1.0 kGy laid a few eggs, but after

the treatment with 1.2 kGy, almost all pairs were infecund. Dose of 0.1 kGy or higher decreased significantly the fecundity of females. Females irradiated with 0.25 kGy laid 87% less eggs than the controls. Mites treated as adults produced eggs over a longer period than those irradiated as deutonymphs. Males and females irradiated with 0.6 or 0.8 kGy produced eggs during the 1st, 2nd and 3d week after the treatment.

Fecundity of irradiated young females and males (1-2 days after last molting) of the mold mite, *Tyrophagus putrescentiae* (Schrank), was greatly affected. The higher the irradiation dose, the higher the percentage of infecund females, i.e., non-laying eggs. Fecundity and fertility of females was decreased considerably. Mites irradiated with 0.6 kGy or higher doses were almost infecund; females produced only a few eggs which were dead. Irradiated females produced eggs during the first weeks, being infecund thereafter.

Results presented in Table I indicate that all untreated females of the bulb mite contained 1-5 eggs within their bodies. Number of eggs visible within the female body decreased considerably after irradiation with 0.2 kGy. There were no females with 5 eggs, and only two with 4 eggs. The number of females without eggs increased during the next weeks after treatment. After the 3d post-treatment week, there were only 2 females with a single egg and four with 2 eggs. After the 5th or 6th week,

TABLE I. NUMBER OF EGGS VISIBLE WITHIN THE BODY OF IRRADIATED FEMALES OF THE BULB MITE, *R. ECHINOPUS*. DATA WERE RECORDED EACH WEEK AFTER TREATMENT.

Dose (kGy)	Week after treatment	Number of females with indicated number of eggs					
		0	1	2	3	4	5
0.0	1	0	2	4	8	4	2
	2	1	2	5	6	5	1
	3	1	3	2	5	6	3
	4	2	5	7	4	1	1
	5	2	1	3	6	7	1
	6	2	1	4	5	6	1
0.2	1	2	5	9	3	1	0
	2	1	11	8	0	1	0
	3	14	2	4	0	0	0
	4	16	3	1	0	0	0
	5	19	1	0	0	0	0
	6	20	0	0	0	0	0
0.3	1	2	9	8	1	0	0
	2	11	4	4	1	0	0
	3	15	3	2	0	0	0
	4	18	2	0	0	0	0
	5	19	1	0	0	0	0
	6	20	0	0	0	0	0
0.6	1	6	6	6	2	0	0
	2	12	3	5	0	0	0
	3	15	4	1	0	0	0
	4	17	2	1	0	0	0
	5	20	0	0	0	0	0
	6	20	0	0	0	0	0

almost all females were without eggs. As the irradiation dose increased, the number of eggs recorded in the body of females decreased. Females irradiated with 0.6 kGy had no more than 3 eggs. After the 4th post-treatment week females had no eggs.

The mold mite was less fecund than the bulb mite, and only exceptionally 5 eggs were found in the body of untreated female. Usually, 2-3 eggs were visible within their bodies. With an increase of dose, the number of eggs in females decreased. Females, which were irradiated with 0.6 kGy and isolated during the 1st week after treatment, had 1-3 eggs. Later, no eggs were found in their bodies (Table II).

The results obtained show that a test based on lack of fecundity of treated mites may be used for detection of irradiated mites.

TABLE II. NUMBER OF EGGS VISIBLE WITHIN THE BODY OF IRRADIATED FEMALES OF THE MOLD MITE, *T. PUTRESCENTIAE*. DATA WERE RECORDED EACH WEEK AFTER TREATMENT.

Dose (kGy)	Week after treatment	Number of females with indicated number of eggs					
		0	1	2	3	4	5
0.0	1	0	5	8	5	2	0
	3	1	4	5	6	4	0
	4	1	3	5	8	2	1
0.2	1	0	3	6	4	2	0
	2	9	2	6	2	1	0
	3	11	3	5	1	0	0
	4	14	3	2	1	0	0
	5	18	1	1	0	0	0
	6	20	0	0	0	0	0
0.3	1	0	3	4	5	2	0
	2	10	1	7	2	0	0
	3	13	2	5	0	0	0
	4	17	1	2	0	0	0
	5	19	1	0	0	0	0
	6	20	0	0	0	0	0
0.6	1	0	5	6	1	0	0
	2	20	0	0	0	0	0
	3	20	0	0	0	0	0
	4	20	0	0	0	0	0
	5	20	0	0	0	0	0
	6	20	0	0	0	0	0

3.2. Locomotor activity of irradiated adults of the grain weevil and the rice weevil

One day after irradiation, the locomotor activity of the grain weevil treated with 0.25-0.5 kGy doses was high and similar to the control. Activity of beetles treated with 0.75 and 1.0 kGy doses was reduced by 36.7% and 80%, respectively. In the next post-treatment days, the locomotion of treated beetles decreased as the dose of radiation increased. However, beetles treated with the lowest dose, 0.25 kGy, were yet as active as the controls.

Weevils irradiated with 1.0 kGy were the most affected. In the first and second day after irradiation, only 50-56% beetles were able to move outside the 20 cm circle during 5 min. In the 5th day after the treatment with 1.0 kGy, grain weevils moved slowly, and none was able to pass the circle. In the 10-12th day after irradiation with 0.5 and 0.75 kGy doses, grain weevils seemed to be moribund, and only a small part of them moved outside the 20 cm circle.

The rice weevil responded to irradiation treatment in a similar way. In the first and second day after irradiation treatment, the locomotor activity of control and treated beetles was similar. In the next days, the effect of radiation dose on the locomotion of insects was evident. In the 5th day after irradiation, only rice weevils treated with 0.25 kGy were as active as the controls. In the 6th-9th day after the treatment, beetles irradiated with higher doses were not able to move out of the circle during 5 min.

Adverse environmental conditions such as lack of food or low temperature experienced by insects before the test had a slight effect on the locomotion of storage beetles. A slight decrease of locomotor activity was noted in the rice weevil and the grain weevil which were starved for a period of 5 and 7 days, respectively. Insects kept at low temperature for 24 hr., and then removed to a room temperature, started to move within 7 minutes, and within the next 30-40 minutes all or almost all beetles were able to move outside the 20-cm circle. A hour after the removal of beetles from a low temperature to a room temperature, all insects were as active as the controls.

3.3. Electron spin resonance (ESR) signal derived from irradiated insects

Results of the introductory experiment, in which nonirradiated adults of the rice weevil were studied, showed that the insects derive a strong natural signal, much stronger than in a bone, spices and herbs, seeds, nuts and other materials.

Natural (native) signals found in the rice weevil and the confused flour beetle were not enhanced by irradiation with doses up to 1.0 kGy. ESR spectrum of nonirradiated adults of the confused flour beetle was very similar to that of insects irradiated with a dose of 1.0 kGy. However, the intensity of the ESR signal induced in irradiated adults of the rice weevil was somewhat smaller than that of unirradiated insects. ESR spectra of adults of the rice weevil and the confused flour beetle were similar, but they differed with the ESR peak intensity.

Values of ESR peak intensity obtained from rice weevils irradiated with doses up to 1.0 kGy are shown in Figure 5. The peak heights decreased by 10-15% as the dose of radiation increased to 1.0 kGy. The peak heights obtained from the confused flour beetle were variable, and are thought to be not affected by irradiation with a dose of 1.0 kGy or lower.

After the irradiation of insects with a 1 kGy dose, a high spin concentration recorded in control beetles of the rice weevil decreased by ca. 5% to 23%, depending on the group of insects studied. After a slight, non-significant decrease of the spin concentration caused by a 1 kGy dose in Group III, it increased considerably after the treatment with a 3 kGy dose. In the other groups of insects, the further decrease of ESR peak height in the rice weevil, even by 31%, was induced by the irradiation treatment with a 3 kGy dose. However, higher doses of gamma radiation produced the gradual increase of the ESR signals, reaching the control value at a dose of 7 kGy.

3.4. Changes in the midgut of the confused flour beetle induced by gamma radiation

3.4.1. Normal structure of the midgut of the confused flour beetle

The midgut is the main site for digestion and absorption of the products of digestion, and is a very metabolically active tissue. The midgut of adults of the confused flour beetle consists of single layer of columnar epithelium placed on a basement membrane. This membrane forms fine folds in the direction to the gut lumen. The next is an inner circular muscle layer and outer bundle of longitudinal muscles.

The epithelium is made up of columnar cells and tiny regenerative cells. Columnar cells have relatively large and oval nuclei, usually present in the central portion of each cell. They have also microvillae on the lumen side that provide a large membrane surface to aid in both secretion of digestive enzymes and absorption. Microvillae are covered with plasmolemma, and create the tight structure called the brush border, which is visible under a light microscope. The peritrophic membrane was not found in the lumen of the gut.

The old epithelium is replaced by new cells produced by the regenerative nidi. Regenerative cells in the midgut of the confused flour beetle are located in small folds of the midgut in an atypical way. They do not lie, as is usually the case, between epithelial cells, but are located in evaginations of the midgut wall, forming pounchlike deverticula known as the regenerative crypts.

The outer layer of the midgut is made up of circular muscles and rare bundles of longitudinal muscles. The longitudinal muscles are less developed than the circular muscles (see also [20]).

3.4.2. Effects of gamma radiation on the midgut of the confused flour beetle

Histopathological changes were found to increase progressively with the increased dose of gamma radiation and with post-radiation time interval.

On the second week post-treatment, the pathological changes in the midgut of *T. confusum* adults treated with 0.1 kGy were evident. The normal and regular structure of the secretory epithelium was disintegrated. The epithelial cells were enlarged and elongated into the lumen of the gut. Walls of epithelial cells were separated one from another, and often disappeared. Cytoplasm from the broken cells flowed into the lumen of the gut. The cytoplasm of the intact cells was vacuolated. The nuclei were irregularly distributed within the cytoplasm, enlarged or damaged, with the chromatin grains found in the cytoplasm. The basement membrane was separated in several places from the muscle layer to form large folds in the direction to the gut lumen. The brush border was absent in many places. The lumen was filled with the damaged epithelial cells and the muscle layer was much thicker than it in non-irradiated insects.

Degeneration of the epithelium was more pronounced in insects irradiated with a 0.3 kGy dose of gamma radiation. Epithelial cells with large vacuoles were present in the lumen together with the fragments of disintegrated cells. Enlarged or destroyed nuclei were present in the gut lumen. The brush border was absent and the regenerative nidi were not visible. The basement membrane was separated in some places from the muscle layer, forming large folds in the direction to the gut lumen. Only a layer of muscles, thicker than in the control, was intact. Diverticula located along the midgut were reduced, and their epithelium was completely destroyed.

A dose of 0.5 kGy of gamma radiation brought about the most severe damage to the midgut of the confused flour beetle. The histopathological changes at this dose were similar to those of the adults treated with a 0.1 kGy dose and studied at the 3rd week after the treatment. The epithelium was completely missing, and as a result the gut lumen was enlarged considerably. Only the muscle layer was well visible with a few fragments of the disintegrated epithelium in the midgut. However, intact muscle layer was much thicker than it in non-irradiated insects. The muscle fibers were loose and swelled. Nuclei of the muscle cells seemed to be enlarged. Changes in the fat body and/or nutrient storage cells occurred as a consequence of the degeneration of the midgut. The fat body was significantly reduced in adults of *T. confusum* within 3 weeks after irradiation with a 0.1 kGy dose or higher (see also [20]).

3.5. A rapid method of detection of irradiated insects: Changes in the midgut of larvae of the Indian meal moth induced by irradiation

3.5.1. Normal structure of the midgut of larvae of the Indian meal moth

Adults of the Indian meal moth are not feeding, so only larval stages are responsible for accumulation of nutrient materials in sufficient amount to support adult life activities and reproduction. The midgut of larvae comprises the largest portion of their alimentary system, and it is the main side for digestion, and is very metabolically active tissue. The midgut of the larvae of the Indian meal moth consists of layer of the epithelium placed on a very thin, but well visible, basement membrane. The next is an inner circular muscle layer and outer bundles of longitudinal muscles, which are weakly developed.

The epithelium is made up of columnar cells, goblet cells, and small regenerative cells. Columnar cells have large, oval nuclei usually present in the central portion of each cell. Apical surface of each columnar cell bordering with the lumen is covered with microvillae, which create the tight structure called the brush border. Goblet cells, usually pear-shaped, are interspersed among the columnar cells. Their cytoplasm is reduced, and the apical border of the cell surface invaginates to form a deep cavity. In this cavity, there are numerous cytoplasmic extensions, similar to the microvillae of the epithelial cells, when are observed under the light microscope. Flat nucleus of the goblet cell is located basally, below its cavity. Small groups of tiny and undifferentiated regenerative cells are scattered along the basal portion of the epithelium, and form structures called the regenerative nidi. The epithelium of the midgut secretes a thin peritrophic membrane which surrounds the food contents in the lumen of the midgut.

3.5.2. Effects of gamma radiation on the midgut of larvae of the Indian meal moth

Histopathological changes in the midgut of larvae of the Indian meal moth were found to increase progressively with the post-radiation time interval. On the second day after the irradiation treatment with a 0.3 kGy dose, some pathological changes in the larval midgut were already evident. Of all structures of the midgut, the regenerative nidi were found to be the most affected. Soon after the treatment, the cytoplasm of regenerative cells vacuolated, and as a result, these cells enlarged. At the second day after irradiation, all regenerative nidi were completely destroyed, and their places in the epithelium were filled up with mass cell fragments. The peritrophic membrane was probably broken, that it was difficult to distinguish this structure in the gut lumen containing food material. The other structures of the midgut were intact. The goblet cells preserved even cytoplasmic extensions in their cavities. It was also noted that nuclei of the columnar cells of the epithelium moved up into their apical part.

On the 7th day after irradiation, the changes in the midgut were very distinct. The peritrophic membrane disappeared in the gut lumen. Numerous epithelial and goblet cells elongated into the midgut lumen. The cytoplasm of the columnar cells was extensively vacuolated, and their nuclei were enlarged. The brush border of the epithelial cells degenerated in many places, but it was yet well visible at those cells which were less affected by the treatment. Cytoplasmic extensions of the goblet cells degenerated, and their fragments were often noted in the cavity of goblet cells. The cavity of some goblet cells enlarged as a result of the disappearance of these cytoplasmic extensions. All regenerative nidi were lost, and the basement membrane formed many folds as a result of the muscle contraction.

On the second week after irradiation, the pathological effects were the most severe. Degeneration of the epithelium was more pronounced than in larvae examined on the 2nd and 7th day after the treatment. The most striking effects included the presence of vacuolated epithelial cells, often without membranes, in the gut lumen. The nuclei were irregularly scattered in the vacuolated cytoplasmic mass. The basement membrane was completely lost, but intact muscle layer was much

thicker than it in non-irradiated insects. All structures of the midgut stained more deeply in irradiated larvae than in the control.

3.6. Alterations in total proteins of irradiated adults of the confused flour beetle

The results of the electrophoretic separation of the proteins revealed several protein bands from homogenate samples of irradiated and control adults of the confused flour beetle. There were so many different protein bands that it was not possible to detect protein bands that show any shifts or separations between the irradiated and control beetles of the confused flour beetle. However, the electropherograms of the irradiated samples exhibited a significant decrease in intensity (density) of most protein bands. The total proteins decreased as the dose of gamma radiation increased.

There was a clear decrease in total proteins of adults of the confused flour beetle following irradiation, especially at the highest dose (0.5 kGy). On the basis of the relative area under the curve generated by densitometric scans, if the control at the 5th day post-treatment is assumed as 1.00, the value of total proteins was lowered by 8% in insects irradiated with a 0.1 kGy dose, and by 48% in insects treated with a 0.5 kGy dose. During this experiment, the total proteins increased in the control, but this value fluctuated in the treated insect, without the tendency for increase. At the 11th day, the value of total proteins in the controls reached the highest level; at the 14th day the value decreased somewhat, and it remained at this level up to the 17th day. At the 20th day it again increased, and decreased at the 26th day after the treatment. The highest values of the total protein content of insects irradiated with 0.1 kGy and 0.3 kGy was observed at the 14th day after treatment; the values decreased after the 17th day and again gradually increased, reaching the initial control value at the 26th day. The relative percentage of total proteins in insects irradiated with a dose of 0.5 kGy was always much lower than in the control or in the treatments with 0.1 or 0.3 doses. The maximal values of total protein content were observed at the 14th and 17th day after irradiation (Table III).

TABLE III. ALTERATIONS IN TOTAL PROTEIN CONTENT (%) OF THE BODY OF THE CONFUSED FLOUR BEETLE, *T. CONFUSUM*, AS A FUNCTION OF DOSE AND TIME ELAPSED AFTER IRRADIATION.

Dose (kGy)	Protein content (%) at days after irradiation*							
	5	8	11	14	17	20	23	26
0.0	1.00	1.03	1.54	1.21	1.24	2.21	2.10	1.73
0.1	0.92	0.98	1.14	1.46	0.87	0.97	0.99	1.02
0.3	0.67	0.71	0.95	1.10	0.88	0.77	0.86	1.05
0.5	0.52	0.44	0.66	0.76	0.75	0.64	0.43	0.56

* The control at the 5th day after the treatment is assumed as 1.00 (100%).

More pronounced alterations in total proteins of the confused flour beetle were found when the density of low (<16 kDa) molecular weight protein fractions were compared with that of medium (16-23 kDa) and/or high (>23 kDa) molecular weight protein fractions (Table IV). In control insects, the relative percent of low (<16 kDa) molecular weight proteins ranged from 15.2-20.7%; proteins of 16-23 kDa comprised 26.2-30.0% of all proteins, while the fraction >23 kDa was represented by 50.0-56.6% proteins throughout observation period.

In irradiated insects, the relative percent of low (<16 kDa) molecular weight proteins was ca. twice that in the control; that of the 16-23 kDa proteins was similar to that in the control, while the >23 kDa fraction decreased considerably. These changes were related to the radiation dose and the time elapsed after the treatment (Table IV).

TABLE IV. PERCENT OF AREA UNDER THE CURVE OF DENSITOMETRIC SCANS COMPRISED BY LOW (<16 kDA), MEDIUM (16-23 kDA) AND HIGH (>23 kDA) MOLECULAR WEIGHT PROTEIN FRACTIONS IN THE CONTROL AND IRRADIATED ADULTS OF THE CONFUSED FLOUR BEETLE.

Dose of gamma radiation (kGy)	Days after irradiation	Percent of area under the densitometric curve comprised by indicated protein fractions		
		< 16 kDa	16-23 kDa	>23 kDa
0.0	5	33.8	27.4	38.8
	8	36.8	28.0	35.2
	11	20.0	30.0	50.0
	14	15.2	29.8	55.0
	17	20.7	29.3	50.0
	20	20.4	27.3	52.3
	23	17.2	26.2	56.6
	26	18.3	27.1	54.6
0.1	5	37.3	26.7	36.0
	8	34.1	25.7	42.2
	11	41.4	26.7	33.9
	14	35.2	24.2	40.6
	17	30.1	26.7	43.2
	20	39.4	24.0	36.6
	23	48.7	24.9	26.4
	26	49.2	23.8	27.0
0.3	5	36.9	24.6	38.5
	8	29.2	29.5	41.3
	11	34.4	23.3	42.3
	14	27.0	20.9	52.1
	17	27.5	26.2	46.3
	20	44.3	27.9	27.8
	23	49.2	28.1	22.7
	26	43.0	31.2	25.8
0.5	5	43.3	20.8	35.9
	8	47.0	24.8	28.2
	11	45.7	22.5	31.8
	14	39.6	25.9	34.5
	17	47.4	23.6	29.0
	20	47.4	25.5	27.1
	23	39.5	32.0	33.5
	26	44.5	29.4	26.1

4. DISCUSSION

Irradiation has proven technically effective and in some cases unique as a quarantine treatment for a number of fruits, vegetables, cut flowers and other products [21]. A research program was initiated in 1985, with the purpose of determining the radiation doses required to provide quarantine security from insects and other pests infesting food and agricultural commodities in trade. After completion of the program, a dose of 0.15 kGy was recommended for fruit fly disinfestation, whereas a dose of 0.3 kGy of gamma radiation was suggested to provide quarantine security against all stages

of other arthropod pests [3]. At these low doses, death of pests is generally not immediate [6]. This can be a disadvantage of the treatment if the product is for immediate export, and there is a zero pest requirement. Particularly in food irradiated for quarantine purposes, it must be ascertained that still living insects are not able to survive or proliferate. 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.

A simple test is needed to ensure the quarantine personnel that the pest has been irradiated and it does not pose a risk. Development of a practical technique for the identification of irradiated pests was recommended by the ICGFI Task Force Meeting on Irradiation as Quarantine Treatment (Chiang Mai, Thailand, Feb. 17-21, 1986) [7].

An ideal method for detection of irradiated insects should be: (1) specific for irradiation and not influenced by other processes, (2) accurate and reproducible, (3) have a detection limit below the minimum dose likely to be applied to agricultural commodity as a quarantine treatment, (4) applicable to a range of pests, (5) quick and easy to perform, and (6) capable of providing an estimate of irradiation dose [3]. None of the developed methods for detection of irradiated insects fulfill all these requirements. 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. Studies on this subject have been limited, and there are a few techniques developed for the identification of irradiated pests [9, 22-26].

A test has been developed on the basis of a radiation-induced distinct decrease in the size of the supraoesophageal ganglion of the treated fruit flies of the family Tephritidae [23, 24]. This test fulfills the objective of estimating the effectiveness of the quarantine treatment, but more work is needed to establish whether these changes are really radiation specific.

Lescano *et al.* [24] examined use of both biochemical and anatomical techniques to detect irradiated larvae and pupae of *Bactrocera tryoni* (Froggatt). They found that electrophoretic protein profiles for control and irradiated larvae were similar, irrespective of their age at which irradiation occurred. They concluded that electrophoretic techniques do not identify consistently either irradiated larvae or pupae of the fruit fly. However, Yulo-Nazarea *et al.* [25] in a study on SDS-PAGE profile of protein from ^{60}Co irradiated fruit fly larvae at a dose of 100 Gy showed the absence of a specific protein band which is an integral part of the bands of proteins that appear only at the pupal and adult stages. They concluded that the absence of G_S protein in the SDS-PAGE gel pattern of pupae could be used as a marker for irradiated fruit flies by the quarantine personnel of the importing country.

Nation *et al.* [26] found that control larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), rapidly melanized, whereas larvae irradiated at $\geq$20 Gy failed to show typical melanization after freezing and thawing. Assays of phenoloxidase in control and irradiated larvae showed greatly decreased enzyme activity. They developed a simple spot test for phenoloxidase that produced a red color with a crushed control larvae and no color with a larva irradiated at $\geq$25 Gy. This simple spot test may be used for identification of Caribbean fruit fly larvae.

4.1. Identification of irradiated mites

A test to verify that a mite pest has been irradiated and is incapable of reproduction may be based on the infecundity of treated females [18, 19]. Acarid mites *(Acaridae)* produce eggs almost throughout their life-span. Irradiated mites produce eggs during the first weeks, being unfecund thereafter. Thus, the counting of any eggs visible within the bodies of females isolated from irradiated or untreated products may help to identify the mites subjected to irradiation. Detection of acarid mites irradiated with 0.3 kGy or higher doses of gamma radiation may be performed as follows:

- isolate about 20 mite females;

- prepare microscopic slides;
- count eggs visible within female bodies.

If females have no eggs within their bodies, it might be assumed that they were irradiated and do not pose a risk to a storehouse. If some (1-6) eggs are visible within female body, one may conclude that they were not irradiated or the dose used was low.

4.2. Locomotor activity of irradiated adults of the grain weevil and the rice weevil

Doses of ionizing radiation ranging from 0.3 to 1.0 kGy are suggested for radiation disinfestation of agricultural products [27]. These sublethal doses not only cause the sterility, accelerated mortality, inhibition of development in treated insects [28], but also affect their behavior. E.g., the complex series of events involved in insect courtship and mating are often interrupted at several points by irradiation [10, 28]. The propensity to fly and duration of each flight is decreased in irradiated insects [29].

The ability to fly and disperse from release sites has been investigated extensively in irradiated insects for the sterile insect release technique (SIRT) needs. The SIRT requires that the mass cultured and irradiated insects should exhibit similar vigor as native. In contrast, the locomotor activities (walking) and dispersal of stored-product pests irradiated with sublethal and/or sterilizing doses have received a little attention [10, 30]. However, it is important to know how pests respond, in terms of locomotor activity, to radiation doses recommended for disinfestation. Therefore, we undertook the study on the effects of gamma radiation on the locomotor activity of storage beetles.

We found that locomotor activities of irradiated insects, and as a consequence, their ability to disperse are greatly affected by gamma radiation. Two related storage beetles, the grain weevil and the rice weevil, respond, in terms of locomotor activity, to gamma irradiation rather in a similar way. Activity of these insects is negatively correlated with both the dose applied and the time elapsed after irradiation. After several post-treatment days, the debilitating effects of radiation reduce activity of the storage weevils to virtually zero.

Changes in the behavior and ability to disperse are of great importance in the application of radiation for the control of stored product insects. Results obtained [30, and this paper] indicate that insects irradiated within the product will not move for a long distance (reduced locomotion activity), and their ability to infest new batches of a product seems to be limited (reduced dispersal).

With data on the percentage of the grain weevils and the rice weevils that moved outside a 20 cm diameter circle during 5 minutes, it is possible to discriminate the insects irradiated with $\geq$0.5 kGy doses from those treated with a 0.25 kGy dose, or untreated. Thus, the locomotor test of Wiygul and Haynes [10] may be used for detection of irradiated stored product insects [30].

Adverse environmental conditions such as lack of food, food quality or low temperature experienced by insects before the test have a slight effect on their locomotion. Because locomotor activity reflects physiological conditions of insects [31], these and other factors should be considered when using the locomotion test for the identification of irradiated insects.

4.3. Electron spin resonance (ESR) signal derived from irradiated insects

Electron spin resonance (ESR, also known as electron paramagnetic resonance, EPR) is a spectroscopic method for detecting unpaired electrons, e.g., free radicals, which are formed during irradiation in heterogeneous biological materials. Generally, free radicals are so short lived that they cannot be readily detected but if trapped in hard, relatively dry components of food, such as bone, shell or seeds, their presence can be confirmed by ESR spectroscopy with high specificity and sensitivity (10^{-7} M). ESR spectroscopy is now established as a qualitative test for the detection of

irradiated fruits, vegetables, spices, herbs and nuts, or food containing bone such as poultry, pork, and fish [32-34].

Recently, Stewart *et al.* [35] have demonstrated the potential of ESR spectroscopy for both the qualitative and quantitative detection of irradiated Norway lobster, *Nephrops norvegicus*, using the signal induced in the cuticle of the tail. The signal in both irradiated and unirradiated cuticle is complex because of the presence of the Mn^{2+} signal. Although the intensity of the signal decreases with storage at 4°C, there is no difficulty in detecting it even 28 days after irradiation. Therefore, it was of interest to investigate if the ESR spectroscopy can be used for detection of irradiated insects.

The aim of the present study was to examine the ESR signal derived from insects or their fragments, and determine the effect of various irradiation doses on the intensity of the signal. Results obtained indicate that non-irradiated insects and their fragments derived a strong natural ESR signal. The natural signal in unirradiated beetles seems to be possibly related to melanin or melanin-type pigments, which are abundant in insects. The natural and strong ESR signals were also found in non-irradiated strawberries, and were also attributed to melanin-type pigments [36].

Peak heights obtained from the confused flour beetle were variable, and are thought to be not affected by irradiation with a dose of 1.0 kGy or lower. The ESR peak heights decreased by 10-15% in the rice weevil as the dose of radiation increased up to 1 kGy. However, this reduction of peak heights (= signal strength) is too small to be used as a criterion for detection of irradiated rice weevils. In contrast, irradiation dose had a highly significant effect ($P<0.001$) on the signal intensity from tail cuticle of the Norway lobster and the overall response was linear within the wide dose range [35].

The results obtained indicate that the ESR spectroscopy cannot be used for detection of stored product beetles, irradiated with doses recommended for radiation disinfestation of agricultural products.

4.4. Changes in the midgut of the confused flour beetle induced by gamma radiation

Ionizing radiation affects dividing cells, and in sufficient doses will sterilize pupae and adults by preventing eggs and sperm development or by introducing lethals into the embryo if sperm production is not completely inhibited. Except for gonads, adult insects have few tissues that undergo cell divisions. There are cells that may increase in size in adult insects, but do not divide. Longer lived insects are likely to have some cell divisions in the midgut to replace old cells of the epithelial lining. The dividing regenerative cells are particularly sensitive to radiation and damage to them may result in loss of midgut epithelium and death [28].

If degenerative changes in the midgut are positively correlated with both the dose and time elapsed after irradiation exposure, a pathological syndrome of effects may be used for a rapid and efficient method to identify irradiated insects. In the present study, the histological studies on the effects of gamma radiation on the midgut of the confused flour beetle, *Tribolium confusum* DuVal., are reported.

Changes in the midgut structure of the confused flour beetle, *T. confusum*, observed after irradiation were: (1) destruction of regenerative nidi; (2) elongation and enlargement of epithelial cells; (3) vacuolization of the epithelial cells; (4) fading of cell boundaries in the epithelium; (5) damage of nuclei (chromatin grains scattered throughout the cytoplasm of epithelial cells); (6) loss of the brush border; and (7) disintegration and further loss of the epithelium. These observations are in agreement with the findings of Jafri and Ismail [37], Ashraf et al. [38], Brower and Ashraf [39], Quereshi et al. [40], Riemann and Flint [41], and Vinson et al. [42], which investigated the midgut of irradiated larvae or adults of *T. confusum, Plodia interpunctella, Tenebrio molitor, Schistocerca gregaria, Anthonomus grandis,* and *Heliothis virescens*, respectively.

The most pronounced effect of irradiation treatment was the destruction of regenerative cells of the midgut which prevented the replacement of the secretory cells of the epithelium. As result, the epithelium disappeared and the gut lumen enlarged. Regenerative nidi of all insects species studied were found to be very susceptible to low doses of irradiation. Cell divisions in regenerative nidi of *T. confusum* larvae were inhibited within 24 hr. after irradiation with a dose of 0.1 kGy [37]. The same effect was observed after 4 days in *P. interpunctella* [38], *S. gregaria* [40], and *A. grandis* [41, 43]. A dose as low as 0.0855 kGy inhibited the cell divisions and enlarged cells of regenerative nidi in the midgut of *Heliothis virescens* [42].

Midgut muscle seems to be very resistant to irradiation. Only the increase of thickness of muscles, probably as a result of their contraction, was observed. This contraction resulted also in formation of basement membrane folds which were more pronounced in irradiated than in non-treated insects. Riemann and Flint [41] suggested also that holes in the epithelium resulting from the destruction of regenerative nidi are often not detectable because of the muscle contraction.

Irradiation of adults of the confused flour beetle with low doses (e.g., 0.1 kGy) resulted in enlarged epithelial cells elongated into the lumen of the gut. These cells were vacuolated. Among them, regenerative nidi were not found. More severe changes in the midgut were observed in *T. confusum* adults treated with a dose of 0.3 kGy. The most important were vacuolization of the epithelial cells, or fading of all boundaries in the epithelium. The gut lumen was filled with disintegrated epithelial cells. The highest tested dose (0.5 kGy) caused the complete disintegration, and loss of the epithelium, damage to the basement membrane, and loss of muscle layer. The damage to midgut of *T. confusum* increased with the intensity of the dose of gamma radiation and with the time elapsed after irradiation exposure [20].

Physical causes other than ionizing radiation producing disintegration of the epithelium in the insect midgut as a result of destruction of regenerative nidi are unknown. Because the degenerative changes in the midgut are positively correlated with both the dose and time elapsed after irradiation exposure, a pathological syndrome of irradiation effects on the midgut may be used for a rapid and efficient method of identification of irradiated insects.

4.5. A rapid method of detection of irradiated insects: Changes in the midgut of larvae of the Indian meal moth induced by irradiation

Ionizing radiation affects dividing cells which are undifferentiated [44, 45]. Except for gonads, insects have few tissues that undergo cell divisions. Adults and larvae have some cell divisions in small groups of undifferentiated cells in the midgut. These cells called the regenerative nidi divide to replace old epithelial cells exhausted by secretory activity. Recently, Szczepanik and Ignatowicz [20] suggested that an indicator that could be used easily for identifying irradiated insects may lie in the post-radiation pathological syndrome of the insect midgut. We have shown that the degenerative changes in the midgut of the confused flour beetle, *Tribolium confusum* DuVal., are positively correlated with both dose and time elapsed after irradiation exposure.

The test for identification of irradiated pests should be rapid, simple and convenient. However, the classical procedures used in studies on the histopathology of irradiated insects [20, 37-43] are time consuming. Midgut tissues are usually fixed for 24 hr. in Bouin's fixative, then dehydrated in a standard alcohol series, and cleared in cedarwood oil before embedding in paraffin [38].

In this study, we tried a rapid method for embedding tissues [12] to determine if this method enables the satisfactory preservation of cell and tissue structures in the midgut of larvae of the Indian meal moth, *Plodia interpunctella* Hübner. This method uses 1,4-dioxane as the final dehydrating agent and Polybed 812 as the embedding medium [13].

Information on the histopathology of the midgut of irradiated larvae of the Indian meal moth was provided by Ashraf et al. [38]. They found that the degenerative changes in the midgut are

positively correlated with the dose. Damage ranged from moderate disruption of epithelial cells 4 days after treatment with 0.05 kGy to almost complete histolysis following a 0.5 kGy dose. The additional objective of this research was to determine if the damage to larval midgut of the Indian meal moth increases with the time elapsed after irradiation exposure.

Results obtained support the opinion that the regenerative nidi are particularly sensitive to radiation and damage to them results usually in loss of midgut epithelium, followed by the insect death [46]. Muscle layers were found to be the most resistant to irradiation.

We noted that all regenerative nidi were destroyed already on the 2nd day after irradiation of larvae with a 0.3 kGy dose, whereas the other structures of the midgut were rather intact. On the 7th day after the treatment, the more changes in the midgut were noted. The brush border was damaged, cytoplasm of the columnar cells was vacuolated, and the muscles were contracted. On the 14th day after irradiation, the pathological effects were the most severe resulting in the total disintegration of the epithelial lining. However, the epithelium was not completely missing, like it was found in larvae of the confused flour beetle, *Tribolium confusum* DuVal., irradiated with a 0.5 kGy dose and examined on the 3rd week after the treatment [20].

The pathological changes in larval midgut of the Indian meal moth increase with the time elapsed after irradiation exposure. Because the degenerative changes in the midgut of larvae of the Indian meal moth are positively correlated with both the dose [38] and time elapsed after irradiation exposure (this paper), a pathological syndrome of irradiation effects on the midgut may be used for an efficient method of identification of irradiated insects. When the destruction of regenerative nidi, lack of brush border, and vacuolated epithelial cells are observed within the transection of the midgut, then one may suspect that the pest was irradiated a few days ago. When the total disintegration of the midgut is observed, one may conclude that the pest was irradiated with a high dose a few days ago, or with a low dose, but several days ago.

A rapid processing technique for dehydrating and embedding the larval midgut involving 1,4-dioxane and Polybed 812 produced consistent results. Using this technique, we were able to process the insect midgut from fresh tissue to sectionable embedded blocks within 5 hours. This technique, which is rapid and convenient, may be used at the quarantine laboratories, when a pathological syndrome of irradiation effects on the midgut is used for detection of irradiated insects.

4.6. Alterations in total proteins of irradiated adults of the confused flour beetle

Separation and identification of macromolecules using polyacrylamide gel electrophoresis (PAGE) show some potential for adaptation as an analytical technique for detecting radiation-induced changes in protein biosynthesis. Biochemical techniques that detect irradiated larvae of fruit flies were considered by Yulo-Nazarea *et al.* [25], Yulo-Nazarea and Manoto [47], and Lescano *et al.* [24]. Additionally, in the current study, we determined quantitative and qualitative changes in total proteins of adults of the confused flour beetle, *Tribolium confusum* DuVal., in order to examine the ability of this biochemical technique to detect irradiated insects. Comparisons between the irradiated and control beetles were made following polyacrylamide gel electrophoresis of whole-body homogenates.

The results of the electrophoretic separation of the proteins (SDS-PAGE) revealed several faint protein bands from homogenate samples of irradiated and control adults of the confused flour beetle, *Tribolium confusum* DuVal. However, it was not possible to detect protein bands that showed any shifts or separations between the irradiated and control beetles of this insect. Lescano *et al.* [24] found that electrophoretic protein profiles for non-irradiated and irradiated larvae of *Bactrocera tryoni* were similar, irrespective of the age at which irradiation occurred.

This study has shown a general depletion of total proteins in irradiated adults of the confused flour beetle, *T. confusum*. In general, the total protein content decreased as the dose of gamma

radiation increased. Irradiation produced major modifications in total proteins of *T. confusum* beetles. The protein electropherograms showed a decrease in intensity of >23 kD protein macromolecules, and an increase in density of <16 kD protein bands. However, these clear changes in electrophoretic patterns cannot be used to distinguish irradiated and non-irradiated insects, as these alterations are not specific for irradiation. Different insect pathogens and parasites produce similar effects.

Newton et al. [48] have shown a general depletion of all protein macromolecules in *T. castaneum* larvae infected with *Nosema whitei*. Glinski and Jarosz [49] revealed a reduction of total proteins in hemolymph of drone brood, *Apis mellifera*, parasitized by *Varroa jacobsoni*. The parasite alters both the electrophoretic patterns and densities of blood proteins, especially of low molecular weight cathodal protein fractions. Weiser and Lysenko [50] found that in the greater wax moth larvae, *Galleria mellonella*, infected with the protozoan *Nosema plodiae* there was an increase of "D peaks" (protein bands) with a decrease of "A peaks". According to these investigators, the infection appeared to cause a transfer of proteins from "A peaks" to "D peaks". A similar protein transfer was detected in the present research involving *T. confusum* adults and the irradiation treatment.

Viral infections also produce major modifications in insect proteins. For example, van der Geest and Craig [51] reported a decrease in total solid protein plasma content of the variegated cutworm, *Peridroma saucia*, during an infection with a nuclear polyhedrosis virus (NPV). Watanabe [52] demonstrated a decrease in the concentration of two protein bands from the hemolymph of the armyworm, *Pseudaletia unipuncta*. Heavy viral infections greatly reduced all of the bands.

Electrophoretic protein spectra vary considerably in individual insects [15], often in relation to differences in their physiological status [53], in different populations of the same insect species, and also during their development and growth [54, 55]. Pupation and emergence of the adult seems to be associated with several changes, preferably in the composition of low molecular weight protein fractions. The great variability of insect protein spectra is an important disadvantage of the biochemical method of detecting irradiated insects.

New protein bands appear in larvae, pupae, and adults of different insects as their development proceeds. For example, the appearance of newly synthesized protein fractions and loss of other hemolymph proteins is associated with the development of the Colorado potato beetle [55]. Proteins specific for certain stages of insect development were found in *Blattella germanica* [56], in *Calliphora* [57], *Pieris brassicae* [58], *Apis mellifera* [54], *Leptinotarsa decemlineata* (Jarosz, 1990), *Dacus dorsalis* [25, 47], and *Bactrocera tryoni* [25].

Yulo-Nazarea et al. [25, 47] found that the SDS-PAGE profile of pupae irradiated as larvae showed the absence of a specific protein band that appeared only during the 1st day of pupation. They suggested that this difference could be used to detect irradiated pupae of *B. dorsalis*. Lescano *et al.* [24] found the same band in *B. tryoni* after the 3rd day of pupation. They concluded, however, that the delayed appearance of this new band in *B. tryoni* makes the electrophoretic procedures unsuitable for detecting irradiated pupae of this species because both irradiated and control pupae have the same banding pattern 2 days after pupation. Furthermore, the lack of a specific protein band within the electrophoretic pattern is not only caused by irradiation treatment. Young and Lowell [59] studied hemolymph of the cabbage looper, *Trichoplusia ni*, during a NPV infection and observed the loss of three normal proteins from the hemolymph with a generalized decrease of all other major protein bands. Thus, the electrophoretic procedures seem to be unsuitable for detecting irradiated insects because (1) they are not specific for irradiation and are influenced by other processes (pathogens, parasites); (2) they are not reproducible due to the great variability in protein patterns generated by individual and population diversity; and (3) they are not quick and easy to perform at the quarantine laboratory.

This study has shown not only a general depletion of total proteins in irradiated adults of the confused flour beetle, but also major modifications in total proteins. These pronounced changes observed in insects irradiated with both high (0.5 kGy) and low (0.1 kGy) doses of gamma radiation

suggest that the irradiation treatment may alter the overall soluble protein composition in treated insects. Changes in the overall soluble protein composition in treated insects are the result of protein depletion, e.g., during post-irradiation starvation of insects caused by the loss of digestive function of the midgut [60]. Also possible are specific biochemical changes in the content of the total proteins induced only by ionizing irradiation. Irradiation may inhibit the production of one or more enzymes, rather than alter the enzymatic reaction involved in the process of protein synthesis. However, further electrophoretic studies on the effects of irradiation on insect protein composition are needed before further conclusions are drawn.

5. CONCLUSIONS

Diverse methods for detection of irradiated insects are considered. We studied the possibility of using the following tests: (a) test for identification of irradiated mites *(Acaridae)* based on lack of fecundity of treated females; (b) test for identification of irradiated beetles based on their locomotor activity; (c) test for identification of irradiated pests based on electron spin resonance (ESR) signal derived from treated insects; (d) test for identification of irradiated pests based on changes in the midgut induced by gamma radiation; and (e) test for identification of irradiated pests based on the alterations in total proteins of treated adults. Of these detection tests, only the test based on the pathological changes induced by irradiation in the insect midgut may identify consistently either irradiated larvae or adults. Further investigations on the other tests are needed before the recommendation on their use for detection of irradiated insects are formulated.

Degenerative changes in the midgut of insect larvae and adults are positively correlated with both the dose and time elapsed after irradiation exposure. Therefore, a pathological syndrome of irradiation effects on the midgut may be used as an efficient method for identification of irradiated insects. When the destruction of regenerative nidi, lack of brush border, and vacuolated epithelial cells are observed within the transection of the midgut, then one may suspect that the pest was irradiated a few days ago. When the total disintegration of the midgut is observed, one may conclude that the pest was irradiated with a high dose a few days ago, or with a low dose, but several days ago. This test is quick and convenient when a rapid processing technique involving 1,4-dioxane and Polybed 812 for dehydrating and embedding the midgut is used. Using this technique, a trained person is able to process the insect midgut from fresh tissue to sectionable embedded blocks within 5 hours.

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AN IRRADIATION MARKER FOR MANGO SEED WEEVIL

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Abstract

The objective of this study was to look for a method to determine whether live mango seed weevil, *Sternochetus mangifera* (Fabricius) present in fruit had been irradiated at a quarantine dose or lower. We looked specifically for anatomical effects on the supra-oesophageal ganglion of larvae and tested a biochemical method for detection of the effects of irradiation on the protein profile of pupae. Neither method was successful. However, because for most international export markets mangoes need only be found free of the pest at inspection sourcing from pest-free production orchards and quality control systems incorporating requisite pest management components could prove practicable and satisfy most markets.

1. INTRODUCTION

The mango seed weevil *Sternochetus mangiferae* (Fabricius) (Curculionidae: Coleoptera), is categorized as a quarantine pest by a number of countries. It develops within the seed and larvae, pupae and teneral adults can all be present when fruit is marketed. They are thus well protected and highly resistant to all quarantine disinfestation methods except irradiation. However because of very slow development, mortality from low dose irradiation is delayed beyond the shelf life of infested fruit. It is therefore important for regulatory authorities to be able to be sure that fruit is free of seed weevil or that any found at inspection have been irradiated a quarantine dose. Otherwise the consignment will be rejected.

2. MATERIALS AND METHODS

2.1. General

For the irradiated insect experiments a sample of 240 'common mangoes', *Mangifera indica* infested with mango seed weevil were collected from trees at Mareeba in North Queensland. These experimental mangoes were collected from aged ornamental trees with a known history of seed weevil infestation. Fruit were visually selected on evidence of oviposition and entry of first instars. They were held at 22^0C until treatment could be done. Then half were separated to be kept as untreated controls. The rest were further divided to 3 equal lots and irradiated at room temperature (ca 25^0C) with a dose of 75, 150 or 300 Gy. This was done using a "Gammacell 220" ™ maintained by the Chemistry Department of the University of Queensland at St Lucia. The dose rate of the irradiator was 420 Gy/hr, the max/min ratio 1.5 and monitoring was with Fricke dosimeters. After treatment all irradiated and control samples were held at 22^0C in plastic containers 35 x 23 x 10 cm with cotton gauze covers and placed in cages with wooden bases and tops and cotton gauze sides to keep them free of *Drosophila spp.* After 15 or 30 days following irradiation the seeds were removed from the fruit and opened to collect larvae, pupae and adults for anatomical and biochemical evaluation.

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2.2 Measurement of supraoesaphageal ganglion and proventriculus

Larvae were dissected under a binocular stereo-microscope and measurement of the ganglion and the proventriculus was attempted.

2.3 Electrophoresis studies of adults and pupae

Each pupa and adult dissected from seeds of irradiated and control mangoes was checked to ensure it was alive before storage at -80^{0}C for subsequent electrophoretic analysis. For this analysis, samples of individual insects were homogenized in 200 µl of grinding buffer (0.02 M Tris, 0.001 M EDTA, 0.01 M NH_4Cl, 0.1 M Glucose, 0.02% NaN_3) with a teflon mortar and pestle, and centrifuged for 10 minutes at 100 000 rpm at 4^{0}C. Ten µl of the resulting supernatant was combined with 10µl of sample buffer (3.3% SDS, 10% 0.1 M Tris-Glycine pH 8.5) and 1 µl Bromophenol blue. Twelve to 14 µl of this mixture was loaded into each well of 1.2% Agarose gel buffered with TAE (2.0 M Tris-Acetate, 0.05 M EDTA pH 8.3).

Electrophoresis was performed at room temperature using an EC 370 Minicell Submarine Gel System and Heathkit Regulated H.V. power supply, model MP-17. The running buffer used was 0.1 M Tris-Glycine pH 8.5 and samples were run at 50 mA until the dye front moved to the end of the gel. Gels were stained for general proteins [1] with naphthol blue black for 1 hr., and destained in a solution of methanol, water and glacial acetic acid (5.5:1) until the background was removed.

3. RESULTS

Of the experimental mangoes, 96% were infested with mango seed weevil. All larvae, pupae, and adults from all unirradiated control and irradiated seeds were alive at this time. Adults were quiescent during the first 1 or 2 hours after removal from seeds but subsequently became active. On the first collection day after treatment (day 15), 111 adult weevils were taken from 60 control seeds, 36 from 20 seeds of mangoes treated at 75 Gy, 22 from 20 seeds of mangoes treated at 150 Gy, and 41 from 20 seeds of mangoes treated at 300 Gy. On the second sampling date (30 days after irradiation) there were 119 adult weevils from seeds of 60 unirradiated control mangoes, 33 from seeds of 20 mangoes irradiated at 75 Gy, 37 from seeds of 22 mangoes irradiated at 150 Gy and 41 from seeds of 20 mangoes irradiated at 300 Gy.

The proportions of each of the stages found in seeds on sampling at 15 and 30 days are shown in Figures 1 and 2. The unirradiated control sample had a higher proportion of adults than irradiated samples with the proportion decreasing with increasing dose. Control fruit seeds had a lower proportion of pupae than seeds of irradiated fruit and the proportion increased as the dose increased. The proportions of larvae showed excessive variation as the dose was increased. Irradiation delayed the development of the immature stages, as observed with the fruit fly *Bactrocera tryoni* (Froggatt) [2]. Figure 3 shows proportions of adults present in samples 15 and 30 days after treatment. Proportions of pupae at sampling which are related to the adult proportions are shown in Figure 4.

Measurement of the supra-oesophageal ganglion in larvae of the seed weevil, as can be done in fruit fly larvae, was not practicable due to anatomical differences. The head and buccal parts were too difficult to dissect for measurement.

No differences were found between the protein profiles of unirradiated and irradiated pupae or adults. However there were differences between pupae and adults whether irradiated or not.

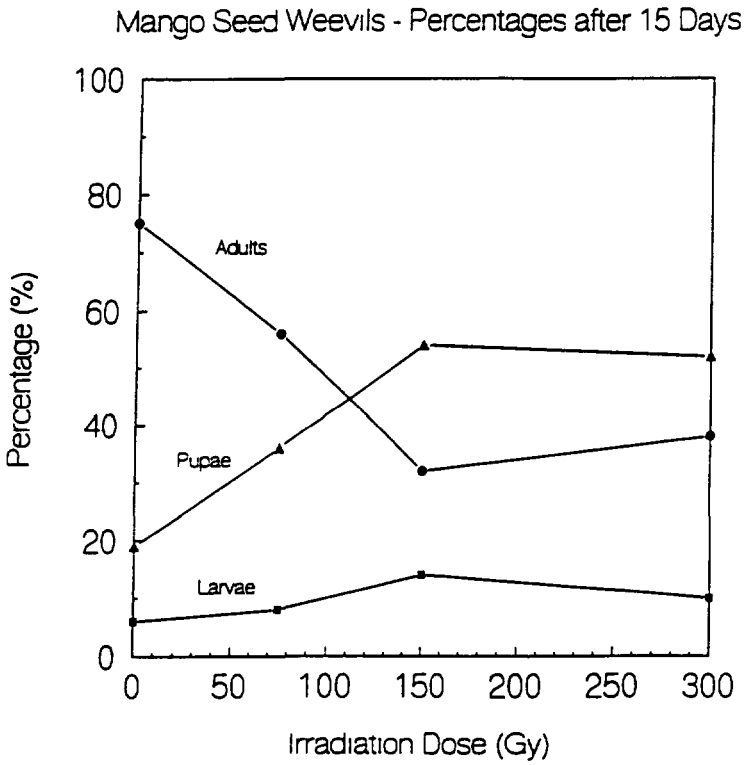

FIGURE 1.

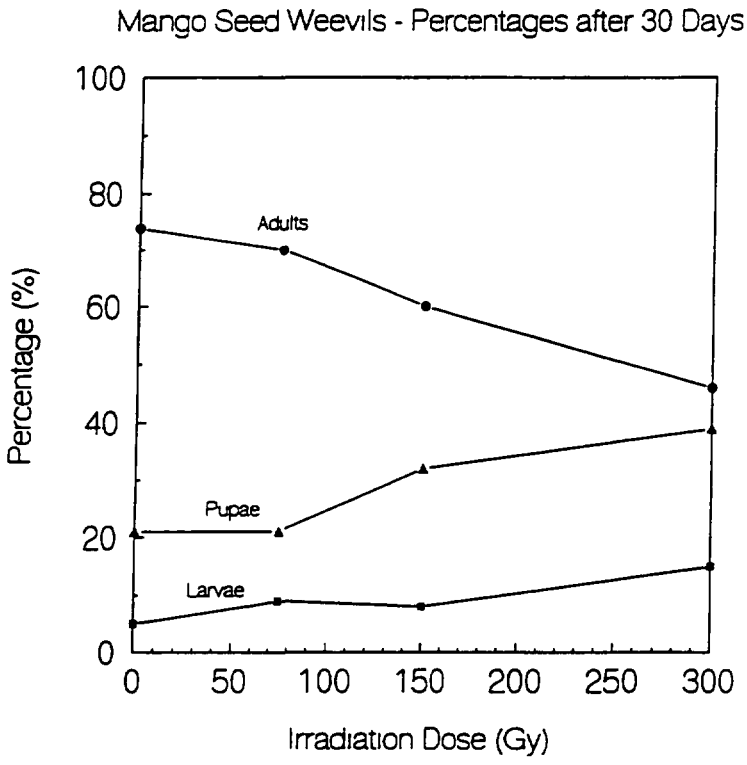

FIGURE 2.

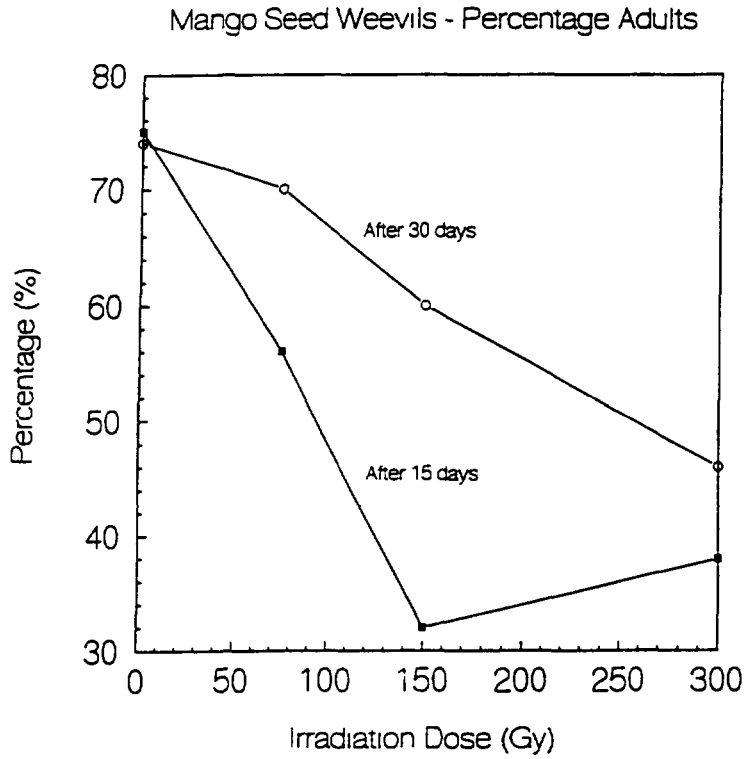

FIGURE 3.

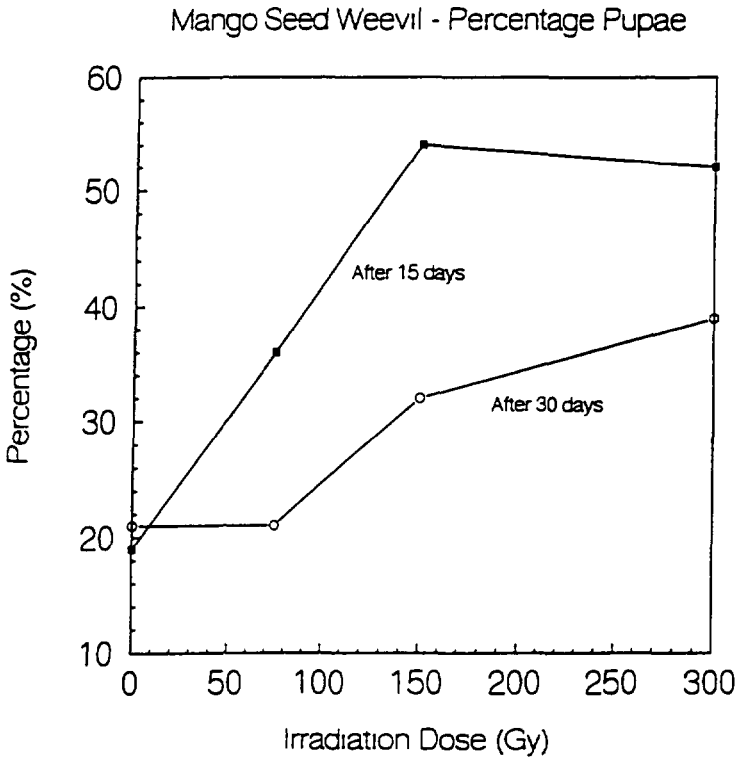

FIGURE 4.

4. DISCUSSION

At present it is still not possible to identify irradiated seed weevil using either anatomical or biochemical methods available to us. The problem is complicated by the need to have a method or methods for larvae, pupae and adults as each of these stages would be likely to be present in infested fruit found at inspection.

On the basis of these negative results other strategies for the market entry of mangoes irradiated against mango seed weevil need to be considered. There is believed to be international agreement that irradiation is an effective disinfestation method for achieving quarantine security against this pest. The concern of some authorities, especially those of USA, centers on the possibility of detection of live insects at post-irradiation inspection and the consequent inability of an inspector to determine whether irradiation had been effective.

However if pest management and quality control systems can achieve freedom of fruit from infestation at an inspection the inspecting authorities need only be concerned with treatment certification based on dosimetry records. In pest management systems such as this it is possible to accept a rejection rate which can be very low and built into the economics of the operation. This rejection rate based on presence of infested fruit at inspection does not compromise quarantine security because the irradiation treatment has been applied. However it satisfies countries which have anachronistic quarantine legislative requirements such as "no live insects".

Acceptable levels of freedom of fruit from seed weevil infestation can be achieved by a Quality Control system which incorporates the following features:

i. Sourcing of exports only from young orchards free of seed weevil. Because of the delay in first fruiting of young mango trees infestations in seeds and trees which are used to produce seedling trees or rootstock and scion material for grafted trees do not live long enough to start an infestation in the new orchard. As mango seed weevil does not disperse actively, orchards can remain free for many years. Freedom from seed weevil in an orchard can be monitored by destructive sampling of fruit before harvest eg fallen fruit which possibly have the highest risk of infestation. A sample of 600 fruit is dissected in Australian mango orchards using this type of certification.

ii. Pest management systems utilizing insecticides for scale and other insect control will have an incidental effect in reducing seed weevil populations. Alternatively, sprays against seed weevil adults sheltering on trunks of trees before the fruit is susceptible (about one quarter grown) could be used to reduce populations below the threshold for detection.

The problem of seed weevil quarantine is complex. Despite the apparent wide agreement on the efficaciousness of irradiation as a quarantine treatment against mango seed weevil its implementation has lacked commercial "drive". For example whilst Australia has seed weevil endemic in most old orchards and may wish to export mangoes to USA sea travel time currently exceeds the commercial life of harvested fruit and air freight is limited by cost and capacity.

There is an additional problem in south-east Asia where the common seed weevil appears to be a different species, *S. olivieri* Faust. This will require at least small scale research to show that it responds similarly to *S. mangiferae.*

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PHENOLOXIDASE AND MELANIZATION TEST FOR MANGO SEED WEEVIL*

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Abstract

This project was initiated to determine whether the phenoloxidase test successfully developed for fruit flies would be applicable to mango seed weevil, *Sternochetus mangiferae* (Fabricius). Mango seed weevil represents a quarantine impediment to the entry of mangoes to mainland USA and some other countries. It is not a destructive pest and rarely causes fruit damage even in late maturing varieties in which adults can emerge from ripe fruit. The main problem with the weevils come from nursery propagators who are concerned about possible effects on germination. It is questionable whether this is adequate justification for the level of quarantine importance with which this pest is currently regarded. It should not be confused with the mango pulp weevil *Sternochetus frigidus* Fabricius which does damage all infested fruit.

1 INTRODUCTION

This project was initiated to determine whether the phenoloxidase test successfully developed for fruit flies [1], [2], [3] would be applicable to mango seed weevil, *Sternochetus mangiferae* (Fabricius) Mango seed weevil represents a quarantine impediment to the entry of mangoes to mainland USA and some other countries It is not a destructive pest and rarely causes fruit damage even in late maturing varieties in which adults can emerge from ripe fruit The main problem with the weevils come from nursery propagators who are concerned about possible effects on germination It is questionable whether this is adequate justification for the level of quarantine importance with which this pest is currently regarded It should not be confused with the mango pulp weevil *Sternochetus frigidus* Fabricius which does damage all infested fruit

Irradiation is the preferred disinfestation treatment, but mango seed weevil is slow to die so a method is required to identify irradiated individuals which may be present and alive at a post treatment inspection The phenoloxidase test relies on reaction of the insect enzyme phenoloxidase with the substrate 2-METHYL-DOPA (2-methyl-3(3,4-dihydroxyphenyl)-DL-alanine) It can be assessed qualitatively as a spot test in which macerated insect tissue is brought into contact with the substrate on a background which will allow color change recognition or tissue homogenate can be added to substrate solution and color development measured quantitatively with a spectrophotometer In tephritid fruit fly larvae irradiated as eggs or 1st instars, irradiation reduces or eliminates melanization that normally occurs after death, and typically greatly reduces phenoloxidase activity in third instars [1], [2], [3]

2 MATERIALS AND METHODS

There is no laboratory method for rearing mango seed weevil, and infestation takes place when mangoes are part grown Thus, infested fruit had to be collected in the field from localities where there was known to be a high incidence of infestation of the weevil Infested fruit can be recognized with reliably by the presence of overgrown oviposition scars For these trials fruit was collected from trees of "Common" and "Kensington" varieties at Bowen, a major coastal production area for mangoes in Queensland (20^0 S)

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* Contract No 9502/RO/Regular Budget Fund.

Irradiation was done on fruit of commercial maturity with equal numbers of each cultivar bulked to single samples for all three tests Doses were 0, 38, 75, 150, and 300 Gy applied on 5 December 1996 with a Gammacell 220 at a dose rate of 260 Gy/hr Twenty fruit were treated at each dose with an equivalent number of untreated fruit for each dosed sample Fruit was then held in a temperature controlled insectary at 27°C until seeds were dissected 2 or 4 weeks after treatment Larvae, pupae, and adults dissected from seeds, put into an ultra deep freeze at -40°C and held until reagents and a suitable spectrophotometer could be accessed

The tests detailed by Nation et al on *Anastrepha suspensa* (Loew) [1] and Mansour and Franz on *Ceratitis capitata* (Wiedemann) [2], [3] were used in my tests on larvae, pupae, and teneral adults of *S mangiferae* to determine melanization, reaction to a colorimetric spot test, and spectrophotometric assays to the extent of the specimens of each stage available for each dose of irradiation

Spectrophotometric assays were conducted with a "DMS 100" spectrophotometer operating at a wavelength of 475nm Larvae, pupae, and adult specimens for assay were macerated individually in 5 ml of 0 1 M phosphate buffer pH 6 5 [1] and centrifuged at 2000g for 10 minutes The supernatant was filtered through a cotton wool plug The substrate used was 2-methyl-3(3,4-dihydroxyphenyl)-DL-alanine (DOPA) which reacts with phenoloxidase by giving a characteristic deep red color that can be measured spectrophotometrically, or visually as a spot test Nation et al used a 1% solution of 2-METHYL-DOPA but we were unable to dissolve more than 0 05% even after prolonged agitation Nevertheless, this gave a satisfactory color reaction

3 RESULTS

Spectrophotometric assays (Table I) revealed no reduction of phenoloxidase activity attributable to irradiation Larvae, pupae, and adults had approximately equal titers but there were some anomalously high results and some in which the activity was zero This was corroborated by phenoloxidase spot tests where 2 adults, one untreated, gave no response In neither test was there any apparent relationship to the irradiation dose Inspection of the incidence of melanization (Table II) showed mostly low level but recognizable development of melanin in larvae and pupae In adults sclerotization would have masked any exhibition of melanin

4 DISCUSSION

The mango seed weevil is a difficult pest for disinfestation with irradiation While assurance of quarantine security based on the criterion of inability to reproduce following irradiation at an appropriate dose is clearly achievable with irradiation at doses that can be tolerated by fresh mangoes at suitable levels of maturity, death of the various stages of the weevil in mango seeds is slow This is almost certainly because of the time spent in each life stage during the extended (1 year) development span Even with fruit fly larvae, death does not always occur until after the change to a subsequent stage such as the pupa [4] The phenoloxidase test appears to be highly reliable for fruit flies [1], [2] However fruit flies can develop from egg to pupa in as few as 5 days and from egg to first instar in 1 to 2 days, with similar time intervals between instars, so nullifying the phenoloxidase system as a result of irradiation in one stage will result in its absence in succeeding stages However, it has been shown that irradiation early in the third instar resulted in only approximately halving of phenoloxidase levels at an assay later in the same stage [1] because the enzyme was synthesized prior to the irradiation

The rapid, short life stages of tephritid fruit flies contrast with the long duration of the life stages of the mango seed weevil Most of the irradiated individuals would not be expected to have changed stage in the 6- or 14-day intervals after irradiation that were sampled, although on the basis of diminution of phenoloxidase levels in fruit flies irradiated early in the third instar some spectrophotometric differences would not have been unexpected The exact time of synthesis of phenoloxidase is the critical factor, however, in whether irradiation can inhibit its synthesis, since in fruit flies the activity of previously synthesized phenoloxidase is not altered by irradiation Although

color development was strong in spot tests, no differences were apparent in these or in spectrophotometric results Longer sampling intervals were not possible because fruit ripened and began to deteriorate at the holding temperature Use of lower temperatures such as the 13°C holding temperature used commercially would slow development of the weevil stages

Even at the highest dose of 300 Gy there was no apparent diminution of phenoloxidase activity attributable to irradiation in larvae pupae or adults Responses were neither dose nor stage dependent This dose was higher than that used for fruit fly The results for spectrophotometric assay, spot testing and melanization were in general agreement

In the absence of a test for irradiated mango seed weevil, greater emphasis will need to be placed on harvesting mangoes from seed weevil-free orchards for the few markets that refuse fruit with seeds containing live larvae This can ensure that infested mangoes will not be present in export consignments Although this alone may not satisfy the quarantine security requirements of some markets, in combination with a generic irradiation dose of 300 Gy it should satisfy the highest security levels required by any market Most orchards are free of mango seed weevil when they first come into production, and provided that there are no infested trees in the near vicinity, they can be maintained pest free for some years with minimal quarantine precautions

TABLE I Spectrophotometrically assayed concentrations of phenoloxidase with L-2-METHYL DOPA 0 05% w/v in water as a substrate for macerated larvae, pupae, and unemerged teneral adults of mango seed weevil 6 and 14 days after gamma irradiation in mangoes at 0-300 Gy followed by freezing at -40°C Where no value is recorded, the stage was absent from the sample

	6 days			14 days		
Dose (Gy)	Larvae	Pupae	Adults	Larvae	Pupae	Adults
0	0 075	0 074	0 023	0 193	0 027	0 012
38		0 025	0 043	0 053	0 044	0 000
75	0 061	0 029	0 006			
150		0 027	0 013	0 063	0 025	0 032
300	0 080	0 000		0 103	0 000	0 021

TABLE II Melanization of larvae and pupae of mango seed weevil thawed following freezing at -40°C 6 or 14 days after gamma irradiation at 38 - 300 Gy (P = Present, N = Absent, ? = Uncertain, No entry = stage not present in fruit sample)

	6 days		14 days	
Dose (Gy)	Larva	Pupa	Larva	Pupa
0	P	N	P	?
38	?	N	N	N
75	?			
150	P	?	N	P
300	P	P	P	

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