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Irradiation as a phytosanitary treatment of food and agricultural commodities

Proceedings of a final research coordination meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture 2002

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IRRADIATION AS A PHYTOSANITARY TREATMENT OF FOOD AND AGRICULTURAL COMMODITIES

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FOREWORD

World trade in fresh horticultural produce, durables and ornamentals continues to grow. Accompanying increased trade in agricultural products is the increased risk for inadvertently transporting quarantine pests to countries or regions where they do not occur. Quarantined pests, including insects such as fruit flies, beetles, moths, scales, mealybugs, thrips, and mites, can seriously disrupt marketing of fresh agricultural products not only between countries, but also between geographical areas within countries (e.g. Florida to California; Hawaii to mainland USA; Queensland to Victoria, Australia; Okinawa to Japan) unless accepted post-harvest quarantine treatments are available. Quarantine or phytosanitary treatments (such as fumigation, heat, cold or irradiation) disinfest host commodities of insect pests before they are moved through market channels to areas where the pests do not occur. Among the phytosanitary treatments, irradiation is recognized as a versatile treatment with broad-spectrum activity against arthropod pests at dose levels that have minimal adverse effects on the quality of most commodities.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, initiated in 1998 a Coordinated Research Project (CRP) on Irradiation as a Phytosanitary Treatment of Food and Agricultural Commodities. This CRP included 16 participants from Australia, Brazil, Chile, China (2), India, Islamic Republic of Iran, Japan, Malaysia, Philippines, Poland, Syrian Arab Republic, Thailand, Turkey and the USA (2). Research coordination meetings were held in Bangkok, Thailand, 29 March–2 April 1999; Fresno, California, 13–16 November 2001; and Vienna, 2–4 November 2002. This CRP built on the achievements of two previous CRPs on Irradiation as a Quarantine Treatment of Fresh Fruits and Vegetables, (1986–1990), and Irradiation as a Quarantine Treatment of Mites, Nematodes and Insects other than Fruit Flies(1992–1997).

This publication presents the research results presented at the final research coordination meeting, where the work completed during the last 5 years (1998–2002) was analysed.

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EDITORIAL NOTE

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SUMMARY

Irradiation as a phytosanitary treatment has gained increasing acceptance in recent years, and the application of irradiation to control arthropods in fresh commodities, stored products and ornamentals has grown. For example, in Hawaii irradiation is an accepted quarantine treatment to control fruit flies in ten fruits and four vegetables and the mango seed weevil in mangoes; and in Florida, sweet potatoes are irradiated to control sweet potato weevil before shipment to California.

Irradiation has several major advantages over other post harvest treatments. Whereas development of heat, cold and fumigation treatments involves generating data for each fruit-pest combination, irradiation treatments are developed for a pest species irrespective of commodity. This is possible because most commodities can tolerate irradiation at doses that kill the pest, while developing other treatments involves finding the balance between killing the pest and minimizing the adverse effects of the process on commodity quality. In fact, Appendix 1 of the IPPC "Guidelines for the use of irradiation as a phytosanitary measure" includes a table which identifies ranges of minimum absorbed dose for pest groups based on treatment research reported in scientific literature.

Moreover, because irradiation is effective against most insects and mites at dose levels that do not affect the quality of commodities, it is the ideal technology for developing "generic" treatments. A generic quarantine treatment is one that provides quarantine security for a broad group of pests. For example, a generic treatment could be applied to all Diptera (flies), or to flies in the family Tephritidae (fruit flies), or to tephritid fruit flies in the genus *Bactrocera*. Before generic treatments can be recommended, information is needed on effective irradiation doses for a wide range of insects within a taxon.

Although generic treatments have been debated over the years, the International Consultative Group on Food Irradiation (ICGFI) was the first group to formalize a recommendation for a generic treatment. In 1986, based on irradiation data for many tephritid fruit fly species and a limited number of other insect pests, they proposed a dose of 150 Gy for fruit flies and 300 Gy for other insects (ICGFI, 1991). To date these doses have not been adopted. However, the concept of a generic treatment has been applied on a limited scale. The California Department of Food and Agriculture accepts a 400 Gy irradiation treatment for control of surface pests on fruits exported from Hawaii. In 2001, the U. S. Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) convened a meeting to establish treatment protocols for Hawaii Pride (a commercial irradiation facility) in Hawaii, and approved generic irradiation doses of 250 Gy for any species of Tephritidae (fruit flies) and Thysanoptera (thrips); and 400 Gy for any species of Coccidae (soft scales), Pseudococcidae (mealybugs), and immature Lepidoptera (moths) infesting eight fruits being exported to the U.S mainland (USDA-APHIS unpublished document). In this case, the doses for non-fruit fly pests were established based on information from studies in Japan and Hawaii on a limited number of species within each taxon. This was the first time USDA-APHIS considered and approved a generic irradiation dose for any group of insects, and signaled an openness by APHIS to approve irradiation for broad groups of pests. Recently, USDA Agricultural Research Service (ARS) prepared a report recommending a generic treatment of 150 Gy for all tephritid fruit flies. Australia has prepared an export submission for irradiation treatment that recommends 150 Gy for fruit flies, 250 Gy for all other insects, and 350 Gy for mites for tropical fruits exported to New Zealand. These reports recommending generic treatments are an outgrowth of the information and ideas generated during the CRPs sponsored by the IAEA from 1986–2002.

The information provided by this and previous irradiation CRPs has been critical in promoting irradiation as a disinfestation treatment.

OBJECTIVES AND ACHIEVEMENTS OF THE CRP

The following research objectives were emphasized:

1. Identify commodity/pest systems amenable to efficient use of irradiation technology.
2. Identify efficacy requirements and data needs acceptable to regulatory agencies.
3. Develop efficacy data for pest/commodities systems.
4. Develop data on commodity tolerance to irradiation.
5. Evaluate whether systems/combinations are amenable to irradiation technology.

The participants worked in a wide variety of pests /commodities. Table 1 summarizes the results obtained.

It is important to note that several participants of this CRP were from countries which are the world's leading producers and exporters of economically important commodities including grapes, pistachios, several other types of dried fruits and nuts, orchids, etc. which require phytosanitary treatments in trade especially by methyl bromide fumigation. These participants are already working on research projects to find alternative treatments to methyl bromide fumigation. Other participants also worked on economically important commodities such as papaya, sweet potatoes, coffee and other stored products that also require phytosanitary treatments to satisfy trade requirements.

In the past, the guiding principle in quarantine treatment research has been the Probit-9 standard for treatment efficacy. The Probit-9 standard (99.9968% mortality) was initially recommended for tropical fruits heavily infested with fruit flies. The Probit-9 approach centers on high mortality of the treated pest population and, for heavily infested commodities, usually provides adequate quarantine security.

Recognizing the principles of plant quarantine, in particular the "technical necessity" for quarantine treatments, the participants considered that Probit-9 mortality may not be an appropriate and/or achievable criterion for non-fruit fly quarantine pests or for fruit flies on poor hosts. Further, the traditional criterion of acute mortality for demonstration of treatment efficacy was not considered appropriate and/or relevant for phytosanitary treatments of food and agricultural commodities by irradiation. Rather, criteria such as inability to reproduce (sterility), non-completion of pest life stages, or non-emergence of adults, were considered by the participants as more relevant to the biological processes involved and would meet quarantine requirements at the same time. The participants therefore agreed that efficacy requirements for the evaluation of phytosanitary treatments using irradiation for non-fruit fly quarantine pests must take into account *inter alia*:

- the appropriateness and relevance of criteria used to measure treatment end-points, and
- the technological feasibility of conducting research, including the production and collection of sufficient pest individuals to be treated per replicate in experiments.

In the absence of clear and precise efficacy requirements for non-fruit fly pests, a Research Protocol was developed by Dr. Neil Heather (Australia), an IAEA consultant who participated in the 1st and 2nd Research Co-ordination Meetings of this CRP.

Phytosanitary treatments for food and agricultural commodities are traditionally demonstrated by research and once approved by relevant authorities, validated on a consignment basis by provision of phytosanitary certification. Currently, importing countries may inspect samples of products on arrival at the points of entry and will release consignments only when any pests that may be found are dead.

Phytosanitary treatment by irradiation, while it satisfies quarantine requirements, does not necessarily need to result in immediate mortality of target pests. Therefore, verification of irradiation as a phytosanitary treatment cannot in most cases be based on dead pests. In other words, rejection of irradiated products that have been correctly treated to meet quarantine requirements but contain live pests at the time of inspection is not scientifically valid.

There are two possible avenues to the regulatory problem of intercepting live pests in irradiated consignments. First, methods could conceivably be developed to verify that live pests have been appropriately irradiated. However, development of such methods for a broad range of target pests has proved to be difficult to date. Therefore, the lack of detection methods to verify irradiated insects should not be an impediment for approval of irradiation as a phytosanitary treatment.

Second, regulatory confidence in the phytosanitary quarantine security of irradiated consignments can be based preferably on procedures to verify the proper implementation of the system used to irradiate foods and agricultural commodities. In addition, procedures are required to ensure and verify that treated consignments have been safeguarded from re-infestation by the target pest(s) or cross-infestation by other pests. In this way, phytosanitary-secure consignments can be released at points of entry based on a phytosanitary certificate or other documentation checks and physical proof of consignment security. Under the principles of the IPPC, inspection of product samples will not be necessary under such a system, so the question of what action to take if live pests are found does not arise.

Dr. Guy Hallman, who was one of the researchers of this CRP, participated in the development of two documents pertaining to the use of irradiation as a phytosanitary treatment. These documents were (1) Guidelines for the Use of Irradiation as a Phytosanitary Measure and (2) Irradiation Phytosanitary Treatment of Imported Fruits and Vegetables.

The first document was initially drafted in 2001 and sent to IPPC member countries for comment. The Standards Committee reviewed the comments and submitted the draft to the International Plant Protection Convention (IPPC) in April 2003 for their vote on approval. Finally, the 5th Session (April 2003) of the Interim Commission on Phytosanitary Measures (ICPM) reviewed and adopted the Guidelines for the Use of Irradiation as a Phytosanitary Measure as part of the International Standards for Phytosanitary Measures (ISPM). The standard provides technical guidance on the application of irradiation as a phytosanitary treatment for regulated pests and does not consider irradiation treatment for purposes of food safety, enhancing commodity quality, inducing mutagenesis, or sterile insect production for pest control.

The importance of the approval of these Guidelines is that they are a legally binding document to the 146 Member Countries of the World Trade Organization. If these countries do not accept irradiation as a phytosanitary treatment, they must show that it is a public health risk, or provide another valid, scientifically substantiated reason to reject it. It is difficult to anticipate the reaction of Member Countries that have previously taken a stance against issuing irradiation permits, such as the European Union and Japan. This is the first time that

the IPPC has drafted a standard for a phytosanitary measure and it may serve as a guide for future standards. Future additions to this standard may include a list of recommended irradiation doses for pests.

The final United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) rule entitled Irradiation Phytosanitary Treatment of Imported Fruits and Vegetables was published in the U.S. Federal Register 23 October 2002. The rule proposes irradiation doses for 11 tephritid fruit flies and the mango seed weevil, providing an alternative to currently approved treatments for these pests. It also sets down monitoring guidelines for foreign irradiation facilities. This groundbreaking rule will give an impetus to the irradiation industry for future growth in the U.S. and abroad in the treatment of fresh commodities, and signals an openness by APHIS to approve irradiation for broad groups of pests. Several of the stipulations contained in the proposed rule published on 26 May 2000, and a supplemental rule published 15 March 2002, were controversial. By the end of the 87-day comment period, 2212 comments had been received, and APHIS changed or amended several sections of the rule after consideration of the comments. In the final rule, the requirement for irradiation indicators on boxes was overturned, and the possibility of importing irradiated fruits and vegetables into three southern U.S. ports (i.e., fruit fly supporting areas) was approved under certain conditions for the first time. APHIS put off for future study recommendations that doses for certain fruit flies should be lowered, and raised the dose for the mango seed weevil from the proposed 100 to 300 Gy due to the limited data on treatment efficacy available for this pest. A section of the rule allows APHIS to be flexible in setting the level of monitoring for irradiation facilities depending on the situations in different countries. Equivalency issues as set forth in the SPS Agreement of the WTO were brought up as a challenge to this approach. APHIS distinguished between equivalency in monitoring procedures and equivalency in phytosanitary protection. APHIS and other countries will jointly develop a framework equivalency plan and negotiate the appropriate conditions to establish and operate the program.

It is important to note that the information provided by this and previous CRPs on the use of irradiation as a quarantine treatment has been critical in the development of the standards and regulations recently adopted.

Excellent progress was made by all participants on their research projects. There was an extensive exchange of views on the findings especially as they related to methodologies for insect rearing and infestation of test commodities, evaluation of treatment effects, control of the irradiation process, and significance of the measure/s and criteria used to establish treatment efficacy for the recommended dose.

The wide experience of some members of the CRP on the control of identical or similar insects in other or similar commodities, and their expertise in relevant areas of insect radiation biology and regulatory approaches to problems in quarantine control and food quality evaluation, also contributed to the productive discussions at the meeting.

The CRP produced a number of “firsts” in the application of irradiation for control of regulatory insect and mite pests.

- The first large-scale confirmatory tests for several non-fruit fly pests (e.g. codling moth) were reported.
- The group reported several extensive studies on the response of mites to irradiation, including the first ever study of an eriophyid mite.

- The methodologies developed to handle these tiny pests are a significant achievement, and the doses found for the different developmental stages of mites are important new findings.
- In one case a combination treatment using irradiation and low temperature was developed for the false red mite, *Brevipalpus chilensis*, under commercial conditions.
- The first commercial shipment of cut flowers using irradiation as part of a quarantine treatment was successfully completed between Thailand and Australia. The treatment combined irradiation with a chemical dip and cold storage to control thrips in Dendrobium orchids.
- Several packaging materials were identified that prevent reinfestation of pistachios and dates by Indianmeal moth and sawtoothed grain beetle.
- One of the projects resulted in the first approval and commercial use of irradiation against a non-fruit fly pest (sweetpotato weevil), and another project produced an approved irradiation treatment for the mango seed weevil, a non-fruit fly pest of international importance. The mango seed weevil irradiation treatment was significant because limited data were used to establish a conservative dose of 300 Gy. In total, the participants conducted research on 28 important quarantine pests.

RECOMMENDATIONS

The discussions identified the importance of the following factors in conducting studies to establish dose levels for irradiation of quarantine pests:

- Recommended irradiation doses should be based on confirmatory studies using a large number of test insects and infestation of test commodities under normal handling conditions.
- Steps must be taken to prevent contamination of irradiated host material during testing, and to ensure test conditions are optimal using the survival and performance of untreated controls as an indicator.
- Pests in the irradiated commodities should be observed until they die to get a full picture of the response to the treatment.
- Careful dose mapping and dosimetry are critical to ensure that test results can be accurately interpreted.
- The tolerance of the commodity to irradiation must be determined at doses sufficient to control the target quarantine pests, taking into account the anticipated dose uniformity ratio of the commercial irradiation facility.

The participants recognized that irradiation is beginning to play an important role as a phytosanitary treatment of agricultural commodities in trade. However, existing data in the literature are not readily accessible, not in uniform format and occasionally conflict with each other. The participants therefore recommended that an International Database on Insect Disinfestation and Sterilization (IDIDAS) be developed by the Joint FAO/IAEA Division, Vienna, which is coordinating this CRP. Such a database should have a uniform format, and be accessible through the Internet. It was agreed that such a database would provide valuable information to both research scientists and regulatory authorities that deal with phytosanitary treatments. This database has been launched and can be accessed at: htpp://www-ididas.iaea.org.

Table I. Minimum irradiation quarantine treatment doses for insect and mite pests

Scientific name	Common name	Major host/ commodity	Most tolerant stage present in/on commodity	Minimum dose required (Gy)	
				To inhibit development of immatures	To sterilize adults
Acanthoscelides obtectus (Coleoptera: Bruchidae)	Dry bean weevil	Beans	Pupa	300	60
Callosobruchus chinensis L. (Coleoptera: Bruchidae)	Cowpea weevil	pulses	—	100	100
Conotrachelus nenuphar (Coleoptera: Curculionidae)	Plum curculio	Pome and stone fruits	Adult	—	92
Cylas formicarius elegantulus (Coleoptera: Curculionidae)	Sweetpotato weevil	sweetpotato	Adult	—	165
Lasioderma serricorne (Coleoptera: Anobiidae)	Cigarette beetle	Various commodities of plant origin	Pupa	120	125
Oryzaephilus surinamensis (Coleoptera: Silvanidae)	Sawtoothed grain beetle	Wheat products	Pupa	700	85
Prostephanus truncatus (Coleoptera: Anobiidae)	Larger grain borer	Corn	Pupa	120	60
Rhizopertha dominica (Coleoptera: Anobiidae)	Lesser grain borer	Grain	Pupa	120	60
Sitophilus granarius (Coleoptera: Curculionidae)	Grain weevil	Grain	Pupa	80	—
Sitophilus oryzae (Coleoptera: Curculionidae)	Rice weevil	Rice, wheat, grain	Pupa	80	—

Scientific name	Common Name	Major host/ commodity	Most tolerant stage present in/on commodity	Minimum dose required (Gy)	
				To inhibit development of immatures	To sterilize adults
Sitotroga cerealella (Lepidoptera: Gelechiidae)	Angoumois grain moth	Grain	Pupa	600	600
Stegobium paniceum (Coleoptera: Anobiidae)	Drug store beetle	Grain, various plant products	Pupa	120	250
Sternochetus mangifera (Coleoptra: Curculionidae)	Mango seed weevil	Mango	Adults	—	300
Tribolium castaneum (Coleoptera: Tenebrionidae)	Flour beetle	Cacao beans and power	Adults	120	—
Trogoderma granarium (Coleoptera: Dermestidae)	Khapra beetle	Cereal, dries fruits, nuts	Pupa	200	200
Cryptophlebia illepida (*Lepidoptera: Tortricidae)*	Koa seedworm	Litchi, longan, rambutan, macadamia	Mature larva	250	125
Cryptophlebia ombrodelta *(Lepidoptera: Tortricidae)*	Litchi fruit moth	Litchi, longan, rambutan, macadamia	Mature larva	250	125
Cydia pomonella (*Lepidoptera: Tortricidae)*	Codling moth	Apples, pears, walnuts, quince	Mature larva	200	—
Ecdytolopha aurantiana (Lepidoptera: Tortricidae*)*	Orange fruit borer	Citrus	Pupa	500	250
Ephestia cautella *(*Lepidoptera: Pyralidae)	Almond moth, fig moth	Dried fig, almond, hazelnut	Larva	300 for eggs1000 for immature	—
Grapholita molesta (Lepidoptera: Tortricidae*)*	Oriental fruit moth	Pome and stone fruits	Mature larva	—	200

Scientific Name	Common Name	Majorhost/ commodity	Most tolerant stage present in/on commodity	Minimum dose required (Gy)	
				To inhibit development of immatures	To sterilize adults
Neoleucinodes elegantalis (Lepidoptera: Pyralidae)	Tomato fruit borer	Tomato	Pupa	400	300
Plodia interpunctella (Lepidoptera: Pyralidae*)*	Indianmeal moth	Nuts	Larva Pupa	450 for eggs 1000 for immature 650 for pupa	350
Tuta absoluta *(*Lepidoptera: Gelechiidae)	Tomato worm	Tomato	Pupa	300	200
Thrips palmi (Thysanoptera: Thripidae)	Melon/orchid thrips	Chrysanthemum, orchid, rose, and other cut ornamentals	Adult	350	300
Brevipalpus chilenisis (Acari Tenuipalpidae)	False red mite	Grapes, cherimoya, citrus, kiwi	Adult	—	300 200 + cold @ 0° for 15 days
Phyllocopreuta oleivora (Acari Eriophyidae)	Citrus rust mite	Citrus	Adult	—	350
Tetranychus piercie Acari: Tetranychidae	Spider mite	Chrysanthemum, orchid, rose, and other cut ornamentals	Deutonypmh	350	280

IRRADIATION QUARANTINE TREATMENTS FOR MANGO SEED WEEVIL AND *CRYTPOPHLEBIA* SPP

P.A. FOLLETT
U.S. Department of Agriculture, Agricultural Research Service,
U.S. Pacific Basin Agricultural Research Center,
Hilo, Hawaii, United States of America

Abstract

Irradiation was explored as a method to prevent adult emergence in, or to sterilize, mango seed weevil. Mixed-age mango seed weevils in mangoes were irradiated with target doses of 50, 100, or 300 Gy and held for adult emergence. The 300 Gy treatment (dose range 180–310 Gy) did not prevent adult emergence. Emerging adults from the 100 and 300 Gy treatments were lethargic and short-lived, and laid no eggs indicating sterility. An irradiation quarantine treatment (300 Gy) to sterilize mango seed weevil in mangoes has been approved. This treatment opens U.S. mainland markets to mango exports from Hawaii. *Cryptophlebia illepida* (Butler) and *C. ombrodelta* (Lower) (Lepidoptera: Tortricidae) are quarantine pests that attack lychee, longan, rambutan, mangoes and other fruits in Hawaii. Studies were undertaken to determine whether irradiation treatment at 250 Gy, an accepted treatment for disinfestation of fruit flies in tropical fruits from Hawaii, would also control the two *Cryptophlebia* species (Follett and Lower 2000). *C. illepida* was determined to be more tolerant of irradiation than *C. ombrodelta* and so *C. illepida* was used in detailed tests. Using the criterion of success in developing to the adult stage, the pattern of tolerance to irradiation in *C. illepida* was generally eggs<early instars<late instars<pupae. The most tolerant stage that could potentially occur in harvested fruits is the late (fourth and fifth) instar. No *C. illepida* larvae receiving an irradiation dose $\geq$125 Gy and emerging as adults produced viable eggs, indicating sterility can be achieved at doses <250 Gy. Large-scale tests in which 11 256 late instars were irradiated with a target dose of 250 Gy resulted in a pupation rate of only 8.4% and no adult eclosion. Therefore, the irradiation quarantine treatment of a minimum absorbed dose of 250 Gy approved for Hawaii's fruit flies will effectively disinfest fruits of any *Cryptophlebia* in addition to fruit flies.

1. INTRODUCTION

With the decline of the plantation agriculture in Hawaii in recent years, vast hectarages have become available for diversified agriculture. A tropical exotic fruit industry is expanding to help fill the void and is expected to be an important part of Hawaii's crop diversification. An important step in this expansion is the development of quarantine treatments to permit exports. Currently, irradiation is an accepted quarantine treatment for export of papaya, carambola, lychee, rambutan, longan, atemoya, sapodilla, and abiu from Hawaii to the U.S. mainland. Irradiation quarantine treatments up to this time have focused exclusively on fruit flies and the approved dose is 250 Gy for the crops listed above. Fruits can undergo irradiation treatment in Hawaii, in non-fruit fly supporting areas of the U.S. mainland, and at three ports in fruit fly supporting areas in the southern U.S. under special conditions.

In addition to fruit flies, many other insect and mite pests are classified as regulatory pests and their appearance can interrupt export shipments. For example, mango seed weevil, *Sternochetus mangiferae* (Coleoptera: Curculionidae) is the key regulatory pest on mango in

Hawaii, and *Cryptophlebia illepida* and *C. ombrodelta* (Lepidoptera: Totricidae) are important regulatory pests of lychee, longan, and rambutan.

The mango seed weevil, *Sternochetus mangiferae* (F.), is a federally quarantined pest that has prevented the shipment of mangos from Hawaii into the continental United States for over 50 years. Mango seed weevil was elevated to the status of quarantine pest because of three beliefs about its impact: (1) infestation reduces the germination capacity of seeds (2) weevil development causes damage to the pulp that renders the fruit unmarketable or unappetizing, and (3) infestation can cause premature fruit drop [1, 2, 3, 4]. However, information on these sources of crop loss is scarce. Pest control research for mango weevil over the years has been largely unsuccessful. Therefore, the industry must depend on a postharvest disinfestation treatment. Postharvest researchers have attempted to kill mango seed weevil in mangoes, while maintaining fruit quality, using heat, cold, and fumigation treatments without success [5]. Irradiation appears to be the best alternative to disinfest mangoes of mango seed weevil.

A typical quarantine treatment for fruit flies is developed by testing hundreds of thousands of lab-reared insects, which are artificially inoculated into the fruit, so-called Probit-9 (99.9968% mortality) level testing. Development of a quarantine treatment for mango seed weevil has been difficult because there is only one generation of weevils per year, and no artificial diet exists to culture the insect in the laboratory. Therefore, Probit-9 level testing is impractical. Instead, we pursued an alternative approach: to show that the weevil is not a serious pest and therefore not a threat to U. S. mainland agriculture [1]. If the risk of mango seed weevil could be put in perspective, regulators could be convinced that a safe quarantine treatment could be developed after testing only a few thousand insects.

Cryptophlebia illepida (Butler) and *Cryptophlebia ombrodelta* (Lower) are internal-feeding pests that typically infest 10-15% of the lychee and longan crop in Hawaii (unpublished data). *Cryptophlebia* spp. also attack rambutan [6] and are not yet listed as a regulatory pest on rambutan but are of concern. Current regulations for quarantine treatment of Hawaii-grown lychee and longan with irradiation to kill fruit flies stipulate that fruits also must be found free of *Cryptophlebia* [7]. Thus, the presence of *Cryptophlebia* spp. can prevent export of lychee and longan from Hawaii, and potentially could interrupt rambutan shipments as well. If the accepted irradiation quarantine treatment for pest fruit flies (250 Gy) is also effective against *C. illepida* and *C. ombrodelta*, these species could be added to the regulation, preventing potential interruption of shipments due to their presence. The objectives of the studies with *Cryptophlebia* spp. reported here were to examine the effects of irradiation on egg, larval, and pupal development, and adult reproductive fitness, and thereby determine the efficacy of the accepted irradiation quarantine treatment against these pests [8].

2. METHODS

2.1. Mango seed weevil

2.1.1. Mango response to mango seed weevil infestation

Natural infestation and artificial damage studies were conducted to test the assumption that weevil-damaged seeds have a lowered germination rate than undamaged seeds [2]. For the natural infestation study, ‘Haden’ (monoembryonic [one seedling per seed]) and ‘Common’ (polyembryonic [multiple seedlings per seed]) mango fruits were included because they potentially respond to weevil injury differently. The fruit pulp and seed husks were removed and naked seeds were assigned to the infested or uninfested treatments by inspecting the dehusked seeds for evidence of larval feeding (tunneling, frass). All infested seeds showed

extensive damage consistent with feeding by one or more late instars. Seeds were planted individually in pots containing potting soil. Seeds in pots were germinated in a black screen shade house.

Artificial damage studies were conducted to examine the effect of different levels of damage on germination rates [2]. 'Haden' mango fruits were weighed and cut open, and only uninfested and undamaged seeds were used in the experiment. The experimental design consisted of four damage treatments created by cutting away 25, 50, or 75% of the seed, or leaving the seed whole as a control. In all treatments the seed cotyledon was the portion cut away and the germ or embryo was left undamaged. Seeds were planted in pots in a shade house for germination as described above.

In a test to determine whether weevils can cause premature drop, a total of 3122 mangoes were collected from an orchard at four times during the season (June-August) from 29 trees [3]. The average size of fruit increased with each successive collection date, but all fruit were immature. On each date, fruit of similar size were first collected off the ground under each tree, and an equal number of similar-sized fruit were harvested from the tree. Mangoes from each position (ground or tree) for each tree were held separately. In the laboratory after fruit were weighed, fruit pulp and seed husks were removed to inspect naked seeds, and all seeds were dissected to determine the number and life stage(s) of any weevils present. Statistical tests applied to the data from the above experiments can be found in the publications [2, 3].

2.1.2. Mango seed weevil irradiation

Harvest-mature 'Haden' mangoes were collected from the Yamada orchard in Kalapana, HI in 1998, and infestation was determined based on oviposition scars [9]. Infested mangoes were transported to the Hawaii Research Irradiator at the University of Hawaii at Manoa and irradiated at target doses of 50, 100, or 300 Gy, or left untreated as controls. The Hawaii Research Irradiator uses a ^{60}Co source of gamma radiation and the dose rate at the time of the tests was 5.3–5.8 Gy min^{-1}. Details on dose mapping, dosimetry, and dose variation are given in Follett and Lower [8]. A sub-sample of fruit was taken beforehand and opened to determine the age structure of weevils in the infested fruit. After irradiation, the fruit flesh was cut away and the seeds in their husks were held over an eight-month period and inspected weekly for adult emergence. Emerging adults were placed in screen cages with immature mango fruits to examine reproduction. The experiment was replicated four times and all emerging adult weevils for a given irradiation treatment (control, 50, 100, 300 Gy) were held in one cage.

2.2 Cryptophlebia irradiation

Irradiation treatment was done at the Hawaii Research Irradiator at the University of Hawaii at Manoa [8]. The Hawaii Research Irradiator uses a ^{60}Co source of gamma radiation and the dose rate at the time of the tests was 5.3–5.8 Gy min^{-1}. The internal dimensions of the treatment chamber are 18 by 53 by 58 cm. For irradiation treatment, eggs, larvae, pupae, or adults in 29 ml plastic cups with or without diet were packed in containers, transported to the Hawaii Research Irradiator on Oahu, and treated at the target dose(s). Density of the packed containers was 0.16 g/cm^3. Gafchromic film dosimeters (ISP, Wayne, NJ), read with a spectrophotometer at 500-nm absorbance, were used to verify dose accuracy in each replicate. Film dosimeters were calibrated using alanine dosimeter standards supplied and quantified by the National Physical Laboratory, Middlesex, United Kingdom. Initially, 900 larvae in individual diet cups were packed into a box equal in volume to the inside of the treatment chamber to maximize the number of individuals treated at the same time. A detailed dose

mapping study midway through our studies indicated that filling the chamber resulted in a high dose uniformity ratio (ratio = 1.8), with highest and most consistent doses in the centre of the chamber and lowest doses at the inside periphery [see figure in 8]. In subsequent tests all individuals to be treated for a particular age or stage were placed in 3.8-liter plastic containers and positioned in the centre of the chamber to minimize variation in irradiation dose (max/min ratio ≈1.3).

All irradiation tests used laboratory-reared *Cryptophlebia* [8]. To identify the more tolerant species between *C. illepida* and *C. ombrodelta,* late (fourth and fifth) instars were irradiated with a target dose of 62.5 Gy. This dose was chosen because it was known from preliminary tests to be sublethal. Each of four replicates had 80 individuals of each species and all replicates were treated on the same day. Emerging adults were classified as 1) normal, 2) with deformed wings, or 3) partially emerged from the pupal case. Percentage adult eclosion was the criterion used for selecting the most tolerant species. A single group of 30 late instars of each species was left untreated and held as controls. Based on the results of this test all subsequent tests focused on *C. illepida* [8].

Eggs (three-day old) and larvae (neonates, second/third instars, and fourth/fifth instars) were irradiated at target doses of 62.5, 125, 250, and 400 Gy [8]. These are the stages that occur in fruit. Although the pupal stage of *Cryptophlebia* is not found in fruits, we tested the effects of irradiation against this stage in the event a larva emerged from a fruit and pupated in a package or shipping container before receiving irradiation treatment. Pupal development to the adult stage is 11 days under our rearing conditions (25 $\pm$ 2^{o}C). Pupae (one to two-day old, four to five-day old, and seven to eight-day old) were treated at 125 and 250 Gy, only. For each larval or pupal age/stage, four-seven replicates were irradiated on different dates, and in each replicate a control group of 25-50 insects was not irradiated.

Irradiation doses were selected based on the accepted irradiation treatment for exporting Hawaii's fruits (250 Gy) [7] and the generic dose established by California for insects other than fruit flies (400 Gy). After treatment, development of each individual in a test was followed until death. For each age-dose combination, individuals that developed to the adult stage were mated *inter se* when possible (i.e., when there was synchronous emergence of male and female moths) to examine irradiation effects on mating success, fecundity, and fertility. Insects received irradiation treatment while on artificial substrates because preliminary artificial infestation tests indicated that lychee, longan, and rambutan were poor hosts for *Cryptophlebia* (i.e., high control mortality in artificially infested fruit) [see also 6]. Eggs were treated naked in plastic cups, and neonates emerging from treated eggs were transferred to diet cups for development. Larvae and pupae were treated in plastic cups with laboratory diet. The efficacy of irradiation treatments against immature *C. illepida* was evaluated based on the number of treated individuals pupating, adult eclosion, and adult female fecundity and fertility.

Pre-mating and post-mating studies were conducted to examine the effects of irradiation of adults on fecundity, fertility, and egg development [8]. In the first study, individuals were isolated in individual plastic cups as pupae, and after eclosion, unmated moths ($\leq$ two-day old) were irradiated with a target dose of 125 or 250 Gy or left untreated. Irradiated and un-irradiated males and females in all combinations were then mated as pairs in individual cups for 24 h. Males were removed after 24 h and eggs were counted daily until adult female death. Actively ovipositing females were transferred to new cups every two days. Egg development was scored every two days using a five-stage maturity rating system: (0) eggs clear (no observable development, or infertile); (1) pink spots appearing on surface; (2) pink coloration

throughout; (3) developing larvae visible within; (4) sclerotized head capsule evident. This test was replicated twice. In a second unreplicated study, a cohort of mated females that had begun ovipositing was irradiated at 250 Gy or left un-irradiated as controls. Moth longevity, the number of eggs laid, and number of eggs eclosing were measured.

All larval dose response tests and the first five replicates of the large-scale confirmatory test used the full volume of the irradiation chamber during treatment [8]. The last six replicates of the large scale confirmatory test, and all pupal and adult mating tests involved placing insects in a 3.8 liter plastic container and placing the container in the center of the chamber.

Adult emergence data for the comparative study between *C. illepida* and *C. ombrodelta* were arcsine transformed and subjected to analysis of variance (ANOVA), and means separation was done using a *t*-test at the 0.05% level of probability. Regression analysis of life stage tolerance to irradiation is discussed in Follett and Lower [8]. Data on percent egg eclosion from the adult reciprocal matings study were subjected to ANOVA and means separation were done for each of the two irradiation doses separately using a Student's t-test at the 0.05% level of probability.

3. RESULTS

3.1 Mango seed weevil

3.1.1 Mango response to mango seed weevil infestation

Germination rates for infested seeds were equal to that of uninfested control seeds in a polyembryonic cultivar ('Common'), whereas germination was significantly reduced for infested seeds of a monoembryonic cultivar ('Haden') compared with uninfested control seeds but germination of infested seeds was still >70% [2]. In the artificial damage study, none of the 25, 50, or 75% excised seed treatments was significantly different from the undamaged controls, indicating that mango seeds can withstand substantial damage and still germinate successfully. Over the two-year period we conducted experiments, only 15 of 5192 mango fruits (0.29%) showed evidence of direct feeding damage to the pulp [2]. Results suggest that *C. mangiferae* is a less serious pest of mangos than previously thought.

In the premature drop study, if weevil-infested fruit were more prone to dropping than uninfested fruit, the prediction was that a higher infestation rate would be found in fruit on the ground compared with fruit on the tree. Average fruit weight was used as an indicator of fruit maturity. The seed infestation rate was significantly higher in fruit collected off the ground compared with fruit collected from the tree in 38 g and 79 g (early season) fruit but not significantly different in 207 g (mid-season) and 281 g (late season) fruit [3]. The age distribution of weevils and the number of insects in infested fruits were similar for ground and tree fruits on all dates. Results from the infestation and premature drop studies suggest that *C. mangiferae* is a less serious pest of mangos than previously thought, however mango seed weevil infestation can increase fruit drop during early fruit development [2, 3].

3.1.2. Mango seed weevil irradiation

Although adult emergence occurred at all irradiation doses, emergence was reduced in irradiated fruits (Table 1) [9]. The 300 Gy treatment did not prevent adult emergence, unlike previous studies in Hawaii [10] and Australia [11]. This is probably explained by dose variation in the treatment chamber: the dose uniformity ratio when using the full volume of the chamber at HRI is 1.6 to 1.8, with the dose distribution skewed toward lower doses

[see 8]. Therefore, the dose range of the 300 Gy treatment was estimated to be 180–315 Gy, and many individuals received a radiation dose less than 300 Gy.

Emerging adults from the 100 and 300 Gy treatments were lethargic and short-lived, and no eggs were laid indicating sterility [9]. Egg laying by untreated control weevils was consistent but at a relatively low rate compared with weevils in the study by Seo et al. [10]. This suggests that cage conditions in our studies were not ideal for mating and/or oviposition. Nonetheless, sterility was achieved in this study with an irradiation dose of 100 Gy (estimated dose range, 60–105 Gy), as it was in the study by Seo et al. [10].

Table 1. EFFECT OF IRRADIATION ON ADULT EMERGENCE AND STERILITY IN MANGO SEED WEEVIL

Target dose (Gy)	No. fruits treated	No. adults emerged	No. adults dead in seed	No. eggs laid	No. eggs hatched
0	255	160	9	190	37
50	330	95	23	57	15
100	331	61	15	0	0
300	335	53	49	0	0

Age structure at treatment: 39% early larvae, 19% late larvae, 18% pupae, and 24% adults

3.2. Cryptophlebia irradiation

Using a diagnostic dose of 62.5 Gy, mean adult emergence was 35.6% (range, 29–40%) for irradiated *C. illepida* and 8.2% (range, 4–11%) for irradiated *C. ombrodelta*, which was a highly significant difference ($t = 8.0$; df = 1,4; $P < 0.0002$) [8]. Therefore, *C. illepida* was the focus for detailed tests. Using the criterion of success in developing to the adult stage, the pattern of tolerance to irradiation in *C. illepida* was generally eggs<early instars<late instars<pupae (Tables 2,3, and 4). The most tolerant stage potentially occurring in harvested fruits was late (fourth and fifth) instars (Table 3). Development to adult was reduced slightly in late instars receiving an irradiation dose of 62.5 Gy, whereas development to adult was dramatically reduced in late instars receiving irradiation doses $\geq$125 Gy. No *C. illepida* larvae receiving an irradiation dose $\geq$125 Gy emerged as adults and produced viable eggs, indicating sterility can be achieved at doses well below 250 Gy (Table 3).

In large scale tests, when 11 256 late instars were irradiated with a target dose of 250 Gy, 951 pupated (8.4%) and none eclosed as adults (Table 3). Actual absorbed doses measured by dosimetry ranged from 150–289 Gy (max/min = 1.9) for the first five replicates, and 210–273 Gy (max/min = 1.3) for the last six replicates. Therefore, the irradiation quarantine treatment of a minimum absorbed dose of 250 Gy approved for Hawaii's fruits should effectively disinfest fruits of any *Cryptophlebia* in addition to fruit flies [8].

Table 2. EFFECTS OF IRRADIATION ON MATURATION OF 3-DAY-OLD *Cryptophlebia illepida* EGGS

Target dose hatched	Reps	*n*	No. eggs	No. pupae females	No. adults	No. reprod.	Total eggs	Total neonates (Gy)
0	6	421	257	141	112	23	1245	1052
62.5	6	647	488	190	0	—	—	—
125.0	6	1047	597	24	—	—	—	—
250.0	5	1082	162	0	—	—	—	—
400.0	6	2144	94	0	—	—	—	—

Table 3. EFFECTS OF IRRADIATION ON MATURATION OF Cryptophlebia illepida LARVAE

Instar	Target Dose Gy	Reps	*n*	No. pupae	No. adults	No. reprod. Females	Total eggs	Total neonates
L1	0	5	255	173	143	24	954	762
	62.5	5	459	212	66	1	20	0
	125.0	5	807	36	2	0	—	—
	250.0	5	873	0	—	—	—	—
	400.0	5	1035	0	—		—	—
L2/3	0	4	204	132	106	12	408	328
	62.5	4	291	159	47	0	—	—
	125.0	4	399	191	0	—	—	—
	250.0	4	489	33	0	—	—	—
	400.0	6	483	0	—	—	—	—
L4/5	0	4	146	119	81	18	812	686
	62.5	4	222	168	78	7	117	1
	125.0	4	414	295	6	0	—	—
	250.0	4	654	318	1	0	—	—
	400.0	4	822	290	0	—	—	—
L4/5	Con-firmatory test	5	696	—	59	169	6858	4441
	0 250	5	11 256	951	0	—	—	—

In general, tolerance of pupae to irradiation treatment increased with increasing age (Table 4). No eggs were produced by surviving females when one to two-day old and four to five-day old pupae were treated with target doses of 125 and 250 Gy. In seven to eight-day old pupae, survival to adult after treatment with target doses of 125 and 250 Gy was 67.7% and 52.4%, respectively. In the seven to eight-day old pupae group treated at 125 Gy, 36 females laid 623 eggs and three were fertile, whereas in the 250 Gy treatment only one female laid six eggs that were infertile (Table 4). Results of regression analysis of life stage tolerance to irradiation are reported in Follett and Lower [8].

Table 4. EFFECTS OF IRRADIATION ON MATURATION OF *Cryptophlebia illepida* PUPAE

Age	Target Dose Gy	Reps	*n*	No. adults	No. reprod. Females	Total eggs	Total neonates
1–2	0	6	165	118	18	730	633
day	125	6	525	147	0	—	—
	250	6	805	4	0	—	—
4–5	0	6	150	136	26	841	677
day	125	6	435	64	0	—	—
	250	6	595	16	0	—	—
7–8	0	7	215	187	25	1238	1065
day	125	7	495	335	36	623	3
	250	7	620	325	1	6	0

Irradiation treatment of adult moths affected successful reproduction (Table 5). In the pre-mating irradiation experiment, when both adults were irradiated (IF x IM) at 125 Gy, 0.7% of eggs laid developed to stage 3 (larva visible), and no eggs reached stage 4 (head capsule visible) or hatched. When both parents were treated at 250 Gy, 0.9% of eggs laid developed to stage 2 (pink color) and no eggs developed farther or hatched. When one parent was irradiated and the other left untreated (IF x UM or UF x IM), egg development and hatch was more common in the 125 Gy treatment compared to the 250 Gy treatment, and more common when the male was irradiated compared to when the female was irradiated (Table 5). The mean number of eggs laid per female was not significantly different among mating treatments at 125 Gy or at 250 Gy (data not shown) [8]. At 125 Gy, eggs laid by females in the UF x IM treatment at 125 Gy had a significantly higher mean percent eclosion ($F = 15.8$; df = 2,1; $P < 0.01$) than in the IF x IM and IF x UM treatments. No eggs eclosed from the pairings treated with 250 Gy. The untreated control groups ($n = 2$, total = 49 females) laid 27.4 (1.4) (mean (SE)) eggs and 39.4 (8.8)% eclosed (Table 5).

Table 5. FITNESS OF *Cryptophlebia illepida* WHEN BOTH PARENTS, ONE PARENT, OR NEITHER PARENT RECEIVE(S) IRRADIATION TREATMENT

Dose (Gy)/ Pairing	No. Pairs	No. female with eggs[a]	Total no. of eggs	Egg development Maturity rating						Mean(±SE) % eggs emerged
				0	1	2	3	4	Emerged	
Untreated	50	49	1 343						529	39.4 (8.8)
125 Gy										
IF x IM	36	28	695a	578	65	46	6	0	0	0.0a
UF x IM	45	31	663a	501	19	22	35	73	13	0.42 (0.07)b
IF x UM	42	30	637a	427	66	105	22	16	1	0.03 (0.03)a
250 Gy										
IF x IM	41	24	384a	348	33	3	0	0	0	0.0a
UF x IM	42	35	368a	342	6	7	3	10	0	0.0a
IF x UM	38	32	414a	373	24	9	6	2	0	0.0a

I = irradiated; U = unirradiated; F = female; M = male.

Means within a column for a dose followed by the same letter are not significant using a Student. *t*-test ($P > 0.05$).

[a]Some pairings resulted in no eggs.

In the post-mating irradiation experiment, irradiation of ovipositing females caused immediate sterilization [8]. No fertile eggs were produced by 134 previously mated and ovipositing females that were irradiated with a dose of 250 Gy, whereas 14 untreated control females laid 527 viable eggs.

4. DISCUSSION

4.1 Mango seed weevil

Mango seed weevil is monophagous and requires mango to complete development. Eggs are laid on the surface of young fruit and neonates burrow down to the developing seed; larvae feed within the seed, which becomes encased in a tough fibrous husk, and development to the adult stage occurs entirely in the seed [2, 5] Therefore, mango seed weevil attack usually goes unnoticed and does not reduce fruit marketability.

The mango industry on the U.S. mainland is small and concentrated in southern Florida. Florida's mango industry focuses on growing mango for seed production in nurseries and orchards. The high-risk quarantine pest status given to mango weevil is mainly in response to concerns from the mango industry in Florida that *C. mangiferae* infestation would reduce seed germination and therefore limit seed production in nurseries and orchards [5]. Our studies suggest that infestation of, and damage to, mango seeds by mango seed weevil does not adversely affect seed germination as previously thought [1, 2] Therefore, mango seed weevil is not a high-risk pest in terms of its potential impact on Florida's mango industry. Nevertheless, mango seed weevil is a pest and may cause some crop loss due to premature fruit drop and damage to fruit pulp [3].

Our irradiation data were submitted to USDA-APHIS for a quarantine treatment along with data from field studies showing that mango seed weevil is a less serious pest of mangoes than previously thought. A final rule was published in the Federal Register on 23 October 2002 approving an irradiation dose of 300 Gy to control mango seed weevil in exported mangoes [12]. This is the first time USDA-APHIS has approved an irradiation quarantine treatment for an insect other than a fruit fly. A final rule was published in the Federal Register 5 February 2003 approving irradiation for use against mango seed weevil and fruit flies in mangoes for export from Hawaii to the U.S. mainland [13].

4.2 Cryptophlebia

Using a single diagnostic dose, *C. illepida* was determined to be more tolerant of irradiation than *C. ombrodelta* [8]. Therefore, the efficacy of irradiation treatments against *C. illepida* is probably applicable for *C. ombrodelta* as well. Using the criterion of success in development to the adult stage, the pattern of tolerance to irradiation in *C. illepida* was generally eggs<early instars<late instars<pupae. For the stages likely to be found in harvested fruits (egg and larvae), fourth and fifth instars (L4/5) were determined to be the most tolerant stage of *C. illepida*. No *C. illepida* larvae receiving an irradiation dose $\geq$125 Gy emerged as adults and produced viable eggs, indicating sterility can be achieved at doses well below 250 Gy. Large-scale tests irradiating fourth and fifth instar *C. illepida* at a target dose of 250 Gy resulted in no adult emergence. *C. illepida* typically leaves the fruit to pupate, so pupae would not normally be encountered in export shipments. An unlikely delay in irradiation treatment after harvesting and packaging of fruit might result in the presence of young pupae. Within the pupal stage, tolerance increased with age; seven to eight-day old pupae treated with an irradiation dose of 125 Gy produced viable offspring, whereas those treated with a dose of

250 Gy produced no viable offspring. Likewise, adult *Cryptophlebia* would not be expected in export shipments. Irradiation of unmated adult pairs before mating, or previously mated adult females, with an irradiation dose of 250 Gy resulted in no viable eggs after treatment. Therefore, the irradiation quarantine treatment of a minimum absorbed dose of 250 Gy approved for Hawaii's fruits will effectively disinfest lychee, longan and rambutan fruit of any *Cryptophlebia* in addition to fruit flies.

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GAMMA IRRADIATION AS A PHYTOSANITARY MEASURE FOR EXPORTED SYRIAN FRESH FRUIT

M. MANSOUR, F. MOHAMAD
Syrian Atomic Energy Commission,
Damascus, Syrian Arab Republic

Abstract

To research the effects of gamma radiation on codling moth, *Cydia pomonella* (L.), eggs and larvae were examined. Eggs of various ages were exposed to doses between 10 and 350 Gy, and the number of hatched eggs and emerging adults was recorded. Mature larvae were also exposed to a series of gamma radiation doses ranging from 50 to 250 Gy, and survival to pupae and adult was examined. Results showed that the effect of gamma radiation on codling moth eggs decreased with increasing age and increased with increasing dose. Eggs irradiated a few hours before they hatched were more tolerant than younger eggs; in older eggs 100 Gy had no significant effect on eggs hatched, and over 56% of treated eggs hatched at 350 Gy. However, when adult emergence was used as the criterion for effectiveness, a 60 Gy dose applied to eggs a few hours before hatching prevented adult emergence. Results with treatment of mature larvae showed that diapausing larvae were more sensitive to irradiation than non-diapausing larvae and females were more sensitive than males. A dose of 150 Gy applied to mature larvae reduced adult emergence to 2%, and a dose of 200 Gy completely prevented adult emergence. Tests in which >100 000 mature larvae in diet and >32 000 mature larvae in apples were irradiated at a dose of 200 Gy resulted in no adult emergence. A dose of 200 Gy had no adverse effects on apple quality. Results show that the use of ionizing radiation as a quarantine treatment for codling moth infesting apples is feasible and requires a relatively low dose.

1. INTRODUCTION

The codling moth, *Cydia pomonella* (L), is a key pest of pome and stone fruits throughout most deciduous fruit growing areas of the world. Its larvae infests apples, pears, walnuts, and many other deciduous fruit crops and causes hundreds of million of dollars in losses to the fruit industry every year. This species is also of quarantine importance in Japan, Korea, Taiwan and part of China (Shel'deshova, 1967) and strict quarantine measures are applied to prevent its entry and establishment in these countries. Codling moth overwinters as a diapausing larva in a cocoon which, sometimes, is present within the shell of in-shell walnuts and in fruit containers. It can also be transmitted as eggs or larvae on or in fruits. As a result, quarantine regulations are imposed on fruits exported to codling moth free countries.

Methyl bromide (MB), a chemical fumigant that is highly effective against this pest, has been identified as an important atmospheric ozone-depleting substance and the industry is looking seriously for an alternative (Ross, 1999). Ionizing radiation has been recognized as an alternative to MB for treating fresh agricultural products in order to overcome quarantine barriers in trade. Reviews of this subject have been presented by several authors during the last decade (Burditt, 1994; Nation and Burditt, 1994; Johnson and Marcotte, 1999; Hallman, 1998, 2000, 2001).

Research has demonstrated that irradiation is an effective disinfestation treatment for stored grain as well as fruit pests (Tilton and Burditt 1983). Investigation of several commodities infested with different agricultural pests showed that gamma irradiation can effectively

control pests without detrimental effects to the commodity (Kadar et al., 1984). A minimum dose of 150 Gy is suggested as a quarantine treatment for fresh fruits and vegetables against fruit flies of the family Tephritidae, and a minimum dose of 300 Gy is suggested against other insect species (Anonymous 1986a). Reviews of this subject have been presented by several authors during the last decade (Burditt, 1994; Nation and Burditt, 1994; Johnson and Marcotte, 1999; Hallman, 1998, 2000, 2001).

Studies with codling moth have indicated that gamma radiation could be an effective quarantine treatment against eggs and mature larvae (Burditt and Moffitt 1985, Burditt et al. 1985). Burditt and Moffitt (1985) projected the dose necessary to prevent 99.9968% emergence of adult codling moth for fifth instar larvae; they calculated that a dose of 225 Gy would be required to meet the Probit-9 (99.9968% mortality) security level for treating diapausing larvae in fiberboard strips, and 206 Gy for non-diapausing larvae in fruits. To the contrary, Burditt (1986) found that diapausing codling moth larvae were more susceptible to irradiation than non-diapausing larvae in walnuts, and estimated that a dose of 230 Gy would be required to prevent adult emergence of non-diapausing larvae. Furthermore, Burditt and Hungate (1989) treated 79 540 non-diapausing larvae of different instars with an irradiation dose of 153 Gy in apples and no adult emerged. However, most of these larvae were still in the second, third, and fourth instars, which are more sensitive to irradiation treatment than fifth instars.

Security levels for insect quarantine treatments are based on the Probit-9 (99.9968% mortality) standard proposed by Baker (1939). At this level, not more than 32 insects are accepted to emerge as adults for each one million treated individuals (Couey and Chew 1986). This level of efficiency for a treatment can also be demonstrated by tests involving 30 000 individuals without survivors, and such tests are required by quarantine officials before irradiation can be accepted as a quarantine treatment.

On the other hand, it should be pointed out that, although the chances of transmitting codling moth larvae in apples are there, it is not very possible as any wormy apples will be removed during packaging. The presence of eggs on fruit, however, is difficult to detect and, consequently, transmitting eggs may be more frequent. The effects of gamma radiation on codling moth eggs have been investigated by several researchers (Proverbs and Newton, 1962; Hough, 1963; Hathaway, 1966; Toba and Burditt, 1992). However, most of these studies were done for the purpose of insect control in the field using the sterile insect technique (Proverbs and Newton, 1962; Hathaway, 1966, Hough, 1963) and, as a result, have little value for quarantine purposes. However, Toba and Burditt (1992) showed that exposure of codling moth eggs at the blackhead stage to an irradiation dose of 100 Gy was sufficient to kill all larvae resulting from treated eggs before reaching the adult stage. It should be noted that geographical strains may differ in their sensitivity to ionizing radiation (Hooper, 1989), making it important to examine the radio-sensitivity of widely separated geographical strains, particularly the Syrian strain which is morphologically different from other strains (Talhouk, 1969).

Furthermore, in a commercial irradiation facility, to guarantee that all parts of the irradiated shipment have received the minimum required dose, parts of the shipment may receive a dose two or even three times higher (Hallman, 1998, 2000). Therefore, studying effects of gamma radiation on the treated commodity is needed before a dose can be recommended as a quarantine treatment against a particular pest on the commodity. Apple fruit were shown to tolerate low levels of gamma radiation, and radiation tolerance varied with cultivar, maturity, and conditions under which the crop was grown (Olsen et al., 1988). Drake et al. (1998) and

Al-Bachir (1999) showed that exposing apple fruit of different varieties to doses of gamma irradiation up to 1000 Gy (1 kGy) had no unacceptable effects on fruit quality.

Reviewing the previous data indicates that more research is needed before a certain dose can be adopted for quarantine security of the codling moth. The objective of this study was to examine the effects of gamma radiation on codling moth (Syrian strain) eggs and mature larvae, and to determine the quarantine dose for this pest. In addition, the effects of the quarantine irradiation dose on the treated commodity was investigated. The specific objectives were to:

1. Study the effects of gamma radiation on codling moth (Syrian strain) eggs and mature larvae.
2. Determine the dose which prevents eggs and mature larvae from developing to adults.
3. Determine the dose necessary to prevent adult emergence from mature larvae reared on an artificial medium.
4. Establish the quarantine dose using large-scale confirmatory tests with infested fruits that provides a probit 9 (99.9968% mortality) level of security (tests that include no less than 30 000 mature codling moth larvae).
5. Determine the quality of apples exposed to the minimum irradiation dose required to provide quarantine security.

2. MATERIALS AND METHODS

2.1. Effects of gamma radiation on codling moth eggs

2.1.1. Eggs

Eggs used in these experiments were obtained from a codling moth colony that has been reared for over 35 generations on an artificial rearing medium similar to that reported by Brinton et al. (1969). The colony originated from moths collected at several locations near the city of Damascus in the summer of 1994. Codling moth males from the local wild populations in the same area were periodically introduced into the colony every summer thereafter. Rearing conditions were maintained at 26 ± 2°C, 50 ± 10% RH, and 16:8 (L:D) photoperiod. Under these conditions, development of the egg stage requires a little over five days, the larval stage takes about one month, and pupae emerge in about a week.

2.1.2. Irradiation

Eggs were exposed to gamma radiation doses from a Co-60 source (Issledova Gamma Irradiator, Techsnabexport Co. Ltd. USR). The average dose rate at the time of irradiation was approximately 44.24 Gy/minute. Rectangular sheets of wax paper (15 x 12 cm) carrying codling moth eggs (300–400) ranging in age from one to 24 h to 97-120 h, at 24 h intervals, were exposed to gamma radiation. Radiation doses ranged, according to the age of the eggs, between 10 and 350 Gy. The whole experiment was repeated four times.

2.1.3. Effect of gamma radiation on egg hatch

After irradiation, eggs were returned immediately to the laboratory, incubated at 26 ± 2 ºC until they reached the blackhead stage (120 h old), and placed on an artificial rearing medium in plastic trays (18 x 15 x 6 cm) keeping the side carrying the eggs in contact with the diet. The plastic trays, each containing about 800 g of the rearing medium, were also incubated under the same conditions. The wax paper was removed four days later and examined under a

binocular microscope. The number of hatched eggs was recorded and percentage egg hatch was calculated.

2.1.4. Effect of gamma radiation on larval survival

Plastic trays containing codling moth larvae from the egg hatch studies, particularly those from eggs irradiated at the blackhead stage, were covered with muslin cloth to prevent emerging adults from escaping and incubated at 26 ± 2º C. After eight weeks of incubation, the trays were opened, the number of emerging adults in each tray was recorded and percentage larval survival to the adult stage was calculated by dividing the number of emerged moths by the number of hatched eggs.

2.2. Effects of gamma radiation on codling moth mature larvae

2.2.1. Insects

Insects used in this research were obtained from the same colony of *C. pomonella* mentioned above. Larvae were reared in plastic trays (18 x 15 x 6 cm), each containing about 800 g of the larval rearing medium. Pupation occurs at the top of the diet in this rearing system. Non-diapausing larvae were reared at 26 ± 2º C, 40–60% RH and 16:8 (L:D) cycle. Under these conditions, development of the larval stage required about one month in our laboratory and rearing on apples takes about the same time. Diapausing larvae, on the other hand, were reared at 24 ± 2º C and 8:16 (L:D) cycle. Corrugated cardboard strips (10 cm long and 1.5 cm wide) were used to collect mature larvae leaving the rearing medium for pupation. After irradiation, all rearing was done at room temperature.

2.2.2. Irradiation

Two Co-60 Gamma irradiation sources were used in this study. The mean dose rate of the first source was approximately 13.5 Gy/minute whereas the dose rate of the second one was approximately 71.0 Gy/minute. Two kinds of tests (small-scale laboratory tests and large-scale confirmatory ones) were conducted. Experiments on the effects of gamma radiation on larval survival in the small-scale laboratory tests were done using the first source. Tests on the effects of gamma radiation on larvae in the large-scale confirmatory tests, however, were done using the second source.

2.2.3. Small scale laboratory tests

2.2.3.1. Irradiating larvae in cardboard strips

Batches of 50 mature larvae were irradiated in plastic vials (5 x 7 cm). A series of gamma radiation doses between 50 and 250 Gy with 50 Gy increment was used and a group of un-irradiated larvae was held as a control. Each treatment was replicated four times. Directly after irradiation, larvae were returned to the laboratory in order to continue their development to the adult stage. The cardboard strips were held for over two months to ensure that the emergence was complete and the number of formed pupae, emerged adults and their sex was recorded.

2.2.3.2. Irradiating larvae in host fruits

‘Golden Delicious’ apples (average weight approximately 200 g) were washed in water, air dried and placed in cardboard boxes. The fruit were infested with codling moth larvae by covering them for about 16 hours with sheets of waxy paper carrying codling moth eggs in the

blackhead stage. Infested fruit was incubated at 26 ± 2º C and examined periodically to remove decaying fruit. Fruit was irradiated four weeks later (late fifth instar). Before irradiation, fruit was divided into five groups of 35 apples each and each group was exposed to a certain dose of gamma radiation. A series of gamma radiation doses between 100 and 250 Gy with 50 Gy increments was applied, and each dose was replicated four times. Irradiated fruit was placed in paper bags containing cardboard strips to collect mature larvae leaving the fruit for pupation. For two weeks, cardboard strips were removed every three to five days and replaced with new ones. Collected larvae were held for over a month and the number of pupae and emerged adults were recorded.

2.2.4. Large-scale confirmatory tests

In order to demonstrate Probit-9 mortality at the 95% confidence level, subsequent large-scale confirmatory tests were carried out. To synchronize larval development for the tests, egg collections were made over 12-16 hours period and egg sheets carrying eggs in the blackhead stage were left on the rearing media for about the same time. The following two experiments were conducted:

2.2.4.1. Irradiating larvae in the larval rearing medium

Plastic trays (18 x 15 x 6 cm) were seeded with codling moth eggs at a rate of about 600 eggs/tray and incubated at 26 ± 2º C for four weeks. At this stage of development (late fifth instar), batches of larvae in the rearing medium were exposed to a dose of 200 Gy on eight different dates. Five trays from each batch were held as controls. Immediately after irradiation, trays were returned to the laboratory and placed in cardboard boxes sealed from the top with clear plastic sheets. For over a month, the boxes were checked every two to three days for any emerging moths resting on the plastic cover, and the trays were examined carefully at the end of the experiment for the presence of any exuviae. The number of treated larvae/replicate was estimated from the number of emerging adults in the controls.

2.2.4.2. Irradiating larvae in apples

Apples (average weight 100 g) were washed in water, air dried and placed in cardboard boxes (45 x 35 x 30 cm). The fruit were infested with codling moth larvae by placing strips of waxy paper carrying codling moth eggs in the blackhead stage among fruit for about 20 hrs. Infested fruit were stored at room temperature (26 ± 3º C) and examined periodically to remove decaying fruits. After four weeks of storage, the fruit were exposed to a dose of 200 Gy; 1,000 fruit were held as a controls. Irradiated fruit were returned to the rearing room and cardboard strips were distributed among them for collecting mature larvae leaving the fruit to pupate. For two weeks, cardboard strips were collected every four to six days and replaced with new ones. Collected cardboard strips were held for over a month and the number of emerging adults was recorded. The total number of treated larvae was estimated from the number of collected larvae in the control.

2.3. Effects of gamma radiation on apple fruit from the local varieties

2.3.1. Apple fruit

Apples used in this experiment were obtained from Orneh (1450 m), southwest of Damascus, in the southern part of the country. ‘Starking’ apples approaching physiological maturity (60–70 mm in diameter) were obtained from the orchards immediately after harvest. Fruit was carefully placed in plastic boxes (40 x 30x 20 cm) and transported to the irradiation facility on the same day.

2.3.2. Irradiation

Irradiation was done in a commercial Co-60 Gamma irradiation facility located in Der Al-Hajer, near the city of Damascus. The mean dose rate of the source, at the time of irradiation, was approximately 78.6 Gy/minute. Fruit packed in boxes were exposed to gamma radiation doses between 100 and 400 Gy at 100 Gy intervals, and a group of boxes was held as a controls. Two boxes were irradiated at each dose level and the whole experiment was replicated four times. Apple boxes were transported immediately after irradiation to a cold storage facility in Deemass, about 25 km northwest of Damascus. Fruit was stored for six months at 1 ± 1° C and 90 ± 5 % RH. Effects of irradiation on weight loss, fruit texture, pH of fruit juice, fruit taste, color and visible injury were examined. Apples in one of the boxes in each treatment/replicate combination were assigned for weight loss determination, colour change and visible injury studies, and fruit in the second box were used to perform the other tests (fruit firmness, pH of fruit juice and taste).

2.3.3. Effects on weight loss

Effects of gamma irradiation on weight loss were examined every 45 days of cold storage. Boxes assigned for this study were weighed and labelled appropriately before irradiation. Boxes were weighed individually using an electric balance and the total weight was recorded. Weight loss was calculated by subtracting the current weight from the previous one and the average weight loss for the four replicates in each treatment was calculated. Fruit in each box was sorted out each time, and any decaying ones were weighed and removed, and the appropriate weight adjustment for the box was made.

2.3.4. Effects on fruit firmness

Fruit texture was measured using a fruit firmness apparatus (Instron 1011) equipped with a strip chart recorder. This was done immediately after irradiation and repeated every 45 days for the duration of the experiment. Each time, the firmness of four to eight randomly selected apples (one or two from each replicate) was measured and averaged.

2.3.5. Effects on pH value of fruit juice

The pH value of fruit juice was measured immediately after irradiation and repeated at 45-day intervals using a pH meter (Hanna Instruments HI 8521). Each time, three randomly selected apples were taken from each replicate, the cortex was peeled off, and the remainder of the fruit was ground in an electric mixer. The pH of the juice was immediately measured and average of the four replicates was calculated.

2.3.6. Effects on fruit taste

Fruit taste was examined by a sensory panel consisted of ten laboratory technicians. Tests were done 135 days after irradiation exposure and repeated 45 days later. Four apples from each treatment (one from each replicate) were cut out into pieces and offered to panel members for tasting. As panel members were unable to distinguish between irradiated and un-irradiated apples, they were asked to evaluate the taste of the fruit on a scale from one (worst) to five (best). All experiments were blind, i.e., panel members did not know treatment levels of apples examined.

2.3.7. Effects on color change and visible injury

Apples were evaluated at 45-day intervals for external appearance and skin injury. The number of injured fruit was recorded and colour changes were visually evaluated.

2.4. Data analysis

Data from these experiments were subjected to analysis of variance. Means were separated by Fisher's protected LSD test.

3. RESULTS

3.1. Results on the effects of gamma radiation on codling moth eggs

Data on the effects of gamma radiation on egg hatch are presented in Tables 1-5. The results indicate that the radio-sensitivity decreased with increasing age of eggs. For instance, Table 1 shows that an irradiation dose of 20 Gy significantly affected egg hatch ($P < 0.05$) in one to 24 h old eggs, and a dose of 60 Gy reduced egg hatch to about 1%. When 25-48 h old eggs were irradiated (Table 2), however, a dose of 20 Gy had no significant effect on egg hatch ($P < 0.05$) and egg hatch at a dose of 60 Gy was approximately 10%. Sensitivity of 49-72 h old eggs was even lower (Table 3); egg hatch was not significantly affected at doses < 80 Gy ($P > 0.05$), and at a dose of 140 Gy egg hatch was about 4%. This percentage increased to about 49% when 73-96 h old eggs were irradiated at 140 Gy (Table 4). Sensitivity was lowest when eggs were irradiated at the blackhead stage (97-120 h old); egg hatch was about 57% at an irradiation dose of 350 Gy (Table 5).

Table 1. EFFECTS OF GAMMA RADIATION ON ONE TO 24H CODLING MOTH EGGS*

Dose (Gy)	No. of Treated eggs	% egg hatch ± SD
0	1542	92.7 ± 2.5[a]
10	1369	93.1 ± 2.7[a]
20	1386	72.8 ± 6.4 [b]
30	1450	14.4 ± 2.7[b]
40	1439	10.4 ± 3.1[b]
50	1279	2.1 ± 0.49[c]
60	1283	1.3 ± 0.42[c]

*Means followed by the same letter within a column are not significantly different ($P > 0.05$, Fisher's LSD test).

Table 2. EFFECTS OF GAMMA RADIATION ON 25-48 H OLD CODLING MOTH EGGS*

Dose (Gy)	No. of Treated eggs	% egg hatch ± SD
0	1328	94.5 ± 2.8[a]
10	1402	92.2 ± 2.4[a]
20	1501	92.5 ± 2.2[a]
30	1519	62.2 ± 4.9[b]
40	1574	22.6 ± 5.6[c]
50	1447	16.4 ± 2.6[d]
60	1339	10.3 ± 2.1[e]

*Means followed by the same letter within a column are not significantly different (P>0.05, Fisher's LSD test).

Table 3. EFFECTS OF GAMMA RADIATION ON 49-72 H OLD CODLING MOTH EGGS*

Dose (Gy)	No. of Treated eggs	% egg hatch ± SD
0	1432	93.4 ± 1.9[a]
20	1387	92.3 ± 2.8[a]
40	1612	89.4 ± 2.4[a]
60	1402	91.3 ± 4.9[a]
80	1486	78.6 ± 8.0[b]
100	1338	48.3 ± 11.9[c]
140	1459	4.0 ± 3.4[d]

*Means followed by the same letter within a column are not significantly different (P>0.05, Fisher's LSD test).

Table 4. EFFECTS OF GAMMA RADIATION ON 73-96 H OLD CODLING MOTH EGGS*

Dose (Gy)	No. of Treated eggs	% egg hatch ± SD
0	1561	91.2 ± 6.4[a]
40	1419	87.6 ± 6.3[a]
80	1483	89.5 ± 3.2[a]
120	1501	78.8 ± 3.3[b]
140	1594	48.9 ± 4.2[c]

*Means followed by the same letter within a column are not significantly different (P>0.05, Fisher's LSD test).

Table 5 also presents data on the effects of gamma radiation on larval survival when eggs in the blackhead stage (97-120 h) were exposed to doses between 40 and 350 Gy. Results show that a dose of 40 Gy significantly reduced larvae survival to the adult stage ($P < 0.05$), and a dose of 60 Gy applied to blackhead stage eggs prevented development to the adult stage. Results from examining the diet showed that all larvae exposed as eggs to a dose of 100 Gy died before pupation.

Table 5. EFFECTS OF GAMMA RADIATION ON EGG HATCH AND LARVAL SURVIVAL IN CODLING MOTH EGGS IRRADIATED IN THE BLACK HEAD STAGE (97-120 H OLD)*

Dose (Gy)	No. of Treated eggs	% egg hatch ± SD	% survival to adults ± SD
0	1393	89.8 ± 2.0^{a}	57.4 ± 1.5^{a}
40	1457	90.9 ± 2.6^{a}	22.1 ± 2.4^{b}
60	1398	91.4 ± 2.9^{a}	0
80	1542	90.1 ± 1.7^{a}	0
100	1461	89.2 ± 2.5^{a}	0
150	1588	72.1 ± 6.3^{b}	0
200	1429	68.0 ± 4.2^{c}	0
250	1507	66.7 ± 4.5^{c}	0
300	1416	66.3 ± 3.9^{c}	0
350	1505	56.8 ± 4.7^{d}	0

*Means followed by the same letter within a column are not significantly different ($P > 0.05$, Fisher's LSD test).

3.2. Results on the effects of gamma radiation on mature coding moth larvae

Results of gamma radiation tests using mature codling moth larvae in cardboard strips are presented in Table 6. The data show a decrease in rates of pupation and adult emergence with increasing radiation dose. At an irradiation dose of 250 Gy, the percentage of pupating larvae decreased from about 90% in the control to 9.5% and 20.5% for diapausing and non-diapausing larvae, respectively. However, when adult emergence was used as a criterion for measuring survival, the effect of gamma radiation was extremely severe. A dose of 150 Gy reduced adult emergence significantly in non-diapausing larvae ($P < 0.001$; df = 5, 3; $F = 942.9$) and 200 Gy completely prevented it. Table 6 also shows that diapausing larvae were more susceptible to irradiation treatment than the non-diapausing larvae. While no adults emerged from diapausing larvae exposed to dose of 150 Gy, 1.5% of the non-diapausing larvae exposed to the same dose completed development to the adult stage. Sexual differences in radio-sensitivity were also noticeable (Fig. 1). Examination of adults that resulted from irradiating non-diapausing larvae showed that larvae destined to become female moths were significantly more susceptible to irradiation injury than males ($P < 0.001$; df = 5, 3; $F = 85.8$). For instance, 64.5% of adults that developed from larvae exposed to 50 Gy dose were males

compared to 48% for adults developed from untreated larvae, and none of the moths developed from larvae exposed to 150 Gy were females. Examination of pupae showed that, at this dose level, female larvae were unable to develop into the adult stage. Female diapausing larvae were even more sensitive to irradiation treatment than non-diapausing larvae (Fig. 1). In diapausing larvae a dose of 50 Gy significantly reduced the number of females ($P < 0.001$; $df = 5, 3$; $F = 346.2$) and 100 Gy completely prevented it.

Table 6. EFFECTS OF GAMMA RADIATION ON MATURE DIAPAUSING AND NON-DIAPAUSING CODLING MOTH LARVAE*

Dose (Gy)	%			
	Diapausing		Non-diapausing	
	Pupation	Emergence	Pupation	Emergence
0	$87.0^{a} \pm 5.3$	$79.5^{a} \pm 3.4$	$94.5^{a} \pm 6.2$	$87.5^{a} \pm 2.4$
50	$77.5^{b} \pm 4.3$	$49.5^{b} \pm 4.7$	$85.0^{b} \pm 4.2$	$65.0^{b} \pm 4.2$
100	$64.5^{c} \pm 3.4$	$09.5^{c} \pm 1.9$	$82.5^{b} \pm 6.2$	$19.3^{c} \pm 2.8$
150	$49.5^{d} \pm 4.4$	$00.0^{d} \pm 0.0$	$69.0^{c} \pm 8.1$	$01.5^{d} \pm 1.9$
200	$11.0^{e} \pm 4.8$	$00.0^{d} \pm 0.0$	$30.5^{d} \pm 7.2$	$00.0^{d} \pm 0.0$
250	$09.5^{e} \pm 3.0$	$00.0^{d} \pm 0.0$	$20.5^{e} \pm 4.4$	$00.0^{d} \pm 0.0$

*Means followed by the same letter within a column are not significantly different ($P > 0.05$; Fisher's LSD test).

When larvae were irradiated inside natural host fruit rather than rearing medium in the small-scale laboratory experiment (Table 7) the results were similar. From an estimated 420 larvae exposed to an irradiation dose of 200 Gy, only 41.6% pupated (compared to 96.8% of the larvae in the control) and none reached the adult stage.

Results of the large-scale confirmatory tests using rearing medium showed that a irradiation dose of 200 Gy completely prevented adult emergence in 460 trays containing an estimated total number >100 740 fifth instar codling moth larvae while the average number of emerging adults in control trays was over 219 moths/tray. Examination of the diet showed that most of the insects died before reaching the pupal stage. Some larvae, however, were able to develop to the late pupal stage, and few pupae contained fully developed adults. (However, these adults were not able to emerge from the pupal case). As the results of irradiating larvae in natural host fruit may differ from irradiating them in the larval rearing medium because of differences in water content and oxygen level, a large-scale confirmatory test with infested fruit was conducted. The results of this test were similar to irradiating larvae in the artificial rearing medium. None of an estimated >32 193 larvae exposed to a dose of 200 Gy in apples reached the adult stage, while 96.3% of the larvae in the controls developed into adults.

3.3. Results on the effects of gamma radiation on local apple varieties

Data on the effects of gamma irradiation on weight loss during six months of cold storage are presented in Table 8. Statistical analysis indicates that irradiation doses up to 300 Gy had no significant effect on weight loss ($P > 0.05$). The effect of 400 Gy dose, however, was significant ($P < 0.05$) at 45 days of storage but not on the other dates.

Table 7. EFFECTS OF GAMMA RADIATION ON THE FIFTH INSTAR NON-DIAPAUSING CODLING MOTH LARVAE IRRADIATED IN APPLES*

Dose (Gy)	%	
	Pupation	**Emergence**
0	$96.8^{a} \pm 4.8$	$90.0^{a} \pm 7.1$
100	$79.9^{b} \pm 10.4$	$18.7^{b} \pm 7.8$
150	$67.8^{c} \pm 4.7$	$04.3^{c} \pm 4.5$
200	$41.6^{d} \pm 9.9$	$00.0^{d} \pm 0.0$
250	$37.4^{e} \pm 3.4$	$00.0^{d} \pm 0.0$

*Means followed by the same letter within a column are not significantly different (P>0.05; Fisher's LSD test).

Table 8. EFFECTS OF GAMMA IRRADIATION ON WEIGHT LOSS IN STARKING APPLES*

Dose (Gy)	% weight loss with time (days)			
	45	90	135	180
0	2.83 ± 0.27^{a}	2.83 ± 0.27^{a}	1.06 ± 0.18^{a}	1.59 ± 0.44^{a}
100	3.08 ± 0.51^{a}	0.69 ± 0.30^{a}	0.87 ± 0.39^{a}	1.26 ± 0.37^{a}
200	2.96 ± 0.53^{a}	0.86 ± 0.34^{a}	0.96 ± 0.45^{a}	1.39 ± 0.30^{a}
300	2.98 ± 0.31^{a}	0.81 ± 0.13^{a}	0.94 ± 0.28^{a}	1.46 ± 0.53^{a}
400	3.95 ± 0.97^{b}	0.74 ± 0.35^{a}	1.17 ± 0.35^{a}	1.29 ± 0.42^{a}

*Means followed by the same letter are not significantly different (P>0.05, Fisher's LSD test).

Table 9 shows results on the effects of gamma radiation on fruit texture. The data show that increasing radiation dose caused a significant decrease in fruit firmness at doses higher than 200 Gy (P<0.05) This effect, however, was only significant after 45 days of storage (P<0.05).

The effects of increasing dosages of gamma radiation on pH value of fruit juice are presented in Table 10. The data indicate that gamma irradiation significantly decreased the pH value of the fruit juice (P<0.001) when measured immediately after irradiation, particularly at the 400 Gy dose. This effect, however, was not clear when the pH measurements were made during storage. In fact, the pH value increased slightly during storage, particularly at the 400 Gy dose, though the effect was not significant.

Table 9. EFFECTS OF GAMMA IRRADIATION ON 'STARKING' APPLE FRUIT FIRMNESS*

Dose (Gy)	Fruit Firmness (Newton/cm^2) after gamma irradiation				
	Day 1	Day 45	Day 90	Day 135	Day 180
0	99.0±09.8^a	64.4±06.6^a	53.0±8.7^a	47.0±5.8^a	46.4±4.4^a
100	95.0±13.1^a	61.3±10.6^a	53.1±6.1^a	45.4±7.8^a	44.5±4.9^{ab}
200	94.3±33.9^a	62.3±10.6^a	51.3±8.8^{ab}	46.6±6.3^{ab}	41.5±6.9^{abc}
300	94.5±17.6^a	59.8±08.2^a	44.5±6.3^b	43.7±4.0^b	38.5±5.7^{bc}
400	91.3±11.7^a	50.6±07.0^a	44.8±3.9^b	41.3±4.3^b	36.3±5.8^c

*Means followed by the same letter are not significantly different (P>0.05, Fisher's LSD test).

Table 10. EFFECTS OF GAMMA IRRADIATION ON PH OF FRUIT JUICE IN STARKING APPLES*

Dose (Gy)	pH value of fruit juice at different storage periods (days)				
	1	45	90	135	180
0	3.70 ± 0.2^a	4.07 ± 0.2^a	4.17 ± 0.1^a	4.10 ± 0.2^a	4.19 ± 0.2^a
100	3.67 ± 0.1^a	4.07 ± 0.1^a	4.19 ± 0.1^{ab}	4.13 ± 0.4^a	4.07 ± 0.3^a
200	3.73 ± 0.1^a	4.12 ± 0.1^a	4.17 ± 0.2^{ab}	4.10 ± 0.1^a	4.10 ± 0.3^a
300	3.69 ± 0.2^a	4.09 ± 0.2^a	4.10 ± 0.2^b	4.19 ± 0.3^a	4.00 ± 0.4^a
400	3.05 ± 0.1^b	4.24 ± 0.2^b	4.35 ± 0.1^{ab}	4.35 ± 0.4^a	4.33 ± 0.5^a

*Means followed by the same letter are not significantly different (P>0.05, Fisher's LSD test).

Data on the effects of gamma irradiation on fruit taste are reported in Table 11. The data show that radiation dosages up to 400 Gy had no detectable effect on fruit taste after 135 days of cold storage. The taste of apples exposed to 400 Gy dose, however, was significantly better after 180 days of storage compared with controls.

Effects of gamma irradiation on skin colour and external injury showed that radiation caused a slight, but inconsistent, change in apple skin colour. The difference between the irradiated fruit and un-irradiated fruit, however, was not sufficiently pronounced to permit easy distinction between the two.

Table 11. EFFECTS OF GAMMA IRRADIATION ON FRUIT TASTE IN STARKING APPLES*

Dose (Gy)	Fruit taste at different storage periods (days)	
	135	180
0	4.65 ± 0.34[a]	4.00 ± 0.33[a]
100	4.75 ± 0.26[a]	4.30 ± 0.48[ab]
200	4.55 ± 0.37[a]	4.05 ± 0.37[ab]
300	4.40 ± 0.64[a]	4.10 ± 0.32[ab]
400	4.45 ± 0.43[a]	4.35 ± 0.47[b]

*Means followed by the same letter are not significantly different (P>0.05, Fisher's LSD test).
Taste scale = 1 (worst) to 5 (best).

4. DISCUSSION

The idea of using gamma radiation as a quarantine treatment for agricultural products is more than 70 years old (Koidsumi, 1930), and a considerable amount of research has been done in this area in the last 50 years. The practical applications, however, were delayed for years due to scientific and technical reasons (Hallman, 2001). Developments in the last decade, particularly those related to the phasing out of MB (Anonymous, 1998), revived interest in this technique, and this method has been recognized as an acceptable alternative to chemical fumigation (Burditt, 1994).

In this report, the effects of gamma radiation on egg hatch and adult emergence from irradiated codling moth eggs and mature larvae from the Syrian strain were examined. In addition, the effect of the quarantine dose on the quality of commercial apple varieties in Syria was examined.

The results of this study show that the radio-sensitivity of codling moth eggs to gamma radiation decreased with increasing age and increased with increasing radiation dose. A 60 Gy dose reduced egg hatch in one to 24 h old eggs to about 1%, where the same dose had no significant effect on 49–72 h old eggs. Similarly, when 49-72 h old eggs were exposed to a 40 Gy dose, egg hatch was reduced to about 4%. However, when 97–120 h old eggs were irradiated with a lot higher dose (350 Gy), egg hatch was over 56%. These results are in general agreement with data reported for a wide range of insect species (Tilton and Brower, 1983), and in particular with data reported for the codling moth (Proverbs and Newton, 1962; Hathaway, 1966; Toba and Burditt, 1992). However, it is different from that reported by Hough (1963) which showed that a dose of 151 Gy did not stop egg hatch in one day old codling moth eggs. The results also show that the Syrian codling moth strain is similar to, or even more sensitive than, the American one to gamma radiation. Our results showed that

exposing eggs in the blackhead stage to a dose of 150 Gy caused significant effect on egg hatch, while the same effect appeared at 200 Gy dose for the American strain (Toba and Burditt, 1992). It should be noted, however, that the dose rate in this study was higher and, consequently, may have been the cause of this increased sensitivity.

These results indicate that preventing codling moth egg hatch, particularly in the blackhead stage, requires relatively high dose (>350 Gy). This dose may be harmful to fruits (Drake et. al. 1998), particularly when applied commercially where the dose uniformity ratio could be higher than 3:1. However, when survival to the adult stage was used as a criteria for effectiveness (Hallman, 2001), the results were very promising. A dose of 60 Gy applied to codling moth eggs a few hours before hatch (blackhead stage) completely prevented any resulting larvae from reaching the adult stage, and at 100 Gy none of the larvae pupated.

Results on the effects of gamma radiation on mature larvae show that increasing radiation dose caused consistent decrease in pupation and adult emergence, and a dose of 200 Gy prevented adult emergence entirely. Results also showed that diapausing larvae were more susceptible to irradiation treatment than non-diapausing larvae which is consistent with results reported in Burditt (1986) and Toba and Moffitt (1996). However, they contradict with data reported by Burditt and Moffitt (1985) which showed that non-diapausing larvae were more sensitive to irradiation treatment than diapausing larvae. Our results also showed that females were more radio-sensitive than males which appears to be generally the case in insects (Hallman, 2000). The results also showed that when lack of pupation was used as a criterion for measuring effectiveness of irradiation treatment, a relatively high dose of gamma radiation (>250 Gy) was required. This dose could be injurious to treated fruits (Drake et al., 1998) when applied on a commercial scale. However, when adult emergence was used as the criterion for effectiveness, the results were more promising. A dose of 200 Gy applied to the fifth instars completely prevented adult emergence and reduced the pupation rate to about 30%. Consequently, the lack of adult emergence should be used as a criteria for effectiveness. Exposure of >100 000 fifth instars in their larval rearing medium to a dose of 200 Gy and >32 000 larvae in apples did not result in a single adult. Therefore, it seems that a dose of 200 Gy applied to the fifth instar codling moth larvae will be adequate for quarantine security of apples against this pest.

Our results on the effects of gamma radiation on the quality of cold stored apple fruit from the southern part of Syria show that the primary effects of irradiation were on weight loss, acidity and firmness. The slight decrease in weight loss, particularly after 45 days of irradiation, could be attributed to the stimulatory effect of irradiation on some metabolic process such as respiration rate and enzyme activity. Massey et al. (1964) found that irradiation stimulated oxygen consumption and respiration rate in several varieties of apples right after exposure to gamma irradiation. Chachin and Ogata (1976) also reported that the respiration rate of apples increased within the first two days of irradiation, then decreased gradually to the level that was maintained before irradiation. Similar results were also reported by Al-Bachir and Sass (1989) and Al-Bachir (1999).

Gamma radiation dosages higher than 200 Gy decreased the firmness of apple fruit 90 days after irradiation with the largest effect at the highest dose. These results are in general agreement with data reported by several other authors previously (Massey et al., 1964; Boyle et al., 1957; Clark, 1968). The decrease in tissue firmness after irradiation may be due to the decrease in protopectin, total pectin, and/or to a change from insoluble pectic materials to soluble forms. Kertesz et al. (1964) found that the dose which caused degradation of cellulose and pectin also caused softening of the tissues. Al-Bachir (1986) also found that the activity

of pectin methyl esterase (PME) increased in 'Jonathan' apples immediately after irradiation with doses ranging from 500–1500 Gy.

The increase in fruit acidity or decrease in pH value of fruit juice when measured immediately after irradiation, particularly at the 400 Gy dose, may be due to increase in some organic acids such as citric and succinic acids. Fernandez and Clark (1962) found that citric acid increased in apple fruit exposed to 1000 Gy of gamma irradiation and Hulme (1962) found that succininc acid increased in irradiated apples. The slight increase in pH value at higher doses (300–400 Gy), particularly at 45 and 90 days of storage, however, may have been caused by an increase in the metabolic activity of irradiated fruit and, consequently, breakdown in some acids. Saito and Igarashi (1971, 1972) and Al-Bachir (1986) found that irradiation accelerated the loss of malic acid during storage of apples.

In summary, this study indicates that the use of ionizing radiation as a quarantine treatment for codling moth infested fruit is feasible and requires a relatively low dose. In irradiating fruit to control codling moth, prevention of moth emergence should be used as a criterion for effectiveness, and the doses providing quarantine security cause no unacceptable adverse effects on fruit quality.

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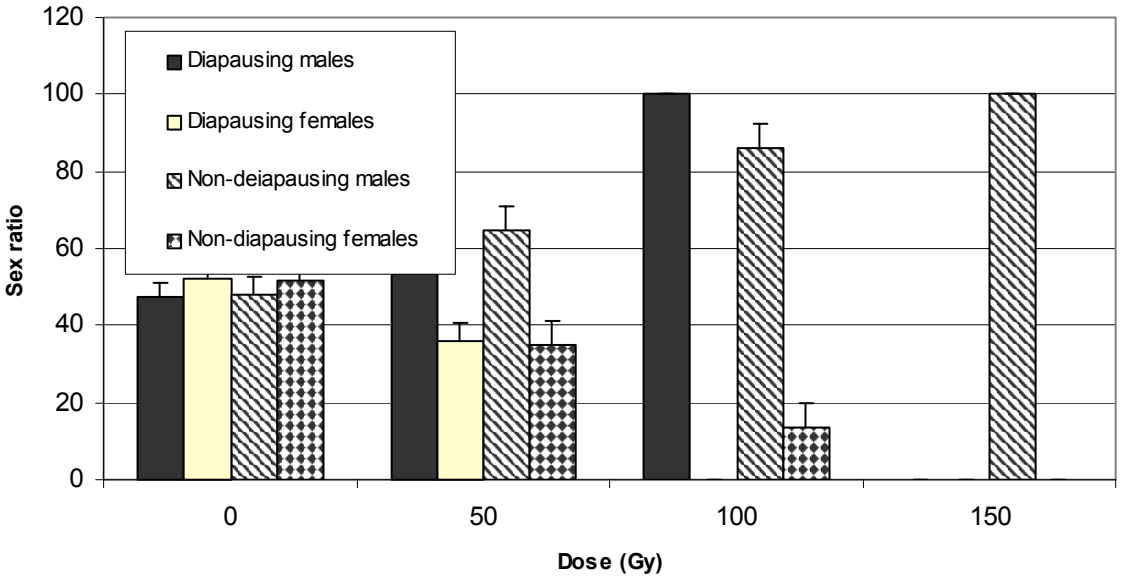
Fig.1. Effects of gamma radiation on sex ratio in mature diapausing and non-diapausing codling moth larvae



IRRADIATION QUARANTINE TREATMENT RESEARCH AGAINST ARTHROPODS OTHER THAN FRUIT FLIES

G.J. HALLMAN
USDA-ARS,
Weslaco, United States of America

Abstract

Irradiation was studied as a treatment to stop development or reproduction of several insects. Reproduction of 25 000 adult plum curculios was stopped with 92 Gy. Reproduction of over 30 000 adult sweetpotato weevils was prevented with 165 Gy. Adult development of over 30 000 last instar oriental fruit moths was stopped with 200 Gy. Preliminary studies showed that at least 350 Gy was needed to prevent reproduction of late pupae of sugarcane borer, southwestern corn borer, and Mexican rice borer. Over 400 Gy was required to stop egg hatch and development past first instar from irradiated adult Indianmeal moth. No reproduction occurred from 220 adult female West Indian sugarcane root borers irradiated with 50 Gy.

1. INTRODUCTION

This report covers irradiation disinfestation research done with arthropods other than fruit flies since this CRP began five years ago. The organisms studied are plum curculio (*Conotrachelus nenuphar* [Herbst]), sweetpotato weevil (*Cylas formicarius elegantulus* [Summers]), oriental fruit moth (*Grapholita molesta* [Busck]), sugarcane borer (*Diatraea saccharalis* [F.]), southwestern corn borer (*D. grandiosella* Dyar), Mexican rice borer (*Eoreuma loftini* [Dyar]), and Indianmeal moth (*Plodia interpunctella* [Hubner]). Cooperative work was done with Walter Gould, then of USDA-ARS-Miami, Florida, on West Indian sugarcane root borer (*Diaprepes abbreviatus*).

Plum Curculio (Coleoptera: Curculionidae)

Plum curculio is native to North America east of the Rocky Mountains and has not become established elsewhere save for an isolated infestation in Utah. Much of the rest of the world quarantines against fruit hosts of plum curculio (pomes and stone fruits) grown in the eastern United States and Canada.

2. METHODOLOGY

Southern strain plum curculios were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service facility in Byron, Georgia. They had originally been collected in the field near Gainesville, Florida. The insects were reared at about 25^{o}C, 70% RH, on immature apples that were picked when about three cm in diameter. Larvae emerging from the apples were placed on sterilized potting soil until adult emergence. Northern strain plum curculios were collected as larvae in apples from the field in Massachusetts and sent to Weslaco as adults.

Immature apples ('Red Delicious', mean diameter 3 cm) that were picked in Washington State in June were infested with southern strain plum curculio by allowing 90–111 adults to oviposit on them in cages for two days (eggs) or five days (larvae). At 26^{o} C, the earliest late fourth instars began emerging from the apples to pupate in the soil at about two weeks. Several apples containing two-day old eggs or approximately third instar larvae (one week after removal from infestation cages) were placed inside cylindrical paper containers

(0.5 liter) that in turn fit into stainless steel perforated cylinders (11.4 cm inside diameter, 50 cm long) and were irradiated with target doses of 0, 20, 40, 60, 80, 100, 150, and 300 Gy. The unirradiated cohort of infested apples was used as the control and to estimate the number of insects present in the apples. There were two and four replicates of 13-17 apples per dose for eggs and larvae, respectively, with an estimated mean of 69 and 243 insects per dose for eggs and larvae, respectively. After irradiation, the apples containing eggs and larvae were placed in plastic containers at about 26°C to allow for continued development of the larvae. Emerging larvae were counted and placed on moist, loose, loamy potting soil for pupation. Apples were opened and examined for additional larvae. Pupation, adult emergence, and reproduction were recorded. Data were subjected to analysis using PROC PROBIT with normal and Gompertz probability density functions.

Fifty early (about seven days after pupation) and 50 late (about two days before first adult emergence was expected) pupae in soil were irradiated with 0, 20, 40, 60, or 80 Gy. Adult emergence and reproduction on small apples were recorded (two replicates).

Actively reproducing (two to three-week old) southern strain adults were irradiated to determine both the dose required for adult mortality and that required to prevent reproduction. In both cases, adults were irradiated with small apples in the same paper and stainless steel cylinders as with larvae. Doses were 0, 80, 150, 300, 500, 1000, and 2000 Gy for mortality determination and 0, 20, 40, 60, 80, 100, 150, and 300 Gy for reproductive determination. After irradiation, adults were maintained on small apples at about 25°C, and mortality was determined every three to four days by placing apparently dead adults on a hot plate at about 60°C to check for movement. The apples were replaced every three to four days and maintained at 25°C for development of any immatures inside. Larvae emerging from apples were collected every two to three days and placed on potting soil for pupation and adult emergence. After larvae were no longer emerging, the apples were opened and any remaining insects collected.

Northern strain adult plum curculios (100, 100, 200, 400, and 970, respectively) with small apples in the same containers used for southern strain adults were irradiated with 0, 20, 40, 60, or 80 Gy in 1997 and 0, 20, or 80 Gy (100, 100, and 395, respectively) in 1998. Two weeks after irradiation, the plum curculios were subjected to a temperature regime to break diapause. Afterwards, dead weevils were counted, and survivors were offered immature apples and allowed to reproduce.

As a confirmatory test, 25 000 two-week old southern strain adults were irradiated with a target dose of 80 Gy and held with small apples in the same manner as before until they died to observe reproduction compared with non-irradiated adults.

2.1. Results

No eggs developed beyond early instars at any of the irradiation doses as no mature larvae emerged from these treatments, while a mean of 69 fully developed fourth instars was produced in the control. Therefore, tests with eggs were halted after two replicates, and it was concluded that the egg stage was quite susceptible to irradiation and would be controlled by doses required to prevent reproduction by adults.

The number of approximately third instar plum curculios that failed to complete larval development after irradiation increased with dose until 97% failed at 150 Gy and 100% failed at 300 Gy. The data fit probit analysis, normal probability density function (PDF) using the

normal logarithm of dose (F value = 9.7; intercept = -6.5 $\pm$ 0.43; slope = 3.7 $\pm$ 0.24; n = 243 larvae/dose), but did not fit the Gompertz PDF or normal PDF without log transformation of dose. The estimated doses for 50 and 99% control were 54 Gy (95% fiducial limits: 49–59 Gy) and 225 Gy (95% fiducial limits: 185–293 Gy), respectively. Forty-seven percent of unirradiated larvae pupated and 34% formed adults, while 48% of larvae irradiated with 20 Gy pupated and 25% formed adults; no larvae exposed to $\geq$40 Gy pupated. Adults that were exposed as larvae to 20 Gy did not successfully reproduce, while control adults did.

Doses $\geq$40 Gy lowered adult emergence of early pupae considerably, but evenly. The highest dose, 80 Gy, did not markedly reduce adult emergence from late pupae. None of the adults irradiated as pupae reproduced.

Two-week old southern strain plum curculio adults lived up to a mean of 5.8 and 24.8 days when irradiated with 2000 and 80 Gy, respectively. The first death from those irradiated with 2000 Gy was at 20 hours and the last at seven days. The data fit the equation: $t = 50.5 + 6.0 \cdot \ln(d)$, where t is days to 100% mortality and d is dose in gray (F statistic = 200; $r^2 = 0.98$). Extrapolation from the data indicates that the dose required to provide near 100% mortality in one day would be about 4 kGy. After 25 days only 13.3 $\pm$ 2.3% of unirradiated weevils had died. None of the irradiated ($\geq$80 Gy) adults reproduced. Adults irradiated with 2000 Gy did not feed. Those irradiated with 500 or 1000 Gy fed very little, while the rest fed more, although less than control weevils.

A mean of 34.4 fourth instar offspring per southern strain female was achieved in the unirradiated control, with progressively less being produced with increasing irradiation dose until only one resulted from a total of 400 adults irradiated at 60 Gy; at $\geq$80 Gy, no fourth instar progeny were produced nor was larval tunneling observed in the apples. Quantity of reproduction was reduced slightly from the first few weeks for the control and adults irradiated at 20 Gy. As in the tests done at higher doses, all irradiated weevils, including those subjected to the lowest dose (20 Gy) had shorter lives than unirradiated weevils.

Although no larvae were observed developing from southern strain adults irradiated with 80 Gy, they laid eggs for up to one week after irradiation, with the number falling off exponentially from the first day to the eighth day.

In 1997, 1770 northern strain plum curculio adults did not reproduce until after being exposed to the temperature regime to end diapause. In 1998, 35 fourth instars resulting in 28 adults were produced by 600 adults in the four weeks prior to irradiation. In the two weeks between irradiation and the initiation of cold treatment, five more fourth instars were produced by 100 control adults and none by 100 or 395 adults irradiated with 20 or 80 Gy, respectively. Upon termination of the temperature regime to end diapause in 1997 and 1998, the survival of adults in the control was 59 and 79%, respectively, and in those irradiated with 80 Gy, survival was 3.7 and 10.1%, respectively.

One week after terminating the diapause-breaking temperature regime, control plum curculio collected in 1997 began to lay eggs and continued for three weeks. One-hundred-eleven fourth instars were produced by the 59 remaining weevils. After that, no more reproduction occurred in the control although they lived up to nine months more. Three weeks after terminating diapause the 33 surviving adults irradiated with 20 Gy produced four fourth instars and did not produce any more, although the last adult died ten days after the last control adult. Adults irradiated with 40–80 Gy did not reproduce. The 74 (of 100) surviving weevils irradiated with 20 Gy in 1998 produced 11 fourth instars over a seven-week period

beginning two weeks after termination of diapause. In 1998, surviving control weevils (79 of 100) produced 381 fourth instars over five and one-half months beginning two weeks after removal from the diapause-breaking temperature regime.

No late instars were found from apples infested by the 25 000 southern strain adults irradiated with a target dose of 80 Gy, while the non-irradiated adults produced a mean of 36.2 fourth instars per female over the three weeks that the irradiated weevils lived. Some eggs were laid soon after adults were irradiated and some appeared to hatch, but no development beyond the first instar was observed. The maximum absorbed dose during this part of the research was 92 Gy, so this dose is the minimum absorbed dose recommended for commercial application. Publication of these results are in press (1).

Sweetpotato Weevil (Coleoptera: Curculionidae)

The sweet potato weevil, is considered the most serious pest of sweetpotatoes, *Ipomea batatas* (L.) Lam., throughout much of the crop's growing range. Female weevils oviposit in sweetpotatoes by chewing a small cavity in the root or stem, depositing an egg, and sealing the hole with frass. In the field, they tend to oviposit near the juncture of the stem and tuber. In storage, sweetpotato weevils infest all over the roots. Sweetpotato-growing areas that do not have the weevil, such as the southwestern United States and the Mediterranean region, prohibit the importation of sweetpotatoes without a treatment that ensures that all weevil stages present are dead.

3. METHODOLOGY

Sweetpotato weevils were collected from a 'boniato' sweetpotato field near Homestead, Florida in the spring of 1999. They were shipped to our laboratory and reared on orange-fleshed sweetpotato roots purchased from the local market and 'boniato' (larger, white-fleshed) sweetpotato roots shipped from Homestead, Florida.

Adult sweetpotato weevils up to three weeks old were placed with pieces of sweetpotato roots in clear plastic cylinders (29 cm x 4 cm diameter) in the center of perforated stainless steel mesh cylinders (11.4 cm inside diameter, 50 cm long) that were placed in the irradiator for sufficient time to achieve the target dose of 125 Gy. Routine dosimetry was used to determine the absorbed dose range. Irradiated weevils and unirradiated controls were placed with sweet potato roots, which were changed every three to four days, until all irradiated weevils were dead. Data recorded were death of weevils and the number of new insects found in sweetpotato roots exposed to both irradiated and unirradiated weevils. In total, 3250 weevils were irradiated at a dose of 125 Gy. All irradiated insects within each replicate were held together to maximize the probability that fertile adults would mate. Subsequently 14 replicates with 1000-3950 adult weevils per replicate (total 30 655) were treated with a target dose of 150 Gy and counts of mortality and reproduction were made as before.

3.1. Results

Weevils irradiated with 125 and 150 Gy died at a faster rate than unirradiated weevils. Complete mortality of irradiated weevils occurred at a mean of 32.6 days at 125 Gy and 31.5 days at 150 Gy. During the research done at 125 and 150 Gy, respectively, 53 and 57% of unirradiated weevils were still alive the day the last irradiated weevils died. Reproduction, based on the number of F_1 adult weevils emerging from sweetpotatoes exposed to irradiated weevils, averaged 0.014 and 0 per female at 125 and 150 Gy, respectively.

The upper range of dosimetry readings when the target dose was set at 150 Gy was 165 Gy; therefore, a minimum absorbed dose of 165 Gy is recommended to achieve quarantine security for sweetpotato weevil adults.

The information from this study was submitted via the Florida Department of Plant Industry to the California Department of Agriculture to enable an irradiation quarantine treatment to be applied to Florida sweetpotatoes, including 'boniatos' for shipment to California. It was approved effective 1 April 2000, and the first shipments occurred in late some weeks later. Although 30 655 adults were eventually irradiated with a target dose of 150 Gy, authorities in California accepted the treatment after research with only 18 800 adults had been completed. California stipulated that sweetpotatoes be packed in cardboard boxes without holes before irradiation to reduce the chance of post-treatment re-infestation. This research has been published (2). The amount of irradiated sweetpotatoes has increased by about 15% each year.

Oriental Fruit Moth (Lepidoptera: Tortricidae)

Oriental fruit moth (OFM) attacks a wide variety of fruit in much of the world. It pupates off the host, so the latest stage that would be found in exported fruit would be last instar. As with fruit flies, it was decided that the measure of efficacy would be prevention of adult emergence to avoid the possibility of adults being found in surveillance traps.

4. METHODOLOGY

A commercial, semi-artificial diet exists for OFM (F9649, BioServ, Frenchtown, New Jersey, USA). It is easier to study OFM using diet-reared instead of fruit-reared insects. Preliminary studies compared radiosusceptibility of last instar OFM in fruit vs. diet at 100 Gy. Because a quarantine treatment should use the lowest dose possible to save resources and reduce potential damage to the commodity, confirmatory testing was initiated at 150 Gy and raised as the dose failed to achieve complete control of a target population of 30 000 last instar OFM.

4.1. Results

Adult emergence was 0.7% for apple-reared OFM and 1% for diet-reared ones, with no statistically significance difference between the two. Therefore, diet-rearing gave the same results as fruit-rearing, and all further research was conducted with diet-reared insects. At 150 and 175 Gy, adult emergence was 0.035 and 0.0055%. A total of >30 000 last instar OFM have been irradiated with a target dose of 200 Gy with no adults emerging. The quarantine treatment dose (200 Gy) is the same as the dose for another tortricid pest, the codling moth, *Cydia pomonella* (3).

Diatraea saccharalis and D. grandiosella (Lepidoptera: Pyralidae)

Work with these two pyralid borers was initiated at the request of a shipper in Florida who saw the market potential of shipping sugarcane stalk pieces to southern California where these borers do not exist. This research was reported during the 2nd RCM in Fresno two years ago, but no further research was done due to limited resources and the complexity of the research. To summarize the findings until now, a quarantine treatment against these insects will require an irradiation dose of >300 Gy (possibly 350Gy) to prevent reproduction from late pupae. However, research was done with these borers in diet only, not in sugarcane. Sugarcane pieces tolerated at least 900 Gy. With recently renewed funding, research will again be taken up and completed with these borers. A preliminary report of this work has been published (4).

Mexican Rice Borer (Lepidoptera: Pyralidae)

This research was also reported during the 2nd RCM in Fresno two years ago and therefore is only summarized here. Mexican rice borer (MRB) is invading the United States (5) and has displaced the sugarcane borer as the major sugarcane pest in southern Texas. Studies with rice borer were initiated as part of the quarantine treatment research program for Florida sugarcane pieces because the insect is expected to eventually invade Florida. MRB already exists in California, so its presence in Florida sugarcane would not be an impediment to shipment of sugarcane pieces to California. However, MRB would prevent the shipment of its crop hosts (sugarcane and others) to other countries. Like *Diatraea*, the MRB pupates in its host, and a quarantine treatment must be effective against all stages of the pest through late pupae. Response of MRB to irradiation is similar to that of other *Diatraea* spp.; i.e., 350 Gy might prevent reproduction from irradiated late pupae. This research was reported in Darmawi et al. (4). No further research is planned with this insect.

Indianmeal Moth (Lepidoptera: Pyralidae)

According to the literature, Indianmeal moth (IMM) may have the highest radiotolerance of any arthropod studied (6) (but see Ignatowicz this volume). The dose required to prevent reproduction of the adult stage with a high degree of security might be as much as 1 kGy or even higher. Although IMM is not of quarantine concern because it is distributed throughout the world, it was studied in order to determine the limits of radiotolerance of insects. As possibly the most radiotolerant arthropod known, determination of the minimum absorbed dose required to prevent its reproduction would offer an estimate of a dose that might serve as the generic dose that would be effective against all arthropods, a sought after goal (7).

5. METHODOLOGY

Pupae of IMM in small rolls of corrugated cardboard were obtained from a colony maintained in the Entomology Department at Oklahoma State University. After the adults had emerged, they were irradiated with 400 or 500 Gy and egg laying and hatch were observed.

5.1. Results

At 400 Gy, 1% of eggs laid from irradiated adults hatched, while at 500 Gy none hatched. Based on these results, the dose required to prevent egg hatch from irradiated adults is probably closer to 500 Gy than 1 kGy.

Lepidoptera often show inherited or F_1 sterility, which is sterility of the F_1 generation after irradiating the parental adults (8). Inherited sterility means that although some reproduction occurs in the irradiated adults, the F_1 generation might be sterile, and this could suffice to achieve quarantine security. For that reason, we are currently evaluating reproductive ability of insects developing from eggs laid by adult IMM irradiated with 400 Gy. Eggs that hatched at 400 Gy began to develop normally, but have not yet been reared to adulthood to see if they can reproduce. This part of the research will continue.

West Indian Sugarcane Root Borer (Coleoptera: Curculionidae)

West Indian sugarcane root borer cannot currently be studied at my laboratory in Weslaco, Texas because, although it has been found in southern Texas, it is not widely distributed, and it was considered too risky to establish a laboratory colony. At my request, the weevil was studied by Walter Gould, formerly of the USDA-ARS in Miami, Florida. The data have not

been published, but a brief summary is reported here. Adult female sugarcane root borers were collected from the field in Homestead, Florida, and irradiated with target doses of 0, 10, 20, 30, 40, or 50 Gy. There were 11 replicates of 20 females each, and numbers of eggs laid and larvae hatched were counted. Weevils laid a considerable amount of eggs at all of the doses, and egg hatch in the control was >95%. Egg hatch was reduced greatly at 10 Gy, was very low at 40 Gy, and was zero at 50 Gy. From these preliminary data, it appears that prevention of adult female reproduction is possible with <100 Gy.

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USE OF GAMMA RADIATION TO CONTROL THREE LEPIDOPTERAN PESTS IN BRAZIL

V. ARTHUR
Centro de Energia Nuclear na Agricultura – CENA,
University of São Paulo – USP,
Brazil

Abstract

Gamma radiation quarantine treatments were developed to control three lepidopteran pests harmful to Brazilian agriculture. All stages of *Ecdytolopha aurantiana* (Lima, 1927) (Lep.: Tortricidae), and all immature stages of *Tuta absoluta* and *Neoleucinodes elegantalis* were treated with gamma radiation using Cobalt-60. All stages of *E. aurantiana*, the orange fruit borer, were irradiated in oranges at target doses of 0 (control), 50, 100, 150, 200, 300, 400, 500, and 600 Gy, and a dose rate between 1.4 and 1.5 kGy per hour. Immature stages of *T. absoluta* and *N. elegantalis*, were irradiated in tomatoes at target doses of 0 (control), 50, 100, 150, 200, 300, 400, 500 Gy, and dose rates of 1.11 and 1.24 kGy per hour, respectively. An irradiation dose of 500 Gy caused 100% mortality in *E. aurantiana* pupae, and an irradiation dose of 300 Gy applied to any life stage was sufficient to prevent development to the adult stage or reproduction. An irradiation dose of 400 Gy applied to *T. absoluta* and *N. elegantalis* was sufficient to prevent reproduction.

1. INTRODUCTION

Besides fruit flies (mainly the medfly, *Ceratitis capitata* but also *Anastrepha* spp), the orange fruit borer is presently the most serious threat to the production of oranges, tangerines, grapefuits, and lemons. In 1999, the losses caused by the orange fruit borer in the State of São Paulo were >1000 tons of fruit with an estimated value of approximately $350 000. Losses were detected only when the fruits were already packaged and ready for shipment to export, so the total losses in citrus to this pest are in reality much higher. The present report discusses results to date with the orange fruit borer, but the overall goal is to identify an irradiation dose that will kill or sterilize orange fruit borer and all the fruit flies. The effects of irradiation of fruit flies has been studied extensively, both in the search for control measures and for use in sterile insect technique programs; it appears that gamma radiation doses around 225 Gy are sufficient to control all fruit flies in Brazil (Arthur, 1998).

Tomato is the second most important vegetable crop in Brazil. Around 65 000 hectares of tomatoes are cultivated in Brazil, with production of >3 million tons per year. This volume puts Brazil among the ten largest producers of tomatoes in the world. About one third of production is destined for processing, and the remainder is sold as fresh produce for consumption in Brazil, but both products require good quality. In some areas losses due to insect pests reach 50% even with insecticde applications. A high percentage of fruit at harvest contain quarantine pests, which poses a problem for export of tomatoes. *Tuta absoluta* (Meyrick, 1917) (Lep.: Gelechiidae) is a serious pest of the tomato leaves, and *Neoleucinodes elegantalis* (Gueneé, 1954) (Lep.: Pyralidae) is an important pest of fruits. Irradiation of packaged tomatoes may be needed to avoid additional losses and to avoid movement of insect pests into importing countries.

2. MATERIALS AND METHODS

The research was carried out in the laboratory of the Radio-entomology and Food Irradiation Section of the Center for Nuclear Energy in Agriculture, CENA, one of the research institutes of the University of São Paulo (USP). The laboratory is situated in Piracicaba, around 160 km from the city of São Paulo. Orange fruit borers were irradiated using a cobalt-60 Gammacell-220 irradiator at a dose rate of 1.4 kGy per hour. The target doses were 0 (control), 50, 100, 150, 200, 300, 400, 500 Gy. Each each life stage, treatment consisted of 10 oranges infested with five eggs, five larvae, or five pupae per fruit. After treatment the oranges were stored into a rearing chamber at 25±3° C and 65-75% relative humidity.

The insects attacking tomatoes were collected in the field, and maintained in the laboratory in a rearing room regulated at 25±3°C and 65–75% relative humidity. Tomato insects were irradiated using the same irradiator but at a dose rate of 1.11 kGy per hour for *T. absoluta* and 1.24 kGy per hour for *N. elegantalis*. Eggs (one to twenty-four hours old) on leaves of both species were placed in a cylinder 10 cm wide and 20 cm high. Eggs were irradiated at doses of 0 (control), 50, 100, 150, 200, and 300 Gy. Each treatment had five replicates with ten eggs each. After irradiation the eggs were transfered to Petri dishes (2.5 cm high and 11 cm in diameter) with moist filter paper and maintained until eclosion. All eggs were examined under a microscope daily for six days to check for emergence (= fertility). Larvae of various ages were irradiated at doses of 0 (control), 50, 100, 150, 200, 300, 400 and 500 Gy. Each treatment had ten replicates with five larvae each. After treatment each larva was held in a glass vial (2.5 cm in diameter and 8.5 cm high), stopped with a cotton ball. Larvae were fed fresh tomato leaves until the adults emerged. The measure of efficacy was prevention of adult emergence. For tests with pupae, last instar larvae were introduced to glass vials with fresh tomato leaves and maintained until pupation. Pupae were irradiated with the same doses as larvae. Each treatment had five replicates of ten pupae, and again the measure of efficacy was prevention of adult emergence. To determine the sterilizing dose for adults, late pupae were irradiated at 0 (control), 50, 100, 150, 200, 300 and 400 Gy and paired after emergence. For each treatment dose, five adult pairs were used. Adults were held with fresh leaves in glass vials similar to those utilized for larvae and pupae. Adult fecundity and viability was determined by examining the oviposition and hatch on tomato leaves.

3. RESULTS AND DISCUSSION

Tables 1, 2, 3 and 4 show the number of irradiated orange fruit borers that developed to the next stage (when treated as eggs) or emerged as adults (when treated as second instars, last instars, and pupae). The lethal doses of gamma radiation for eggs in oranges was 150 Gy, and the lethal dose for second instars and last instars was 200 Gy. The sterilization dose for pupae was 300 Gy, but their lethal dose was 500 Gy. A dose of 500 Gy resulted on total mortality of 15-day old pupae, however, pupae were sterilized at a dose of 300 Gy. Large-scale tests involving irradiation of 1000 12-day old pupae at each dose confirmed that 500 Gy prevented adult emergence (Table 5), and a minimum absorbed dose of 300 Gy was sufficient to prevent adult reproduction (data not shown). These results were consistent with preliminary results as part of this coordinated research project where orange fruit borer was irradiated in artificial diet. Similar results were also found with several other moths including [1, 2, 5, 9 10], who irradiated all phases of *Sitotroga cerealella*, *Plodia interpunctella*, *Corcyra cephalonica* and *Tuta absoluta*. The results are also consistent with irradiation research [4, 5, 6, 7] with larvae and pupae of *Diatraea saccharalis*, *Spodoptera frugiperda*. We also observed that there was no difference in radio-sensitivity of any stage of the orange fruit borer when irradiated in artificial diet or in artificially infested oranges for.

Results for the *Tuta absoluta* and *Neoleucinodes elegantalis* are shown in Tables 6-13. The data suggest that the lethal dose for eggs of the two species is 100 Gy (Tables 6 and 10). For larvae of both species the lethal dose of 200 Gy prevented adult emergence (Tables 7 and 11). For pupae of *T. absoluta* 300 Gy prevented adult emergence (Table 8), and for *N. elegantalis* 400 Gy prevented adult emergence (Table 12). The sterilizing dose for adults resulting from irradiated pupae was 200 Gy and 300 Gy for *T. absoluta* and *N. elegantalis*, respectively (Tables 9 and 13). The results are consistent with previous irradiation studies with *Sitotroga cerealella*, *Plodia interpunctella*, *Corcyra cephalonica* and *Tuta absoluta* [1, 2, 8, 9, 10]. The results are also consistent with studies with larvae and pupae of *Diatraea saccharalis* and *Spodoptera frugiperda* [4, 5, 6, 7].

In conclusion, an irradiation dose of 400 Gy was lethal to all life stages of the two tomato pests. An irradiation dose of 500 Gy prevented reproduction in the three insects species studied, and could be recomended as a suitable quarantine treatment for citrus fruits and against all species of lepidopterans and fruit flies.

Table 1. NUMBER OF ECLOSED LARVAE FROM IRRADIATED EGGS OF ORANGE FRUIT BORER, *Ecdytolopha Aurantiana*, IN ARTIFICIALLY INFESTED ORANGES

Dose (Gy)	No. eggs	No. larvae	% eclosion
0	50	45	90%
100	50	3	6%
150	50	0	0%

Table 2. NUMBER OF ECLOSED ADULT ORANGE FRUIT BORER LARVAE IRRADIATED AS SECOND INSTARS IN ARTIFICIALLY INFESTED ORANGES

Dose (Gy)	No. larvae	No. adults	% adult eclosion
0	50	41	82%
100	50	10	20%
150	50	1	2%
200	50	0	0%

Table 3. NUMBER OF ECLOSED ADULT ORANGE FRUIT BORER LARVAE IRRADIATED AS LAST INSTARS IN ARTIFICIALLY INFESTED ORANGES

Dose (Gy)	No. larvae	No. emerged adults	% emergence
0	50	43	86%
50	50	10	20%
100	50	1	2%
150	50	0	0%

Table 4. NUMBER OF ECLOSED ADULT ORANGE FRUIT BORER LARVAE IRRADIATED AS 12-D-OLD PUPAE IN ARTIFICIALLY INFESTED ORANGES

Dose (Gy)	No. pupae	No. emerged adults	% Emergence
0	50	44	88%
200	50	20	40%
300	50	39	18%
400	50	1	2%
500	50	0	0%

Table 5. LARGE-SCALE TEST: NUMBER OF ECLOSED ADULT ORANGE FRUIT BORER LARVAE IRRADIATED AS 12-DAY OLD PUPAE IN ARTIFICIALLY INFESTED ORANGES

Dose (Gy)	No. pupae	No. emerged adults	% Emergence
0	1000	901	90.1%
300	1000	141	14.1%
400	1000	18	1.8%
500	1000	0	0.0%

Table 6. PERCENT VIABILITY OF IRRADIATED EGGS OF *Tuta Absoluta*

Dose (Gy)	No. eggs	No. larvae	% Eclosion
0	50	49	98%
50	50	10	20%
100	50	0	0%

Table 7. PERCENT EMERGENCE AS ADULTS OF IRRADIATED LAST INSTARS OF *Tuta Absoluta*

Dose (Gy)	No. larvae	No. adults	% Emergence
0	50	41	82%
50	50	30	60%
100	50	15	30%
150	50	1	2%
200	50	0	0%

Table 8. PERCENT EMERGENCE AS ADULTS OF IRRADIATED PUPAE OF *Tuta Absoluta*

Dose (Gy)	No. pupae	No. adults	% Emergence
0	50	48	96%
50	50	36	72%
100	50	30	60%
200	50	26	52%
300	50	0	0%

Table 9. TOTAL NUMBER AND VIABILITY OF EGGS LAID BY ADULTS OF *Tuta Absoluta* WHEN IRRADIATED AS PUPAE

Dose (Gy)	No. eggs	Viable eggs	% Viability
0	131	124	94.7%
50	96	81	84.3%
100	37	15	40.5%
200	0	0	0.0%

Table 10. VIABILITY OF EGGS OF *Neoleucinodes Elegantalis* LAID BY IRRADIATED ADULTS

Dose (Gy)	No. eggs	Viable eggs	% Viability
0	50	48	94%
50	50	9	70%
100	50	0	0%

Table 11. PERCENT ADULT EMERGENCE OF *Neoleucinodes Elegantalis* IRRADIATED AS LAST INSTARS

Dose (Gy)	No. pupae	No. adults	% EMERGENCE
0	50	47	94%
50	50	35	70%
100	50	11	22%
150	50	2	4%
200	50	0	0%

Table 12. PERCENT ADULT EMERGENCE OF *Neoleucinodes Elegantalis* IRRADIATED AS PUPAE

Dose (Gy)	No. pupae	No. adults	% Emergence
0	50	47	94%
50	50	38	76%
100	50	31	62%
200	50	22	44%
300	50	9	18%
400	50	0	0%

Table 13. TOTAL NUMBER AND PERCENT VIABILITY OF EGGS LAID BY ADULTS OF *Neoleucinodes Elegantalis* IRRADIATED AS PUPAE.

Dose (Gy)	No. eggs	Viable eggs	% Viability
0	251	235	93.6%
50	198	165	83.3%
100	86	31	36.0%
200	5	0	0.0%
300	0	0	0.0%

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IRRADIATION AS AN ALTERNATIVE TO METHYL BROMIDE FUMIGATION OF AGRICULTURAL COMMODITIES INFESTED WITH QUARANTINE STORED PRODUCT PESTS

S. IGNATOWICZ
Agricultural University of Warsaw,
Warsaw, Poland

Abstract

A generic gamma radiation dose of 0.3 kGy completely inhibits the development of immatures and sterilizes adults of beetle pests of stored products. Irradiation at the lower doses may be applied to some commodities. Species-specific doses of gamma radiation lower than 0.3 kGy are identified, and can be used as a quarantine treatment of commodities infested with a single species of beetles. Stored-product moths, however, are more resistant to the sterilizing effects of irradiation than beetles. A dose as high as 0.6 kGy is suggested for quarantine treatment of commodities infested by immatures and adults of lepidopteran pests.

1. INTRODUCTION

Many species of stored-product pests are cosmopolitan, but other serious pests such as the khapra beetle, *Trogoderma granarium* Ev., the larger grain borer, *Prostephanus truncatus* (Horn), the lesser grain borer, *Rhizopertha dominica* F., the rice weevil, *Sitophilus oryzae* (L.), the corn weevil, *S. zeamays* Motsch., the Angoumois grain moth, *Sitotroga cerealella* Ol., the almond moth, *Cadra cautella* Wlk., and various bruchids (*Bruchidae*) and mites (*Acaridae*) are not. These stored-product pests are included on the list of quarantined pests for many countries (e.g., Polish list A2). Some studies have been performed on the possible use of irradiation as a potential quarantine treatment for these pests, and the radiation biology of these pests is known [4]. However, data on the effectiveness of irradiation on these quarantine pests, based on criteria acceptable to regulatory authorities, should be developed.

The aim of this study was to (1) identify an irradiation dose that will prevent development of immatures and/or sterilize adults of some stored product pests, (2) to confirm a dose that prevents development of immatures and/or sterilizes adults of these pests by testing large numbers of insects at the most tolerant stage to irradiation, and (3) to perform large-scale tests to confirm a dose that prevents development of immatures and/or sterilize adults of the pest.

2. MATERIAL AND METHODS

All radiation experiments were conducted using the Warsaw Agricultural University irradiator located at the Veterinary Faculty, Warsaw. The source was Cobalt-60 and the dose rate was approx. 20 Gy/min., as determined by a Fricke or alanine dosimetry. Irradiation was applied to insects kept on their food medium.

2.1. Experiments with the dry bean weevil, *Acanthoscelides obtectus* Say

Stock cultures of the bean weevil have been maintained on beans (cv. Bia'a Wyborowa) in darkness at 25±1°C and 75±5% relative humidity (RH). Beans infested with ca. 100 eggs of the bean weevil were exposed to one of four doses of gamma radiation one, two, three, or four weeks after infestation. Treated material was stored in a controlled environment cabinet. After 50 days, the beetles that emerged were counted, and the percentage of insects that had completed their development was estimated.

One- or two-day old adults of the bean weevil were collected from stock cultures and exposed to gamma radiation. After treatment, insects were placed in Petri dishes (5 cm diameter, five weevils per dish) containing a few bean seeds. Dishes with insects were stored in a controlled environment cabinet. Observations of mortality were made every one to two days until the last treated beetle died. Total number of eggs laid and number of eggs that hatched (= empty egg shells) were determined, and fertility of treated beetles was estimated.

A large-scale experiment was conducted to confirm a dose that would prevent development of immatures and/or sterilize adults of the bean weevil. About 5 kg of beans (*cv*. Bia'a Wyborowa) heavily infested by all developmental stages of the bean weevil were irradiated with one of the following doses of gamma radiation: 0.0 (control), 0.1, 0.2, and 0.25 kGy. After the treatment, the beans were placed into 3 L jars (1.6 kg of infested beans per jar) and stored in a cabinet. Every one to three days the irradiated beans were checked to determine the period after which all beetles were dead. Then, the beans were sieved thoroughly and dead weevils were discarded. After another two weeks, beans were sieved again, and the number of progeny was recorded.

2.2. Experiments with the larger grain borer, *Prostephanus truncatus* (Horn), and the lesser grain borer, *Rhizopertha dominica* (F.)

Cultures of the larger grain borer, *Prostephanus truncatus* (Horn), were obtained from the Plant Protection Institute, Poznan, and cultures of the lesser grain borer, *Rhyzopertha dominica* F. have been maintained in our laboratory for several years. Stock cultures of the larger grain borer and the lesser grain borer were maintained in darkness at 30±1°C and 75±5% RH.

Food medium (500 grams of maize grain for *P. truncatus*, and 500 grams of wheat grain for *R. dominica*) infested with eggs of beetles was exposed to one of the following doses: 0 (control), 40, 60, 80, 100, and 120 Gy of gamma radiation one, two, three, or four weeks after infestation. Insects irradiated one week after infestation were treated as old eggs and/or young larvae; insects irradiated two and three weeks after treatment were treated as larvae; those irradiated four weeks after infestation were old larvae and pupae.

Treated material was stored in a controlled environment cabinet (30±1°C, 75±5% RH). After 30 days, adult beetles that emerged were counted, and the percentage of insects that completed their development was estimated. Results of this experiment were used to identify an irradiation dose that prevents development of immatures of the larger grain borer and the lesser grain borer.

To determine a sterilizing dose for *R. dominica*, adult borers less than one week old were collected from rearing dishes (5 cm diam.) and placed into small glass vials (2 x 1 cm) with crushed wheat grain. These cultures were irradiated with one of the following doses: 0 (control), 20, 40, 60, 80, and 100 Gy. After irradiation treatment, the insects within the medium were placed into rearing cabinets (30±1°C, 75±5% RH). Females laid eggs individually or in batches of several eggs on grains or on sides of vials. These eggs were counted and transferred to separate dishes every three days to observe hatch.

Because *P. truncatus* females construct tunnels and side chambers in grain for oviposition [9], different methods were adopted to determine a sterilizing dose of gamma radiation. Adult borers less than one week old were separated from the culture and irradiated with one of the following doses: 0 (control), 20, 40, 60, 80, and 100 Gy. After irradiation treatment insects

were placed into 0.33 L jars with maize. Each treatment was replicated three times, and each replication contained 20 insects. After two weeks, adults were sieved from the medium and the maize was placed back into the rearing chamber for four more weeks. After this period, borers (= progeny of irradiated beetles) were segregated from the breeding stock with sieves of different mesh sizes. These borers were counted and the fertility of irradiated *P. truncatus* adults was estimated.

About five kg of food medium (maize for the greater grain borer, and wheat grain for the lesser grain borer) heavily infested by all developmental stages (egg, larvae, pupae, adults) of the greater grain borer or the lesser grain borer was thoroughly sieved to remove adults and then divided into two equal parts. One half of the grain used an untreated control, and the second half was irradiated with one of the following doses of gamma radiation: 0 (control), 20, 40, 60, 80 and 100 Gy. After the treatment, irradiated and control grain was stored in a cabinet (30±1°C, 75±5% RH). After six weeks grain was sieved to remove adults. The number of adult borers (= progeny) was recorded.

2.3. Experiments with the cigarette beetle, *Lasioderma serricorne* (F.), and the drug store beetle, *Stegobium paniceum* (L.)

Drug store beetles were fed a mixture of wheat grain, spices, dry fruits and herbs. The food medium for the cigarette beetle was tobacco. All test insects were reared in laboratory cultures under a controlled environment of 28±1°C and 75±5% RH.

Food medium infested with eggs of anobiids was exposed to one of five doses of gamma radiation one (mostly eggs), two, three (larvae), or four weeks (mostly pupae) after infestation. Treated material was stored in a controlled environment cabinet (28±1°C and 75±5% RH). After 50 days, adult beetles that emerged were counted, and the percentage of insects that completed their development was estimated. Results of this experiment were used to identify an irradiation dose that prevents development of immatures of the cigarette beetle and the drug store beetle.

To determine a sterilizing dose for the cigarette beetle and the drug store beetle, adults less than 1 week old were isolated from rearing dishes (5 cm. diam.) and placed into small glass vials (2 x 1 cm) with food medium. These cultures were irradiated with one of the following doses: 0 (control), 25, 50, 75, 100, 125, 150, 200, 250 and 300 Gy. After irradiation treatment, the insects within the medium were placed into the rearing cabinets (30±1°C, 75±5% RH). Females laid eggs individually or in batches of several eggs on food or on sides of vials. These eggs were counted and transferred to separate dishes every three days to observe hatch.

About five kg of food medium heavily infested by all developmental stages (egg, larvae, pupae, adults) of the cigarette beetle or the drug store beetle was thoroughly sieved to remove adults, and then divided into two equal parts. One half of food medium was used as an untreated control, and the other half was irradiated with one of the following doses of gamma radiation: 0 (control), 30, 60, 90, 120 and 150 Gy. After treatment, irradiated and control food medium was stored in a cabinet (30±1°C, 75±5% RH). After six weeks, the food medium was sieved to remove adults. The number of adult anobiids (= progeny) was recorded.

2.4. Experiments with the grain weevil, *Sitophilus granarius* (L.), and rice weevil, *Sitophilus oryzae* (L.)

About 3 kg of wheat grain heavily infested by all developmental stages (egg, larvae, pupae) of the grain weevil or the rice weevil was divided into two equal (1.5 kg) parts. One half of grain

was used as the untreated control, and the other half was irradiated with one of the following doses of gamma radiation: 0 (control), 20, 40, 60, 80 and 100 Gy. After treatment, the grain was stored in a cabinet. Every week grain was sieved to remove adults. The number of adult weevils (progeny) was recorded.

2.5. Experiments with the Angoumois grain moth, *Sitotroga cerealella* Ol.

Stock cultures of the Angoumois grain moth were maintained in darkness at 25±1°C and 75±5% RH. Samples of wheat grain (100 g) were placed in Petri dishes and exposed to a heavy population of adults of the Angoumois grain moth for two to three days to obtain infestations of insects of known ages. After exposure, wheat was removed from the chamber and stored in glass jars in cabinets. The Petri dishes were refilled with fresh wheat from the same original lot and exposed to moths. This process was repeated until moths began to emerge from the first infested sample. At this time, the material was exposed to one of four doses of gamma radiation. Treated material was stored in a controlled environment cabinet. After 40 days, the adult moths that emerged were counted, and the percentage of insects that completed their development was estimated; using these data an irradiation dose that prevents the development of immatures of the Angoumois grain moth was determined.

In the next experiment, one or two-day old adults of the Angoumois grain moth were collected from stock cultures and exposed to gamma radiation. After treatment, insects were placed in Petri dishes (5 cm diameter, 10 moths per dish) containing food medium (wheat grain). Dishes with moths were stored in a controlled environment cabinet. Observation of mortality was made every one to two days until the last treated moth died. The total number of eggs laid and the number of eggs that hatched were determined, and fertility of treated Angoumois grain moths was estimated. An irradiation dose that sterilizes adults of the Angoumois grain moth was determined.

A large-scale experiment was performed to confirm a dose that would prevent development of immatures of the Angoumois grain moth. About 5 kg of food medium (wheat grain) heavily infested by all developmental stages of the Angoumois grain moth was irradiated with the lowest dose which was found to prevent development of immatures and/or sterilize adults. After the treatment, irradiated material was placed into glass jars. The surface of grain was observed for moth emergence. After four weeks the number of emerged adults of the Angoumois grain moth was recorded.

3. RESULTS

3.1. Experiments with the dry bean weevil, *Acanthoscelides obtectus* Say

Low doses of gamma radiation affected the development of immature stages of the bean weevil. When beans infested with eggs were irradiated with 0.1 kGy or higher doses one to three weeks after infestation, no adults emerged. No emergence was also noted when beans were given a dose 0.3 or 0.35 kGy at the fourth week after infestation. Thus, the dose of 0.3 kGy is considered to be lethal for all larvae and pupae of the bean weevil. At lower doses, some immature weevils completed their development. A dose of 0.25 kGy and lower doses did not prevent development of the older stages (last instars and pupae) to adults, but killed young larvae (Table 1).

Of all stored product pests, the bruchids (Bruchidae) appear to be the most sensitive to the sterilizing action of ionizing radiation. Data presented in Table 2 support this opinion. When young adults of both sexes were irradiated with a dose of 0.06 or higher, no eggs hatched. A

0.06 kGy dose was the sterilizing dosage of gamma radiation for the bean weevil, because at lower doses females produced some viable eggs (e.g., at 0.04 kGy there were about 5% viable eggs).

Different investigators have determined various sterilizing doses for the bean weevil. Pesson [10] found that adults treated with 0.10-0.18 kGy were partially sterilized, and that 0.2 kGy was required for complete sterility. Andreev [1] reported that young beetles irradiated with a dose as low as 0.01 kGy produced only a few progeny, but 0.6 kGy was required for complete sterilization. Later on, Andreev et al. [2] considered 0.12-0.15 kGy as fully sterilizing dosages. Cavalloro and Bonfonti [5] found that sterilizing doses for males and females irradiated four days before the end of the pupal stage were 0.04 and 0.06 kGy, respectively, while on the day of emergence 0.05 and 0.1 kGy were required. According to Jermy [7] both sexes were sterile when young beetles had been irradiated with 0.1 kGy. Arthur et al. [3] found that pupae irradiated with the dose of 0.03 kGy were sterile. According to Rosada [12], a dose of 0.05 or 0.06 kGy sterilized males and females of the bean weevil (see also [8]). This considerable variation in results on sensitivity of the bean weevil to ionizing radiation seems to be caused by many factors, including age, sex, strains, food, temperature, humidity, type of radiation, dose rate, and dosimetry. Most investigators agree that males and females of the bean weevil irradiated with a dose of 0.15 kGy under normal conditions would be sterile.

Results of the large-scale experiment to confirm a dose that prevents development of immatures and/or sterilizes adults of the bean weevil are shown in Table 3. In the control, more than 80 000 progeny per replicate was obtained from non-irradiated bean seeds whereas no progeny was found in beans irradiated with a dose 0.1 kGy and higher. This means that no progeny were discovered because of complete mortality of immatures during their development. However, at this dose adults are partially sterile (Table 1).

Data in Tables 1–3 indicate some controversy, but suggest that a dose higher than 0.1 kGy could be used to produce an acceptable level of quarantine security. Thus, the large-scale experiment conducted to confirm a dose that prevents development of immatures and sterilizes adults of the bean weevil will be repeated to indicate the dose of gamma radiation which provides quarantine security.

Table 1. EFFECT OF GAMMA RADIATION ON MORTALITY OF IMMATURE STAGES OF THE BEAN WEEVIL

Dose (kGy)	Weeks after infestation	No of emerged adults	No. of "windows"*
0.0 (control)	—	320	0
0.1	1	0	0
	2	0	10
	3	0	65
	4	96	218
0.2	1	0	0
	2	0	3
	3	0	23
	4	37	202
0.25	1	0	0
	2	0	0
	3	0	7
	4	1	291
0.3	1	0	0
	2	0	0
	3	0	4
	4	0	317
0.35	1	0	0
	2	0	0
	3	0	1
	4	0	266

Beans infested with eggs were irradiated 1, 2, 3, or 4 weeks after infestation, and number of emerged adults (number of "windows")* was recorded.

* Number of "windows" = number of immatures that dies within seeds after forming a "window";

The "window" is a "greasy" spot visible on the surface of the seeds.

Table 2. HATCHABILITY OF EGGS PRODUCED BY THE BEAN WEEVILS IRRADIATED WITH 0-0.08 KGY OF GAMMA RADIATION

Dose (kGy)	**"Egg Wave"***	**No of eggs**	**No. of hatched eggs**	**Hatch-ability (%)**
0.0	1st	245	213	86.9
	2nd	130	109	83.8
	3rd	34	25	73.5
	4th	0	0	—
	Total	409	347	84.8
0.02	1st	741	41	5.5
	2nd	214	33	15.4
	3rd	56	2	3.5
	4th	4	0	—
	Total	1015	76	7.5
0.04	1st	382	5	1.3
	2nd	152	10	6.6
	3rd	48	1	2.1
	4th	6	0	—
	Total	588	16	2.7
0.06	1st	218	0	0.0
	2nd	110	0	0.0
	3rd	5	0	0.0
	4th	3	0	0.0
	Total	336	0	0.0
0.08	1st	292	0	0.0
	2nd	49	0	0.0
	3rd	5	0	0.0
	4th	0	0	0.0
	Total	346	0	0.0

*The treated beetles were kept in Petri dishes containing beans for three to four-day periods, after which they were transferred into new dishes. The obtained "egg-waves" were incubated for one week in a cabinet, then the number of eggs laid and the number of hatched eggs were determined.

Table 3. EFFICACY OF IRRADIATION AS A QUARANTINE TREATMENT OF BEANS INFESTED WITH THE BEAN WEEVIL

Dose (kGy)	Replicate	Period after which all beetles were dead (days)	No. of progeny
0.25	1	22	0
	2	22	0
	3	22	0
0.02	1	26	0
	2	28	0
	3	26	0
0.01	1	30	0
	2	33	0
	3	33	0
Control	1	live beetles	83 360
	2	live beetles	104 200
	3	live beetles	93 780

3.2. Experiments with the larger grain borer, *Prostephanus truncatus* (Horn), and the lesser grain borer, *Rhizopertha dominica* (F.)

Results presented in Table 4 demonstrate that the developmental stage of the borers determines its susceptibility to gamma radiation. The most susceptible stage was young eggs and/or young larvae (one week after infestation). The dose of 80 Gy completely inhibited their development. The most resistant stage is the pupal stage (four weeks after infestation), as a dose of 100 Gy did not prevent all pupae from any further development; one or a few adults emerged from immature borers that were irradiated with 100 Gy four weeks after infestation. However, a dose of 120 Gy completely inhibited the development of all immature borers. Thus, a dose in the region of 100 Gy seems to be the dose that prevents development of immatures of both borer species.

Results presented in Table 5 indicate that *R. dominica* adults are highly susceptible to gamma radiation. Doses in the region of 20 and 40 Gy reduced the hatchability of eggs to 8.8% and 2.6%, respectively. Beetles irradiated with a dose of 60 Gy or higher laid eggs that did not hatch. Thus, the lowest sterilizing dose of gamma radiation for the lesser grain borer seems to be 60 Gy. The findings of Watters and MacQueen [14] that a dose of 62.5 Gy completely sterilized *R. dominica* adults are in agreement with the present work. Singh and Liles [13] found a somewhat lower dose (50 Gy) that sterilized the lesser grain borer.

Ramirez and Ramos [11] reported that the exposure of *P. truncatus* adults to a dose of 150 Gy produced 100% mortality within 15 days; in the meantime, only 3.3% of irradiated females laid eggs and only 1.5% of these eggs hatched. Results obtained in the present study (Table 6) indicate that the sterilizing dose for the pest is much lower. Doses as low as 20 and 40 Gy caused a high degree of sterility in greater grain borer adults, and at a dose of 60 Gy or higher resulted in no progeny. Thus, the lowest sterilizing dose of gamma radiation for the lesser grain borer seems to be 60 Gy, as with the lesser grain borer.

Low doses of gamma radiation caused high mortality for immatures of the larger grain borer and the lesser grain borer. A dose as low as 20 Gy induced ca. 82% mortality in eggs, larvae and pupae of the larger grain borer and the lesser grain borer, indicating high susceptibility of immatures borers to radiation.

Doses of 40 and 60 Gy allowed several adult borers to complete their development (Table 7). Thus, mortality rates ranged for these doses between 96.72% and 99.76%. At a dose of 80 Gy few adults completed their development, and at a dose of 100 Gy no immature completed its development.

Thus, a dose of 100 Gy could be suggested for quarantine treatment of maize and wheat grain infested with the larger grain borer and the lesser grain borer, respectively, with assumption that the non-completion of pest life stages is a measure for the efficacy of radiation as a quarantine treatment.

Table 4. EFFECTS OF GAMMA RADIATION ON THE DEVELOPMENT OF IMMATURES OF THE LESSER GRAIN BORER AND THE LARGER GRAIN BORER

Dose (Gy)	**Weeks after infestation**	**Number of progeny adults recorded**	
		P. truncatus	*R. dominica*
0	1	367	568
(control)	2	320	453
	3	451	187
	4	239	648
40	1	56	78
	2	135	345
	3	278	378
	4	220	439
60	1	4	1
	2	27	45
	3	59	41
	4	40	39
80	1	0	0
	2	7	5
	3	35	6
	4	64	19
100	1	0	0
	2	0	0
	3	0	0
	4	1	6
120	1	0	0
	2	0	0
	3	0	0
	4	0	0

Table 5. FERTILITY OF IRRADIATED ADULTS OF THE LESSER GRAIN BORER

Dose	No. of eggs observed	Hatched eggs		Sterility index (%)
		No.	%	
0	450	447	99.3	—
20	317	28	8.8	91.2
40	382	10	2.6	97.4
60	316	0	0.0	100.0
80	130	0	0.0	100.0
100	76	0	0.0	100.0

Table 6. FERTILITY OF *P. truncatus* ADULTS IRRADIATED WITH GAMMA RADIATION

Dose (Gy)	No. progeny borers*	Sterility index (%)
0	472	—
20	23	95.1
40	7	98.5
60	0	100.0
80	0	100.0
100	0	100.0

*Pooled results of three replicates.

Table 7. MORTALITY OF IMMATURES OF THE LARGER GRAIN BORER AND THE LESSER GRAIN BORER AFTER IRRADIATION TREATMENT

Dose (Gy)	Pest species	Estimated number of irradiated eggs, larvae and pupae*	Number of adults that completed development	Mortality of immatures (%)
100	Larger grain	3974	0	100.00
80	borer	4018	2	99.95
60	(*P. truncatus*)	3741	9	99.76
40		3189	48	98.49
20		4790	875	81.73
100	Lesser grain	6361	0	100.00
80	borer	5845	9	99.85
60	(*R. dominica*)	4960	43	99.13
40		5429	178	96.72
20		6094	1052	82.73

*Emergence of adults of the larger grain borer and the lesser grain borer from control (non-irradiated) immatures is assumed to be 100% (mortality of immatures = 0%).

3.3. Experiments with the cigarette beetle, *Lasioderma serricorne* (F.), and the drug store beetle (*Stegobium paniceum* (L.)

The results presented in Table 8 indicate that the most susceptible developmental stage of these anobiid beetles is the egg, followed by the larva and pupa. A dose of 120 Gy completely inhibited the development of immatures of the cigarette beetle and the drug store beetle to the adult stage. These results are in agreement with data presented by Tilton et al. (1966). They found that some eggs and larvae irradiated with a dose ≥132 Gy successfully molted to the next developmental stage, but they never became adults. Thus, a dose in the region of 120 Gy seems to be the dose that prevents development of immatures of both anobiid species.

Results presented in Tables 9 and 10 indicate that adults of the cigarette beetle are more susceptible to the sterilizing effects of gamma radiation than the drug store beetle. A dose as low as 125 Gy resulted in complete sterility when both sexes of the cigarette beetle were treated. Brower and Tilton [4] reported that the cigarette beetle females are sterilized by 175 Gy and males by 250 Gy.

Table 8. EFFECTS OF GAMMA RADIATION ON THE DEVELOPMENT OF IMMATURES OF THE CIGARETTE BEETLE AND THE DRUG STORE BEETLE.

Dose (Gy)	Weeks after infestation	Number of progeny adults recorded	
		L. serricorne	*S. paniceum*
0 (control)	1 (eggs)	658	327
	2 (larvae)	630	396
	3 (larvae)	721	415
	4 (pupae)	459	461
30	1 (eggs)	47	35
	2 (larvae)	39	17
	3 (larvae)	34	10
	4 (pupae)	31	18
60	1 (eggs)	4	8
	2 (larvae)	15	12
	3 (larvae)	24	25
	4 (pupae)	28	17
90	1 (eggs)	0	0
	2 (larvae)	0	3
	3 (larvae)	5	4
	4 (pupae)	14	9
120	1 (eggs)	0	0
	2 (larvae)	0	0
	3 (larvae)	0	0
	4 (pupae)	0	0
150	1 (eggs)	0	0
	2 (larvae)	0	0
	3 (larvae)	0	0
	4 (pupae)	0	0

Table 9. FERTILITY OF IRRADIATED AND CONTROL ADULTS OF THE CIGARETTE BEETLE*

Dose (Gy)	Number of eggs Observed	Hatched eggs No.%		Sterility index (%)
0	400	386	96.5	3.5
25	400	353	88.3	11.7
50	398	280	70.4	29.6
75	260	164	63.1	36.9
100	116	37	31.9	68.1
125	60	0	0.0	100.0
150	65	0	0.0	100.0

* Pooled results of five replicates.

Table 10. FERTILITY OF IRRADIATED AND CONTROL ADULTS OF THE DRUG STORE BEETLE*

Dose (Gy)	Number of eggs observed	Hatched eggs No. %		Sterility index (%)
0	300	290	96.7	3.3
25	300	286	95.3	4.7
50	290	283	97.6	2.4
75	200	160	80.0	20.0
100	165	45	27.3	72.7
125	180	32	17.8	82.2
150	180	27	15.0	85.0
200	160	11	6.9	93.1
250	140	0	0.0	100.0
300	128	0	0.0	100.0

* Pooled results of five replicates.

Adults of the drug store beetle irradiated with a dose of 250 Gy or 300 Gy laid eggs that did not hatch. Thus, the lowest sterilizing dose of gamma radiation for the drug store beetle seems to be 250 Gy. Brower and Tilton [4] mentioned that females of the drugstore beetle are sterilized by 300 Gy and males by doses greater than 300 Gy. In their experiment, however, males and females were irradiated separately, and paired with untreated opposites after the treatment.

The large-scale experiment (Table 11) was conducted to confirm the dose that prevents development of immatures of the cigarette beetle and the drug store beetle. Results obtained indicate that low doses of gamma radiation cause high mortality of immatures of the cigarette beetle and the drug store beetle. Doses as low as 30 Gy caused 93-94% mortality in eggs, larvae and pupae of these anobiids.

At a dose of 60 or 90 Gy several immatures of the cigarette beetle developed to the adult stage, whereas no drug store beetles reached the adult stage after irradiation with a dose of 90 Gy. At a doses of 120 Gy and higher no immatures of both species completed their development (Table 11). Thus, a dose of 120 Gy could be suggested for quarantine treatment of stored products infested with the cigarette beetle and the drug store beetle, with the assumption that the non-completion of the pest life stages is a measure of efficacy for radiation as a quarantine treatment.

Table 11. MORTALITY OF IMMATURES OF THE CIGARETTE BEETLE AND THE DRUG STORE BEETLE AFTER IRRADIATION TREATMENT (LARGE-SCALE EXPERIMENT)

Dose (Gy)	Pest species	Estimated number of irradiated eggs, larvae and pupae*	Number of adults that completed development	Mortality of immatures (%)
150	Cigarette beetle	6853	0	100.0
120	(*L. serricorne*)	5906	0	100.0
90		5763	7	99.9
60		5642	46	99.8
30		6064	364	94.0
150	Lesser grain borer	3467	0	100.0
120	(*S. paniceum*)	3953	0	100.0
90		3349	0	100.0
60		3766	31	99.1
30		2904	178	93.9

*Emergence of anobiid adults from control (non-irradiated) immatures is assumed to be 100% (mortality of immatures = 0%).

3.4. Experiments with the grain weevil, *Sitophilus granarius* (L.), and rice weevil, *Sitophilus oryzae* (L.)

Low doses of gamma radiation caused high mortality of immatures of the grain weevil and the rice weevil (Table 12). A dose of 20 Gy induced 84% mortality in eggs, larvae and pupae of the grain weevil, and 91% mortality in the rice weevil, indicating possibly higher susceptibility of immatures of the latter species to radiation.

Few adults completed their development after irradiation at doses of 40 and 60 Gy; at these doses mortality rates ranged between 99.58 and 99.94%. At doses of 80 and 100 Gy no immatures of either species completed their development (Table 12).

Table 12. MORTALITY OF IMMATURES OF THE GRAIN WEEVIL AND THE RICE WEEVIL AFTER IRRADIATION TREATMENT

Dose (Gy)	Pest species	Estimated number of irradiated eggs, larvae and pupae*	Number of adults that completed development	Mortality of immatures (%)
100	Grain weevil	2670	0	100.00
80	(*S. granarius*)	3138	0	100.00
60		1581	1	99.94
40		2643	4	99.85
20		3609	573	84.12
100	Rice weevil	4548	0	100.00
80	(*S. oryzae*)	9771	0	100.00
60		12 165	11	99.91
40		14187	59	99.58
20		7656	688	91.01

* Emergence of adults of the grain weevil and the rice weevil from control (non-irradiated) immatures is assumed to be 100% (mortality of immatures = 0%).

Thus, a dose of 80 Gy could be suggested for quarantine treatment of cereals infested with weevils with the assumption that the non-completion of pest life stages is the criterion for efficacy to provide quarantine security.

3.5. Experiments with the Angoumois grain moth, *Sitotroga cerealella* Ol.

Results presented in Table 13 indicate that the most susceptible developmental stage of the Angoumois moth is the egg, followed by the larva and pupa. A dose of 0.1 kGy inhibited the development of eggs, and young- and intermediate-aged larvae. A dose of 0.2 kGy almost completely prevented the emergence of adults from treated eggs, larvae and young pupae. At a dose of 0.3 and 0.4 kGy moths emerged from intermediate and late pupae. Thus, doses >0.4 kGy seem to be required prevent development to the adult of larvae and pupae of the Angoumois grain moth.

Cogburn et al. [6] reported that 1 kGy was nearly sterilizing to males and females, but that both males and females were fertile after pupae were treated with 1 kGy. Thus, the Angoumois grain moth is apparently the most radio-tolerant species of stored-product pest that has been studied. The results presented in Table 14, although different from Cogburn et al. [6], support this conclusion.

The longevity of moths was not affected by gamma radiation doses of 0.1–0.8 kGy, however, mortality of eggs laid by irradiated females mated to irradiated males increased with increasing dose. At a dose of 0.5 kGy only 7.7% of eggs hatched. At higher doses, however, all eggs were dead. A dose of 0.6 kGy is considered to be a sterilizing dose when both sexes are irradiated. This was confirmed with large-scale tests (Table 15): no progeny were produced after irradiating an estimated 57 013 moths at a dose of 0.6 kGy.

Table 13. MEAN NUMBERS OF ANGOUMOIS GRAIN MOTHS THAT EMERGED FROM INFESTED WHEAT SAMPLES TREATED WITH GAMMA RADIATION

Age of insects (days)	Probable stages present*	Control *vs.* treatment	Dose (kGy) 0.1	0.2	0.3	0.4
0–2	E	C	249	271	194	216
		T	0	0	0	0
2–8	E, YL	C	253	303	289	270
		T	0	0	0	0
8–12	YL, IL	C	316	187	265	278
		T	0	0	0	0
12–16	YL, IL, ML	C	217	244	327	280
		T	6	0	0	0
16–19	IL, ML, YP	C	339	328	290	300
		T	7	1	0	0
19–24	ML, YP, IP	C	267	279	265	198
		T	13	8	0	0
28–31	IP, MP	C	216	383	284	372
		T	98	25	13	3

* E = eggs, L = larvae, P = pupae, Y = young, I = Intermediate, M = mature.

Table 14. LONGEVITY, FECUNDITY AND FERTILITY OF ADULTS OF THE ANGOUMOIS GRAIN MOTH TREATED WITH GAMMA RADIATION

Dose (kGy)	Longevity of moths (days)	Number of eggs observed	% Eggs hatched
0 (control)	6.5a	300	92.3a
0.1	6.5a	300	91.3a
0.2	6.3a	298	87.3b
0.3	6.4a	300	60.3c
0.4	6.2a	310	29.7d
0.5	6.0a	300	7.7e
0.6	6.0a	300	0.0
0.7	5.8a	260	0.0
0.8	5.9a	217	0.0

Data means within a column were separated by Duncan's multiple range test at the 5% level of confidence. Means followed by the same letter did not differ significantly.

Table 15. EFFICACY OF IRRADIATION AS A QUARANTINE TREATMENT OF WHEAT GRAIN INFESTED WITH THE ANGOUMOIS GRAIN MOTH.

Dose (kGy)	Replicate	Period after which all moths were dead (days)	Number of progeny
0.6	1	6	0
	2	7	0
	3	6	0
0.4	1	8	45
	2	6	216
	3	6	179
Control	1	live moths	13 570
	2	live moths	24 690
	3	live moths	18 753

4. CONCLUSIONS

(1) A generic dose of 0.3 kGy completely inhibited the development of immatures to the adult stage of the stored products beetles tested.

(2) Species-specific doses of gamma radiation lower than 0.3 kGy can be used as a quarantine treatment of commodity infested with a single species of beetles.

(3) Stored-product moths are more resistant to the sterilizing effects of irradiation compared with stored-product beetles.

(4) A dose as high as 0.6 kGy is suggested for quarantine treatment of commodities infested by immatures and adults of lepidopteran pests.

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SOFT-ELECTRON TREATMENT AS A PHYTOSANITARY MEASURE FOR STORED PRODUCT PESTS

T. HAYASHI,* T. IMAMURA, S.S. TODORIKI, A. MIYANOSHITA, H .NAKAKITA
National Food Research Institute,
Kannondai, Tsukuba, Japan

Abstract

Developmental stages of four stored product insect pests, *Tribolium castaneum* (Herbst), *Plodia interpunctella* (Hübner), *Callosobruchus chinensis* (L.) and *Sitophilus zeamais* (Mothschulsky) were treated using "soft-electrons" (low energy electrons) with an energy of 60 keV. Soft-electrons at 60 keV effectively inactivated eggs, larvae and pupae of *T. castaneum* and *P. interpunctella* and eggs of *C. chinensis* at a dose of 1 kGy. The adults of *T. castaneum* and *P. interpunctella* were inactivated by electron treatment at 5.0 kGy and 7.5 kGy, respectively. Adults of *C. chinensis* survived at 7.5 kGy, but were inactivated having lost the ability to walk at 2.5 kGy. Soft-electrons at 60 keV could not completely inactivate the larvae of *C. chinensis* and smaller larvae (second instars) of *S. zeamais* inside beans and grains because the electrons with low penetration did not reach larvae inside the host commodity. However, soft-electrons at 60 keV inactivated eggs, larger larvae (fourth instars), and pupae of *S. zeamais* in rice grains, which indicated that *S. zeamais* was exposed to electrons even inside the grains. Grains and pulses infested with insects such as *Tribolium castaneum* (Herbst) and *Plodia interpunctella* (Hübner) are sometimes disinfested with methyl bromide (MeBr) in Japan. However, the usage of methyl bromide is to be phased out for most purposes due to its ozone depleting potential by 2005 and 2015 in developed and developing countries, respectively. Development of an alternative treatment is urgently needed. Irradiation with gamma rays or high-energy electron beams is one alternative method but sometimes adversely affect the quality of host commodities. Soft-electrons (low energy electrons at energies lower than 300 keV) with a low penetration capacity have been reported to effectively disinfect grains, pulses, spices, dehydrated vegetables, tea leaves, and some sprout seeds with considerably less quality deterioration than other disinfection techniques such as heating and irradiation with gamma-rays [1-7]. Electrons with such low energies do not require a thick safety shield due to their low penetration capacity, thus enabling less expensive in-line disinfection at food processing plants. The dose required for insect disinfestation is expected to be lower than that for disinfection. Disinfestation of grains and pulses with soft-electrons potentially will have less deleterious effects on commodity quality than irradiation with gamma rays or high-energy electrons. It is expected that soft-electrons could disinfest grains and pulses with minimal quality deterioration. The purpose of this study was to determine the applicability of soft-electrons to the inactivation of different developmental stages of various stored-product insect pests: the red flour beetle (*T. castaneum*) and the adzuki bean weevil (*Callosobruchus chinensis*) as Coleoptera, and Indianmeal moth (*P. interpunctella*) as Lepidoptera. We also investigated the effects on the maize weevil, *Sitophilus zeamais* inside rice grains.

*Present address: Japan International Research Center for Agricultural Sciences, Ohwashi, Tsukuba, Ibaraki, Japan

2. MATERIALS AND METHODS

2.1. Test insects

T. castaneum, *P. interpunctella* and *S. zeamais* were reared at 30°C, 70% relative humidity (RH), while *C. chinensis* was reared at 25°C, 70% RH. *T. castaneum* was reared on whole wheat flour, *P. interpunctella* and *S. zeamais* on brown rice, and *C. chinensis* on adzuki beans (*Vigna angularis*).

2.2. Soft-electron treatment of external feeding insects (*T. castaneum* and *P. interpunctella*)

Eggs, larvae, pupae and adults of *T. castaneum* and *P. interpunctella* were exposed directly to electrons at an acceleration voltage of 170 kV and a beam current of four μA for different periods at ambient temperature (about 20°C) in air using a Van de Graff electron accelerator. Samples were confined in open Petri dishes under the scanning horn of the accelerator at a distance of 15 cm. The dose rate was estimated to be 0.5 kGy/min based on dosimetry with an RCF film dosimeter (Far West Co. Ltd.)[8]. The energy of electrons at a distance of 15 cm from the window (50 μm thick titanium) of the scanning horn was estimated at 60 keV, based on the mass stopping powers of air and titanium [9].

2.3. Soft-electron treatment of *C. chinensis*

Fifteen adzuki beans, each with one egg of *C. chinensis*, were fixed on plastic tape with the egg side turned up in a petri dish with a netted cover and treated with soft-electrons at an accelerating voltage of 170 kV. Twenty adzuki beans, each with one larva of *C. chinensis* inside, were treated with soft-electrons in netted Petri dishes under rotation to expose the entire surface of the beans to the electrons. The adults of *C. chinensis* were directly exposed to soft-electrons.

2.4. Soft-electron treatment of rice grain infested with *S. zeamais*

Adults of *S. zeamais* were mixed with rice grains and were allowed to oviposit freely for two days. Eight grams of brown rice infested with eggs, 10-day old larvae, 17-day old larvae or 22 to 24-day old pupae were placed on the sample tray of a grain rotator [2,3] and treated with soft-electrons at 60 keV under rotation. After the treatment, the samples were maintained at 30°C and 70% RH.

2.5. Observation of the effects on DNA in cells of insect

DNA strand breakage in individual cells of *P. interpunctella* was examined by alkali-comet assay according to the method of Singh et al. [10].

3. RESULTS

The results are shown as averages of three replicates in Table 1.

3.1. Effects on *T. castaneum*

The number of larvae emerged from 20 untreated eggs was 15, while no larva emerged from eggs treated with electrons at 1 kGy or higher. Similar results were obtained for adults that emerged from the treated larvae and pupae. The numbers of adults developed from 20

untreated larvae and 15 untreated pupae were 19 and 13 respectively, while no adult developed from the larvae or pupae treated at 0.5 kGy or higher. Adults survived even after treatment at 2.5 kGy, but all adults died at 5 kGy. The results indicated that eggs, larvae, pupae and adults of *T. castaneum* could be inactivated with soft-electrons with an energy of 60 keV or higher.

3.2. Effects on *P. interpunctella*

The effects of soft-electrons on *P. interpunctella* were similar to those on *T. castaneum*. In untreated samples, 16 larvae emerged from 20 eggs, five adults developed from 20 larvae, and nine adults developed from 10 pupae. Eggs, larvae and pupae treated with soft-electrons did not develop to larvae or to adults, even following 1 kGy electron-treatment. The adults required 7.5 kGy for inactivation.

3.3. Effects on *C. chinensis*

The number of larvae emerged from 15 untreated eggs was 12, while in the treated samples no larva emerged even at 0.5 kGy. The number of adults from 20 soft-electron treated larvae was about three, irrespective of exposure time, while 19 adults emerged from untreated larvae. The adults survived even at 7.5 kGy, but all adults lost their ability to walk at 2.5 kGy.

3.4. Effects on *S. zeamais* in rice grain

No eggs or pupae developed to adults after soft-electron treatment at 5 kGy, while 57 and 54 adults developed from untreated rice with eggs and larvae, respectively. Smaller larvae (second instars) inside grains were not completely inactivated with soft-electrons at 60 keV at any dose between 0.5–7.5 kGy, whereas larger larvae (fourth instars) were completely inactivated by treatment even at 0.5 kGy. Eggs, larvae and pupae were located inside holes that the adults produced to lay eggs. Soft-electrons would get into the holes, resulting in the inactivation of eggs, larvae and pupae. All the adults died several days after treatment even at 0.5 kGy.

Some of the smaller larvae (second instars) of *S. zeamais* and the larvae of *C. chinensis* would move to the areas where soft-electrons getting into the holes produced by insects did not reach. However, apparently larger larvae (fourth instars) and pupae of *S. zeamais* were sufficiently large that soft-electrons could hit some parts of their bodies, wherever they were located in the holes.

3.5. Effects on DNA

The cell nuclei from whole bodies of *P. interpunctella* larvae were collected and subjected to electrophoresis for alkali-comet assay. Fig.1a shows the comet images for cells obtained from untreated larvae. Almost all the cells showed comet images of round shape with very small tails, indicating little or no DNA damage. Fig.1c shows comet images for cells obtained from larvae irradiated at 100 Gy with gamma-rays, where comets with very large tails were uniformly observed, indicating that the cells of whole body were homogeneously damaged and DNA strand breakage occurred. Fig.1b shows the example of comet images from the soft-electron treated larvae. Most comets had large tails, but comets with short or medium tails were occasionally observed, indicating that the DNA damage occurred non-homogenously in cells of the larvae exposed to soft-electrons.

These results indicate that the effect of soft-electrons at 60 keV is not limited to the surface layers of larvae and internal damage of inside cells should not be ruled out.

4. CONCLUSIONS AND FUTURE WORK

The results of these studies indicate that soft-electrons at 60 keV effectively inactivated the eggs, larvae, pupae and adults of *T. castaneum* and *P. interpunctella* and the eggs and adults of *C. chinensis*, when the samples were directly exposed to the soft-electrons. The eggs, larger larvae (fourth instars) and pupae of *S. zeamais* were effectively inactivated with soft-electrons, even if the samples were located inside grains. The results indicated that the insects were exposed to electrons entering the holes produced by insects. However, some of the larvae of *C. chinensis* in pulses and those of *S. zeamais* in grains were not inactivated by soft-electrons, which indicated that the larvae moved to areas where electrons did not reach in the holes. These facts suggest that insect pests residing inside grains and pulses cannot be completely inactivated by soft-electrons with low penetration depending on the locality of the insects inside grains and pulses.

It has been reported that irradiation with gamma rays causes sterilization of insect pests [11-13]. Because DNA damage occurred in cells of the soft-electron treated larvae as shown in Fig.1, it is expected that soft-electrons cause sterilization of insects.

A Japanese company has developed a “Soft Electron Processor”, a commercial-scale machine for soft-electron treatment, which can disinfect grains at 0.5-2 tonnes/h with a dose of 28 kGy and a beam current of 50 mA [14,15]. The size of the machine is 1.6 m (W) x 2.7 m (L) x 2.1 m (H) and the width of scanning beam is 45 cm. Other Japanese companies are developing different types of soft-electron machines.

Further studies are required to clarify the efficacy of soft-electron treatment for infested grains and pulses, especially on the inactivation of internal feeding insects, by using a commercial-scale soft-electron machine. We will investigate the sterility of adults treated with soft-electrons and those emerged from treated eggs, larvae and pupae, as well.

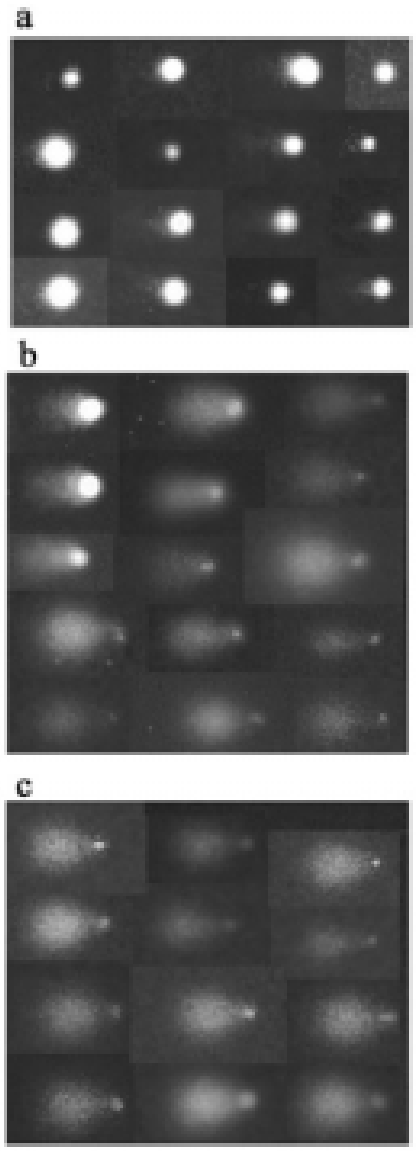

Fig. 1. DNA comet assay for the cells of *P. interpunctella* larvae.

a. Comet images of individual cells from untreated larvae;

b. Comet images of individual cells from the larvae treated with electrons at an acceleration voltage of 60 keV for 10 sec;

c. Comet images of individual cells from larvae treated with gamma rays at 100 Gy.

Table 1. SURVIVAL RESPONSES OF STORED PRODUCT INSECT PESTS AFTER EXPOSURE TO LOW ENERGY ELECTRONS

	Stage	No. of individuals	Day after exposure	Number of survivors per replicate					
				Exposure time (min)					
				Control (0 kGy)	1 (0.5 kGy)	2 (1.0 kGy)	5 (2.5 kGy)	10 (5.0 kGy)	15 (7.5 kGy)
T. castaneum	Egg	20	5	15 [a]	—	0 [a]	0 [a]	0 [a]	0 [a]
	Larva	20	17	19 [b]	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]
	Pupa	15	7	13 [b]	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]
	Adult	20	7	19 [c]	19 [c]	19 [c]	16 [c]	0 [c]	0 [c]
P. interpunctella	Egg	20	6	16 [a]	—	0 [a]	0 [a]	0 [a]	0 [a]
	Larva	20	20	5 [b]	—	0 [b]	0 [b]	0 [b]	0 [b]
	Pupa	10	8	9 [b]	—	0 [b]	0 [b]	0 [b]	0 [b]
	Adult	10	3	9 [c]	9 [c]	7 [c]	3 [c]	3 [c]	0 [c]
C. chinensis	Egg	15	7	12 [a]	0 [a]	0 [a]	0 [a]	0 [a]	—
	Larva	20	35	19 [b]	3 [b]	4 [b]	3 [b]	3 [b]	3 [b]
	Adult	10	5	10 [c] (10 [d])	10 [c] (9 [d])	10 [c] (7 [d])	9 [c] (0 [d])	3 [c] (0 [d])	3 [c] (0 [d])
S. zeamais	Egg	— [e]	50	57 [b]	1 [b]	0 [b]	0 [b]	0 [b]	0 [b]
	Larva(2^{nd})	— [e]	40	63 [b]	6 [b]	7 [b]	7 [b]	8 [b]	7 [b]
	Larva(4^{th})	— [e]	33	60 [b]	0 [b]	0 [b]	0 [b]	0 [b]	0 [b]
	Pupa	— [e]	27	54 [b]	26 [b]	18 [b]	2 [b]	0 [b]	0 [b]
	Adult	30	0.1 (2hr)	30 [c]	30 [c]	30 [c]	0 [c]	0 [c]	0 [c]
	Adult	30	7	30 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]

[a] Number of hatched larvae
[b] Number of emerged adults
[c] Number of surviving adults
[d] Number of active adults (able to walk)
[e] Free oviposition on 8 g of brown rice for two days

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IRRADIATION AS A PHYTOSANITARY TREATMENT FOR *TROGODERMA GRANARIUM* EVERTS AND *CALLOSOBRUCHUS CHINENSIS* L. IN FOOD AND AGRICULTURAL PRODUCTS

MEIXU GAO, CHUANYAO WANG, SHURONG LI, SHENGFANG ZHANG
Department of Food Irradiation,
Institute for Application of Atomic Energy,
Chinese Academy of Agricultural Sciences,
Beijing, China

Abstract

An effective irradiation quarantine treatment results in suppression of the F1 generation based on an inability to reproduce or non-completion of development of immature stages. To meet this goal, the irradiation sensitivity of different development stages of khapra beetle (*Trogoderma granarium*), large-scale testing of a proposed irradiation quarantine treatment dose, and literature evaluation of the organoleptic properties of irradiated products were investigated. From the experimental results and literature investigation, irradiation doses as low as 48 Gy prevented egg hatch; and 60 Gy prevented the development to pupae for young larvae and 100 Gy for old larvae and diapausing larvae. No successful reproduction was found after irradiating older larvae, pupae, and adults of khapra beetle with a dose of 200 Gy; therefore, the effective quarantine irradiation dose for khapra beetle was 200 Gy. The treatment efficacy at 200 Gy was 99.7% at 95% CL. The irradiation efficacy at a dose of 100 Gy for *C. chinensis* was higher than 99.95% at 95% CL. Literature also supported an effective dose of irradiation quarantine treatment for khapra beetle at 200 Gy, and 100 Gy for *C. chinensis.*

1. INTRODUCTION

Methyl bromide (MB), the most widely used fumigant to control insects in food and agricultural commodities, is being phased out globally in the near future. Agricultural products and food that are possibly infested by quarantine pests need an alternative measure to maintain trade. Irradiation offers promise as a disinfestation method for agricultural products and food. There are considerable data on irradiation as a quarantine treatment against tephritid fruit flies, but little data on the use of this technology against other arthropod pests of quarantine importance.

Among stored-product pests, khapra beetle, *Trogoderma granarium* Everts is a high priority pest. This beetle is a serious quarantine pest that occurs on stored cereals and cereal products, dried fruits, nuts, and a rather wide variety of foodstuffs. It can cause complete destruction of grain and pulses in a short time under hot, dry conditions. The larvae can diapause for two to eight years under unfavorable conditions. Chemical control of the khapra beetle with both contact insecticides and fumigants is difficult [1]. All life stages infest the commodity. Because this beetle has limited mobility, it spreads principally through the agency of man [2]. Khapra beetle is considered an important quarantine pest in China, and is often intercepted at the ports of entry.

The weevil *Callosobruchus chinessis* L. is another common pest in China and causes serious damage to stored pulses. *C. chinensis* is a quarantine pest for exports to Russia and other countries. The pest has four to six generations per year in northern China. All life stages infest the commodity. It takes about 20–21 days for one generation under favorite conditions of 31±1^{o}C and 70±5% RH [3].

An effective irradiation quarantine treatment results in suppression of the F1 generation based on an inability to reproduce or non-completion of development of immature stages for khapra beetle and *C. chinensis* [4]. To meet this goal, the irradiation sensitivity of different development stages of khapra beetle, large-scale testing of a proposed irradiation quarantine treatment dose, and literature evaluation of the organoleptic properties of irradiated products were investigated.

2. MATERIAL AND METHODS

2.1. *T. granarium*

2.1.1. Establishing lab rearing method of khapra beetle

The khapra beetle colony used in this research was started from individuals intercepted at a port of entry by the quarantine department. The pest was reared in the plant quarantine lab of the Institute of Plant Quarantine. Rearing follows Yinon (1968) [5]. The pest was reared with broken wheat in 12×20×5cm enamel trays with enamel covers. The trays were kept in a darkened incubator at 35± 1^{o} C and 40±5% RH. The results of observations showed that it takes about 26 days for the development from egg to old larva, 31 days for the development to pupae, and 40 days for the development to adults.

2.1.2. Collecting different development stages of khapra beetle

Khapra beetle eggs and young larvae were too small to be counted; therefore, unknown numbers of egg and young larvae were treated. The samples were prepared as follows: about 30 female adults and several male adults were placed in covered plates, then removed after two days of oviposition. Eggs samples were prepared after two days culture, and samples of young larvae after ten days [6]. The number of insects in each sample was between 50–150.

For old larvae, pupae, and adults of khapra beetle, samples were prepared by introducing adults on a layer of broken wheat in trays. After two days the adults were removed. The trays were kept at the above-mentioned rearing conditions, and cultured for 26, 31 and 40 days to collect the development stages of old larvae, pupae, and adult. Each sample had 50 beetles, and two replicates were conducted for each treatment [7].

For the diapausing larvae of khapra beetle, samples were produced by holding instars two to four in a culture plate, without media, at 15±1^{o}C and 20±5% RH for seven days. Each sample had 50 beetles, and each treatment was replicated twice.

All samples were transferred to tubes (15×200 mm) with broken wheat for irradiation.

2.1.3. Large-scale experiments

Ten pairs of adults were placed on a layer of broken wheat in Petri dishes to collect eggs of khapra beetle and removed after three days. This was repeated at 0, 9, 18, and 27 days, and irradiation was applied at 32 days rearing so that it should include all development stages (egg, larvae, pupae) of khapra beetle. One dish was the control and the other four dishes were irradiated at 200 Gy. After irradiation, the samples were kept in an incubator at rearing conditions. Every day the samples were checked and adults removed and recorded. The experiment was repeated twice.

2.2. *C. chinensis*

2.2.1. Establishment of lab rearing method

C. chinensis were collected from infested mung bean at a local market in May 2001. An expert from the quarantine department identified the pest.

Mung bean irradiated at 1 kGy (to make the mung bean insect free) was used as the lab culture material. Samples were prepared as follows: male and female adults of *C. chinensis* were transferred to the culture material and cultured at 31±1^{o}C and 70±5% RH until the mung beans were heavily infested.

2.2.2. Investigation of Effective Irradiation quarantine dose for C. chinensis

Thirty adults were placed on mung beans in Petri dishes to collect eggs of *C. chinensis*, and then removed after three days. This was repeated at 0, 5, 10 and 15 days. Irradiation was applied at 20 days rearing, so that all development stages of the pest would be included. Two dishes were kept as controls and four dishes each were treated with 100 and 150 Gy. After irradiation, both control and irradiated samples were kept in an incubator at the above conditions. Every day the samples were checked and adults removed and recorded. The experiment was repeated twice.

2.2.3. Large-scale test

About 2 kg of mung beans heavily infested by all developmental stages of *C. chinensis* was divided into twelve parts, each containing 155 g. Each part was kept in an 8 cm-diameter glass bottle with the top covered by screen. Two bottles were controls and the other ten were irradiated at 100 Gy. After irradiation, both control and irradiated samples were cultured at the above conditions. Every day the samples were checked, and the adults in the samples were removed and recorded. The experiment was repeated three times.

2.3. Irradiation

All irradiation experiments were conducted using a Co-60 research irradiator at the Institute for Application of Atomic Energy, Chinese Academy of Agricultural Sciences. Fricke dosimeters were used for dose calibration of the irradiator. The dose mapping followed national standards. The absorbed dose rate was between 10-15 Gy/min.

3. RESULTS AND DISCUSSION

3.1. *T. granarium*

3.1.1. Eggs

No larvae emerged among irradiated samples (Table 1); ten days after irradiation, the number of hatched eggs in untreated samples was 82. Irradiation at the dose of 100 Gy stopped egg hatch. The dose for stopping emergence of adults should be lower than 100 Gy. Ahmed (1975) reported that 38.8-48.5 Gy was 100% lethal to the eggs [8].

3.1.2. Young larvae

Young larvae were irradiated with broken wheat. One day after irradiation, about half of the larvae in the 500 Gy treatment became dark (Table 2). Irradiation at 500 Gy killed larvae within three days. Three days after irradiation, the death rate of the 100 Gy-treated samples was 50%. Live larvae could be found in the 100 Gy samples 12 days after irradiation, but the larvae developed slower than in the control.

More detailed results are shown in Table 3 and Figures 1 and 2. The survival rate and survival time decreased with the increase of irradiation dose when young larvae were treated at the dose range of 60-150 Gy. Compared with the control, irradiation at 30 Gy slowed the development of young larvae to pupae and adults, but did not stop the development to adult. Irradiation at 60 Gy stopped the development of young larvae to pupae.

Table 1. SURVIVAL OF KHAPRA BEETLE EGGS AFTER IRRADIATION

Days	0	100 Gy	200 Gy	300 Gy	500 Gy
10	82	0	0	0	0

Table 2. SURVIVAL RATE OF YOUNG LARVAE OF KHAPRA BEETLE AFTER IRRADIATION

Days	0	100 Gy	200 Gy	300 Gy	500 Gy
1	100%[1]	90%	90%	80%	50%
3	100%	50%	30%	10%	0
10	100%	33%	18%	0	0
12	100%	10%	2%	0	0

Table 3. EFFECTS OF IRRADIATION ON THE DEVELOPMENT OF YOUNG LARVAE OF KHAPRA BEETLE

Dose Gy days	0			30			60	90	120	150
	larvae	pupae	adults	larvae	pupae	adults	larvae	larvae	larvae	larvae
8	—[1]	—	—	—	—	—	73.9[2]	61.0	55.8	57.7
15	4.8	47.5	2.7	98.0	1.0	1.0	48.0	14.6	0	0
22	32.6	39.1	28.3	92.7	6.1	1.2	34.3	0	0	0
29	5.4	17.4	77.2	64.4	26.9	8.7	0	0	0	0
35	—	—	—	27.4	2.5	70.1	0	0	0	0

1. the data in the table was the percent of survival insect.

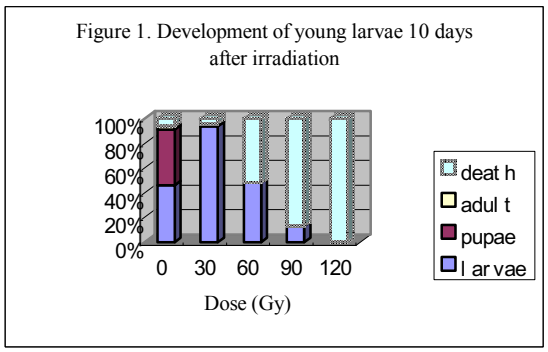


Figure 1. Development of young larvae 10 days after irradiation

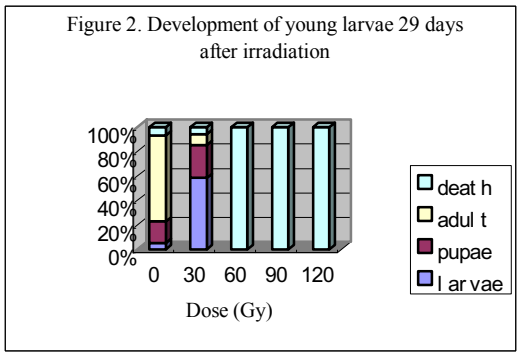


Figure 2. Development of young larvae 29 days after irradiation

3.1.3. Old larvae

Older larvae were irradiated with broken wheat. Seven days after irradiation, the ability of larvae to move and eat after treatment with a dose of 1000 Gy was obviously decreased. But there were no obvious differences between untreated and other treated samples. Twenty-one days after irradiation (Table 4), adults were only found in the control. Two to five pupae were found in the 200–800 Gy treatments, and no pupation occurred at 1000 Gy. There were still live larvae in both treated and untreated samples. It seemed that irradiation at 200–800 Gy affected development of larvae to pupae. 1000 Gy irradiation could kill the larvae within 25 days.

More detail results are shown in Table 5 and Figures 3 and 4. The survival rate and the survival time decreased with increasing irradiation dose when young larvae were treated at 50–300 Gy. Compared with the control, irradiation with a dose of 50 Gy slowed the development of old larvae to pupae and adults, but could not stop the development to adult. Irradiation at 100 Gy stopped the development of young larvae to pupae.

Table 4. DEVELOPMENTAL STAGE OF OLDER LARVAE OF KHAPRA BEETLE 25 DAYS AFTER IRRADIATION

Irradiation dose (Gy)		0	200	400	600	800	1000
25	Larvae	17	30	32	38	13	0
days	Pupae	5	3	4	4	5	0
	Adults	17	0	0	0	0	0

Table 5. THE DEVELOPMENT OF OLDER LARVAE OF KHAPRA BEETLE AFTER 0–300 GY IRRADIATION

Dose Gy days	0			50			100	150	200	300
	larvae	pupae	adults	larvae	pupae	adults	larvae	larvae	larvae	larvae
5	57.4	42.6	0	86.0	14.0	0	91.0[1]	76.0	60.0	57.0
8	8.0	69.3	22.7	60.0	27.0	6.0	—	—	—	—
15	12.0	4.0	84.0	47.0	7.0	39.0	80.0	47.0	17.0	9.00
22	—	—	—	24.0	2.0	47.0	66.0	29.0	10.0	5.0

[1]The data in the table was the percent of survival insect.

3.1.4. Diapaused larvae

Diapaused larvae were irradiated with broken wheat. The survival rates of individuals in treated samples decreased with the increasing of irradiation dose (Table 6). There was no pupation when the irradiation dose was >150 Gy and the pupation rate was only 2.5% at 100 Gy. Adult eclosion happened only in the 50 Gy and control treatments. But eclosion at 50 Gy was delayed about three weeks, and the eclosion rate was lower than in the control.

The irradiation effects on the eclosion of old larvae and diapausing larvae are similar (Figures 5 and 6). When these two samples were treated at 50 Gy, the final eclosion rate of both samples was about 40-50%; however, the response time for diapausing larvae was delayed for almost 30 days.

3.1.5. Pupae

The eclosion rate of pupae six days after irradiation is shown in Table 7 and Figure 6. The eclosion rate decreased with increasing irradiation dose. There was no eclosion in the 1000 Gy treatment. All of the samples were cultured at lab rearing conditions for 35 days, and young larvae were found only in untreated samples; therefore, an irradiation dose of 200 Gy applied to pupae prevented reproduction. For the irradiation effect on the pupae of Khapra beetle, Afify and Gharib (1995) reported that the sterilizing dose of gamma radiation was 200 Gy for male pupae and 60 Gy for female pupae [9].

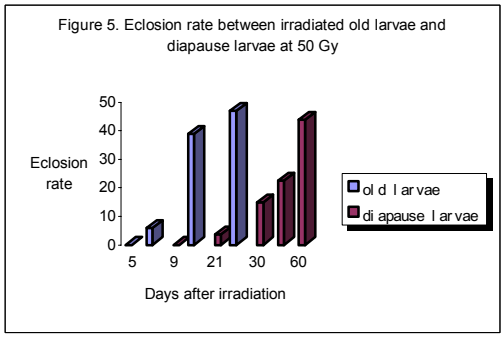
Table 6. THE DEVELOPMENT (%) OF DIAPAUSED KHAPRA BEETLE LARVAE AFTER 0–300 GY IRRADIATION

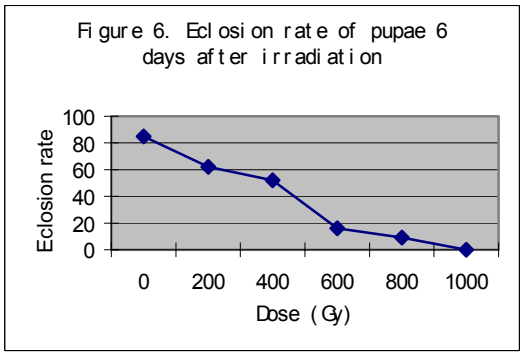
Dose Gy / Days	0			50			100		150	200	300
	larvae	pupae	adults	larvae	pupae	adults	larvae	pupae	larvae	larvae	larvae
9	93.3	6.6	0	86.3	11.3	0	97.5	2.5	100	100	100
21	76.7	23.3	0	85.0	8.75	3.75	78.8	2.5	68.8	46.3	56.3
30	—	30.2	56.7	—	18.3	15.0	38.7	2.5	25.0	28.9	30.0
42	—	—	71.1	69.0	24.7	22.5	20.0	2.5	6.25	7.5	15.0
60	—	—	—	—	—	43.8	—	—	—	—	—

The data in the table was the percent of survival insect

Table 7. ECLOSION OF IRRADIATED OF KHAPRA BEETLE PUPAE (NUMBERS)

Irradiation dose (Gy)		0	200	400	600	800	1000
Days	3	5	6	8	5	1	0
	6	35	31	26	8	5	0
	15	38	36	26	8	5	0





3.1.6. Adults

The irradiation effects on adults are shown in Table 8. There was no obvious difference in mortality between treated and untreated adults after six days. Twenty days after irradiation, young larvae were found only in the control and not in treated samples. Carney [10] reported that sterilizing doses for male and female adults of *Trogoderma granarium* Everts was 160 Gy and 60 Gy, respectively. Our results suggested irradiation at 200 Gy could prevent reproduction of adults.

3.1.7. Effective quarantine irradiation dose for khapra beetle

From the experimental results and literature investigation, a irradiation dose as low as 48 Gy could stop the hatching of eggs; and 60 Gy irradiation for young larvae and 100 Gy for old larvae and diapause larvae could prevent the development to pupae. It seems that 200 Gy was sufficient for sterilization, because irradiation at a dose of 200 Gy resulted in no older larvae, pupae, and adults; therefore, the effective quarantine irradiation dose for khapra beetle was 200 Gy. The irradiation sensitivity between old larvae and diapausing larvae was almost identical, but the development of diapausing larvae was slower than nondiapausing larvae in untreated controls and lower dose irradiation treatments [11].

3.1.8. Large number test

Adults continued emerging from the control sample for eight weeks. The number of emerged adults from untreated controls was 320 for experiment I, and 384 for experiment II (Table 9). No emergence was observed from irradiated samples.

Table 8. EFFECTS OF IRRADIATION ON KHAPRA BEETLE ADULTS

Irradiation dose (Gy)	0	200	400	600	800	1000
No. of live adults after irradiation 6 days	50	50	50	50	50	50
No. of larvae after irradiation 20 days	Many	0	0	0	0	0

Table 9. LARGE-SCALE EXPERIMENT WITH KHAPRA BEETLE

Time (week)	1	2	3	4	5	6	7	8	Total No. - one dish	Total No. - four dishes
Experiment I	2	12	6	7	8	2	25	18	80	320
Experiment II	2	15	9	16	23	4	21	6	96	384

From the above results, there were no survivors from 604 treated beetles at 200 Gy. Because khapra beetle is an important quarantine pest in China, there is difficulty in obtaining larger numbers of pests to test. Overall, the treatment efficacy was 99.7% at CL 95%, i.e., no survivors from 604 pests [7].

3.2. C. chinensis

3.2.1. Literature review

The irradiation effects on the development of *C. chinensis* have been studied since the 1970s [11]. Yoshida [12] reported that the sterilizing dose of irradiation for *C. chinensis* was 90 Gy. The paper concluded that irradiation at 150 Gy for larvae and pupae could decrease emergence but could not prevent it [13]. Bhuiya et al. [14] found that 50 Gy irradiation caused the mortality of the fourth instar. Gill and Pajni [15] found that adults and older pupae were not killed by irradiation treatment at 80 Gy but were rendered completely sterile. Yang [16] studied the effects of irradiation on adults and found that 110 Gy applied to female adults decreased oviposition by 97.4% compared with the control. The literature review suggested that irradiation at about 100 to 150 Gy might be sufficient to prevent reproduction and stop development of immature stages of *C. chinensis*.

3.2.2. Effective irradiation quarantine dose for C. Chinensis

No survivors occurred in the 100 Gy and 150 Gy irradiation treatments, whereas about 180.5 adults appeared in the control sample; therefore, irradiation at 100 Gy will be effective in causing non-completion of the development of immature stages of *C. chinensis*.

Table 10. EFFECTIVE IRRADIATION QUARANTINE DOSE FOR *C. CHINENSIS*

Time (days)	3	4	5	6	7	8	9	10	11	12	13	Total
Control	2.0	11.5	26.0	29.0	26.5	25.0	27.0	9.5	21	1.5	0.5	180.5
100 Gy samples	0	0	0	0	0	0	0	0	0	0	0	0
150 Gy samples	0	0	0	0	0	0	0	0	0	0	0	0

Table 11. NUMBER OF DEAD *C. Chinensis* ADULTS AFTER IRRADIATION TREATMENT WITH 100 GY

No.	1	2	3	4	5	6	7	8	9	10	Average per sample
Experiment I	177	283	159	214	199	219	271	363	146	199	223.0
Experiment II	107	42	93	135	75	181	75	31	28	119	88.6
Experiment III	80	85	136	81	171	238	66	118	87	219	128.1

Table 12. NUMBER OF ECLOSING *C. Chinensis* ADULTS IN THE CONTROL TREATMENT

Time (days)	2	4	6	8	10	12	14	16	18	20	22	24	Total
Experiment I	250	40	40	12	40.5	44	72	97.0	97	72	31.5	18.5	820.5
Experiment II	56	34	74	149	318.0	648	654	2.4	74	70	23.0	1.0	2335.0
Experiment III	37	64	30	23	17	65	78	52.0	16	8	2.0	—	447.0

Table 13. ESTIMATED NUMBER OF *C. Chinensis* KILLED BY 100 GY FOR THE THREE EXPERIMENTS

	Total no. adults in control	Average no. dead adults in treated samples	No. insects surviving	No. pests not surviving for 10 replicates	Total pests not surviving for the large-scale test
Experiment I	820.5	223.0	597.5	5975	
Experiment II	2335.0	88.6	2246.4	22 464	31 628
Experiment III	447.0	128.1	318.9	3 189	

3.2.3. Large-scale experiment

Large-scale experiments were repeated three times. The experiment continued for 20 to 24 days until no more adults emerged in the control samples. The adults in treated samples were dead eight to eleven days after irradiation, and no survivor was observed for all treated samples.

Because newly eclosed adults stay inside the mung bean for some time, it is difficult to remove all of the adults before the experiment. All samples contained egg, larvae, pupae, and adult development stages. The number of dead adults in irradiated samples was counted 20 days after irradiation (Table 11). The numbers of adults removed from control samples for 24 days are in Table 12. From the data in Table 11 and Table 12, the total number for confirming that irradiation of 100 Gy was an effective dose for *C. chinensis* was calculated in Table 13. The total number for large-scale test was 31 628. The treatment efficacy was higher than 99.95% at CL 95%.

LITERATURE EVALUATING THE ORGANOLEPTIC QUALITY OF IRRADIATED PRODUCTS

The safety of irradiated food has been thoroughly investigated and accepted by many countries and international organizations. Nowadays there are about 20 countries with standards and regulations for irradiated cereals and legumes and their products for the purpose of disinfestation. More than half of the countries have approved an irradiated dose for grains and legume of 1 kGy, while other countries have approved treatments between 0.3–0.7 kGy. Considering the uniformity of irradiation processing, 100 Gy and 200 Gy irradiation treatments are accepted for the two quarantine pests.

In terms of the organoleptic properties of irradiated wheat and wheat products, Zhu Chengxiang [17]. reported that 1 kGy irradiation has almost no effect on the baking properties of wheat. Rao et al. [18] found that the baking properties of Indian wheat could be improved by irradiation under 2 kGy.

For the organoleptic properties of irradiated legumes, Rao et al. [19] reported that the water-absorption capacity of irradiated legume samples increased. Irradiation at 2.5–10.0 kGy of legume samples caused a significant reduction in cooking time compared with controls. Sensory evaluation of cooked un-irradiated and irradiated samples at 5 kGy revealed no significant differences in acceptability [19]. Machaiah et al. [20] reported that irradiation at insect disinfestations dose levels improved the digestibility and nutritional quality of mung beans by reducing the content of oligosaccharides responsible for intestinal gas production. Myung-Woo Byun and II-Jun Kang [21] claimed that an irradiation dose of 5 kGy caused an increase in yield of soya milk and tofu while having very little effect on their quality; the properties of tofu prepared with the soya beans irradiated at 2.5–5 kGy showed no significant difference from the non-irradiated control [21].

All the literature relevant to the quality of irradiated cereal and legumes has the consistent results that irradiation at disinfestations dose has no obvious effect on the organoleptic properties of irradiated products. The applied quarantine dose is equal or lower than disinfestations dose, it could be concluded that 100 Gy irradiation for legumes and 200 Gy irradiation for cereal and its products will have no effects on the organoleptic properties of irradiated products.

4. CONCLUSIONS

From the experimental results and literature, irradiation doses as low as 48 Gy could stop the hatching of eggs; irradiation at 60 Gy for young larvae and 100 Gy for old and diapausing larvae prevented development to pupae. Irradiation at 200 Gy prevented reproduction of pupae and adults. Therefore, the effective quarantine irradiation dose for khapra beetle was 200 Gy. In a large-scale test for khapra beetle, an estimated 604 insects were irradiated at a dose of 200 Gy with no survivors (efficacy = 99.7% at CL 95%).

For *C. chinensis,* the literature indicated that 100 Gy might be an effective quarantine dose. In our large-scale test, an estimated 31 628 insects were irradiated at 100 Gy with no survivors (efficacy = 99.95% at CL 95%).

The literature supported our finding that the effective irradiation quarantine treatment doses for khapra beetle and *C. chinensis* were 200 Gy and 100 Gy respectively.

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INHIBITION OF EGG AND LARVAL DEVELOPMENT OF THE INDIANMEAL MOTH *PLODIA INTERPUNCTELLA* AND FIG MOTH *EPHESTIA CAUTELLA* BY GAMMA RADIATION OF DECORTICATED HAZELNUTS

B. AKINBINGOL, E. DENLI, E. IC, M. ALABAY
Ankara Nuclear Research Centre for
Agriculture and Animal Science,
Turkish Atomic Energy Authority, Ankara, Turkey

Abstract

The effects of irradiation on inhibition of egg, larval, and pupal development of the Indianmeal moth and fig moth in hazelnuts were investigated. Irradiation doses required to inhibit development of eggs of *P. interpunctella* and *E. cautella* were 450 Gy and 300 Gy, respectively. In large-scale tests, irradiation treatment of a composite of immature stages of Indianmeal moth or fig moth at a dose of 1 kGy resulted in no adult emergence. Adults emerging from samples irradiated at 0.25 kGy and 0.5 kGy produced no viable eggs, indicating sterility. The low-dose irradiation treatment of decorticated hazelnuts did not cause excessive oxidative deterioration, and no significant differences were found in organoleptic properties after treatment at doses of 0.5–3.0 kGy; however, total tocopherol content decreased depending on irradiation dose.

1. INTRODUCTION

Turkey is the largest producer of hazelnuts, producing more than two thirds of the world supply. Ninety-five percent of production is exported. Hazelnut orchards are widespread along the Black Sea shores of Anatolia, and this region alone produces 70% of the world's hazelnuts. Turkey exported mainly shelled and kernel forms to 32 countries before 1980. In recent years, Turkey has exported hazelnuts and processed forms of hazelnut products to 75 countries. Total hazelnut exports from Turkey were 543 million dollars in 2000 [1].

Besides its economic value, hazelnuts are also nutritious. Hazelnuts contain amino acids; vitamins such as Vit. B_1, Vit. B_2, Vit. B_6, pentatonic acid, niacin, and Vit. E; minerals such as Fe, Ca, Mg, Mn, K, Zn, Cu, P; and monounsaturated fatty acid (oleic) and polyunsaturated fatty acids (linoleic and linolenic). Hazelnuts are also a source of MUFAs, PUFAs, and natural sterols. Hazelnuts are mainly consumed as a major raw material in the chocolate, confectionary, and baking industries, and an ingredient in edible nut mixes [2–6].

The largest union of producers and a private company purchase, process, and pack most of the hazelnuts produced in Turkey. The package size varies from 100 g to 75 kg. Package materials used are vacuumed polyethylene and jute sacks. One of the main problems in the export trade is infestation by stored product insects. The fig moth, *Ephestia cautella* (Walk.) is a quarantine pest, and the Indianmeal moth, *Plodia interpunctella* (Hubner) damages hazelnuts, lowering the quality of the product, and limiting the exports due to its presence as a contaminant. Infestations are seen between May and October. Infestation by these pests results in repeated fumigations, usually with methyl bromide, to comply with the requirements of importing countries. Methyl bromide fumigation rates are 48, 32 and 24 g/m^3 at temperatures of 4–9, 15–20 and 25° C, respectively. Irradiation could offer an important and attractive alternative to methyl bromide or other chemicals that leave chemical residues [7–8]. The objectives of the present study were to determine the irradiation doses to inhibit egg hatch and development of immature stages of *Ephestia cautella* and *Plodia interpunctella*.

2. MATERIALS AND METHODS

Adults and larvae of Indianmeal moth and fig moth were collected from the Integrated Hazelnut Processing Plant in Fiskobirlik (Union of Agricultural Cooperatives for Sale of Hazelnuts), established in Giresun province in the Black Sea Region of Turkey. Indianmeal moth (*Plodia interpunctella)* and fig moth (*Ephestia cautella)* were reared in an insectarium at 27 ± 2° C and 70 ± 5 % relative humidity on natural and blanched whole hazelnut kernels.

2.1. Determining an irradiation dose that inhibits egg hatch

A two-part container was used for egg production. Insects obtained from the culture medium were placed in the first container, which had a plastic sieve at the bottom, then sieved into the second container after 24 hours. Eggs were then placed individually into micro-wells (3 mm in diameter) of Plexiglas plates by means of a thin brush using a stereoscopic microscope, and covered with a piece of plastic stretch film. The dose range was identified in a preliminary "range finding" test by exposing eggs to 0–1.5 kGy using a Cobalt 60-source, 4870 Ci irradiator with a dose rate of 3.02 kGy/h (as determined by Fricke dosimetry). An equal number of eggs were left untreated as controls in each treatment. Preparation and irradiation took place at ambient temperatures. Forty-eight-hour old eggs were irradiated at 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 Gy. The test was repeated three times at each dose. Eggs were inspected for hatch each day for seven days.

Mortality data were subjected to analysis of variance after correcting for control mortality using Abbott's formula. Means were separated using the Duncan's Multiple Range Test ($P < 0.05$). Dose-mortality response lines were estimated using probit analysis in SPSS.

2.2. Determining an irradiation dose that inhibits the development of immature stages

To determine and confirm the irradiation dose inhibiting the development of immature stages of moths in decorticated hazelnuts, the dose range (0 kGy, 0.25 kGy, 0.5 kGy, 0.75 and 1 kGy of gamma radiation doses) was identified by preliminary tests. Five jam jars, each containing approximately 500 grams of crashed hazelnuts, were used for the irradiation tests. Ten moths were placed in each jar every week for four weeks. At the end of the fourth week, jars containing immature stages of moths (eggs, various stages of larvae, and pupae) were treated with radiation at doses of 0, 0.25, 0.5, 0.75 and 1 kGy. Jars were inspected for moths each day for 30 days after irradiation.

2.3. Preparation and processing of hazelnut samples for quality analysis

The effect of irradiation on the organoleptic quality of hazelnuts was investigated. Roasted 'Tombul' hazelnuts were supplied by the Hazelnut Research Institute (1999 and 2000 harvest seasons; Giresun, Turkey). To prevent rancidity, the samples were packaged in vacuum pouches (30 µ DURETHANE PK-38 (polyamide), 10 µ SURLYN ionomer, 70 µ polyethylene) using the Multivac Vacuum Sealer Model A 300/22. The bags were divided into six groups: one bag of the group was chosen as a control, and the others were irradiated at 0.5, 1.0, 1.5, 2.0, or 3.0 kGy. All treatments were at ambient temperature and at a fixed dose rate of 2.72 kGy/h using a Co-60 gamma irradiator (PX-γ-30 Isslodovateji). After treatment, the unirradiated and irradiated samples were stored at 15±2^{o}C and 50±3 % relative humidity, and analysed after 0, three, and six months storage. All analyses were performed in triplicate.

2.4. Chemical analyses

Moisture, ash, protein (NX5.30), crude fat, and crude fiber content were determined using AOAC Standard Methods 925.40, 900.02, 955.04, 948.22, and 962.09, respectively [9].

A cold extraction method was used to obtain hazelnut oil. For this purpose, a 250 g homogenized sample was transferred into a 1 liter flask, and 500ml of pure n-hexane was added. The solution was left at room temperature overnight and filtered through a filter paper. The n-hexane was removed by rotary evaporator before analysis.

Total free fatty acidity values were determined using the AOCS Official Method [10]. The acetic acid-chloroform method was used to determine peroxide values [11]. The Wijs method was used to determine iodine values [12]. The Kreiss rancidity test, based on measurement of a red color resulting from the reaction with phloroglucinol, was carried out using the AOAC Standard Method [9]. Total tocopherol content was determined at 520 nm using a UNICAM UV-200 spectrometer [13].

2.5. Sensory evaluation

Sensory evaluation of unirradiated and irradiated hazelnut samples was carried out after 0, three, and six months storage [14]. Samples were presented to a panel of ten judges selected from the staff of our research center. The judges were asked to evaluate all samples for their visual colour, aroma as determined with their nose, and texture as determined by mouth feel, on a scale of 1 to 5, with 5 being the most preferred score. The average score for these parameters was calculated and used as a general score for statistical analysis.

2.6. Statistical analyses

Experiments were replicated at least three times. Data for each attribute were subjected to analysis of variance using the GLM procedure in SAS. Mean differences were determined using the Least Significant Difference (LSD) procedure at $P<0.05$ [15].

3. RESULTS AND DISCUSSIONS

3.1.Results of egg experiments

The results of irradiation of eggs are summarized in Table 1 for *P. interpunctella* and in Table 2 for *E. cautella.* For both species, egg hatch decreased with increasing dose. For *P. interpunctella* the percentage of unhatched eggs was 66.8% at 100 Gy, 92.41% at 300 Gy, and 100% at 450 and 500 Gy. Fig moth eggs were more sensitive to radiation than Indianmeal moth eggs; the percentage of unhatched eggs was 97-98% between 50 Gy to 250 Gy, and 100% (total mortality) at 300 Gy. It was noted that non-viable eggs were darkened (dark orange in colour) and sunken on one side due to irradiation.

Table 1. EFFECT OF IRRADIATION ON EGGS OF *Plodia interpunctella*

Dose (Gy)	**REPLICATES**	**Number of** eggs	**Number of hatched eggs on 7th day**	**Rate of hatchability (%)**	**Means of hatchability (%)**	**Total mortality * (%)**
0	1	50	47	94.00	90.63±9.3 **a**	10.34 **a**
	2	56	45	80.36		
	3	54	53	98.15		
	total	**160**	**145**			
50	1	50	21	42.00	50.97±8.63 **b**	45.50 **b**
	2	51	26	50.98		
	3	54	32	59.26		
	total	**155**	**79**			
100	1	50	18	36.00	30.38±7.66 **c**	66.8 **c**
	2	55	12	21.82		
	3	53	18	33.96		
	total	**158**	**48**			
150	1	50	10	20.00	20.26±3.52 **cd**	73.79 **cd**
	2	50	12	24.00		
	3	53	9	16.98		
	total	**153**	**31**			
200	1	50	10	20.00	15.58±5.23 **de**	83.44 **de**
	2	51	5	9.8		
	3	53	9	16.98		
	total	**154**	**24**			
250	1	50	4	8.00	10.46±7.24 **ef**	88.96 **ef**
	2	48	2	4.17		
	3	55	10	18.18		
	total	**153**	**16**			
300	1	50	4	8.00	7±2.66 **fg**	92.41 **fg**
	2	51	2	3.92		
	3	56	5	8.93		
	total	**157**	**11**			
350	1	50	2	4.00	5.9±3.13 **fg**	93.79 **fg**
	2	50	2	4.00		
	3	53	5	9.43		
	total	**153**	**9**			
400	1	50	2	4.00	3.18±2.78 **g**	96.55 **g**
	2	51	0	0.00		
	3	56	3	5.36		
	total	**157**	**5**			
450	1	50	0	0	0.00 **h**	100 **h**
	2	52	0	0		
	3	51	0	0		
	total	**153**	**0**			
500	1	50	0	0	0.00 **h**	100 **h**
	2	50	0	0		
	3	55	0	0		
	total	**155**	**0**			

* Calculated by Abbott's formula

Means in a column with the same letter are not significantly different at $P < 0.05$, Duncan's Multiple Range Test.

Table 2. EFFECT OF IRRADIATION ON EGGS OF *Ephestia Cautella*

Dose (Gy)	**REPLICATES**	**Number of eggs**	**Number of hatched eggs on 7th day**	**Rate of hatchability (%)**	**Means of hatchability (%)**	**Total mortality * (%)**
0	1	134	130	97.02	92.58 ± 5.6 **a**	0.7 **a**
	2	130	110	84.62		
	3	128	123	96.09		
	total	**392**	**363**			
50	1	133	3	2.26	2.72 ± 0.3 **b**	97.24 **b**
	2	134	4	2.99		
	3	103	3	2.91		
	total	**370**	**10**			
100	1	131	8	6.11	3.35 ± 2.1 **b**	96.42 **b**
	2	111	1	0.90		
	3	132	4	3.03		
	total	**374**	**13**			
150	1	131	5	3.82	2.11 ±1.2 **b**	97.79 **b**
	2	130	2	1.54		
	3	104	1	0.96		
	total	**365**	**8**			
200	1	135	4	2.96	2.12 ± 0.6 **b**	97.79 **b**
	2	133	2	1.50		
	3	105	2	1.90		
	total	**373**	**8**			
250	1	130	4	3.08	1.84 ± 0.9 **b**	98.07 **b**
	2	108	1	0.92		
	3	131	2	1.53		
	total	**369**	**7**			
300	1	135	0	0	0 **c**	100 **c**
	2	106	0	0		
	3	103	0	0		
	total	**344**	**0**			
350	1	128	0	0	0 **c**	100 **c**
	2	113	0	0		
	3	105	0	0		
	total	**344**	**0**			
400	1	130	0	0	0 **c**	100 **c**
	2	131	0	0		
	3	104	0	0		
	total	**365**	**0**			
450	1	131	0	0	0 **c**	100 **c**
	2	122	0	0		
	3	105	0	0		
	total	**358**	**0**			
500	1	131	0	0	0 **c**	100 **c**
	2	101	0	0		
	3	102	0	0		
	total	**334**	**0**			

* Calculated by Abbott's formula

Means in a column with the same letter are not significantly different at $P < 0.05$, Duncan's Multiple Range Test.

3.2. Results of large-scale tests

Results of large-scale tests with immature stages of *P. interpunctella* and *E. cautella* are shown in Tables 3 and 4. For both species, emergence of adults decreased with increasing irradiation dose. No adults emerged in samples irradiated at 1 kGy for either moth species; however, adults emerging from samples irradiated at 0.25 kGy and 0.5 kGy produced no viable eggs, indicating sterility.

Table 3. INHIBITING RADIATION DOSES ON THE DEVELOPMENT OF IMMATURE STAGES OF *P. interpunctella* BY TESTING LARGE-SCALE TEST

Treatment dose (kGy)	No. of adult emerged
control	5386
0.25	359
0.50	203
0.75	2
1.00	0

Table 4. INHIBITING RADIATION DOSES ON THE DEVELOPMENT OF IMMATURE STAGES OF *E. cautella* BY TESTING LARGE-SCALE TEST

Treatment dose (kGy)	No. of adult emerged
control	4868
0.25	32
0.50	22
0.75	3
1.00	0

Cogburn et al. studied effects of six gamma radiation dosages from five to 100 krad on all metamorphic stages of *Cadra cautella.* Irradiation doses of 30 and 20 krad prevented development to the adult stage from treated eggs and larvae, respectively. Some adults emerged from treated pupae at all irradiation levels except 100 krad, but radiation greatly reduced their lifespan [16]. Brower and Tilton reported that, irradiation is an approved method of direct control for stored product insects in wheat and wheat flour in many countries, and indications are that it will soon be approved for all grain, grain products, and other dry food commodities. Traditional quarantine treatments are designed to produce rapid mortality, but irradiation is only minimally effective in this regard. However, irradiation is very effective in preventing insect development and in producing sterility. In addition, they emphasized that lepidopteran stored product pests are relatively resistant to sterilizing effects of irradiation, and doses greater than 500 Gy may be required to sterilize a population [17]. Johnson and Vail found that adult emergence of *P. interpunctella* was reduced or eliminated at 14.4–92.1 krad. Females from pupae of Indianmeal moth irradiated with 26.9–31.9 krad were completely sterile, whereas this dose only partially sterilized males. Progeny of irradiated parents showed a high degree of sterility [18]. Ahmed et al. reported that when 1 day old male pupae of the Indianmeal moth were irradiated at 0, 5 and 9 krad and held at 25^{o} C, percent

male emergence was 84.0, 53.3 and 24.0%, respectively, while it was 82.7, 44.0 and 10.7% at 30°C, respectively. When female pupae were irradiated at 0.5 and 7 krad and held at 25°C, the percent emergence was 86.0, 45.3 and 28.0%, respectively, while it was 80.7, 34.0 and 14.7% at 30°C [19]. An irradiation dose of 0.7 kGy was more effective than commercial methyl bromide fumigation for *C. cautella* larvae [7]. The lifespan of adults of the Indianmeal moth, appeared to be shortened after treatment with 0.8 kGy; however, adults were sterilized with 0.2 kGy. Complete kill of eggs was obtained with 0.4 kGy, whereas 0.8 kGy was required to prevent development of larvae [21]. A minimum irradiation dose of 0.3 kGy and 1.2 kGy and were required to kill fig moth eggs and larvae, respectively, ten days after treatment. Adults of *C. cautella* developing from emerging larvae of eggs exposed to 0, 50, 100, and 150 Gy were 81, 40, 31 and 27%, respectively [22].

In practice, the goal of an irradiation quarantine treatment is usually to cause mortality of immature stages before development to the adult, or, where the stage present is an adult, to prevent further reproduction.

The above-mentioned studies show that the irradiation dose required to inhibit the development of immature stages to adults is around 1 kGy, and that reproduction could be stopped at 0.25–0.5 kGy. This is accordance with our findings.

3.3. Chemical and sensory analysis of irradiated and stored decorticated hazelnuts

Table 5 summarizes the composition of hazelnut samples used in this study. The two samples (harvests of 1999 and 2000) did not differ significantly with respect to various constituents.

Table 5. THE COMPOSITION OF ROASTED HAZELNUT SAMPLES

Harvest season	Moisture (%)	Ash (%)	Protein (%, Nx6.25)	Fat (%)	Crude fiber (%)
1999	2.04	2.20	14.96	62.4	3.65
2000	2.66	2.28	19.39	66.7	2.14

Total free fatty acid, peroxide value, iodine value, and total tocopherol content (1999 harvest season) are presented in Table 6. Free fatty acid and iodine values (= degree of unsaturation) of unirradiated and irradiated samples after one-month storage were not significantly different ($p>0.05$). The same results were obtained at the end of three and six months storage; however, total free fatty acid increased in both unirradiated and irradiated samples (especially at 0.5, 1.0, and 1.5 kGy) during storage. Iodine values were not influenced by storage time. Peroxide values increased significantly after irradiation ($p<0.05$) and one and three months storage; but after six months storage the differences disappeared. This suggests that products containing a high amount of oil and fats are susceptible to rancidity. Free fatty acid content is an important criterion in determining the quality and taste of hazelnuts. Free fatty acid values above 1% indicate rancidity. Moreover, roasting can significantly affect free fatty acid and peroxide values [23–24]. All free fatty acid and peroxide values obtained from this study generally were below the unacceptable limit for processed hazelnut samples [25]. Storage time had no consistent effect on the peroxide value.

Total tocopherol amounts were lower than that of results obtained in previous studies (Table 6). Compositional differences due to variety, soil composition, and cultural practices

such as use of fertilizer and irrigation might affect stability and subsequently quality of hazelnuts [26]. Total tocopherol content of both unirradiated and irradiated samples were shown to decrease markedly during storage ($p<0.05$). As compared with the control sample, total tocopherol content generally decreased with increasing irradiation dose (Table 6). Previous reports support these findings. When products are irradiated and stored in the presence of air, the surface area exposed is important. Whole hazelnuts treated with 1 kGy of radiation in the presence of air and analyzed the following day had lost 19% of their vitamin E concentration whereas ground hazelnuts lost 32% under the same conditions. Vitamin E is the most radiation sensitive of the fat-soluble vitamins; however, this sensitivity depends on the presence of oxygen. When rolled oats were irradiated with at 1 kGy and stored at room temperature in the presence of air, they had 44% less vitamin E than the unirradiated control after six months of storage; however, vacuum packaging reduced the loss to 14%, and nitrogen packaging to 7% [27–28]. The observed fluctuations in total tocopherol content may have arisen from experimental error.

The results for sensory evaluation of unirradiated and irradiated samples (1999 harvest season) are shown in Fig. 1. No significant differences ($p>0.05$) were found between samples for general sensory attributes during the storage. In papers discussing packaging, it has been stated that irradiation disinfested products look, taste, and smell the same as the good quality untreated products [29].

The same results were obtained for hazelnut samples harvested in the 2000 season (Table 7). The doses of irradiation applied had no significant affect on the free fatty acid content ($p>0.05$); however, storage time had a significant effect on the free fatty acid content for all samples ($p<0.05$). Irradiation dose significantly affected the peroxide values ($p<0.05$) after one-month storage but not after three and six months storage. Storage time significantly affected peroxide values in unirradiated samples and in the samples irradiated at a dose of 0.5 kGy ($p<0.05$). Neither irradiation dose nor storage time markedly affected iodine values (Table 7). There was a significant decrease in total tocopherol content with increasing irradiation dose ($p<0.05$). Storage time affected total tocopherol content of the samples irradiated at 0.5,1.0, 1.5 kGy and in the unirradiated sample (Table 7). There were no significant changes in sensory attributes of samples after any of the three storage periods. Irradiated hazelnut samples were found to be organoleptically acceptable (Figure 2). The Kreiss rancidity values were not significantly different among samples (1999 and 2000 harvest seasons).

In conclusion, the low-dose irradiation treatment does not seem to cause excessive oxidative deterioration of hazelnuts. Irradiation could be used as a disinfestation treatment in stored hazelnuts as an alternative to chemical fumigants.

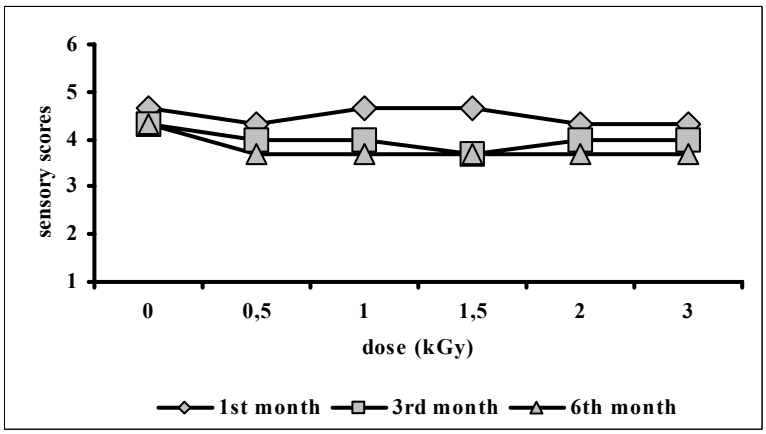

Figure 1. Sensory scores of the unirradiated and irradiated hazelnuts (1999) during storage at room temperature.

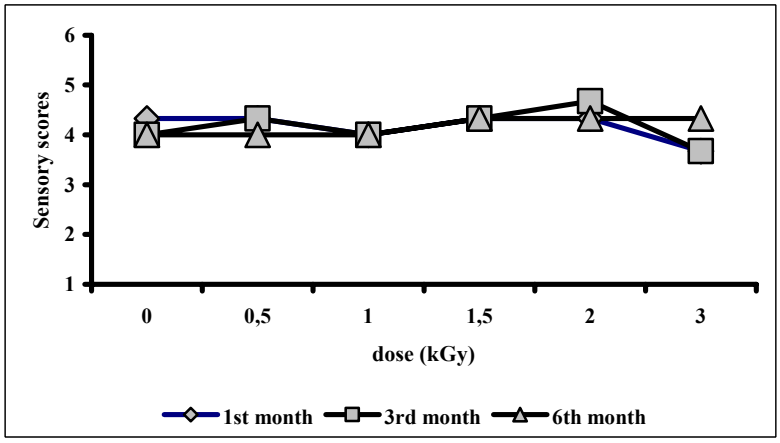

Figure 2. Sensory scores of the unirradiated and irradiated hazelnuts (2000) during storage at room temperature.

Table 6. THE EFFECT OF IRRADIATION ON CHEMICAL PROPERTIES OF HAZELNUT OIL (1999) DURING STORAGE AT ROOM TEMPERATURE

Dose (kGy)	Free Fatty Acid (%)			Peroxide Value (meq/kg)			Iodine Value			Total Tocopherol (ppm)		
	1st mo	3rd mo	6th mo	1st mo	3rd mo	6thmo	1st mo	3rd mo	6th mo	1st mo	3rd mo	6th mo
Control	0.21^{b}	0.29^{a}	0.28^{a}	1.31^{B}	1.54^{B}	1.72	79.45	75.20	78.68	213.31^{Aa}	170.49^{Ab}	144.17^{ABc}
0.5	0.24	0.30	0.29	1.58^{AB}	1.91^{AB}	2.15	76.22	70.93	78.40	169.70^{Ba}	148.32^{ABb}	147.49^{Ab}
1.0	0.22^{b}	0.29^{a}	0.28^{ab}	1.68^{AB}	1.98^{AB}	1.79	82.07	78.89	72.76	158.51^{BCa}	144.48^{Ba}	121.31^{Cb}
1.5	0.24^{b}	0.26^{b}	0.31^{a}	1.83^{A}	2.27^{A}	2.17	76.93	69.66	75.01	156.88^{BCa}	138.90^{Bb}	128.36^{BCb}
2.0	0.23	0.27	0.27	1.88^{A}	2.05^{AB}	2.17	80.47	73.09	74.59	146.19^{CD}	134.36^{B}	133.39^{ABC}
3.0	0.25	0.28	0.27	1.81^{A}	2.26^{A}	2.14	79.33	79.43	75.58	138.88^{D}	131.47^{B}	132.91^{BC}

^{a,b,c,} Means in a row within an attribute not having a common superscript letter are different ($p<0.05$)

^{A,B,C} Means in a column not having a common superscript letter are different ($p<0.05$)

Table 7. THE EFFECT OF IRRADIATION ON CHEMICAL PROPERTIES OF HAZELNUT OIL (2000) DURING STORAGE AT ROOM TEMPERATURE

Dose (kGy)	**Free Fatty Acid (%)**			**PeroxideValue (meq/kg)**			**Iodine Value**			**Total Tocopherol (ppm)**		
	1st mo	**3rd mo**	**6th mo**	**1st mo**	**3rd mo**	**6thmo**	**1st mo**	**3rd mo**	**6th mo**	**1st mo**	**3rd mo**	**6th mo**
Control	0.19^{b}	0.21^{ab}	0.24^{a}	1.25^{Bc}	1.77^{b}	2.28^{a}	84.82	84.45	81.91	223.97^{Aa}	197.24^{Ab}	196.30^{Ab}
0.5	0.17^{b}	0.23^{a}	0.25^{a}	1.40^{ABb}	2.23^{a}	2.23^{a}	83.02	79.15	80.37	205.98^{Ba}	194.66^{Aa}	166.64^{BCb}
1.0	0.17^{b}	0.21^{a}	0.23^{a}	1.83^{AB}	2.04	2.10	82.80	81.53	75.85	204.67^{Ba}	156.16^{Cc}	166.22^{BCb}
1.5	0.17^{c}	0.21^{b}	0.24^{a}	1.81^{AB}	1.99	2.19	84.99	79.40	78.44	191.26^{Ca}	167.43^{BCb}	160.49^{Cb}
2.0	0.17^{c}	0.20^{b}	0.23^{a}	1.90^{A}	1.82	2.22	83.84	78.35	80.86	180.21^{D}	176.79^{B}	172.81^{B}
3.0	0.17^{b}	0.21^{a}	0.23^{a}	1.93^{A}	2.33	2.29	84.40	79.31	79.48	165.85^{E}	165.55^{BC}	164.28^{BC}

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IRRADIATION TO CONTROL *PLODIA INTERPUNCTELLA* AND *ORYZAPHILUS SURINAMENSIS* IN PISTACIOS AND DATES

H.R. ZOLFAGHARIEH
Food Irradiation and Pest Control Group, Agriculture Section,
Nuclear Research Center for Agriculture and Medicine,
Atomic Energy Organization of Iran,
Karaj, Islamic Republic of Iran

Abstract

The effect of irradiation on *Plodia interpunctella* and *Oryzaephilus surinamensis* survival and reproduction in pistachios and dates was investigated. Eggs, larvae, pupae and adults of *P. interpunctella* and *O. surinamensis* were treated with irradiation doses ranging from 0.05–0.8 and 0.05–0.7 kGy, respectively. Results indicated that an irradiation dose of 0.7 kGy can control all developmental stages of both species, and the sterilizing doses were 0.35 kGy for *P. interpunctella* and 0.085 kGy for *O. surinamensis*. In determining the damage caused by *P. interpunctella* and *O. surinamensis* on pistachios, results showed that a dose of 0.45 kGy for pistachios and 0.55 kGy for dates prevented damage by *P. interpunctella,* and a dose of 0.075 kGy for pistachios and 0.065 kGy for dates prevented damage by *O. surinamensis*. In a test of the puncture resistance of different packaging materials, results indicate that PET, PET/PVDC and OPP/PE were resistant to *P. interpunctella* and *O. surinamensis*. PET 80 micron was superior to other packaging materials in terms of tensile strength, sealing qualities, puncture resistance, and keeping products fresh. Irradiation at 0.5 and 1.0 kGy had no effect on the texture, taste, odour, and colour of pistachios and dates during 1 year of storage. In some cases, pistachios irradiated with a dose of 1.0 kGy were preferred to unirradiatied pistachios. Pistachios and dates irradiated at 0.7 kGy showed no differences in protein, fat, or sugar content compared with unirradiated controls.

1. INTRODUCTION

Iran is the largest producer of pistachios and dates in the world, with an average total production from 1991–1994 of 206 000 and 640 000 tonnes, respectively [10]. Approximately 120 000 tonnes of pistachios are exported each year to Europe, Arabic countries, and Japan. Harvesting of pistachios begins the last week of August and continues through the first week of December. After harvesting, pistachios are delivered to processing factories for peeling, cleaning, drying, size grading, and packaging. The product is packed mostly in polypropylene woven sacks weighing 60-70 kg.

Different varieties of dates are produced in Iran, a few of which are exported to Europe and Japan. Approximately 107,000 tonnes are exported annually. The harvest period is October through November.

According to FAO, approximately 10% of stored products in Iran are lost due to insect infestation. Fumigation is normally practiced to prevent post-harvest losses of food in storage. Phostoxin and methyl bromide are the most common fumigants for insect disinfestation in storage. Fumigants leave residues in treated products, and, due to the ozone depleting properties of fumigants such as methyl bromide, they are under great pressure to be phased out in the near future. Therefore, it is necessary to find environmentally friendly techniques for insect disinfestation to replace fumigants. Two important stored-pests of nuts are *P. interpunctella* (Hubner) and *O. surinamensis (L)*. Irradiation is a hygienic and safe alternative to fumigation.

2. MATERIALS AND METHODS

2.1. Determining lethal and sterilizing doses for *P. interpunctella* and *O. surinamensis*

P.interpunctella was reared on artificial diet containing two parts wheat, one part oats, one part bran, one-half part dry yeast powder, and 15 ml glycerol/100 g dry ingredient. The diet was decontaminated at 60º C for six hours. For egg collection, adults were put in a funnel and placed over radiology film for 24 hours. Eggs on the film were transferred to diet in plastic jars (17×17×25 cm) and held in an incubator at 28 ± 2° C and 55 ± 5% RH to rear various life stages. The dimension of the plastic jars was 17x17x25 cm. *O.surinamensis* was reared on a diet of ground wheat. The diet was decontaminated at 60° C for six hours. Adults were placed on diet in plastic jars (17×17×25 cm) and held in an incubator at 29 ± 2 º C and 60 ± 5% RH.

When the population of insects grew sufficiently large, four developmental stages of each insect (egg, larvae, pupa and adult) were irradiated. The lethal and sterilizing radiation doses for each developmental stage ranged between 0.05–0.8 kGy for *P. interpunctella* and 0.05–0.7 kGy for *O. surinamensis*, respectively. Dose response tests for each species were based treating 50 individuals of each developmental stage, replicated four times. In both species, for egg and pupa, the criterion was the ability to develop to the next stage, i.e., larvae and adults, respectively. In the case of larvae and adults, sampling was done at specific intervals after treatment to judge mortality and the results were recorded.

2.2. Determining the level of destruction of pistachios and dates due to *P. interpunctella* and O.*surinamensis*

To determine damage caused by *P. interpunctella* and *O. surinamensis*, 0.5 kg of pistachios and dates were packed separately and each contaminated with ten fourth instars each species separately. The test was replicated four times. The range of radiation doses was 0.05–0.55 kGy for *Plodia* and 0.025-0.085 kGy for *Oryzaephilus*. Sampling was done monthly and compared with untreated controls.

2.3. Evaluation of sensory quality for irradiated and non-irradiated pistachios and dates

A test was conducted to evaluate the sensory quality of pistachios and dates irradiated at doses of 0.5 and 1.0 kGy in terms of texture, taste, odour, and colour. Samples were packed in polypropylene woven sacks and sealed conventionally (pistachios and dates) or with vacuum (pistachios only, dates are damaged by vacuum packaging). The test was replicated six times for each packaging material. Each sample contained 200g of pistachios or dates. The samples were stored in 10 ± 1° C and 45 ± 5% RH. Six experienced referees evaluated the samples at two-month intervals for 12 months. Samples were judged as "very bad, bad, moderate, good, or very good".

2.4. Evaluation of locally available packaging materials for pistachios and dates

Eight types of packaging materials were tested for their resistance to irradiation at a minimum absorbed dose of 1.0 kGy. Packaging types included BOPP 30 micron, PVC28, PP/PP50, OPP/PE70, PP/PS80, PET80, PE/PS80, and PET/PVDC80. The test was replicated four times. Each replication involved treating one square meter of material. The different irradiated and non-irradiated packaging materials were sent to Poushineh Plastic Co. for quality analysis and comparison of thickness, density, haze, transmittance, opacity, gloss, sealing strength, seal initiation temperature, treatment type, treatment intensity, tensile, elongation, elasticity modulus, static friction coefficient, shrinkage, and charge decaying rate.

The second part of the research was to determining the resistance of the different packaging materials to penetration and re-infestation by *P.interpunctella* and *O.surinamensis*. Ten first instars were introducing to the inside of the packaging material and packages were sealed. The packages were then placed in a large plastic jar with 100 g uncontaminated pistachios to determine insect penetration and puncturing outward to the product. This test was also done in the opposite way with insects outside and pistachios or dates inside. The number of holes created by insects determined the package's resistance and durability. Each test was replicated four times, and the samples were kept under the same controlled rearing conditions of *P. interpunctella* and *O. surinamensis* colonies. Sampling was conducted weekly for eight weeks.

2.5. Chemical analysis of irradiated and non-irradiated pistachios and dates

Irradiated and unirradiated pistachios and dates in packages were sent to a chemical analysis laboratory to determine protein, sugar, and fat content.

2.6. Large-scale test

A large-scale confirmatory test is being conducted with the best packaging material (PET 80 micron) and irradiation treatment using the dose determined to control *P.interpunctella* and *O.surinamensis*. The experiment is being replicated six times using one kg packages of pistachios and dates which will be infested with eggs, fourth instars, pupae and adults of each species. All the samples will be stored at 25 ± 2° C and 60 ± 5% RH. Samples will be examined monthly for insect disinfestation and packaging resistance to insect punctures for six months.

3. RESULTS

3.1. Determining lethal and sterilizing doses for *Plodia interpunctella* and *Orzaephilus surinamensis*

For all life stages, *Plodia* was more tolerant of irradiation than *Oryzaephilus* (Table 1). There was no emergence for eggs of either species at an irradiation dose of 0.05 kGy. The sterilizing dose for eggs was in the range of 0.035–0.05 kGy. *Plodia* larvae showed 100% mortality 14 days after irradiation with a dose of 0.65 kGy, and 35 days after irradiation with a dose of 0.2 kGy. Mortality was 100% for *Oryzaephilus* larvae seven days after irradiation with a dose of 0.09 kGy. The sterilizing dose for controlling larval stages of the two species was in the range of 0.03–0.09 kGy. *Plodia* pupae were controlled with irradiation doses between 0.35–0.65 kGy, and *Oryzaephilus* pupae were controlled with irradiation doses between 0.6–0.7 kGy. The sterilizing dose for *Plodia* pupae was 0.35 kGy and emerging adults were malformed and could not lay eggs. The sterilizing dose for *Oryzaephilus* was 0.085 kGy. Mortality was 100% after 14 days in *Plodia* adults irradiated at a dose of 0.5–0.65 kGy compared to 0.115–0.145 kGy for *Oryzaephilus* adults. The sterilizing dose for adults of *Plodia* and *Oryzaephilus* was 0.35 kGy and 0.085 kGy, respectively.

Table 1. COMPARING LETHAL AND STERILE DOSES FOR *Plodia* (P) AND *Oryzaephilus*(O)

Dose (kGy)	Egg		Larvae		Pupa		Adult	
	P	O	P	O	P	O	P	O
Lethal	0.05-0.2	0.025-0.05	0.2-0.65	0.09-0.12	0.35-0.65	0.6-0.7	0.5-0.65	0.115-0.145
Sterile	0.05	0.035	0.09	0.03	0.35	0.085	0.35	0.085

3.2. Determining the degree of destruction of products having insects surviving different radiation doses

Irradiation treatment dramatically reduced product damage by *O. surinamensis* and *P. interpunctella* (Tables 2 and 3). The maximum percentage loss to pistachios due to *O.surinamensis* damage was 82.5% for untreated controls, but at 0.075 kGy damage was zero. *O.surinamensis* had 87.5% damage to dates in the case of untreated controls but at 0.065 kGy it was zero.

P. interpunctella caused 92.5% damage to pistachios in untreated controls compared to zero percent damage at 0.45 kGy. Damage to dates created by this insect was 99.5% in the case of untreated controls and zero percent at 0.55 kGy.

Table 2. PERCENT DESTRUCTION OF PISTACHIOS AND DATES CAUSED BY *O.surinamensis*

Dose (kGy)	**Control**	**0.025**	**0.035**	**0.045**	**0.055**	**0.065**	**0.075**	**0.085**
Pistachios	82.5	60	41	29.0	18.0	10	0	0
Dates	87.5	61	45	22.5	7.5	0	0	0

Table 3. PERCENT DESTRUCTION OF PISTACHIOS AND DATES CAUSED BY *P.Interpunctella*

Dose (kGy)	**Control**	**0.05**	**0.15**	**0.25**	**0.35**	**0.45**	**0.55**
Pistachios	92.5	75	55.00	45	15.0	0	0
Dates	99.5	80	71.25	45	32.5	10	0

3.3. Determining the sensory quality of irradiated pistachios and dates subjected to a minimum dose of 0.5 and 1.0 kGy

There was no significant difference in the quality of irradiated and non-irradiated pistachios. In the case of dates, there was some indication that nuts in the 0.5 and 1.0 kGy treatments had better quality compared with controls. This demonstrates that packaging materials can be effective in preserving dates but packaging is less important with pistachios.

There was no significant difference among packaging materials among treatments (control, 0.5 and 1.0 kGy) for texture, taste, smell, and colour of pistachios and dates (Duncan's, P=0.05); however some packaging materials were preferred in terms of resistance to punctures made by insects, sealing properties, etc. The sequence of preference are listed in Table 4:

Table 4. COMPARING PACKAGING MATERIALS: THE FIRST FOUR PACKAGING MATERIAL ARE ACCEPTABLE AND THE SECOND FOUR ARE UNACCEPTABLE

Pistachios (conventional sealing)	**Pistachios (vaccuum sealing)**	**Dates (conventional sealing)**
PET	PET	PET
OPP/PE	OPP/PE	OPP/PE
PET/PVDC	PET/PVDC	PET/PVDC
PP/PP	PP/PP	PP/PP
PE/PS	PE/PS	PE/PS
PP/PS		PP/PS
BOPP		BOPP
PVC		PVC

3.4. Evaluation of the packaging materials for suitability, resistance to pest infestation, and resistance to irradiation at 1.0 kGy

Table 5 shows a comparison of the different packaging materials in terms of physical and mechanical characteristics. For the tests done at the Poushineh Plastic Co., there was no significant difference between irradiated packaging materials and untreated controls, except that the static friction and kinetic friction coefficients of PET/PVDC were improved in irradiated samples compared to non-irradiated samples. This is important in the choice of packaging materials.

The effectiveness of different packaging materials in excluding pests was determined by counting the number of punctures created by insects and the percentage of infestation of the material. PE/PS, PP/PS, PVC, PP/PP and BOPP were not resistant to *P.interpunctella* larvae, but PET, PET/PVDC and OPP/PE were resistant (Table 6). All the packaging materials except PP/PS and BOPP were resistant to *O.surinamensis* larvae (Table 7).

In light of the results shown in Tables 5, 6, and 7, and the results of sensory quality, PET, OPP/PE, and PET/PVDC were the best packaging materials among those tested.

Table 5. COMPARING DIFFERENT PACKAGING MATERIALS IN TERM OF PHYSICAL AND MECHANICAL CHARACTERISTICS

	PET	**OPP/PE**	**PET/ PVDC**	**PP/PP**	**PE/PS**	**PP/PS**	**BOPP**	**PVC**
Haze	Very good	Good	Good	Good	Bad	Bad	Moderate	Bad
Transmitance	"	"	"	"	"	"	Good	"
Gloss	"	"	"	"	"	"	"	"
Opacity	"	"	"	"	"	"	"	Good
Sealing strenght	"	"	Very good	"	Good	"	"	"
Sealing initiation temp.	"	"	"	"	"	"	"	"
Treatment type	"	"	"	"	Moderate	"	"	Bad
Treatment intensity	"	"	"	Bad	"	"	"	"
Tensile	Good	"	Bad	Good	Bad	Moderate	"	Very bad
Elongation	"	"	"	Moderate	Moderate	"	"	"
Elasticity modulus	"	"	Good	Good	"	"	"	"
Static friction coeficient	"	"	"	"	Good	Bad	"	Good
Kinetic friction coeficient	"	"	"	"	"	"	"	"
Shrinkage	"	"	Bad	Very good	Very good	Good	"	Very bad
Charge decaying rate	Moderate	"	"	Moderate	"	"	"	"

Table 6. NUMBER OF PUNCTURES AND DAMAGE PERCENTAGE PRODUCED BY *P.Interpunctella* LARVAE

Position of infested pistachio	Number of replications	Packaging material															
		OPP/PE		PP/PP		PET/ PVDC		PET		PE/PS		PP/PS		PVC		BOPP	
Outside packaging	4	_	_	3	50%	1	_	_	_	3	50%	7	75%	1	75%	11	100 %
Inside packaging	4	_	_	_	_	1	_	_	_	_	_	3	50%	_	_	_	_

Table 7. NUMBER OF PUNCTURES AND DAMAGE PERCENTAGE PRODUCED BY *O.Surinamensis* LARVAE

Position of infested pistachio	Number of replications	Packaging material															
		OPP/PE		P/PP		PET/ PVDC		PET		PE/PS		PP/PS		PVC		BOPP	
Outside packaging	4	_	_	_	_	_	_	_	_	_	_	2	100%	_	_	_	_
Inside packaging	4	_	_	_	_	_	_	_	_	_	_	_	_	_	_	3	100%

3.5. Conducting ingredient analysis of irradiated and non-irradiated pistachios and dates

No significant differences were observed in the composition of irradiated and non-irradiated pistachios and dates (Table 8).

3.6. Conducting pilot scale experiments to confirm results under laboratory condition

Six months after irradiation, no insect contamination or packaging punctures have been observed, compared to 35% contamination in untreated control packages.

Table 8. COMPARING ANALYSIS OF IRRADIATED AND NON-IRRADIATED PISTACHIOS AND DATES IN TERMS OF NUTRITIONAL COMPOSITION

	Pistachios		**Dates**	
	Control	**Irradiated**	**Control**	**Irradiated**
Glycine	8.70	8.60	0.45	0.47
Serine	9.97	9.87	0.37	0.35
Phenyl-alanine	7.16	7.08	0.22	0.21
Lysine	10.44	10.39	0.92	0.87
Lusine	9.31	9.33	0.23	0.26
Isolusine	6.04	6.10	0.13	0.11
Valine	9.68	9.48	0.26	0.23
Tyrosine	4.14	4.30	0.15	0.14
Proline	8.77	8.82	1.24	1.26
Alanine	9.48	9.36	1.12	1.06
Treonine	6.30	6.50	0.33	0.32
Argenine	8.70	8.58	0.52	0.47
Histidine	5.39	5.47	0.27	0.25
Methionine	1.30	1.28	0.04	0.04
Aspartic-acid	11.29	11.30	1.63	1.59
Glutamic-acid	41.80	41.53	1.68	1.63
Fat	0.475	0.485		
Sugar			0.721	0.75

4. CONCLUSIONS

Plodia interpunctella and *Oryzaephilus surinamensis* are the most destructive pests of dried fruit and nuts in Iran. Radiation sensivity of the two insects was determined at different developmental stages. Results show that all developmental stages of both pests are controlled by the lethal dose of 0.7 kGy and the sterilizing dose of 0.35 kGy. Consequently the dose of 0.7 kGy is recommended for disinfestation of pistachios and dates [9].

a) Studies to determination of the degree of destruction show that the doses to prevent destruction of pistachios and dates by *O.surinamensis* are 0.075 kGy and 0.065 kGy, respectively. The doses to prevent destruction of pistachios and dates by *P.interpunctella* are 0.4 5 kGy and 0.55 kGy, respectively.

b) In terms of selecting suitable packaging materials with regard to irradiation and resistance to *P.interpunctella* and *O.surinamensi,* the three most favourable packaging materials are PET, OPP/PE, and PET/PVDC. In view of tensile, sealing, puncture resistance, and keeping product fresh, PET80 micron is more favourable than the two others.

c) Sensory quality evaluation showed that there is no significant difference between irradiated and non-irradiated pistachios; however, irradiation, especially with 1 kGy dose, could be effective in preserving pistachios quality and in some cases irradiated pistachios were more delicious and fresher. In case of dates, radiation decreased crystallization of sugar significantly. Both products show less fungal and insect contamination when irradiated compared unirradiated controls.

d) Pistachios and dates in PET80 micron packaging and irradiated with a dose of 0.7 kGy showed no important measurable differences in protein, fat, or sugar content compared with untreated controls [8].

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IRRADIATION AS AN ALTERNATIVE TREATMENT TO METHYL BROMIDE FOR DISINFESTATION OF *TRIBOLIUM CASTAÑEUM* IN STORED CACAO

M.U. GOCHANGCO, E.M. SAN JUAN, A.O. LUSTRE
Food Development Center, National Food Authority,
FTI Complex, Taguig, Metro Manila,
Philippines

Abstract

Effects of irradiation on red flour beetle, *Tribolium castañeum*, eggs, larvae, pupae, and adults were examined. Data was taken on mortality, adult emergence, and sterility after irradiation at doses between 0.02 and 0.16 kGy. Mature eggs were more resistant to irradiation than young eggs; development to adults was prevented at a dose of 0.08 kGy for one to two day old eggs but was not stopped for the three to four day old eggs at doses as high as 0.16 kGy. Larvae were the most sensitive to irradiation. Young larvae appeared to be more resistant to irradiation than older larvae. Pupae were the most resistant to irradiation, and older pupae were more resistant than younger pupae. Development to adults was not prevented in both types of pupae up to 0.16 kGy. Survivors were not observed after four weeks for adults receiving a dose of 0.10 kGy or higher. Adults emerging from irradiated eggs, larvae, and pupae were sterile, in some cases, at slightly higher doses than those required to prevent adult emergence. Adults from irradiated eggs and larvae were sterile at 0.06 kGy. For pupae, sterility was achieved at 0.06 and 0.12 kGy for the one to two days and four to five days old pupae, respectively. Adult sterility was achieved at 0.12 kGy. As expected, when one member of a mating pair was left unirradiated, the dose requirements were higher. The effect of mating combination is still being analyzed. Data are still being collected and analyzed for *Ephestia cautella*, another major pest of stored cacao bean. Tests with T. *castañeum* and *E. cautella* must be completed before a dose can be recommended for commercial treatment.

1. INTRODUCTION

Methyl bromide is a widely used fumigant for the control of insect infestation in many agricultural commodities. As methyl bromide has the potential to destroy the ozone layer in the environment, alternative methods have to be sought to replace its use in insect control.

Cacao bean is one of the major commodities fumigated with methyl bromide for insect control in the Philippines (Bayani, 1997). The major stored product pests associated with the commodity are the red flour beetle, *Tribolium castañeum* (Herbst) and cocoa moth (*Ephestia cautella* (Walker). Several studies have been carried out on the relationship between irradiation dose and sterility and mortality of various metamorphic stages of *T. castaneum.* Minimum sterilization doses of 0.06 kGy (Blanco et. al. 1981) and 0.1 kGy (Muda et.al, 1991) have been recommended. Our work aims to validate these studies, calculate the LD values, and establish similar data for the cocoa moth (*Ephestia cautella* (Walker).

The Philippines produces 42% of its cacao requirements, equivalent to about 15 335 metric tons, and imports the remainder. It exports cacao to various countries in the form of cocoa powder and by-products. Whether for storage or trade, cocoa beans and powder undergo some form of insect control, primarily methyl bromide fumigation. For irradiation to be evaluated as a feasible substitute for methyl bromide, the optimum dose requirements for both *T. castaneum* and *Ephestia cautella* have to be established.

The first phase of this project established the LD 99 and LD99.9 values for mortality of various developmental stages of *T. castaneum*. This work continues from that earlier phase of the project. Our objective is to evaluate dose requirements to prevent adult emergence or sterilize emerging insects at doses of 0.02 to 0.16 kGy. Information on the LD99 and LD99.9 values for insect sterility is also presented. There was a six-month delay in the project due to the repair of the irradiation facility.

2. METHODOLOGY

2.1. Observations on effects of irradiation on eggs, larvae, pupae and adults

2.1.1. Materials Used

Insects

The adult insects of *Tribolium castañeum* Herbst came from a stock culture maintained at the Food Development Center. The insects were originally obtained from a Manila warehouse a year before the experiments started. The insects were maintained with the artificial diet described below at ambient temperature of 30 ± 2°C and 70 ± 2% relative humidity.

Culture Medium

The artificial diet used for rearing the red flour beetle (*T. castañeum)* was a mixture of white all-purpose flour (47.5%), corn meal (47.5%) and wheat germ (5%).

2.1.2. Number and ages of developmental stages treated

Two ages for each developmental stage were produced namely: one to two and three to four-day old eggs; 14 to 15 and 20 to 21-day old larvae; one to two and four to five-day old pupae, and four to five and seven to eight-day old adults. The different developmental stages were exposed to 0.0 (control) 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14 and 0.16 kGy doses of ionizing radiation, chosen based on the results of the study of Blanco *et al.* (1981). Thirty insects were treated per dose per developmental stage. There were three replicates and three trials per dose or 270 insects per dose for the three trials and 2430 insects for nine doses.

2.1.3. Irradiation treatment

Irradiation was administered in a Cobalt-60 irradiator (Gamma Cell 220) having a dose rate of 0.124 kGy per hour at the Philippine Nuclear Research Institute of the Department of Science and Technology. The dose rate was measured using a Fricke dosimeter. The reading was within 2% of the International Dose Assurance Service (IDAS) dosimeter of IAEA. The dose rate was established as of August 2001 when the experiment was conducted.

2.1.4. Collection and observation of insects

For each test of radio-sensitivity for a given developmental stage, a new culture cycle of *T. castañeum* was started from 200 parents. Insects were grown using the culture medium; eggs were collected every 24 hours by sieving the medium. Eggs obtained from sieving were collected in 3.5 oz. plastic cups with cover containing culture medium.

Eggs for treatment were held for one to two days and three to four days at ambient conditions prior to irradiation to produce the two age groups. Treated and untreated eggs were placed in

separate plastic cups with cover for assessment. These were evaluated weekly for six weeks, to determine the percentages of eggs that hatched, and the number of larvae, pupae and adults that emerged after irradiation. The number of eggs hatching was obtained by counting the number of eggs laid every day by a pair of adults, and transferring the eggs to a separate plastic cup with cover to determine hatch seven days later.

Larvae for treatment were obtained from a new culture cycle as described for eggs and kept at ambient temperature. The larvae collected were held for 14-15 days (third instars), and 20–21 days (fifth and sixth instars) prior to irradiation. Irradiated and unirradiated controls were held separately in plastic cups with cover and evaluated weekly for four weeks to determine the number of emerging pupae and adults.

Pupae to be irradiated were obtained from a new culture as described for eggs. They were held for one to two days and four to five days in the culture medium prior to irradiation to produce the two age groups. Irradiated and unirradiated pupae were checked weekly for two weeks to determine the number of emerging adults.

Adults from the irradiated and control pupae and larvae were observed for longevity for a period of four weeks. Those showing marked deformity or which could not free themselves from their pupal case were considered non-survivors. One week after exposure to gamma radiation, four to five-day old and seven to eight-day old adults were paired into different mating combinations with unirradiated adults to observe their egg laying capacity and to evaluate the effects of irradiation on sterility as measured by the percentage of egg hatch. Pairing of the adults within the same age group and developmental stage was as follows: (1) irradiated male (IM) and irradiated female (IF), (2) irradiated male (IM) and unirradiated female (UF), (3) unirradiated male (UM) and irradiated female (IF), and (4) unirradiated male (UM) and unirradiated female (UF or control). There were nine pairs per dose. The mean percentage of egg hatch was used as the index of fertility.

The percentage of egg hatch, percentage of adult emergence, the reduction in fertility (as a percentage of control), and mortality were calculated as follows:

a. percentage egg hatch = $\frac{\text{number of eggs hatch}}{\text{number of eggs laid}} \times 100\ \%$

b. percentage adult emergence = $\frac{\text{total actual number of emerged metamorphic stage}}{\text{total number of original metamorphic stage}} \times 100\%$

c. percentage reduction = percentage egg hatch of control - percentage egg hatch of irradiated samples in fertility (as percentage of control)

d. Corrected mortality = $\frac{X - Y}{X} \times 100\%$ (IAEA, 1982),

where X = percentage alive in the control, and Y = percentage surviving in the treated sample.

2.2. Determination of LD99 and LD99.9 values for sterility

The study determined the LD values for sterility of *T. castañeum* using irradiation. The data on eggs laid and eggs hatched was evaluated by probit analysis to establish the LD99 and LD99.9 values for sterility of males and females (Institute of Math and Statistics of the University of the Philippines at Los Baños, Laguna).

2.3. Comparison of the LD99 and LD99.9 Values for Mortality of *Tribolium castañeum*

The first phase of this project established the LD99 value for mortality using irradiation doses of 0.2, 0.4 0.6, 0.8 and 1.0 kGy (dose range based on the work of Brower and Tilton 1973). The effect of irradiation on mortality was determined by counting live and dead insects. The data were evaluated using probit analysis with fiducial limits at 95% confidence level. The above previously established data for determining the LD99 value for mortality of *T. castaneum* were statistically re-analyzed at the Institute of Math and Statistics of the University of the Philippines at Los Baños to get the corresponding LD99.9 values.

3. RESULTS AND DISCUSSION

3.1. Response of the developmental stages of *Tribolium castañeum* to irradiation

Eggs

Table 1 shows that after six weeks of observation, the three to four-day old eggs had a higher percntage of egg hatch and percentage of adult emergence than the one to two-day old eggs. Emergence to adults was prevented at a dose of 0.08 kGy for one to two-day old eggs, but was not stopped for the three to four-day old eggs at the highest dose used in the experiment of 0.16 kGy. The adult emergence from irradiated three to four-day old eggs was very low (in some cases only one adult) at the higher doses.

Larvae

Table 2 shows that larvae were sensitive to irradiation and suggests larvae were the most sensitive developmental stage. Emergence of adults was prevented at 0.08 kGy for the 14–15 day old larvae and at 0.06 kGy for the 20–21 day old larvae. The greater sensitivity of the more mature larvae was unexpected. Sokoloff (1977) had indicated that larvae near the pupal stage, which was the 20–21 day old larvae used in this study, are more sensitive to irradiation due to damage in the imaginal discs which is responsible for the formation of the digestive system, the muscular tissues, the wings and other tissues and organs in the adult. This might explain this finding.

Pupae

Table 3 shows that pupae were more resistant to irradiation than eggs and larvae. Development to adults was not prevented in both the one to two-day old and four to five-day old pupae at all doses including the highest dose used in this study of 0.16 kGy within the two weeks of observation, although the percentage generally decreased with dose applied. The data indicate greater tolerance to radiation of four to five days old pupae compared to the younger pupae.

Adults

Irradiated adults were observed to survive up to four weeks at all doses. After four weeks, survivors were no longer observed for those adults receiving a dose of 0.10 kGy or higher. Survivors of both ages one week after irradiation were paired singly into different mating combinations to observe their egg laying capacity. Effects of irradiation on sterility of adults as measured by the percentage of egg hatch, is discussed in section 3.2 below.

3.2. Effects of irradiation on the sterility of Tribolium adults emerging from irradiated eggs, larvae and pupae

The egg laying capacity of adults irradiated as immatures (eggs, larvae, or pupae) is shown in Tables 4–7.

Adults which emerged from eggs and larvae

The oviposition rate of adult females that emerged from eggs and larvae was reduced when treated with gamma radiation. Hatching of eggs was prevented at 0.06 kGy for eggs and larvae irrespective of age (IM x IF) (Tables 4 and 5); however, egg hatching was observed at 0.06 kGy for one to two-day old eggs with the female unirradiated (IM x UF) (Table 4) and for 14–15-day old larvae with the male unirradiated (UM x IF) (Table 5). A more systematic evaluation of the effect of mating combinations could not be done because of the inadequate number of adults produced for some dose levels.

Adults which emerged from pupae

The egg laying capacity of adult females irradiated as pupae was reduced. Adult emergence was prevented at 0.06 kGy for one to two-day old pupae and at 0.12 kGy for four to five-day old pupae (IM x IF) (Table 6). When one member of the pair was left unirradiated, fertility was prevented in four to five-day old pupae only at 0.14 kGy (IM x UF, UM x IF).

Adults

When adult beetles were irradiated, the percentage egg hatch was higher for the seven to eight-day old adults than the four to five-day olds (IM x IF), and the percentage of egg hatch decreased with increasing dose (Table 7). Fertility was stopped at 0.12 kGy, but when the female was left unirradiated, egg hatch was not prevented even at doses as high as 0.16 kGy. When the male was left unirradiated fertility was prevented at 0.16 kGy in seven to eight-day old adults but not in four to five-day old adults.

3.3. LD Values for Sterility

The irradiation dose required to achieve LD99 and LD99.9 varied with life stage and maturity within each life stage. Results for eggs could not be obtained because there were not enough adults produced. The results shown in Table 8 indicate that the lethal doses for 99.9% sterility of 0.15 to 1.72 kGy are higher than doses recommended in the literature for the irradiation of *T. castaneum*. The log-dose probit lines (Fig. 1) calculated for larval, pupal and adult stages show the trends in sensitivity. There are clear differences in radiation sensitivity between 14–15-day and 20–21-day old larvae with the former being more sensitive. Adults (four to five days old) were slightly more radiation resistant. The LD values for other mating combinations are still being evaluated.

The dose indicated in this study for the endpoints specified below are the following:

	No adult emergence or 100% mortality	**Emerging adults from irrad. pairs, sterile**	**LD 99.9 for sterility**
Eggs			
1–2 days old	0.06 kGy	0.06 kGy	----------
3–4 days old	>0.16 kGy	0.06 kGy	----------
Larvae			
14–15 days old	0.08 kGy	0.06 kGy	0.15 kGy
20–21 days old	0.06 kGy	----------	----------
Pupae			
1–2 days old	>0.16 kGy	0.06 kGy	0.16 kGy
4–5 days old	>0.16 kGy	0.12 kGy	0.51 kGy
Adults			
4–5 days old	0.10 kGy	0.12 kGy	0.45 kGy
7–8 days old	0.10 kGy	0.12 kGy	1.72 kGy

N/A: Not applicable; ---------- Not enough adults obtained for observation

3.4. Comparison of LD99 and LD99.9 values for mortality of *T. castaneum*

The LD99 values for the different developmental stages of *T. castaneum* were previously established using irradiation doses of 0.2, 0.4, 0.6, 0.8 and 1.0 kGy. LD99 values were found to be 0.73 kGy for eggs four weeks after irradiation, 0.50 kGy for larvae four weeks after irradiation, 0.42 kGy for pupae 17 days after irradiation, and 0.40 kGy for adults three weeks after irradiation. The statistical analysis of the data used to determine the LD99 value was re-analyzed to determine the corresponding LD99.9 value for mortality (Table 9). The difference in the LD99 and LD99.9 values for each developmental stage was minimal, and different by approximately 0.001 to 0.007 kGy.

Table 1. PERCENTAGE ADULT EMERGENCE AND MORTALITY OF IRRADIATED EGGS OF *Tribolium Castañeum* SIX WEEKS AFTER IRRADIATION

Dose applied (kGy)	Age of Eggs							
	1 to 2-day old eggs				3 to 4-day old eggs			
	No. of eggs used[a/]	% Egg hatch[b/]	% Adult emerg-ence [b/]	% Morta-lity	No. of eggs used[a/]	% Egg hatch[b/]	% Adult emerg-ence [b/]	% Morta-lity
0 (control)	270	81.5	38.1	61.9	270	81.9	49.6	50.4
0.02	270	13.0	4.8	95.2	270	48.5	27.4	72.6
0.04	270	2.6	2.2	97.8	270	40.7	7.8	92.2
0.06	270	1.8	1.1	98.9	270	18.9	2.2	97.8
0.08	270	1.1	0	100.0	270	2.2	1.5	98.5
0.10	270	1.1	0	100.0	270	1.5	1.1	98.9
0.12	270	0	0	100.0	270	2.2	1.1	98.9
0.14	270	0	0	100.0	270	0.4	0.4	99.6
0.16	270	0	0	100.0	270	0.4	0.4	99.6

[a/] Total for three trials consisting of three replicates per trial (1 replicate=30 insects)
[b/] Average of three trials

Table 2. PERCENTAGE ADULT EMERGENCE AND MORTALITY OF LARVAE OF *Tribolium Castañeum* FOUR WEEKS AFTER IRRADIATION

Dose applied (kGy)	Age of Larvae					
	14 to 15-day old larvae			20 to 21-day old larvae		
	No. of larvae used[a/]	% Ault emergence [b/]	%Mortality	No. of Lrvae used[a/]	% Ault emergence [b/]	%Mortality
0 (control)	270	82.9	17.1	270	60.0	40.0
0.02	270	64.8	35.2	270	35.2	64.8
0.04	270	20.0	80.0	270	3.3	96.7
0.06	270	1.8	98.2	270	0	100.0
0.08	270	0	100.0	270	0	100.0
0.10	270	0	100.0	270	0	100.0
0.12	270	0	100.0	270	0	100.0
0.14	270	0	100.0	270	0	100.0
0.16	270	0	100.0	270	0	100.0

[a/] Total for three trials consisting of three replicates per trial (1 replicate=30 insects)
[b/] Average of three trials

Table 3. PERCENTAGE ADULT EMERGENCE AND MORTALITY OF PUPAE OF *Tribolium Castañeum*, TWO WEEKS AFTER IRRADIATION

Dose applied (kGy)	Age of Pupae					
	1 to 2-day old pupae			4 to 5- day old pupae		
	No. of pupae used[a/]	% Adult emergence [b/]	%Mortality	No. of pupae used[a/]	% Adult emergence [b/]	%Mortality
0 (control)	270	94.8	5.2	270	81.1	18.9
0.02	270	70.7	29.3	270	75.8	24.2
0.04	270	57.8	42.2	270	58.9	41.1
0.06	270	55.9	44.1	270	53.2	46.8
0.08	270	31.5	68.5	270	36.6	63.4
0.10	270	33.7	66.3	270	38.9	61.1
0.12	270	24.8	75.2	270	41.9	58.1
0.14	270	15.6	84.4	270	48.5	51.5
0.16	270	7.4	92.6	270	35.1	64.9

[a/] Total for three trials consisting of three replicates per trial (1 replicate=30 insects)
[b/] Average of three trials

Table 4. STERILITY EXPRESSED AS PERCENT EGG HATCH AFTER FIVE WEEKS OF *Tribolium Castañeum* ADULTS THAT EMERGED FROM IRRADIATED EGGS

Mating combination	Dose applied (kGy)	Sterility of adults which emerged from eggs							
		Adults from 1 to 2-day old eggs				Adults from 3 to 4-day old eggs			
		Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)	Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)
UM x UF (control)	0	390	344	88.2		411	352	85.6	
IM x IF	0.02	458	270	59.0	29.2	314	210	66.9	18.7
	0.04	16	7	43.8	44.4	156	65	41.7	43.9
	0.06	0	0	0	88.2	8	0	0	85.6
	0.08	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
IM x UF	0.02	248	150	60.5	27.7	290	202	69.7	15.9
	0.04	168	90	53.6	34.6	142	72	50.7	34.9
	0.06	22	6	27.3	60.9	31	0	0	85.6
	0.08	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
UM x IF	0.02					254	154	60.6	25.0
	0.04		Not enough adults obtained for mating			267	112	41.9	43.7
	0.06					(-)	(-)	(-)	(-)
	0.08					(-)	(-)	(-)	(-)
	0.10					(-)	(-)	(-)	(-)
	0.12					(-)	(-)	(-)	(-)
	0.14					(-)	(-)	(-)	(-)
	0.16					(-)	(-)	(-)	(-)

(-) = no adult emerged at this dose
IM = irradiated male
IF = irradiated female
UM = unirradiated male
UF = unirradiated female

Table 5. STERILITY EXPRESSED AS PERCENT EGG HATCH AFTER FIVE WEEKS OF *Tribolium Castañeum* ADULTS THAT EMERGED FROM IRRADIATED LARVAE

Mating combination	Dose applied (kGy)	Sterility of adults which emerged from larvae							
		Adults from 14 to 15-day old larvae				Adults from 20 to 21-day old larvae			
		Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)	Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)
UM x UF (control)	0	531	459	86.4		446	369	82.7	
IM x IF	0.02	294	137	46.6	39.8	461	221	47.9	34.8
	0.04	100	42	42.0	44.4	4	1	25	57.7
	0.06	26	0	0	86.4	(-)	(-)	(-)	(-)
	0.08	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
IM x UF	0.02	300	174	58.0	28.4	407	196	48.2	34.5
	0.04	78	28	35.9	50.5	13	0	0	82.7
	0.06	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.08	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
UM x IF	0.02	281	145	51.6	34.8	227	83	36.6	46.1
	0.04	48	16	33.3	53.1	9	0	0	82.7
	0.06	72	2	2.8	83.6	(-)	(-)	(-)	(-)
	0.08	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	0.16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

(-) = no adult emerged at this dose
IM = irradiated male
IF = irradiated female
UM = unirradiated male
UF = unirradiated female

Table 6. STERILITY EXPRESSED AS PERCENT EGG HATCH AFTER FIVE WEEKS OF *Tribolium Castañeum* ADULTS THAT EMERGED FROM IRRADIATED PUPAE

Mating combination	Dose applied (kGy)	Sterility of adults which emerged from pupae							
		1 to 2-day old pupae				4 to 5-day old pupae			
		Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)	Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)
UM x UF (control)	0 (control)	815	604	74.1		461	395	85.7	
IM x IF	0.02	894	271	30.3	43.8	231	138	59.7	26.0
	0.04	170	7	4.1	70.0	42	10	23.8	61.9
	0.06	32	0	0	74.1	39	9	23.1	62.6
	0.08	20	0	0	74.1	24	5	20.8	64.9
	0.10	0	0	0	74.1	9	1	11.1	74.6
	0.12	0	0	0	74.1	0	0	0	85.7
	0.14	0	0	0	74.1	0	0	0	85.7
	0.16	0	0	0	74.1	0	0	0	85.7
IM x UF	0.02	896	526	58.7	15.4	376	235	62.5	23.2
	0.04	332	111	33.4	40.7	162	85	52.5	33.2
	0.06	472	119	25.2	48.9	391	146	37.3	40.4
	0.08	350	65	18.6	55.5	172	60	34.9	50.8
	0.10	562	99	17.6	56.5	163	53	32.5	53.2
	0.12	505	55	10.9	63.2	97	20	20.6	65.1
	0.14	495	32	6.5	67.6	0	0	0	85.7
	0.16	437	24	5.5	68.6	0	0	0	85.7
UM x IF	0.02	819	366	44.7	29.4	300	203	67.7	18.0
	0.04	250	34	13.6	60.5	217	105	48.4	37.3
	0.06	49	1	2.0	72.1	50	19	38.0	47.7
	0.08	30	0	0	74.1	46	16	34.8	50.9
	0.10	27	0	0	74.1	156	46	29.5	56.2
	0.12	0	0	0	74.1	4	1	25.0	60.7
	0.14	0	0	0	74.1	0	0	0	85.7
	0.16	0	0	0	74.1	0	0	0	85.7

IM = irradiated male
IF = irradiated female
UM = unirradiated male
UF = unirradiated female

Table 7. STERILITY EXPRESSED AS PERCENT EGG HATCH AFTER FIVE WEEKS OF *Tribolium Castañeum* ADULTS

Mating combination	Dose applied (kGy)	Sterility of adults							
		4 to 5-day old adults				7 to 8-day old adults			
		Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)	Total no. of eggs laid	Total no. of egg hatch	% Egg hatch	Reduction in fertility (% of control)
UM x UF (control)	0 (control)	1518	1280	84.3		1520	1306	85.9	
IM x IF	0.02	925	645	69.70	14.6	604	364	60.3	25.6
	0.04	563	283	50.30	34.0	638	272	42.6	43.3
	0.06	494	108	21.90	62.4	202	53	26.2	59.7
	0.08	231	27	11.70	72.6	90	18	20.2	65.7
	0.10	80	5	0.06	84.2	41	6	14.6	71.3
	0.12	62	0	0	0	17	0	0	85.9
	0.14	27	0	0	0	0	0	0	85.9
	0.16	0	0	0	0	0	0	0	85.9
IM x UF	0.02	1058	780	73.7	10.6	922	646	70.1	15.8
	0.04	848	553	65.2	19.1	593	339	57.2	28.7
	0.06	836	493	58.9	25.4	566	242	42.8	43.1
	0.08	627	333	53.1	31.2	617	229	37.1	48.8
	0.10	923	382	41.4	42.9	438	142	32.4	53.5
	0.12	833	318	38.2	46.1	762	216	28.3	57.6
	0.14	255	81	31.8	52.5	519	124	23.9	62.0
	0.16	532	135	25.4	58.9	598	1117	19.6	66.3
UM x IF	0.02	1176	838	71.3	13.0	921	615	66.8	19.1
	0.04	658	372	56.5	27.8	826	406	49.2	36.7
	0.06	680	311	45.7	38.6	569	212	37.3	48.6
	0.08	510	128	25.1	59.2	426	144	33.8	52.1
	0.10	198	38	19.2	65.1	94	26	27.7	58.2
	0.12	44	6	13.6	70.7	103	21	20.4	65.5
	0.14	85	12	14.1	70.2	78	13	16.7	69.2
	0.16	67	7	10.4	73.9	10	0	0	85.9

IM = irradiated male
IF = irradiated female
UM = unirradiated male
UF = unirradiated female

Table 8. COMPARISON OF LD99 AND LD99.9 VALUES FOR STERILITY OF *Tribolium castaneum* AFTER IRRADIATION

Parameters	LARVAE			PUPAE						ADULT					
	14–15 day old larvae			1–2 day old pupae			4–5 day old pupae			4–5 day old adult			7 - 8 day old adult		
	Weeks after irradiation			Weeks after irradiation			Weeks after irradiation			Weeks after irradiation			Weeks after irradiation		
	3	4	5	1	2	5	1	2	5	3	5	7	3	5	7
Dose required to achieve LD 99	**0.1782**	**0.3256**	**0.0933**	**0.0896**	**0.0502**	**0.0891**	**0.1049**	**0.2014**	**0.2770**	**0.1763**	**0.3032**	**0.2426**	**0.1649**	**0.2312**	**0.6605**
95% confidence limit for effective dosage															
Lower limit	0.0786	0.6832	0.0449	0.0465	0.0370	0.0525	0.017	0.0747	0.1328	0.1373	0.1506	0.1443	0.1123	0.1285	0.2536
Upper limit	1.0063	—	—	0.2767	0.1292	0.6350	0.6670	0.8197	1.2110	0.2521	1.6735	0.8546	0.3398	0.9493	1.6511
Dose required to achieve LD 99.9	**0.3263**	**0.8029**	**0.1541**	**0.1548**	**0.0672**	**0.1603**	**0.1394**	**0.3550**	**0.5097**	**0.2873**	**0.6116**	**0.4469**	**0.2732**	**0.4392**	**1.7265**
95% confidence limit for effective dosage															
Lower limit	0.1132	0.0994	0.0585	0.0632	0.0450	0.0764	0.0846	0.1024	0.1975	0.2080	0.2477	0.2236	0.1656	0.2026	0.4810
Upper limit	0.6081	—	—	0.4836	0.2378	1.5809	1.7577	1.5100	1.6582	0.4582	2.6751	2.5135	0.7085	2.8543	3.0270

— = Probit analysis did not generate upper 95% confidence limits for effective dosage

Figure 1. Dose required for 99.9 % sterility in irradiated pairs of *Triboleum castañeum*

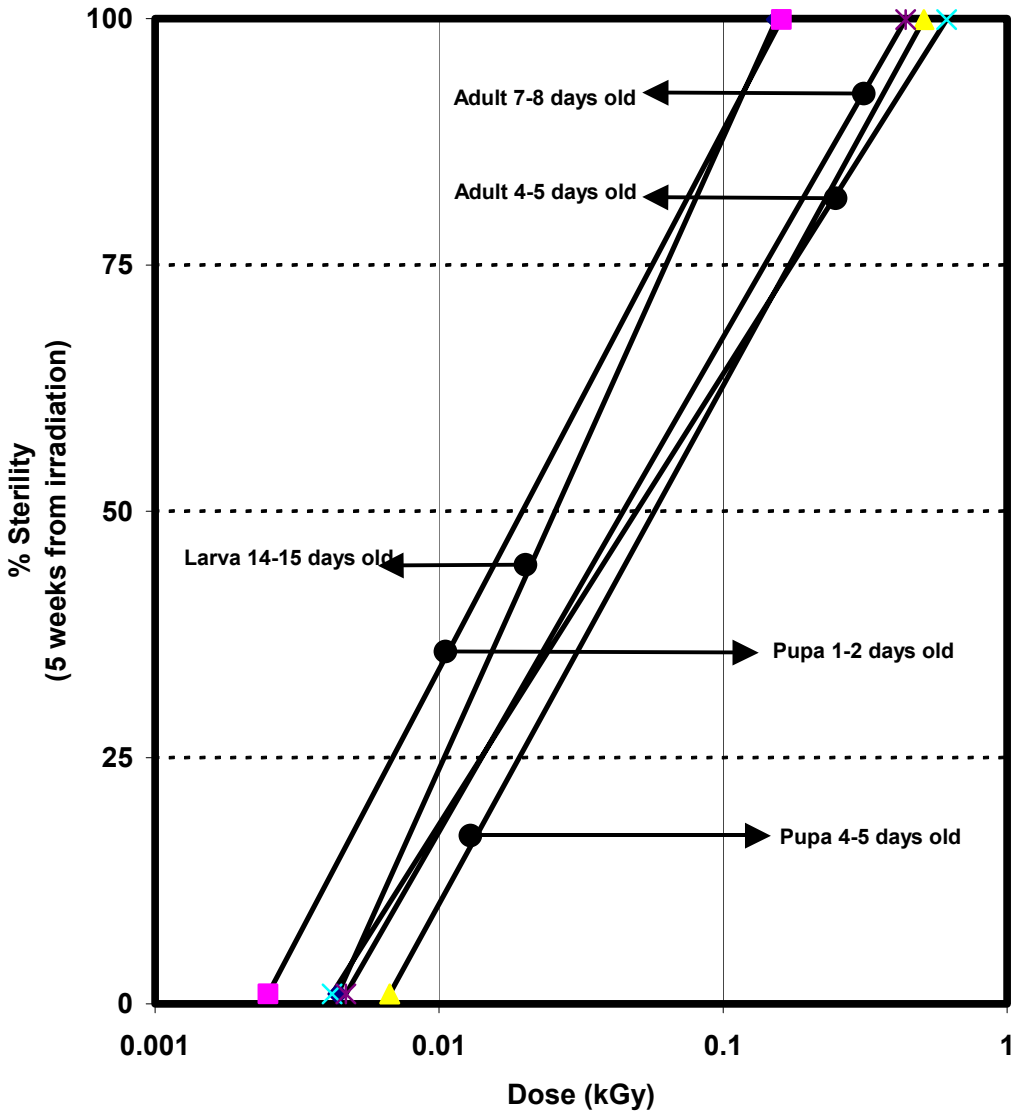

Table 9. COMPARISON OF LD99 AND LD99.9 VALUES FOR MORTALITY OF DIFFERENT DEVELOPMENTAL STAGES OF *t. Castañeum* AFTER IRRADIATION

	EGGS 3–6 days old				LARVAE 21–25 days old				PUPAE 2–5 days			ADULT 4–7 days old		
	Days after irradiation				Days after irradiation				Days after irradiation			Days after irradiation		
Parameters	7	14	21	28	7	14	21	28	7	14	17	7	14	21
Dose required to chieve LD99	**3.4764**	**1.4052**	**1.0590**	**0.7263**	**2.9192**	**1.0945**	**0.7884**	**0.5045**	**3.3537**	**0.9050**	**0.4227**	**2.404**	**1.2352**	**0.3966**
95% confidence limit for effective dosage														
Lower limit	2.9908	1.2700	0.9282	0.6420	2.4164	0.9662	0.424	0.0045	2.8960	0.7976	0.3704	2.0944	1.1011	—
Upper limit	3.0639	1.5816	1.2161	0.8159	4.0450	1.2309	1.0742	0.0961	4.2318	1.0289	0.4869	2.9294	1.4080	—
Dose required to achieve LD99.9	**3.4800**	**1.4072**	**1.0608**	**0.7277**	**2.9227**	**1.0966**	**0.7911**	**0.5070**	**3.3582**	**0.9066**	**0.4234**	**2.4063**	**1.2369**	**0.4010**
95% confidence limit for effective dosage														
Lower limit	2.9952	1.2740	0.9340	0.6454	2.4506	0.9717	0.4335	0.0100	2.9315	0.8022	0.3728	2.1059	1.1072	—
Upper limit	4.2866	1.5787	1.2140	0.8154	4.0192	1.2297	1.0739	0.8541	4.2062	1.0275	0.4859	2.9228	1.4054	—

--- = Probit analysis did not generate upper and lower 95% confidence limits for effective dosage

4. SUMMARY AND RECOMMENDATIONS

1. Mature eggs were more resistant to irradiation than young eggs. Emergence to adults was prevented at a dose of 0.08 kGy for one to two day old eggs but was not stopped for three to four day old eggs at doses as high as 0.16 kGy

2. Larvae were the most sensitive to irradiation. Young larvae however were more resistant to irradiation than older larvae.

3. Pupae were the most resistant stage to irradiation. Older pupae were more resistant than younger pupae. Emergence to adults was not prevented in both types of pupae up to 0.16 kGy.

4. Survivors were not observed after four weeks for adults receiving a dose of 0.10 kGy or higher.

5. Adults emerging from irradiated eggs, larvae, and pupae were sterile, in some cases, at doses slightly higher than those required to prevent adult emergence. Adults from irradiated eggs and larvae were sterile at 0.06 kGy. For pupae, sterility was achieved at 0.06 and 0.12 kGy for the one to tw- day and four to five-day old pupae respectively; and for adults sterility was achieved at 0.12 kGy. As expected, when one member of a mating pair was left unirradiated, the dose requirements were higher. The effect of mating combination is still being analyzed. A comparison was not always feasible due to the inadequate number of data.

6. LD 99 and LD 99.9 values for sterility have not been reported before for this insect. A major problem encountered was the inability to get an adequate number of adult survivors from eggs and larvae to carry out Probit analysis because the use of high dose levels resulted in low numbers of adults. For pupae and adults on the other hand, the dose levels used were too low to establish the dose at which egg hatch would be zero. The data are undergoing further analysis. Tests with the other major storage pest in cacao beans, *Ephestia cautella* also have to be completed before making a decision on a recommended dose. The dosage to be used in commercial treatment should be based on the most tolerant species, stage, and age. Phase 2 of this project involving an evaluation of the effect of dose on the functional properties of the beans and the sensory properties of its products and by-products will be started as soon as disinfestation dose requirements are established.

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IRRADIATION AS A QUARANTINE TREATMENT AGAINST CITRUS RUST MITE (*PHYLLOCOPTRUTA OLEIVORA*)

MEI-YING HU, XIU-QIONG LIU, LI-JUAN ZHOU, XUE-MEI LO, REN-HUAN HOU, QUN-FANG WENG
Department of Plant Protection,
Agricultural University,
Guangzhou, China

Abstract

Irradiation experiments with various stages of the citrus rust mite, *Phyllocoptruta oleivora*, showed that eggs were the most sensitive stage; although acute mortality was low, no nymphs were found after irradiation with a dose of 100 Gy. Protonymphs were also sensitive to irradiation, and 100 Gy was the lethal dose. An irradiation dose of 300 Gy controlled deutonymphs. Adults were sterilized with an irradiation dose of 350 Gy, and 450 Gy applied to one-day old adults resulted in 100% mortality after sevendays. Experiments on the effect of irradiation combined with low temperatures showed that an irradiation dose of 400 Gy and storage at 5 ± 1^{o} C and 70% RH prevented adult reproduction. Nutrient analysis of irradiated oranges showed that doses $\leq$ 400 Gy had minimal effect on ascorbic acid content and total acids. An irradiation dose of 400 Gy will provide complete control of citrus rust mite with no adverse effect on selected nutrients of oranges.

1. INTRODUCTION

Citrus has been grown in China for a many centuries. Hongjiang sweet orange (red orange) is a new cultivated variety in China with high Vitamin C. It has a beautiful shape and many desirable attributes, such as a thin peel, red flesh, and flavorful, nutritious juice. It has become a favorite variety for many consumers. Hongjiang sweet orange won awards during the Mandarin oranges/tangerines competition in 1987, and was honored with the name "The King of the Orange". It is exported from China to Hongkong, Singapore, Malaysia and other countries in Southeast Asia. In 1997, the Chinese government invested more than 10 million yuan in a project to plant Hongjiang sweet orange in Guangdong province in order to increase production and exports of the orange to Southeast Asian countries.

Phyllocoptruta oleivora Ashmead, the citrus rust mite, is an important insect pest of citrus in China. The mite causes direct losses of fruit in the orchard, and reduces the quality of fresh fruit. Citrus rust mite damages the fruit by puncturing the epidermal cells of the rind with its piercing mouthparts. The outer layer of cells is destroyed causing the injured area to turn russet and black in color; in addition, the rind of the fruit becomes thicker than normal and infested fruit are typically smaller. The grade of mite-damaged fruit is, of course, greatly reduced. Heavy rust mite populations on leaves and twigs causes leaves to turn light gray and finally brown, and the myriad cast skins may give the tree a dusty or powdery appearance. It is imperative to develop methods for quarantine treatment of this pest on fruit to prevent damage in transit and the spread of *P. oleivora* to other countries.

In the past, the major impetus for the development of irradiation as a quarantine treatment has been for the control of various species of fruit flies (Tephritidae) on fresh fruits and vegetables. Radiation disinfestation has many characteristics of a good quarantine

treatment: it is a rapid process that does not unduly delay shipment of the commodity, it is highly effective at the proper doses, it produces no undesirable or toxic residues, it usually results in no adverse effects to product quality, it allows sealed packages and containers to be treated effectively without being opened, and it is equally effective during cold weather and when insects are diapausing or in the egg stage.

In this paper, we examine (1) the effects of gamma irradiation on eggs, protonymphs, deutonymphs and adults of the citrus red mite, (2) test the effects of combining irradiation and cold temperature as a disinfestation method for the mite, and (3) measure the effect of irradiation on ascorbic acid and total acids content.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Radiation Source

Insects were irradiated using research-scale irradiators (designed by Nordion Company) located in the Biophysics Building, South China Agricultural University. The irradiators use a cobalt-60 source of gamma radiation; the sources during the period of the experiments were 2.14×10^{11} Bq and 3.70×10^{15} Bq and the dose rate was 11.90 Gy/min and 26.2 Gy/min respectively.

2.1.2. Original adult mites

Citrus rust mites were obtained from a colony maintained on citrus seedlings without insecticides in the greenhouse at the Laboratory of Insect Toxicology, Department of Plant Protection, South China Agricultural University, Guangzhou, People's Republic of China.

2.1.3. Eggs

Citrus leaves were detached from the seedlings, washed with water and dried with a paper towel. Each leaf was placed on a wet cotton pad in a Petri dish (12 cm diam.). The leaf petiole was covered with wet cotton. Approximately 500 yellow adult mites were carefully transferred onto each leaf. Adults were removed after they had oviposited about 300 eggs.

2.1.4. Protonymphs

Citrus leaves were removed from seedlings and prepared as described above. Then, 300-400 yellow adult mites were carefully transferred onto each leaf. Adults were removed after they had oviposited about 200 eggs. After three days, eggs that had not hatched were removed.

2.1.5. Deutonymphs

Citrus leaves were prepared as described above. About 400 adults were transferred onto leaves and removed after they had oviposited about 200 eggs. After seven days, all life stages except deutonymphs were removed.

2.1.6. Adults

Citrus leaves were prepared as described above. Then, 100 adult mites were carefully transferred onto each leaf.

2.2. Methods

2.2.1. Irradiation of eggs

Leaves with eggs were immediately irradiated at doses of 0, 100, 200, 300 or 400 Gy. There were six replications. After treatment, Petri dishes with leaves and eggs were placed at 25±1^{o} C, 70% RH and a photoperiod of 12:12 (L:D) h. Egg eclosion was counted at one, three, and five days after the treatment and the neonates from treated eggs were also counted at five and seven days after treatment.

2.2.2. Irradiation of protonymphs

Leaves with protonymphs were immediately irradiated at doses of 0, 100, 150, 200, 250 and 300 Gy. There were six replications and each consisted of four leaves with about 120 protonymphs on each. After treatment, Petri dishes with leaves and mites were placed at 25±1^{o}C, 70% RH and a photoperiod of 12:12 (L:D)h. Mortality was assessed by prodding each mite with a tiny brush-pen to stimulate movement. Individuals that did not move appendages vigorously were considered dead.

2.2.3. Irradiation of deutonymphs

Leaves with deutonymphs were immediately irradiated at doses of 0, 100, 150, 200, 250, 300 and 400 Gy. There were six replications and each consisted of about four leaves with about 100 deutonymphs on each. Mortality was assessed using the method described for protonymphs.

2.2.4. Irradiation of adults

Adult mites (25-30) were carefully transferred onto each leaf, and infested leaves were immediately irradiated at doses of 0, 200, 250, 300, 350 and 400 Gy. There were six replications. Adult mortality was assessed using the method described for protonymphs. Adult progeny (eggs, protonymphs, and deutonymphs) were counted at 1one, three, five, seven, and ten days after treatment.

2.2.5. Combined treatment using irradiation and cold temperature

Leaves with adults were immediately irradiated at doses of 100, 200, 300, 400 and 500 Gy. After irradiation, at each dose was coupled with 15±1^{o}C & 70% RH, 10±1^{o} C & 70% RH, or 5±1^{o} C & 70% RH and photoperiod of 12:12 (L:D) h also. Each dose and temperature had six replications. Adult mortality was assessed using the method described for protonymphs. Adult progeny (eggs, protonymphs and deutonymphs) was also counted at one, three, five, and seven days after the treatment.

2.2.6. Analysis of nutrients of orange

2.2.6.1. Determination of ascorbic acid

Ascorbic acid content was determined by titration against 2,6-dichlorophenol indophenal.

2.2.6.2. Determination of total acids

Titratable acidity was determined by titration against 0.1N NaOH.

3. RESULTS AND DISCUSSION

3.1. Effect of irradiation on eggs

Irradiation of eggs at various doses showed that the mortality of eggs increased with increasing dose (Table 1). Eggs treated at 400 Gy showed mortality of 91.90% five days after treatment whereas eggs treated at 100Gy showed 55.37% mortality (control mortality was 4.68%). Five days after the treatment, 1598 eggs hatched and developed normally in the control, while no nymphs were found five and days after the treatment at 100–400 Gy.

3.2. Effect of irradiation on protonymphs

Protonymphs were highly susceptible to irradiation. Protonymphs treated at 300 Gy showed mortality of 100 % five days after treatment whereas protonymphs treated at 100 Gy showed 82.30% mortality (control mortality was 4.41%) (Table 2). Ten days after treatment, protonymphs treated at 100 Gy showed mortality of 100%, while control mortality was 8.66%. Therefore, an irradiation dose of 100 Gy will disinfest fruit of citrus rust mite protonymphs.

3.3. Irradiation on deutonymphs

Deutonymphs treated at 300 Gy showed mortality of 43.71% one day after treatment whereas protonymphs treated at 400 Gy showed 60.95% mortality (control mortality was 1.07%) (Table 3). Deutonymphs treated at 300 Gy showed mortality of 97.08% five days after treatment whereas deutonymphs treated at 400 Gy showed 100% mortality (control mortality was 4.83%). Ten days after treatment, deutonymphs treated at 300Gy showed 100% mortality while the mortality of control was 19.80%. Therefore, an irradiation dose of 300 Gy will disinfest fruit of citrus rust mite deutonymphs.

3.4. Irradiation of adult mites

3.4.1. Lethal effect of irradiation against 1-day-old adults

Adult mites exhibited low mortality three days after irradiation at 200–400 Gy (Table 4); however, seven days after irradiation at doses of 300, 350, 400 and 450 Gy, mortality was 76.76%, 86.21%, 97.03%, and 100%, respectively, whereas control mortality was 18.52%.

3.4.2. Sterilizing effect of irradiation against 1-day-old adults

The number of eggs produced by decreased with increasing dose between 100-300 Gy. Adult mites treated at 350-500 Gy produced no viable progeny (Table 5); therefore, 350 Gy was the sterilizing dose.

3.5. Irradiation coupled with different temperature on adults

In general, higher mortality resulted with storage at 5^{o} C than 15^{o} C after irradiation. Seven days after irradiation at a dose of 400 Gy and storage at 5^{o} C, mortality of adults was 97.32% and no eggs were found (Table 6). When adults were treated at a dose of 500 Gy and stored at 5^{o} C, mortality was 100% after five days and no eggs were found. Irradiation at 400–500Gy coupled with extended cool storage at 5^{o} C could disinfest citrus of adult citrus rust mites.

3.6. Measurement of nutrients of oranges irradiated at various dosages

3.6.1. Content of ascorbic acid of orange after irradiation

Ascorbic acid levels of oranges irradiated at 300 and 400 Gy were not significantly different from untreated control fruit after 7, 20, 40 or 60 days storage (Table 7). Oranges irradiated at 500 and 600 Gy showed a slight but sometimes significant decrease in ascorbic acid content compared to untreated controls. Fruit in all irradiation treatments showed a decrease in ascorbic acid content during storage. In the 400 Gy treatment, average ascorbic acid content decreased from 46.25 to 42.24 mg/100 ml during 60 days storage.

3.6.2. Content of total acids of orange after irradiation

Fruit in all irradiation treatments showed a decrease in total acids content during storage. After 20 days storage, total acids of fruit irradiated at 500 and 600 Gy were significantly lower than that of fruit treated at 300 and 400 Gy and the untreated control fruit; however the difference disappeared at 40 days storage and fruit irradiated at 500 and 600 Gy actually had significantly higher total acids than fruit treated at lower doses after 60 days storage.

4. CONCLUSIONS

1. Experiment on the effect of irradiation on eggs of citrus rust mite showed that though acute mortality was low, no nymphs were found seven days after the treatment even at the lowest dose of 100 Gy.

2. Experiment on the effect of irradiation on protonymphs of citrus rust mite showed that protonymphs were also sensitive to radiation, and 100 Gy prevented development to adults (100% mortality after 10 days).

3. Experiment on the effect of irradiation on deutonymphs of citrus rust mite showed that 300 Gy was lethal (100% mortality after 10 days).

4. Experiment on effect of irradiation on adults of citrus rust mite showed that 350 Gy was a sterilizing dose.

5. Experiment on effect of irradiation combined with cold temperature storage showed that 400 Gy and 5 ± 1^{o} C and 70% RH prevented reproduction of adults.

6. Irradiation doses sufficient to control citrus rust mite should have no effect on ascorbic acid or total acid content or oranges.

All in all, according to the sterilizing and lethal doses determined for different stages of citrus rust mite, and considering the effect of irradiation on the ascorbic acid and total acid content of the oranges, we conclude an irradiation dose of 400 Gy should be an effective dose to control citrus rust mite with no adverse effects on selected nutrients.

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DEVELOPMENT OF IRRADIATION AS A QUARANTINE TREATMENT OF MITES ON CUT FOLIAGE AND ORNAMENTALS

HAMIDAH SULAIMAN, MOHD. SHAMSUDIN OSMAN[1],
ZAINON OTHMAN[2], MOHD. RIDZUAN ISMAIL[3]
[1]Malaysian Agricultural Research and Development Institute (MARDI), Kuala Lumpur, Malaysia.
[2]Malaysia Institute for Nuclear Technology Research (MINT) Kajang, Malaysia.
[3]Department of Agriculture, Serdang, Selangor, Malaysia.

Abstract

Cut flowers are an important export commodity of Malaysia in international trade, and are often subjected to infestation by various pests such as mites, scales, and thrips. The use of low ionizing radiation has been suggested as an alternative to methyl bromide fumigation, the current pest disinfestation treatment for cut flowers but which is being phased out due to environmental concerns. The criterion for efficacy of radiation as a quarantine treatment will be the inability of treated mites to reproduce at a new location rather than causing immediate mortality. Irradiating the red spider mite, *Tetranychus piercie* at a dose of 300 and 400 Gy produced sterile female adults from irradiated protonymphs and deutonymphs, respectively. A lower dose of 200 Gy induced sterility in female adults that developed from irradiated eggs and larvae. Deteriorating effects caused by irradiation treatment were reflected in immatures by their reduced emergence rate/mortality in subsequent developmental stages. A dose of 280 Gy prevented reproduction in female adults of *Tetranychus piercie* by inducing sterility, whereas a much higher dose of 5 kGy is required to produce acute mortality. A dose of 350 Gy was required to sterilize *T. piercie* deutonymphs. Based on the results obtained, gamma irradiation with dose in the range of 300–400 Gy may be applied as a quarantine treatment for *Tetranychus piercie*. Quality tests suggest this dose range is suitable for chrysanthemums (in 4% sucrose solution) but not roses, carnations, and orchids, which showed phytotoxic symptoms within the dose range of 100–400 Gy.

1. INTRODUCTION

Malaysia exported RM 79 million of floricultural produce to 42 countries in 2001. This includes RM 11 million of cut orchids, RM 18 million of temperate flowers in particular chrysanthemums, RM 19 million of dried flowers and foliages, RM 7 million of fresh cut foliage, and RM 23.5 million of ornamental potted plants (1). Government support and commitment in establishing Malaysia as a global center for floriculture products under the Third National Agricultural Policy (1998–2010) will further increase the growth of this sector.

The economical significance of red spider mites of the family Tetranychidae as pest species has increased considerably during recent decades because of their ability to develop resistance to a wide variety of pesticides used in the field. The current methods of disinfestation are insecticidal dips and fumigation with methyl bromide; however, fumigation with methyl bromide is a technology that is being phased out due to environmental concerns. This effective fumigant will be banned in developed countries by 2005 and in developing countries by 2015 under the Montreal Protocol.

Irradiation has been demonstrated as an effective method to replace the fumigants to overcome quarantine barriers against fruit flies in trade of fresh fruits and vegetables (2). The advantages of irradiation include the absence of undesirable residues, and the short treatment time compared to fumigation. Radiation doses of 1–3 kGy resulting in immediate mortality of mites are not recommended because they cause phytotoxicity to horticultural produce. Hence, lower doses that are friendly to the produce should be considered. The criterion for efficacy of radiation as a quarantine treatment will thus be the inability to reproduce at a new location rather than causing immediate and complete mortality.

2. MATERIALS AND METHODS

2.1. Survey and identification of major pests of cut ornamentals for export

Surveys of the major pests of cut ornamentals were conducted on the West Coast of Peninsular Malaysia. The areas visited were in Johore, Selangor, Perak and Pulau Pinang. They constitute the major ornamental growing areas in the country. Plants were examined for the presence of pests and diseases, and the extent of damage was recorded. Insect stages were brought back to the laboratory for identification or rearing to the adult stage for identification.

2.2. Development of rearing methods to obtain various development stages of mite for irradiation studies

Spider mites were obtained from Post Entry Quarantine Station, Serdang of the Department of Agriculture. The mites were reared on *Arachis pintoi* plants in pots. For experiments, mites were kept on a detached leaf culture, consisting of five to seven *Arachis pintoi* leaves placed on cotton wool kept saturated with water in a Petri dish and maintained in the laboratory under rearing condition of temperature 27^{o}C, 70% RH. Female and male adults were allowed to mate and the duration of the development of different life stages produced was monitored.

2.3. Effect of irradiation from 0.1 to 0.6 kGy on different developmental stages of mites

Mites of different developmental stages (eggs, larvae, protonymphs, deutonymphs, and adult females and males) on detached leaves were irradiated with doses ranging from 0–600 Gray (Gy). After irradiation, the mites were held at rearing conditions until adult emergence. Development to the next stages was monitored and viability of offspring was recorded. Sex ratio of their progeny was also noted. Fecundity, fertility and mortality of irradiated females were also determined. The dose required to prevent production of viable offspring was determined.

2.4. Efficacy test using large numbers of most resistant stage of mites to ensure no production of viable offspring

The deutonymph was found to be the most resistant stage; however, adult mites were also irradiated. Thus a total of 20 000 mites, 10 000 for each stage were irradiated at 240 Gy. The development of any viable offspring was determined.

2.5. Tolerance study on the cut ornamentals

Tolerance dose is the highest dose that can be applied to the plant without any visible or measurable injuries in terms of quality and appearance (phytotoxicity). Cut flowers, namely orchids, roses, chrysanthemums and cut foliages, were commercially packed and irradiated at the range of 0-1200 Gy. The cut ornamentals were placed at ambient temperature (25–28^{o}C) in sucrose solutions to determine its potential shelf life. Quality evaluation was based on visual observation of display characteristics such as colour, firmness of stems, foliage, and weight loss. Any phytotoxic symptoms were recorded. The shelf life of irradiated and non-irradiated cut ornamentals was compared.

Irradiation of mites was carried out at a cobalt-60 facility installed at MINT (Shepherd Model 109-68, activity 23 kCi, dose rate11.8-13.5 kGy/hour). Irradiation of cut ornamentals was conducted using the commercial cobalt-60 facility MINTec Sinagama (activity 0.9 Mci, dose rate 1.8kGy/hour). Fricke dosimeters were used for calibration.

3. RESULTS AND DISCUSSION

3.1. Location survey

The surveys were divided into three locations. In the north, six ornamental nurseries were visited in Tapah, Sungkai, and Seberang Perai. Two orchid nurseries were surveyed at Ipoh and Seberang Perai. In the central region, two orchid nurseries and seven other ornamental nurseries were surveyed in the Sungei Buloh area of Selangor. Six ornamental nurseries and two orchid nurseries were surveyed at Layang-layang, Ulu Tiram, Muar, and Senai in Johore.

3.2. Plants surveyed and pests identified

Three categories of plants were surveyed including potted and landscape plants, cut foliage, and cut flowers. The pests found are tabulated below.

Table 1. ARTHROPOD PESTS OF CUT ORNAMENTALS

Pest	Host	Status
Mites (*Tetranychus sp)*	*Dracaena godseffiana* Finger palm Yellow palm	Severe
Mites (*Tenuipalpus sp)*	Grametophylum (orchids) Dendrobium	Severe
Thrips	Rhapis exelsa Finger palm	Minimal infection
Snails (*Sublina sp)*	Cordyline sp.	Severe
Scale insects	Dracaena sp.	Minimal infection
Mealy bugs	Dracaena sp.	Minimal infection

3.3. Life cycle of *Tetranychus piercie*

The life stages of tetranychid mites are the egg followed by three active immature stages - a six-legged larva, and the eight-legged stages of protonymph and deutonymph. The active stages undergo a final resting stage before the adult emerges. Under the above rearing conditions, the life cycle of the mite obtained is as shown in Figure 1. The mite species was identified as *Tetranychus piercie* McGregor by researchers in the Entomology and Zoology Division of Thailand Department of Agriculture. The average longevity of adult mite is nine days.

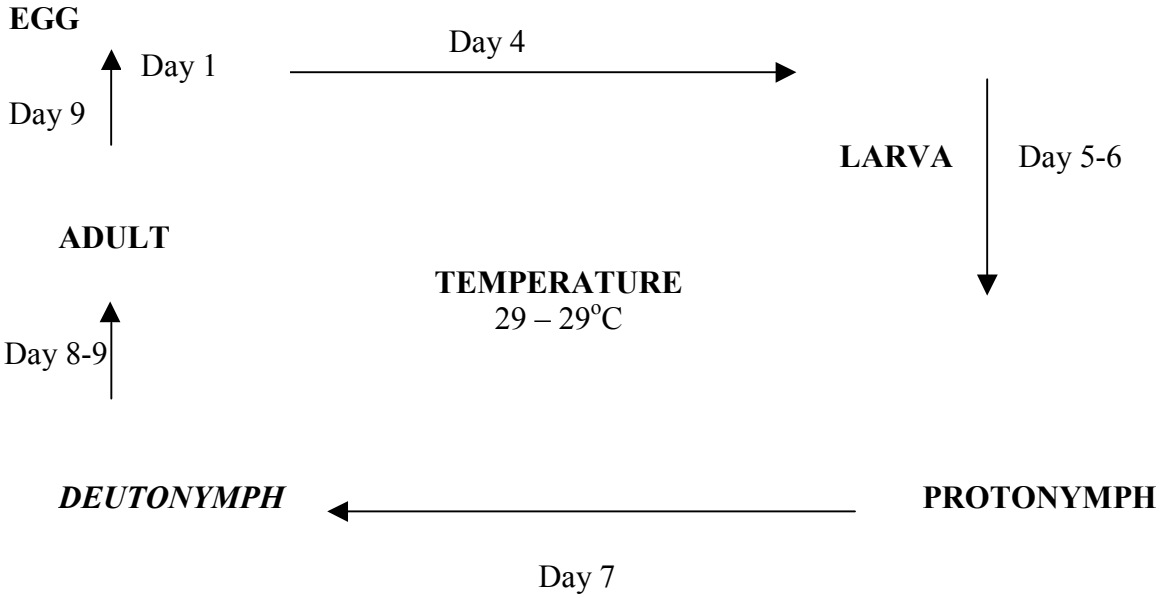

Fig. 1. Life cycle of *T. piercie.*

3.4. Effects of gamma irradiation on developmental stages of *T. piercie*

Susceptibility of egg stage (one-, two, three, and four-day old) to irradiation is shown in Table 2. Both one-day old and two-day old eggs were killed completely at 150 Gy while 41.3% of three-day old eggs and 99.0% of four-day old eggs still hatched at 600 Gray. The results showed that *T. piercie* eggs varied in its tolerance to irradiation with embryonic development, whereby tolerance increased with age of eggs. At the doses tested, tolerance remarkably increased three days after oviposition, when eyespots were observed.

A similar tendency was reported for gamma irradiation against *Tetranychus urticae* (3). Four-day old eggs were the most tolerant of all egg stages. There was no significant difference in hatchability between control and four-day old eggs irradiated at 500 Gy; however, irradiation reduced emergence rate in subsequent stages (Fig. 2). Adults developing from eggs irradiated at 0.2 Gy or higher were all female and sterile (did not lay eggs). Eggs fated to be male were obviously susceptible to irradiation. The females were inactive compared to control mites, and malformation of the legs was noticed in adults from irradiated three- and four-day old eggs.

Table 2. EFFECTS OF IRRADIATION ON HATCHABILITY OF DIFFERENT AGES OF *T. piercie* EGGS[1]

Dose (Gy)	1-day old	2-day old	3-day old	4-day old
0	99.3 (277)[(2)]	100 (205)	96.9 (228)	94.6 (257)
100	3.4 (237)	0.42 (479)	88.2 (262)	97.7 (261)
150	0 (263)	0 (176)	–	–
200	0 (193)	0 (346)	82.0 (250)	99.0 (314)
300	0 (135)	0 (200)	75.4 (285)	98.7 (309)
400	–	–	56.3 (192)	93.8 (288)
500	–	–	58.9 (265)	94.9 (295)
600	–	–	41.3 (293)	88.9 (279)

[1] Mated adult females were allowed to oviposit on *Arachis pintoi* leaves for 24 hours. Five females per leaf were used.

[2] Value in parenthesis is a sum of tested eggs in five replications.

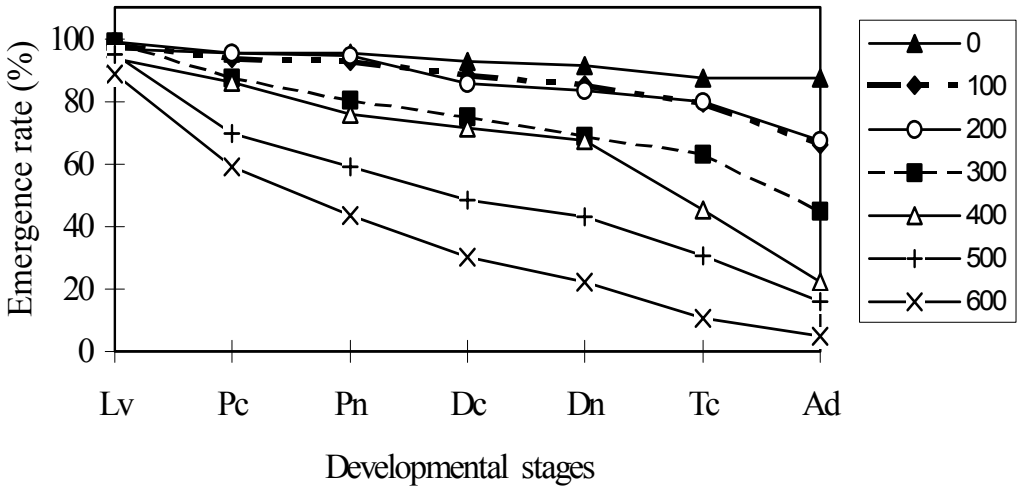

Fig. 2. Emergence rate of immature stages from irradiated 4-day old eggs

Lv = larva,
Pc = protochrysalis
Pn = protonymph
Dc = deutochrysalis
Dn = deutonymph,
Tc = teleiochrysalis
Ad = adult

Figure 3 shows the effect of irradiation on the inability of immature stages to develop to adult stage. Radiation treatment resulted in reduction of adult emergence and/or delayed adult emergence. The deutonymph was the most tolerant stage to irradiation compared to the four-day old egg, larva, and protonymph stages, while the four-day-old egg was the most sensitive to irradiation. At 600 Gy, 42.1 % of deutonymphs failed to develop into adult as compared to 95%, 66.7% and 50.9% for eggs, larvae, and protonymphs, respectively. A radiation dose of 300 Gy produced sterile female adults from irradiated protonymphs, while 400 Gy produced sterile adults from irradiated deutonymphs. A lower dose of 200 Gy was shown to induce sterility in female adults developed from irradiated four-day old eggs and larvae. Deteriorating effects caused by irradiation treatment were reflected in immatures by their reduced emergence rate / mortality in subsequent developmental stages.

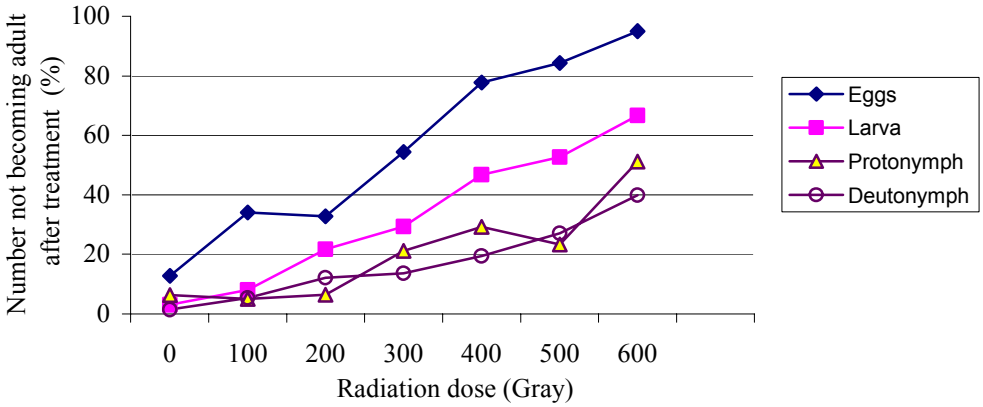

Fig. 3. Effect of irradiation on various developmental stages of *T. piercie.*

3.5. Effect of irradiation on adult mites

Eggs laid by irradiated females were reared on *Arachis pintoi* leaves until the adult stage and their development was recorded. Viability of eggs laid by irradiated females was significantly reduced (Table 3). Female mites treated with a dose of 200 Gy produced >99% non-viable eggs compared to 10% in control mites. A dose of 300 Gy produced 100% mortality of eggs. To determine the sterilising dose of adult females, mites were irradiated with doses of 160, 200, 240, 280 and 320 Gy. Hatchability of eggs produced by irradiated mites indicated that a dose of 240 Gy is the lowest dose causing total sterility in female adults of *T. piercie.* The dose required to sterilise *T. urticae* by gamma irradiation was reported to be 300 Gy [3].

Adult mites were irradiated at doses of 1, 2, 3, 4 and 5 kGy to determine the dose causing acute mortality. Instant mortality was only observed at 5 kGy as shown in Table 4. However, this high dose will certainly produce deleterious effects in the horticultural commodity and therefore is not suitable as a quarantine dose. When large-scale confirmatory testing was performed with adult mites (n = 10 000), it was found that 280 Gy is the quarantine dose for adult mites. In the case of deutonymphs, when large-scale confirmatory testing (n = 10 000) was performed it was found that 350 Gy is the quarantine dose; no viable offspring were observed at this dose.

Table 3. EFFECT OF IRRADIATION ON DEVELOPMENT OF EGGS FROM IRRADIATED FEMALE ADULTS

Dose (Gy)	Number of female adults[(1)]	Total eggs observed	Egg to Larva	Egg to Proto-nymph	Egg to Deuto-nymph	Number of F1 Adult	Sex ratio female:male
0	145	2424	2173 (89.6)	2118 (87.4)	2075 (85.6)	2032 (83.8)	4.99
100	166	2023	264 (13.0)	241 (11.9)	197 (9.7)	195 (9.6)	3.15
200	155	3371	20 (0.6)	20 (0.6)	19 (0.6)	19 (0.6)	0.90
300	155	2911	0	0	0	0	0
400	145	2890	0	0	0	0	0
500	145	2396	0	0	0	0	0
600	145	2016	0	0	0	0	0

[(1)] Sum of treated female adults in five replications

Table 4. MORTALITY DOSE OF FEMALE ADULT *T. Piercie*

Dose (kGy)	No. of adult observed	Mortality after treatment (%)		
		Day 1	Day 4	Day 6
0	64	0	4.7	14.1
1	71	0	12.7	23.9
2	74	0	97.3	100.0
3	77	3.0	100.0	
4	64	81.8	100.0	
5	55	100.0		

The effect of irradiation on sex ratio is critical to mite population growth rate. Because daughters determine growth, the proportion of offspring that are female affects the rate of population increase. Sex ratio of progeny developed from irradiated eggs, larvae, protonymphs, deutonymphs, and adults is as shown in Table 5. The sex ratio of progeny from irradiated immature stages was skewed towards females. In contrast, the sex ratio of progeny developed from irradiated adult was skewed towards males.

Table 5. SEX RATIO OF PROGENY DEVELOPED FROM IRRADIATED IMMATURE STAGES AND ADULTS

Dose (Gy)	Female: male ratio of progeny from irradiated stages				
	Egg	Larva	Protonymph	Deutonymph	Adult
0	3.76	5.19	4.41	4.57	4.99
100	6.82	5.81	4.63	4.36	2.78
200	All female (sterile)	35.0 (sterile)	3.49	4.09	1.00
300	All female	All female	11.28 (sterile)	5.94	No F1
400	All female	All female	57.6	7.0 (sterile)	No F1
500	All female	All female	124.5	18.21	No F1
600	All female	All female	-	22.8	No F1

3.6. Tolerance dose of cut ornamentals

Tolerance dose was determined for roses, carnations, chrysanthemums, orchids, and cut foliage, and the results obtained are shown in Table 6.

Table 6. MAXIMUM IRRADIATION DOSE TOLERATED BY VARIOUS CUT ORNAMENTALS

Type	Normal shelf-life (days)	Radiation tolerance dose (Gy)
Roses	4-6	100
Carnation	12	200
Orchids	14–21 (depends on variety)	100–300 (depends on variety)
Chrysanthemum (in 2 % sucrose)	14–21 (depends on variety)	200–400 (depends on variety)
Chrysanthemum (in 4% sucrose)	14–21 (depends on variety)	>750
Cut Foliage	14–28	600–1200 (depends on variety)

The results indicate that roses and carnation are not suitable for irradiation as they exhibit phytotoxic effects at doses lower than those required for quarantine security (280 Gy). The phytotoxic effects caused by irradiation include withering/ browning of flowers and leaves and bending of petioles. Some varieties of orchids and chrysanthemum are quite tolerant to irradiation. Holding cut chrysanthemums in sucrose solution following irradiation has been shown to prevent radiation-induced deterioration of the flower (4). When irradiated chrysanthemums were held in 4% sucrose solution, they showed no phytotoxic symptoms at doses higher than 750 Gy. This was also observed for certain cut foliages (depending on the variety).

4. CONCLUSIONS

The pest survey indicated the importance of mite infestation of cut foliage and ornamental potted plants. In adults, hatchability of eggs laid by irradiated mites indicated that a dose of 240 Gy is the lowest dose causing sterility in female adult of *Tetranychus piercie*. No viable offspring were produced when 10 000 adult mites were irradiated at 280 Gy, and no viable offspring were produced when 10 000 deutonymphs were irradiated at 350 Gy. Only chrysanthemum and cut foliage can withstand irradiation at 280-350 Gy without loss of quality. Further studies on the relative tolerance on cut foliage varieties are in progress.

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IONISING RADIATION AS A QUARANTINE TREATMENT FOR CONTROLLING *BREVIPALPUS CHILENSIS* (ACARINA: TENUIPALPIDAE) IN THOMPSON SEEDLESS GRAPES

D. CASTRO
Fundación para el Desarrollo Frutícola,
Area Cuarentenaria,
Sargento Aldea, Chile

J. ESPINOSA, M. VARGAS
Comision Chilena de Energia Nuclear,
La Reina, Chile

Abstract

Irradiation was investigated as a possible quarantine treatment against the false red vine mite, *Brevipalpus chilensis*, on Thompson Seedless grapes. The most resistant stage of *B. chilensis* was the adult. Irradiation doses required to cause 90% mortality of adults, nymphs and eggs were 1307, 970, and 328 Gy, respectively. The viability of eggs from irradiated adults decreased as irradiation dose increased. At doses between 450 and 600 Gy, adult females laid eggs but they were not viable. In dose response tests at 250, 300 and 350 Gy using adult mites on grapes, the minimum irradiation dose that prevented adult reproduction was 300 Gy. A large-scale confirmatory study demonstrated the effectiveness of 300 Gy using 8 042 adult mites. An irradiation dose of 200 Gy combined with 15 day cold treatment (simulating commercial shipping conditions) was also shown to be sufficient to stop reproduction in the mite, and this treatment combination was later confirmed with 5088 adult mites. The organoleptic properties of Thompson Seedless grapes irradiated at 600 Gy were not affected, nor were ripeness parameters altered relative to soluble solids and acidity; no phytotoxicity was detected in either berries or the stem.

1. INTRODUCTION

1.1. General background

The major quarantine problem for exportation of Chilean table grapes to North America is the false red vine mite, *Brevipalpus chilensis* Baker, a native pest belonging to the Order Acari and to the family Tenuipalpidae. This mite is found on a variety of fruits and ornamentals from the IV to the IX region of Chile. The main fruit hosts are wine grapes (primarily the cultivars Cot Rouge, Semillon, Sauvignon, Cabernet and Ribier among others), lemon, orange, cherimoya, and in kiwi plantations close to grapevine cultivation. It is also found on ornamental plants such as Chinese privet (*Ligustrum sinensis*), chrysanthemum, and cardinal flower. The false red vine mite is not found in the United States, and for this reason the U.S. Department of Agriculture has it under strict quarantine, especially in fresh fruit shipments originating from Chile (Gonzlález, 1989).

Chile exports some 170 000 000 fruit and vegetable boxes annually, with table grapes being the main export product with 67 000 000 boxes. Of the total grape exports, 55% is channelled to the United States market, some 37 000 000 boxes, and this has a value close to $220 000 000 FOB.

Currently, table grapes and lemons exported by Chile to the United States must be fumigated with methyl bromide according to protocols and regulations given in the Quarantining Treatments Manual of the USDA, Treatment Schedule T-101(a). Methyl bromide fumigation can be done upon entry into United States ports, or in Chile as a pre-shipment phytosanitary treatment (USDA, 1998).

Methyl bromide has been used for many years as an effective treatment for a great range of agricultural products, and in some cases it is the only available quarantine treatment; however, at the moment methyl bromide is being questioned because it is highly destructive to the ozone layer, according to findings established in the Montreal protocol of which Chile is a member. The Montreal Protocol has established a schedule for a gradual reduction in the production and use of methyl bromide beginning the year 2001 with a 25% reduction, followed by a 50% reduction by 2005 and a 100% reduction by 2010. In the last session of this organization the complete phase out of methyl bromide was set for 2010 in developed countries, and 2015 in developing countries. Thus far, quarantine uses of the fumigant are exempt from this schedule; however, the U.S. Environmental Protection Agency (EPA) under the auspices of the Clean Air Act (CAA) decreed the total prohibition of the consumption and production of methyl bromide beginning in 2005.

If methyl bromide is banned or becomes unavailable for quarantine uses, Chile would experience serious difficulties meeting phytosanitary requirements for exporting fruit, in particular table grapes, to the North American market. This would produce a great economic and social hardship for the agriculture sector. For this reason, it is necessary to evaluate and implement diverse alternatives, among which ionising irradiation is a potential option.

Recent research in physical quarantine treatments includes the use of technologies such as irradiation, controlled atmosphere, cooling, and heat (either by hot forced air or steam), which have been successful in the control of Mediterranean fruit fly and other insects and mites.

1.2. Irradiation of insects and mites

There are many studies concerning the use of radiation as a quarantine treatment. The objective of the treatment need not be mortality, but rather the prevention of the emergence of insects capable of flight in the case of immature pests found in the fruit, or sterility in the case of adults that may be present in the fruit.

Ionising radiation used for the disinfestation of fruits and vegetables is at the present time one of the best substitutes for fumigants. Since low doses are required for control of quarantine pests, possible adverse changes in the fruit may be insignificant.

The ICGFI (International Consultative Group on Food Irradiation) in 1984 established that irradiation could be used effectively as a phytosanitary treatment for fresh fruit and vegetables. The sensitivity of insects or mites is variable according the stage of development at the moment of treatment. In general, the juvenile stages have greater sensitivity as they have a greater active cellular division. This activity is higher in the egg stages and lower in the adult stages (ICGFI, 1991).

Heather (1990) pointed out that mites in general have a response to irradiation similar to insects. Studies carried out by Ignatowicz (1990, 1997) determined that for nymphs and

adults of *Tyrophagus putrescentiae*, doses between 1.5 and 2 kGy achieved 100% mortality, but that a dose of 260 Gy were effective in preventing further reproduction. For other mites such as *Rhizoglyphus achinopus* (bulb mite) a dose between 500 and 600 Gy is required to stop reproduction (Ignatowicz cited by Hallman, 1998). For the eggs of *Panonychus ulmi* a dose between 400 and 600 Gy is adequate to prevent hatch; however, *P. ulmi* nymphs turned out be more resistant to the radiation than larvae and eggs, but had problems completing development (Ignatowicz, 1997). The same research indicated that sterility in both sexes is achieved with a dose of 320 Gy. Similarly, in the case of *Tetranychus urticae* and *Panonychus citri,* sterility is achieved with doses between 200 and 300 Gy and 160 and 320 Gy, respectively (Ignatowicz, 1999; Hennebeny and Beavers *et al.*, 1971, cited by Hallman, 1998). In studies carried out with eggs of *Acaro siro* (grain mite), and nymphs and adults of *Brevipalpus destructor* (in grapes), an irradiation dose of 300 Gy was sufficient to cause sterility (RIDID February, 2000). In Chile, some research was previous carried out on the effectiveness of irradiation for quarantine control of *Brevipalpus chilensis* with respect to mortality and reproductive capacity. The research was conducted using Chinese privet (*Ligustrum* sinensis) as a host.

The first study in Chile on irradiation as a quarantine treatment was carried out in 1984 between INTEC-Chile and the Comisión Chilena de Energía Nuclear, CCHEN (Chilean Nuclear Energy Commission) using various insects and mites of quarantine importance in table grapes and lemons. Later in 1994, a cooperative study between Dr. T. Rubio (CCHEN) and Mr. Díaz and Mr. Vargas (Degree Thesis of the Universidad de Chile) was conducted on the effects of ionising radiation as a quarantining treatment for the control of *Brevipalpus chilensis*. This study generated information on artificial breeding of the mites, as well as the effect of radiation on different developmental stages. An irradiation dose of 500 Gy caused 100% mortality in *Brevipalpus chilensis* eggs. Adults treated with 500 Gy showed high mortality, and the survivors generated some non-viable eggs. To obtain 100% mortality in juvenile and adult stages, doses of 1500 Gy were suggested (Díaz and Vargas, 1994. U de Chile, CCHEN).

1.3. Effects on fruit and tolerance to irradiation

In general, the quality of most fruits is not affected at irradiation doses between 100 and 300 Gy (e.g. avocado is an exception). Apples have a maximum tolerance of 900 Gy, and grapefruit greater than 300 Gy (RIDID, February, 2000). According to Drake and Neven (USDA-ARS, 1998), apricots, cherries and peaches show a minimal change in texture and firmness after irradiation. Anjou pears treated with an irradiation dose of 600 Gy showed an increase in scald and loss of firmness; however, treatment with a dose of 300 Gy did not affect its quality. Studies carried out between INTEC-CHILE and CCHEN in 1994, indicate good tolerance of table grapes to irradiation at doses up to 1000 Gy.

2. OBJECTIVE

To determine through dose-response studies and large-scale confirmatory tests the minimum irradiation dose required to interrupt the life cycle of *Brevipalpus chilensis* (i.e., prevent reproduction).

3. METHODOLOGY

3.1. Artificial breeding of *Brevipalpus chilensis*

To obtain the mites needed for the irradiation tests, artificial breeding of *Brevipalpus chilensis* was established on Chinese privet (*Ligustrum sinensis*) under controlled environmental conditions of 25 ± 2ºC, 40–60% RH, and photoperiod of 14:10 (L:D).

3.2. Experiments

Preliminary tests demonstrated that the adult as the most resistant stage. The present study involved both dose-response studies and large-scale confirmatory tests using irradiation alone. Similar studies were also conducted to test the effectiveness of irradiation combined with cold treatment.

3.2.1. Dose-response test with low doses

The dose-response studies consisted of irradiating adult mites (the most resistant stage) with doses of 250, 300 and 350 Gy. For each dose, grapes were infested with 200 mites, and tests were replicated three times. Untreated controls were included in each replicate. Grapes were infested in the laboratory using a fine brush to place mites on the fruit. Infested grapes were held for 12 to 18 hours at 25 ± 2º C, 40–60% RH, and a photoperiod of 14:10 (L:D). Before treatment, the infested cluster was wrapped in paper and placed in the center of a box (30x50x14 cm) with fresh (uninfested) grapes previously washed with water and detergent. Approximately 8.2 kg of fruit was irradiated at each dose in each replicate. After irradiation treatment, the fruit with the mites was kept under rearing conditions for 13 days.

3.2.2. Irradiation plus cold temperature test

The irradiation plus cold temperature test consisted of irradiating adult mites at doses of 150, 200 and 300 Gy as described above, then placing treated fruit in a refrigerated chamber at 0º C +/- 1º C for 15 days, followed by holding the fruit at 25º C and 40–60% RH for another seven days before evaluation.

3.2.3. Dosimetry

Irradiation was performed at the Comisión Chilena de Energía Nuclear CCHEN (Chilean Nuclear Energy Commission), using an irradiator with a Cs-137 source. The Gray (Gy) was used as the absorbed dose unit, which is equivalent to a Joule of absorbed energy per kilogram of sample. The dosimeter used in this study was the Fricke dosimeter, which consists of an Iron II (Fe^{+2}) solution, which is oxidized to Iron III (Fe^{+3}) because of the ionizing radiation. A dosimeter was placed at the center of each box of irradiated grapes. After irradiation, dosimeters were taken out of the boxes and read with a spectrophotometer.

3.2.4. Evaluation

In the laboratory, mites were removed from the grape cluster by flushing the bunch with soapy water through two sieves - one was 20-mesh (top) and the other 200-mesh (bottom). Mites were washed from the 200-mesh sieve with the aid of a wash bottle into a Petri dish, and then live and dead adults and any eggs present were transferred to a dry Petri dish

containing leaves of Chinese privet. All life stages were counted after 13 days to determine egg hatch. Under conditions of 25ºC, the time for eggs to hatch averages nine days (Gonzalez, 1958).

Percent hatch and mortality data were normalized using arc-sin square root transformation. Means separations were done using the Duncan multiple-range test ($P \leq 0.05$).

3.2.5. Large-scale confirmatory test

Once having determined the irradiation dose that achieved interruption of the life cycle, a large-scale confirmatory test was carried out. General methods were as described above. For the irradiation only test, grape clusters were infested with adult mites and treated at 300 Gy; five replicates included 1,240; 1,687; 865; 1,347; and 2,903 mites for a total of 8,042 adult mites. Untreated control mites totaled 1,233. After irradiation treatment, adults were washed from the fruit and held for 13 days before evaluation as described above.

The irradiation plus refrigeration test was performed in a similar way as described previously, but with only three replications to achieve about 5 000 treated adult mites.

4. RESULTS AND DISCUSSION

4.1. Dose-response tests with low doses

4.1.1. Effect on adult mortality

In Table 1, the mortality of adult *B. chilensis* at 13 days after irradiation at various doses is shown. There were no significant differences in mortality among the dose treatments. With doses of 300-350 Gy, mortality was 40.5–46.6%, compared with 38.0% mortality in untreated controls.

Table 1. MORTALITY OF *Brevipalpus Chilensis* - ADULTS IRRADIATED 13 DAYS POST TREATMENT

Treatment Doses (Gy)	Nº treated adult mites	Nº mites		Mortality %
		Alive	Dead	(1)
0	381	236	145	38.0(a)
250	494	306	188	38.0(a)
300	438	248	190	43.3(a)
350	414	245	169	40.8

Nº of replications = 3

(1) values followed by same letter do not differ statistically, according to Duncan test for 95% CL

4.1.2. Effect on oviposition

Irradiation caused a significant reduction in *B. chilensis* oviposition (Table 2). At a dose of 350 Gy, the number of eggs laid was 0.12 per female compared with 0.59 per female in the control treatment.

Table 2. OVIPOSITION OF *Brevipalpus chilensis* - ADULTS IRRADIATED 13 DAYS POST TREATMENT

Treatment Doses (Gy)	N° treated adult females	Egg-laying	
		N° Eggs	N° Eggs/females
0	381	228	0.59(a)
250	494	106	0.21(b)
300	438	132	0.30(b)
350	414	51	0.12(b)

N° of replications = 3

(1) values followed by same letter do not differ statistically, according to Duncan test for 95% CL

4.1.3. Effect on egg viability

Irradiation caused a significant reduction in *B. chilensis* egg hatch (Table 3). No of egg hatch occurred when adults were treated with an irradiation dose of 300 Gy.

Table 3. HATCHED EGSS OF *Brevipalpus chilensis* IRRADIATED 13 DAYS POST TREATMENT

Treatment Doses (Gy)	N° treated adult mites	N° observations Eggs	Hatched	
			N° Eggs	%
0	381	228	72	31.5(a)
250	494	106	6	5.6(b)
300	438	132	0	0.0(b)
350	414	51	0	0.0(b)

N° of replications = 3

(1) values followed by same letter do not differ statistically, according to Duncan test for 95% CL

4.2 Confirmation test

Results of the confirmatory test show that irradiation treatment with a dose of 300 Gy prevents any reproduction (table 4); 8042 irradiated adult mites laid 1853 nonviable, whereas 1233 untreated control mites laid 623 eggs and 207 hatched.

Table 4. CONFORMITY TEST OF *Brevipalpus chilensis* IRRADIATED WITH 300 GY 13 DAYS POST OVIPOSTURE

Treatment Dosesn (Gy)	Replication	N° treated adult mites	N° observations Eggs	Hatched	
				N° of eggs	%
	1	1240	380	0.0	0.0
	2	1687	328	0.0	0.0
300	3	865	270	0.0	0.0
	4	1347	450	0.0	0.0
	5	2903	425	0.0	0.0
	Total	**8042**	**1853**	**0.0**	**0.0**
Control		1233	623	207	33.2

4.3 Irradiation plus refrigeration test

Mortality increased with increasing dose and the application of cold temperature in the tests using a combination treatment of irradiation plus refrigeration at 0ºC. Mortality of adult mites at ambient temperature was 48.4%, and rose to 69.9% with the application of 15 days cold storage alone. Irradiation with a dose of 300 Gy plus cold storage increased mortality to 91.8% (Table 5).

Oviposition was reduced to 0.09 eggs per female in the 300 Gy combined with cold storage treatment, compared with 0.22-0.23 eggs per female in unirradiated control treatments at ambient temperature or cold temperature alone (Table 6). Egg hatch was prevented with an irradiation dose of 200 Gy plus cold temperature for 15 days (Table 7). This result was confirmed later with the large-scale confirmatory test using 5088 adult female mites (Table 8).

Table 5. MORTALITY OF *Brevipalpus chilensis* - ADULTS IRRADIATED 15 DAYS COLD STORAGE PLUS 7 DAYS TEMPERATURE POST TREATMENT

Treatment Doses (Gy)	N° treated adult mites	N° mites		Mortality %
		Alive	Dead	(1)
Ambient temperature	766	394	372	48.5(a)
Cold storage	639	188	451	70.5(a)
150	808	104	704	87.1(c)
200	724	83	641	88.5(c)
300	785	66	719	91.6(c)

N° of replications = 3

(1) values followed by same letter do not differ statistically, according to Duncan test for 95% CL

Cold storage: 0° =/- 1°

Ambient temperature: 25° C

Table 6. OVIPOSITION OF *Brevipalpus chilensis* - ADULTS IRRADIATED 13 DAYS AFTER 15 Days OF COLD STORAGE PLUS 7 DAYS AMBIENT TEMPERATURE POST TREATMENT

Treatment Doses (Gy)	No observed Adult Females	Egg laying	
		N° of eggs	N° of eggs/female
Ambient temperature	394	93	0.23(a)
Cold storage	188	42	0.22(a)
150	104	27	0.26(a)
200	83	5	0.06(a)
300	66	6	0.09(a)

N° of replications = 3

(1) values followed by same letter do not differ statistically, according to Duncan test for 95% CL

Cold storage: 0° =/- 1°

Ambient temperature: 25° C

Table 7. HATCHED EGGS OF *Brevipalpu chilensis* IRRADIATED 13 DAYS AFTER 15 DAYS COLD STORAGE PLUS 7 DAYS AMBIENT TEMPERATURE POST TREATMENT

Treatment Doses (Gy)	N° treated adult mites	N° observations Eggs	Hatched	
			N° of eggs	%
Ambient temperature	394	93	47	50.5(a)
Cold Storage	188	42	16	38.1(a)
150	104	27	9	33.3(a)
200	83	5	0	0.0(b)
300	66	6	0	0.0(b)

Table 8. CONFIRAMATORY TEST OF *Brevipalpu chilensis* IRRADIATED WITH 200 GY 13 KAYS AFTER 15 DAYS COLD STORAGE PLUS 7 DAYS AMBIENT TEMPERATURE POST TREATMENT

Treatment Doses (Gy	Replication	N° treated adult mites	N° observations Eggs	Hatched	
				N° of eggs	%
	1	1693	0	0	0.0
200	2	1645	0	0	0.0
	3	1750	0	0	0.0
	Total	**5088**	**0**	**0**	**0.0**
CONTROL (cold storage)	1	850	66	19	28.8
	2	744	41	13	31.7
	3	996	54	23	42.6
	Total	**2590**	**161**	**55**	**34.2**
CONTROL (ambient temperature)	1	808	143	57	39.9
	2	877	111	44	39.6
	3	933	74	44	59.5
	Total	**2618**	**328**	**145**	**44.2**

5. CONCLUSIONS

1. The oviposition capacity of *Brevipalpus chilensis* decreased with increasing irradiation dose. At a dose of 350 Gy, the number of eggs laid was 0.12 per female compared with 0.59 per female in the control treatment.
2. Eggs laid by adults irradiated with a dose of 300 Gy were not viable.
3. Irradiation with a dose of 200 Gy plus refrigeration for 15 days at 0ºC also prevented reproduction.

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COMBINATION TREATMENTS WITH IRRADIATION FOR CONTROLLING ORCHID THRIPS, *THRIPS PALMI*

K. BANSIDDHI, S. SIRIPHONTANGMUN, S. RUMCHIAPIKUL,
P. KIENMEESUKE, S. PRASONGSAB, U. JAIPETCH, S. KASA,
P. ONBOON, V. KAMNUNGSAK
Division of Entomology and Zoology,
Department of Agriculture,
Jatuchack , Bangkok, Thailand

Abstract

A combination treatment including an insecticide dip (imidachloprid 10% SL), irradiation (350 Gy) and cold storage (15^{o}C) was developed as part of a systems approach to disinfest Good Agricultural Practices (GAP)-grown orchids of orchid thrips, *Thrips palmi* Karny. In large-scale confirmatory tests, the combination treatment was sufficient to prevent development to the next growth stage in immature thrips, and adults were sterilized at this dose. Irradiation treatment delivered a minimum dose of 300 Gy and a maximum of 380 Gy. The first trial shipment through commercial channels of irradiated orchids using the quarantine treatment against orchid thrips was conducted in March 2002 between Thailand and Australia. In the commercial-scale trial, the combination treatment controlled orchid thrips and did not cause significant damage to exported GAP-grown orchid cut flowers. The systems approach proved to be an effective and economical treatment for control the orchid thrips.

1. INTRODUCTION

Due to the multiplicity of insect pests infesting fresh fruits, vegetables and ornamentals entering internationally trade, these products are frequently unacceptable without an approved disinfestation treatment. Orchid thrips, *Thrips palmi* Karny, is considered the most destructive sucking insect of ornamental crops, especially of orchid cut flowers (*Dendrobium spp.*), in Thailand. It is regulated by quarantine inspection both at the port of exit and entry. When there is a risk of rejection of orchid cut flowers due to the presence of thrips, methyl bromide is applied as a disinfestation treatment. Total trade of orchid cut flowers from Thailand was 11 680 tons in 1998. In 1997, many shipments of orchids exported to the European Union (EU) were rejected and destroyed because of thrips infestation. Loss of access to methyl bromide could seriously jeopardize this trade, and there is an urgent need for alternatives. Irradiation is a preferred option; however, irradiation, chemical dips, fumigation, and temperature manipulation when used alone are unlikely to be an acceptable treatment to support a quarantine treatment proposal. Therefore, irradiation is being studied as part of a multiple or combination treatment with other phytosanitary measures.

The project objectives were:

1) To determine the effectiveness of combination treatments to inhibit development and prevent reproduction of the orchid thrips.

2) To conduct a trade trial with Australia using the combination treatment to disinfest orchids of orchid thrips.

2. METHODS

2.1. Large-scale confirmatory test to establish efficacy of a combination treatment

Two large-scale confirmatory tests were conducted in the laboratory under 15°C and 70–80% RH humidity controlled conditions at the Division of Entomology & Zoology, Department of Agriculture, Bangkok, Thailand, during Dec 2001-Feb 2002. Orchid thrips were reared to sufficient numbers and 800 eggs, 2500 second-stage nymphs and 2500 adults were randomly released onto 5000 export quality *Dendrobium* sp. orchid inflorescences (this is approximately 60 000 flowers). Insects were held on inflorescences for 60 minutes in a pre-packing house (15°C) to allow thrips to feed, then dipped into an insecticide solution (imidacloprid 10% SL) at 100 ppm for five seconds. After dipping, orchids were air dried for approximately 15 minutes and packed in 80 cardboard boxes (size 36x70x7 cm, the same package used for exporting orchids). Cardboard boxes containing 65 inflorescences (1450 flowers) each were irradiated at the Thai Irradiation Center (TIC) at a dose of 350 Gy (min 300, max 380 Gy). Inflorescences were evaluated daily for the number of live and dead insects in each life stage, and egg fertility. Vase life effects were also evaluated using the grading index: 1 = fresh, no damage, no difference in quality between treated and untreated flowers; 2 = slight damage symptoms, 1–5% of treated flowers (opened and unopened) showing symptoms of wilting; 3 = moderate damage symptoms, with 6–25% flowers wilted and beginning to yellow; 4 = slightly severe damage symptoms, with 26–50% flowers wilted; and 5 = severe damage, with >51% flowers wilted, and flowers or buds completely drooped over and unacceptable level to consumers. Levels 1-3 are considered acceptable to consumers and meet export standards.

2.2. Trade trial with Australia

A commercial trade trial was conducted with Australia. Export quality orchid cut flowers grown under "good agricultural practices" (GAP) and orchid cut flowers not grown under GAP (non-GAP) were treated with a combination treatment including an insecticide dip (imidacloprid 10 % SL), irradiation with cobalt-60 at a dose of 350 Gy (min 300 Gy, max 380 Gy), and storage at 15°C. The project was coordinated with scientists and regulators with the Australia Quarantine Inspection Service (AQIS) to ensure acceptance of the quarantine treatment data and to meet all AQIS requirements. Thai researchers contacted AQIS officers Dr. Niranjani Saverimuttu and Mr. Gary Luckman for the "Permit to Import Quarantine Material", and established close contacts with voluntary trading partners or commercial buyers in Sydney, namely Don J-Dee Co, Ltd. "Phytosanitary Certificates" were issued by Thai Quarantine officials and an "Irradiation Certificate" was issued by the Thai Irradiation Center (TIC). Reservations for airfreight (Thai Airlines) to Australia (importing country) were made through commercial channels.

The export consignment included 5000 inflorescences (45 000 flowers) that had been collected from both certified-GAP and non-GAP producers. Orchids were held in the exporting company's packing unit under cold room condition (15°C), until they were dipped into an insecticide solution (imidacloprid 10% SL) at 100 ppm for five seconds and air dried for approximately 15 minutes. Orchids were then packed in 60 cardboard boxes (36x70x7 cm). Each cardboard box containing 65 inflorescences was irradiated at the Thai Irradiation Center at the dose of 350 Gy (min 300 Gy, max 380 Gy). Within 24 hours after irradiation, the consignment had been certified by DOA- PQ, TIC and the Thai Customs Unit, and was *en route* to Australia via airfreight. The irradiated orchid cut flowers arrived

at Sydney airport and AQIS on Saturday, 16 March 2002. The consignment was inspected upon arrival (in Australia) 1) to determine the effectiveness of the combination treatment in preventing development and reproduction of thrips (by counting live and dead insects, following the development of survivors, and determining egg hatch); 2) to evaluated vase-life effects of irradiation and shipping, and 3) to conduct market testing in Sydney.

3. RESULTS AND DISCUSSION

3.1. Large-scale confirmatory test to establish efficacy of a combination treatment

Results of the two large-scale confirmatory experiments on the effectiveness of the combination treatment are shown in Tables I and II. Egg hatch was 1.0% (8/800) and 1.25% (10/800) in the two tests but all emerging neonates were dead 48 hours after emergence. At three and seven days after irradiation (DAT), no nymphs were observed, and only 0.76% (19/2500) and 0.6% (15/2500) live adults were found in the two experiments. Surviving adults were transferred to fresh plants to examine reproduction. After 10 days, 10 and 12 adults were alive in the two tests but laid no fertile eggs. These data suggest a dose of 350 Gy (min 300 Gy, max 380 Gy) is sufficient to inhibit development or induce sterility in eggs, nymphs and adults of orchid thrips. Evaluation of vase life effects after the combination treatment showed no difference between orchids receiving the combination treatment and untreated controls. No symptoms of wilting occurred at 10 DAT and orchids were considered acceptable for export (Tables 1 and 2). Additional large-scale confirmatory tests after the trade trial with Australia (discussed below) showed similar results (data not shown).

Table 1. EFFECT OF COMBINATION TREATMENTS ON DEVELOPMENT STAGES OF *Thrips palmi* AND VASE LIFE EFFECTS (1ST EXPERIMENT)

	1st EXPERIMENT				
	48hr AT	**3 DAT**	**5 DAT**	**7 DAT**	**10 DAT**
% Eggs hatching	1.0 % (8 eggs)	–	–	–	–
Nymphs	X	(0)	(0)	(0)	(0)
Adults - Dead	X	(0)	(7)**	–	–
Adults - Live	X	0.76% (19)	0.48% (12)	0.48% (12)	(12)***
Vase life effects	X	1.00	1.035	1.375	2.34

X = not investigated
( ) = number of thrips
** = completely dead 5 DAT
*** = number of live adults not reproducing.

Table 2. EFFECT OF COMBINATION TREATMENT ON DEVELOPMENT STAGES OF *Thrips palmi* AND VASE LIFE EFFECTS (2ND EXPERIMENT)

	2nd EXPERIMENT				
	48hr AT	**3 DAT**	**5 DAT**	**7 DAT**	**10 DAT**
% Eggs hatching	1.25 % (10eggs)*	–	–	–	–
Nymphs	X	(0)	(0)	(0)	(0)
Adults - Dead	X	(0)	0.2 % (5)**	(0)	(0)
Adults - Live	X	0.6% (15)	0.4% (10)	0.4% (10)	0.4% (10)***
Vase life effects	X	1.00	1.16	1.59	2.25

X = not investigated
( ) = number of thrips
* = completely dead after 48 hours (48 hr AT)
** = completely dead 5 DAT
*** = number of live adults not reproducing.

Vase life grading index levels between 1–3 are considered acceptable to consumers and at the export standard.

3.2. Trade trial with Australia

The trade trial arranged between Thailand and Australia was conducted in March 2002. The shipment was a significant success, and all consumer feedback was positive when the irradiated orchids were sold at local markets in Sydney.

The vase life evaluation at 7 DAT showed that quality was acceptable (Table 3), and no difference was observed in quality between orchids receiving the combination treatment and untreated controls.

Table 3. EFFECT OF THE COMBINATION TREATMENT ON ORCHID CUT FLOWER QUALITY AFTER EXPORT TO AUSTRALIA, MARCH 2002

	Vase life effects from combination treatments			
	3 DAT	**5 DAT**	**7 DAT**	**10 DAT**
GAP orchids	1.00	1.10 *	1.52	2.76
Non-GAP orchids	1.00	1.11 *	1.59	2.91
Untreated	1.00	1.15 *	1.58	2.96

Note : Grading index levels between 1–3 are considered acceptable to consumers and meet export standards.
* = 3-5% of flowers showed damage.

A few arthropods (thrips and spiders) were intercepted during inspection of the non-GAP grown orchids. AQIS inspectors reported that, "The pest intercepted was a spider and the entomologists in New South Wales were not able to go any further than that in identifying as the organism was very immature and was not in a state for further observation for analysis" (Dr. Niranjani Saverimuttu, 19 August 2002. No arthropods were found in GAP-

grown orchids (Table 4). AQIS inspectors recommended fumigating the shipment with methyl bromide, even with the research results in hand from Thailand indicating that the irradiation treatment would prevent reproduction in surviving orchid thrips.

Table 5. EFFECTIVENESS OF THE COMBINATION TREATMENT AGAINST ORCHID THRIPS IN THE EXPORT TRIAL TO AUSTRALIA, MARCH 2002

	GAP Products		**Non-GAP Products**	
	LIVE	**DEAD**	**LIVE**	**DEAD**
No. thrips				
- Nymph stage	0	0	2	5
- Adult	0	0	0	3
Number of Spider	0	0	2	0

We collected the same spider from non-GAP production areas in Thailand and identified it as *Oxyopes spp*., a beneficial predator common in orchid nurseries. Other spiders are rare, suggesting comparatively low species diversity in orchid plantations. A similar trend in arthropod interceptions was observed in subsequent shipments of irradiated orchids to Australia (May-July, 2002). This brings up the problem for regulatory authorities when live interceptions are made in irradiated products: although living organisms are present they are expected die before developing to the adult stage or to be sterile as adults.

An economic analysis of the trade trial is shown in Table V. The total cost of the trade trial (using commercial channels) was approximately $1735.34. The net income after marketing the 4500 orchid inflorescences in Sydney was $4500. The positive net income was $2764.66 or a 159% return on the investment. The fixed costs showed in Table 5 could be reduced. For example, the irradiation costs at TIC were calculated for treating 5000 orchid inflorescences with a one hour minimum, although the treatment facility is capable of treating 20 times this many orchids in one hour.

Items	**US dollars**	**Thai Baht**
1. Production costs of 5,000 orchid inflorescences for export	714.28	30 000
2. Irradiation costs (1 hr)	107.14	4500
3. Air freight (216.5 kg)	479.40	20 135
4. Expense of processing in Thailand	59.52	2500
5. Fumigation at Sydney, Australia	75.00	3300
6. Import Tax at Sydney	150.00	6300
7. Other expenses at destination	150.00	6300
Total costs	**1735.34**	**72 884.52**
Earnings when marketed in Sydney (US$4500 x 2 = AU$9000)	4500	189 000
Total net income	**2764.66**	**116 115.48**

Note : The fixed costs showed in items 1, 2, 3 and 6
The exchange rate, 1 US$ = 2 AU$ = 42 Baht. (15 March 2002)

In conclusion, research indicates the combination treatment including irradiation at a dose of 350 Gy (min 300 Gy, max 380 Gy) is sufficient to prevent development to the next growth stage in immature thrips and sterilize adults of orchid thrips. The experience from the trade trial of irradiated orchid cut flowers exported to Australia from Thailand indicates it is an effective and economical disinfestation treatment against thrips; *Thrips palmi*, and useful to countries exporting orchids in Southeast Asia (SEA). A nominal dose of 350 Gy applied on a commercial scale did not cause significantly damage to exported orchid flowers and was well received in the marketplace in Australia. Some countries still have government policies prohibiting irradiation as a phytosanitary treatment for food and agricultural products. It is expected however that importing countries will eventually accept and support the use of irradiation as a technology.

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COMPARATIVE EFFECTS OF IRRADIATION AND HEAT QUARANTINE TREATMENTS ON THE EXTERNAL APPEARANCE OF LYCHEE, LONGAN, AND RAMBUTAN

P.A. FOLLETT
U.S. Department of Agriculture, Agricultural Research Service,
U.S. Pacific Basin Agricultural Research Center,
Hilo, Hawaii, United States of America

Abstract

Irradiation and heat quarantine treatments are used to disinfest lychee (*Litchi chinensis*), longan (*Dimocarpus longan*) and rambutan (*Nephelium lappaceum*) of fruit flies and other pests before export from Hawaii to the U.S. mainland. For each fruit type, fruit of various cultivars were subjected to a) hot water immersion at 49.0°C for 20 min or vapor heat at 47.2°C, b) irradiation treatment at a minimum absorbed dose of 250 or 400 Gy, or c) left untreated as controls. Fruit were then stored at 2-5°C (lychee and longan), or 10°C (rambutan) and quality attributes were evaluated after one to three weeks. The external appearance of fruit treated with hot water immersion (lychee and longan) or vapor heat (rambutan) was generally less acceptable than irradiated or untreated fruits. Overall, under the experimental conditions tested, irradiation was superior to hot water immersion and vapor heat as a quarantine treatment on the basis of fruit quality maintenance.

1. INTRODUCTION

In Hawaii, lychee, longan and rambutan are the main crops of an expanding tropical specialty fruit industry, and there is substantial commercial interest in exporting these fresh fruits to the U. S. mainland and elsewhere. Lychee, longan and rambutan, like many other tropical fruits grown in Hawaii, are under a federal quarantine because the fruits are potential hosts of the Mediterranean fruit fly, *Ceratitis capitata,* and the oriental fruit fly, *Bactrocera dorsalis*. These pests are not established in the continental United States, and commodity quarantine treatments ensure that the risk of exporting them from Hawaii is minimized.

Irradiation with a minimum absorbed dose of 250 Gy is an approved treatment by the U.S. Dept. of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS) for disinfestation of fruit flies in lychee, longan and rambutan [2]. Since 1995, various tropical fruits, including lychee, have been flown from Hawaii to the U.S. mainland for irradiation treatment and subsequent distribution and sale. This practice is expensive because of the limited number of treatment facilities and their distances from major markets. An e-beam/converted x-ray facility has recently been constructed in Hawaii, and other irradiation facilities may be forthcoming if market interests grow. Irradiation with a minimum absorbed dose of 400 Gy is accepted by the California Department of Food and Agriculture for the treatment of insects other than fruit flies [4]. The commercial irradiation facility in Hawaii typically treats all tropical exotic fruits at this level to avoid rejections due to the presence of non-fruit fly quarantine pests (scales, mealybugs, thrips, etc.). A hot water immersion treatment of 49.0 °C for 20 min, is another USDA-APHIS-approved treatment for lychee and longan from Hawaii [1, 3], and vapor heat, consisting of heating fruit to a seed surface temperature of 47.2°C in not less than 1 h and holding for 20 min, is a USDA-APHIS-approved treatment for rambutan [3]. Hawaii has numerous vapor heat treatment facilities, and one hot water immersion treatment facility undergoing certification.

A major problem in marketing lychee and rambutan is rapid pericarp browning, which makes them unattractive although the aril remains edible. Much of the postharvest research for these fruits has been directed at prevention of pericarp browning. Pericarp darkening after harvest in longan is less of a problem because longan is naturally brown in color. Although multiple disinfestation treatments have been developed for the three fruits no information on the effects of the treatments on fruit quality was generated. Different treatments may have different effects on fruit quality. The purpose of this study was to make a direct comparison of the effects of irradiation and heat quarantine treatments on lychee, longan and rambutan quality during storage under simulated commercial conditions.

2. METHODS

2.1. Experimental design

Lychee, longan and rambutan were obtained from various growers in Hawaii during commercial harvests, removed from the panicles, and stored in the laboratory in perforated plastic bags in fiberboard boxes at 25 $\pm$ 1^{o}C [5, 7, 8]. Fruit were harvested ripe from the tree on successive weeks, and each harvest date constituted a replicate of the experiment. One day after harvest, undamaged fruit were randomized for treatments, and baseline quality analyses (described below) were performed on fruit samples before initiation of quarantine treatments. Fruit were subjected to: a) hot water immersion at 49.0^{o}C for 20 min (lychee and longan) or vapor heat at a seed surface temperature of 47.2^{o}C (rambutan), b) irradiation treatment at a minimum absorbed dose of 250 (rambutan) or 400 Gy (lychee and longan), or c) left untreated as controls. A factorial design consisting of three quarantine treatments (control, irradiation, heat) and various storage temperatures and times was used.

2.2. Hot water immersion treatment

Tests were conducted in a 70 L circulating bath heated by two electric heaters to a constant 49 $\pm$ 0.2^{o}C [6, 7]. For hot water immersion treatment of lychee and longan, 2.3 kg of fruit were placed in a nylon mesh bag and immersed in water at 49^{o}C for 20 min. After heating, the fruit were immediately placed into a 70 L ambient (~ 21^{o}C) water bath to cool for an additional 20 minutes. Seed surface temperatures were recorded from a sample of fruit of various sizes using thermocouples and a data logger. Fruit were then air-dried in shade for ~1.5 h before repacking in perforated plastic bags and fiberboard boxes for cold storage.

2.3. Vapor heat treatment

Vapor heat treatment of rambutan was performed using a computer-controlled chamber that could be programmed for a ramp to a desired target internal fruit temperature while regulating humidity via injection of water vapor from a steam generator [5]. The treatment used in our study involved heating the fruit to a seed surface (fruit center) temperature of 47.2^{o}C in one hour and then holding the seed surface temperature at 47.2^{o}C for 20 min. Eight large rambutan fruit of each replicate were probed individually with thermocouples at the seed surface to monitor fruit center temperature. Additional thermocouples were used to monitor the temperature of air entering and exiting the vapor heat chamber, and the temperature at the outer surface of the pericarp of the fruit.

2.4. Irradiation treatment

For treatment with irradiation, 4.5 kg of fruit in a perforated bag inside a fiberboard box were treated at a nearby commercial x-ray facility (Hawaii Pride, Keaau, Hawaii) using an electron linear accelerator (lychee and longan), or at the Hawaii Research Irradiator (University of Hawaii at Manoa) which uses a ^{60}Co source of gamma radiation and had an average dose rate of 5.3 Gy·min^{-1} (rambutan) [4, 5, 7, 8]. After extensive dose mapping, dosimeters were placed at multiple locations inside and outside the box. The dose uniformity ratio in all studies was ≤1.3, and the minimum absorbed dose was at or above the target dose, simulating commercial conditions.

2.5. Quality determination

Twenty-five fruit were evaluated per replicate (harvest date) on the day of fruit arrival (1one day after harvest), and 25 fruit/treatment/replicate were evaluated after various storage intervals. Quality evaluations included colorimeter measurements, percentage of soluble solids content, pH, total acidity, and visual ratings of external appearance, and sensory evaluations of peel and pulp texture, and taste [5, 7, 8]. Only external appearance ratings are reported here. For lychee and rambutan, external appearance ratings were based on the degree of browning of the pericarp: 1 = (best rating) red (with green) and no darkening of outer pericarp, 2 = red with ≤25% surface area darkened, 3 = 26–50% surface area darkened, 4 = 51–90% surface area distinctly darkened, 5 = (worst rating) 91–100% darkened outer pericarp. For longan, external appearance ratings were also based on the degree of darkening of the pericarp: 1= (best rating) green to light-brown and not darkened, 2 = loss of green, but without darkening, 3 = ≤50% surface area darkened, 4 = > 50% surface area distinctly darkened, 5 = (worst rating) 100% darkened outer pericarp. Formal grades and standards are not used for lychee, longan and rambutan in Hawaii, but an external appearance rating of 3 or higher would probably indicate reduced commercial acceptability.

2.6. Data analysis

A two-way analysis of variance (ANOVA) procedure using the standard least squares model was performed on fruit averages to test for differences in treatment, storage temperature or interval, and the treatment x storage temperature [11]. When the effect of quarantine treatment was significant, a means separation was done using the Tukey-Kramer HSD test at $P \leq 0.05\%$ [5, 7, 8].

3. RESULTS

Complete results of the fruit quality experiments comparing irradiation and heat treatments for lychee, longan, and rambutan have been published [5, 7, 8]. The external appearance of the fruit is usually the most important factor to the consumer. I report here only external appearance rating data to illustrate the differential response to irradiation and heat treatments by each fruit type.

After eight days of storage, lychee fruit treated by hot water immersion were rated as significantly less acceptable than untreated (control) fruit for pericarp appearance when held at 2 or 5°C (Table 1). Pericarp appearance was more acceptable in irradiated fruit compared to hot water immersion fruit at both storage temperatures but the results were not significant. In another experiment examining the rate of color loss, pericarp appearance ratings for lychee fruit treated by hot water immersion at 49°C were highest

(the least desirable) on all days (Table 2). Fruit treated by hot water immersion at 49° C were rated as unacceptable (ratings ≥ 3) after one day of storage at 4°C, whereas irradiated and untreated fruit were rated as acceptable after eight days storage at 4°C (Table 2).

After 14 and 21 days of storage, the external appearance of 'Chompoo' longan fruit treated by hot water immersion was rated as significantly less acceptable than those treated by irradiation or left untreated (Table 3). After 21 days of storage, the external appearance of 'Biew Kiew' fruit treated by hot water immersion were rated as significantly less acceptable than those treated by irradiation, and both were less acceptable than untreated control fruit (Table 3).

In the rambutan experiment, the most significant result was that the external appearance of 'R134' fruit treated with vapor heat was unacceptable after four days of storage whereas irradiated fruit remained acceptable for four to eight days of storage after treatment (Table 4). External appearance for all treatments was unacceptable (ratings ≥ 3) after 12 days of storage. External appearance ratings for vapor heat-treated 'R167' rambutan fruit were numerically highest (the least desirable) on all dates. External appearance for all treatments was acceptable after four and eight days of storage but unacceptable (ratings ≥ 3) after 12 days.

Table 1. EXTERNAL APPEARANCE OF 'KAIMANA' LYCHEE FRUIT AFTER HOT WATER IMMERSION OR IRRADIATION TREATMENT (400 GY) AND 8 DAYS STORAGE AT 2°C or 5°C. THE INITIAL EXTERNAL APPEARANCE RATING WAS 1.1

	Storage Temperature	
Treatment	2° C	5° C
Control	1.9a	1.6a
Hot water	3.2b	3.1b
Irradiation	2.3zb	2.0

Table 2. EXTERNAL APPEARANCE OF 'KAIMANA' LYCHEE FRUIT AFTER HOT WATER IMMERSION OR IRRADIATION TREATMENT (400 GY) AND VARIOUS INTERVALS OF STORAGE AT 4°C

	Days of storage					
Treatment	1	2	5	7	8	9
Control	1.2 (0.08)	1.5 (0.23)	2.1 (0.33)	2.4 (0.48)	2.7 (0.50)	3.1 (0.52)
Hot water	3.2 (0.08)	3.5 (0.30)	3.7 (0.28)	4.0 (0.28)	4.0 (0.05)	4.3 (0.10)
Irradiation	1.1 (0.03)	1.6 (0.12)	2.1 (0.10)	2.3 (0.05)	2.7 (0.30)	3.1 (0.20)

Table 3. EXTERNAL APPEARANCE OF 'CHOMPOO' AND 'BIEW KIEW' LONGAN FRUIT AFTER HOT WATER IMMERSION OR IRRADIATION TREATMENT (400 GY) AND 7, 14, OR 21 DAYS STORAGE AT 10°C

		Cultivar	
Days	Treatment	Choompoo	Biew Kiew
Initial		1.0_	1.1_
7	Control	1.2a	1.4a
	Hot Water	1.7a	1.8a
	Irradiation	1.7a	1.9a
14	Control	1.8a	1.8a
	Hot Water	2.9b	3.1a
	Irradiation	2.2a	2.1a
21	Control	2.6a	2.1a
	Hot Water	4.9b	5.0c
	Irradiation	2.6a	2.4b

Table 4. EXTERNAL APPEARANCE OF 'R134' and 'R167' RAMBUTAN FRUIT AFTER VAPOR HEAT OR IRRADIATION TREATMENT (250 GY) AND 4, 8, OR 12 DAYS STORAGE AT 10°C

		Cultivar	
Days	Treatment	R134	R167
Initial		1.5_	1.8_
4	Control	1.8a	2.0a
	Vapor heat	3.1b	2.2a
	Irradiation	1.9a	2.1a
8	Control	2.2a	2.2a
	Vapor heat	3.4b	2.6a
	Irradiation	2.3a	2.3a
12	Control	3.7a	3.7a
	Vapor heat	3.9a	4.0a
	Irradiation	3.3a	3.6a

The two quarantine treatments compared in our study were developed to kill Hawaii's fruit fly pests prior to export of fruit, and both irradiation and heat treatment protocols are approved for exporting lychee, longan and rambutan. The protocol for the hot water immersion treatment for lychee [12] contains warnings about the limited research on fruit quality after treatment application and varying tolerance among different cultivars but, until now, no quantitative information was available. No research was available on fruit quality after treatment for longan and rambutan. External appearance of lychee and rambutan treated with heat was rated as unacceptable after relatively short periods of cold storage. However, taste remained acceptable (data not shown) [5, 7, 8]. Hot water immersion (lychee) and vapor heat (rambutan) greatly accelerated pericarp browning, and irradiation was superior to both heat treatments based on maintenance of fruit appearance.

Alternate quarantine treatments are always desirable to prevent interruption of exports in the event a treatment or treatment facility is lost. The availability of two quarantine treatments, hot water immersion or vapor heat and irradiation, to control fruit flies in lychee, longan and rambutan serves this purpose in Hawaii. However, the heat treatments may not be acceptable alternatives to irradiation due to problems with fruit quality and other alternatives should be pursued [8]. For example, quarantine cold treatments are available for export of lychee from China, Taiwan and Israel to the U.S. The duration of the cold treatment depends on the temperature and pest species to be controlled. The cold treatment for China and Taiwan is 1^{o}C for 15 days or 1.4^{o}C for 18 days to control oriental fruit fly (*Bactrocera dorsalis*) and litchi fruit borer (*Conopomorpha sinensis*). McGuire [9] reported that cold treatment of 15 days at 1.1^{o}C caused minimal loss of quality in Florida lychees and compared favorably with irradiation up to 300 Gy (with 6 days storage at 5.0^{o}C). Hawaii might consider a lychee cold treatment as an alternative to irradiation. Cold treatments are already approved for control of Mediterranean fruit fly and oriental fruit fly in carambola and avocado exported from Hawaii to the U.S. mainland. In addition to these fruit flies, a lychee cold treatment for Hawaii would need to control two other quarantine pests, koa seedworm (*Cryptophlebia illepida*) and litchi fruit moth (*C. ombrodel*ta) [4, 6]. Although they require more time to complete, cold treatments can be applied in transit in marine containers and refrigerated trucks. Surface transport is cost effective compared with the air transportation now used for movement of lychee from Hawaii to the US mainland.

In general, lychee and rambutans have a short storage life under ambient conditions. Desiccation with the accompanying loss of red color and development of browning can occur rapidly after harvest (<72 h). Browning renders the fruit hard to sell, therefore, prolonging the shelf life could be commercially advantageous. This is less of a problem with longan because the skin is naturally green to brown, although the skin will darken after harvest. Lowering the storage temperature is proven to extend the shelf life, and various other techniques have also been tested including packaging, fungicides, chemical treatments, and modified atmosphere with reportedly impressive results [10]. Research is needed to identify treatments or procedures to slow the rate of quality loss in lychee, longan, and rambutan after hot water immersion or vapor heat to make the use of these quarantine treatments practical. Until then, quality considerations will probably outweigh other market factors, and irradiation will be the quarantine treatment of choice for Hawaii's lychee, longan, and rambutan.

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GENERALIZED QUARANTINE DISINFESTATION RESEARCH PROTOCOL

N.W. HEATHER
School of Land and Food,
University of Queensland,
Gatton Collage, Queensland, Australia

1. INTRODUCTION

The purpose of a quarantine disinfestation treatment is to prevent the establishment of a pest associated with a commodity to be imported into a country or region where it does not already occur or where its presence is restricted. Irradiation is particularly suited to this purpose with applications over a wide range of commodities and pests. It meets the current consumer requirement of freedom from chemical residues that are associated with fumigation and insecticide treatments.

Disinfestation treatments may need to gain the specific approval of quarantine authorities in each importing country. Therefore the supporting data required may differ from country to country. Such differences are usually minor and this generalised protocol will provide most of the information required by most importing countries.

This protocol is intended to meet the broad quarantine treatment requirements of the following countries: Japan, USA, Australia and New Zealand. At present irradiation is likely to prove acceptable only to USA. This can be expected to change as public perception of irradiation matures and confidence develops. Additional countries for which the methodology should prove generally acceptable include the other ASEAN countries, China, Korea, Taiwan, India, South Africa and most South American and Central American countries. Signatories to GATT have agreed to abide by the principle of equivalence so there should be some flexibility in how the work is done including existing data obtained in an equivalent manner.

2. SCOPE

This protocol applies particularly to pests of fresh horticultural produce that can be maintained in laboratory cultures (e.g. fruit flies and mites), but it can be adapted for use with field-collected insects provided that adequate numbers of known stages can be obtained.

3. RESEARCH REQUIREMENTS

3.1. Entomology

3.1.1. Pest Species

It is essential to define the pest taxonomically and to ensure that the appropriate species is used in disinfestation tests. The broadest possible applicability should be negotiated with the importing country from the outset. Where the pest is highly variable or occurs as a number of geographic races and is likely to be redesignated in a taxonomic subdivision (e.g. oriental fruit fly), "voucher" specimens of the test colony should be lodged in a secured tenure collection against the possibility of future dispute. This can also provide the benefit of extension of the research to other pests subsequently shown to be physiologically similar, with minimal additional research.

3.1.2. Host Status of Commodities

Ideally, disinfestation testing should be done on the host cultivar ("variety") in which the pest is most tolerant of the treatment. This involves comparative testing usually made difficult by major natural variation. Where no difference can be identified statistically at the 95% Confidence Level (CL), arithmetic values can be used although these can be misleading and there is considerable value in these circumstances in using the cultivar which makes up the greatest proportion of trade or the cultivar at greatest risk of infestation. Some countries (eg. New Zealand and Japan) have in the past required testing of all host "varieties". Some of these so-called "varieties" are simply from unique seed lines, often hybrids, so wherever possible the definition of a cultivar should avoid these because of their short commercial life. Where the host commodity has a low susceptibility to infestation or is only infested under specific conditions, field host records, cage tests and field susceptibility tests may enable treatments of lower efficacy to be negotiated compared to those required for high-risk cultivars.

Much of this information could be available from pest risk analyses now done or required by quarantine authorities of most countries. The procedure for establishing non-susceptibility of a host may be specified by the importing country (e.g. New Zealand, Ministry of Agriculture and Fisheries Standard for Determination of Fruit Fly Host Status).

3.1.3. Seasonal Incidence

Basic information on seasonal incidence of a pest relative to production times of the cultivar to be exported can identify high and low risk times, where they occur.

3.1.4. Disinfestation Testing

3.1.4.1. Laboratory Culturing of Pest

Ideally, laboratory cultures should be sourced recently from field infested host material. Collections should be made throughout the geographic range of the host and the pest species. Ideally, cohorts of >200 females should be collected to ensure a high probability of sampling the genetic variation in a population. Care should be taken not to restrict the sample to one or a few pieces of nearby host fruit as they might be infested by progeny of a single female. Where there is a possibility of more than one species in a host fruit or where parasitism is suspected, it is wise to isolate each pupa (or final instar or nymphal stage) in a vial until the adult ecloses. Viable adults of the same species can then be transferred to a cage for breeding subsequent generations. For fruit flies, a guide to culturing on a routine basis is given in the method of Heather and Corcoran for *Bactrocera tryoni*, [1].

It is usual practice to supplement the genetic diversity of laboratory cultures by the addition of field-collected individuals, adults or juveniles, on a regular basis, e.g. annually. They should be collected and handled as for the original source of the culture and should not be added until one or more generations have passed in isolation to ensure that the identity is correct, and that the sample is free of parasites or disease.

Finally, cultures should be managed so that pests available to infest the commodity used in the experiment are at peak vigour. This can be accomplished by rearing multiple cohorts, and replacing cohorts after their peak has passed. The duration of peak vigour need to be confirmed for each pest species.

As a further guide to the vigour of culture populations, the following parameters should be monitored each generation:

- egg to pupa proportion
- mean pupal weight for appropriate species
- development time
- eclosion percentage
- sex ratio of eclosed adults
- fecundity.

Where culture methods for the pest species are not available or where the importing country requires treatments to be tested on field pest populations it may be possible to do this by collecting field infested fruit in large quantities. These can either be treated directly, or, F_1 adults can be used to infest fruit for disinfestation testing.

3.1.4.2. Determination of most tolerant stage

Determination of the most tolerant stage can be done using:

- naked insect stages (in vitro)
- artificially infested fruit
- naturally infested fruit.

Whichever method is chosen the experimental numbers to be used must be decided in consultation with a biometrician and confirmed with the reviewing authorities of the importing country if possible.

A pre-requisite for this testing is knowledge of development times for each stage in culture medium or in association with the host, according to the method to be used. It will be found that even where oviposition time is short, overlapping of instars will occur and that the test sample may contain some stages other than that which is the object of the test. Sometimes these can be selected manually on morphological characters but the age within the stage will not be known with certainty.

Naked insect stages

For pests of which more than one stage may be present on the commodity at export, the responses of eggs, instars, nymphs, pupae or adults to the treatment need to be compared as mortalities (or survivors) to determine whether any stage is more tolerant than another.

If the response of the population being tested is homogeneous, i.e. chi-squared value/degrees of freedom ≈ 1 or less, a regression analysis model is appropriate. If the response is heterogeneous then regression analysis can still be undertaken but wide fiducial limits are likely to be indicative of no significant differences. Here, best results can often be obtained by identifying a discriminating dose at which survival occurs and applying an ANOVA model (as described below) for testing differences in mortality using naturally infested fruit.

Transformation of data is necessary to achieve a linear regression model. Probits are normally used for mortality and a log transformation may or may not be appropriate for the dose or time of exposure. The methods of Finney [2] are well tried and highly regarded. For each stage in each cultivar to be tested five evenly spaced doses on the

response line, in addition to an untreated control sample, are desirable for adequate degrees of freedom in analyses. The dose or time range will probably need to be identified in a preliminary "range-finding" test. A minimum of 50 individuals in each sample, replicated three times, should be tested at each of the five doses. Whole line comparison of stages can be done only if response lines are parallel and for this a separate test of parallelism is necessary, see Finney [2]. Otherwise, comparisons are only valid at equivalent points (e.g. LD_{50}). Finney [2] should also be consulted to ensure that all of the basic assumptions on which the model is based are met. These analytical methods are available in statistical software "packages" such as Genstat and SAS.

Natural infestation

This is the method that is closest to operational reality of a commercial disinfestation treatment. It has the advantage that eggs are oviposited in the same position in the fruit as would occur naturally. With strong vigorous colonies of the pest, narrow age limits of cohorts are possible.

Disadvantages include an inability to accurately control numbers in each fruit (which can be overcome to some extent for fruit flies by "pin holing" (0.4 mm) a number of oviposition sites), the possibility that at low doses numbers surviving can exceed control survival due to uneven numbers of eggs, and that estimation of control mortality is not possible although it can be expected to be lower than for artificial infestation.

For regression analysis this method requires a different statistical model because it only permits estimation of survivors. A proven model is the "Wadley's problem" method described by Finney [2] and a number of statistical software packages can undertake this type of analysis provided that fiducial limits are calculated correctly.

Alternatively, the ANOVA model used in conjunction with the "discriminating dose" approach may be used. This involves identification of a dose at which there are significant numbers of survivors e.g. 30–50% in all or all but the most tolerant stage. Replicated tests are then done on each stage and compared on the basis of survival using a means separation test. This model is very useful for natural infestation tests because of characteristically high variance caused by uneven pest numbers in or on each host unit (piece of fruit or flower stem) and the difference in survival due to unit-to-unit variation. It is more or less analogous to point-wise comparisons necessary when regression lines for stages or cultivars are not parallel. Again, use of precise experimental irradiation practices is essential.

Prediction of dose needed to achieve treatment efficacy required

The quarantine security level required of a treatment will need to be ascertained from the importing country. Although USA uses 99.9968% extensively (probit-9) other countries have lower levels (e.g. 99.99% or 99.5% for fruit flies and 99.5% to 95% for other pests). These security levels may be assured by treatments with appropriate minimum efficacy levels which can be confirmed at a required confidence level by testing against appropriate numbers of pest individuals. Tables are available to enable the calculation of either the number to be tested or the efficacy indicated by the number tested together with survivors [3]. Sometimes the security required is implicit in an inspection level required (e.g. 600 units free of pests assures 99.5% freedom at the 95% C L).

The dose required to achieve a specific treatment efficacy can be calculated from the probit regression line taking into account fiducial limits but at higher efficacies extrapolation may be required and reliability may be poor. Care needs to be exercised in using probit analyses as most software programs will calculate a line even if data is not linearly distributed which is frequently the case. This can result in an over-dose or an under-dose but usually in an over-dose.

Some countries advise a small-scale test (e.g. 3000-5000 insects) to ensure that the treatment chosen can achieve the required efficacy. This is often unnecessary if good probit regression analyses have been done on adequate data. Also, there is no certainty that tests on 3000-5000 will identify the dose which is needed to satisfy the criteria of no survivors from 30 000 (99.99% efficacy at the 95% CL) or from 100 000 (99.9968% at the 95% CL) treated individuals. These tests are required by some countries (e.g. Japan, New Zealand) to be done as replications of >7 500 or >10 000 individuals which is frequently more practical to use for the purpose.

If successful it then becomes a replicate of the large-scale confirmatory test for the required efficacy. This implies an iterative approach and failure at one dose level can provide assurance that the successful dose is no more than necessary to achieve the required efficacy.

At this stage it is necessary to ensure that the treatment to be confirmed will not cause commodity damage. If injury tests have been done previously the limits of damage will be known, otherwise they will need to be done at this stage. (See 3.2)

3.1.4.3. Large-scale Confirmatory trials

These trials confirm the efficacy of the treatment at a given confidence level. Numbers of insects to be tested will be advised by the proposed recipient country. For an explanation and calculations see Couey and Chew [3]. For the purposes of operational usage it will be the upper limit of the max/min ratio in these trials, which will need to be used as the minimum operational dose. This is because it is not possible to be certain that insects present in the portions receiving the highest dose levels would have met the criteria for quarantine security had they been in the portions which received a lesser dose than the maximum. Usually, the tests will be required to be done on the most tolerant life stage in the cultivar in which they were found to be most tolerant of the treatment. Where the stages or cultivars are not found to be statistically different, arithmetic differences have been used, but preferably the stages and cultivars constituting the greatest risk should be used.

Infestation should be done so that the untreated commodity units are representative. Randomisation designs such as a Latin square may lead to unrepresentative numbers if a factor such as the light source influences the density of infestation. Depending on the stage to be treated infested commodity units must be held at known temperature and preferably humidity so that the development to the stage to be treated can be predicted and subsequently to ensure maximum survival after treatment. Untreated and treated commodity units must be held in different rooms or incubators after treatment to avoid any possibility of cross- or re-infestation.

For fruit flies, drainage slits must be cut if necessary to avoid drowning of larvae and to enable escape of CO_2 from insect metabolism and fruit breakdown. Fruit should be held on gauze covered receptacles to keep larvae out of drained fruit juices and residue-free

moistened sawdust should be provided in preference to sand, which gives a slightly lower survival.

Commercial trials

These should only be required to confirm the efficacy of the treatment for the irradiation geometry of the irradiator and for the product and packaging to be used. They are usually done on the basis of dosimetry and insect infested samples are not required. However where insect samples are required the rules for infestation and incubation for survivors would apply.

Computation of data

This should be done using recognised computing software (e.g. Genstat, SAS). However it is necessary to take biometrical advice to ensure that the correct analysis methods are used (e.g. use probit analysis only where the number of control insects and their mortality is accurately known otherwise a "Wadley's problem" type of analysis must be done as this is designed for data where only survivors can be assessed). For probit analysis it is essential to use programs that calculate the fiducial limits correctly, as non-overlap of fiducial limits may be taken as evidence of statistical difference although this is not conclusive. The data to be computed can include the chi-squared value and slope of the line as well as a number of indicator LD values (e.g. LD_{50}, LD_{99}, $LD_{99.9}$ as well as the LD value for the efficacy required of the treatment), together with fiducial limits at 95 and 99% CL. Most programs will also print out probit lines showing data points. Programs for comparison of probit lines are valid only if lines are parallel although most will compute an answer regardless. Therefore, a test for parallelism must be done and found to be positive before any comparative test of regression lines is done.

Where an ANOVA design is used on data from survivors and low dose survivors exceed those from untreated controls they should be treated as "missing values". This is valid and it avoids "negative" mortality and prevents bias at higher values.

3.2. Post-harvest Physiology

It is essential that post-harvest physiology studies be done in conjunction with insect disinfestation testing. These studies should include the production history of the product as this can affect its response to irradiation.

The product should be subjected to a range of doses, if necessary using different max/min dose geometry within the irradiator, temperatures, and modified atmospheres. Electron beam irradiation response should be compared experimentally with gamma- or x-radiation if it is intended to use that method but previous research had been done with the other methods. Doses should be extended to a level where injury occurs to identify the threshold. This would then become the upper limit permitted for the max-min ratio.

3.2.1. Assessment parameters

Following are parameters that should be considered for the assessment of changes in the product as a result of irradiation:

- colour development or changes,
- softness of fruit or flower tissue,

- pH of fruit juice,
- brix (total solids) of fruit as a measure of maturity,
- ascorbic acid content of fruit,
- ethylene production patterns with time,
- CO_2 production patterns with time,
- visible injuries – appearance,
- chlorophyll fluorescence,
- citric acid content of fruit,
- eating quality of fruit,
- appearance of cut flowers.

Data on these parameters should be collected using biometrically sound experimental designs and should be analysed accordingly. It should be done over more than one season - ideally three - and should take the form of comparisons with un-irradiated fruit otherwise held identically.

3.3. Disease Assessment

Post-harvest pathology observations should be done to ensure that there are no unknown disease status changes. While at doses for insect disinfestation irradiation is not likely to have much positive effect on disease control it can make a substrate favourable for saprophytic organisms where tissue is rendered non-viable and loses its antibiotic activity.

Measures for the reduction of post-harvest diseases such as hot water dipping can affect some pest mortality. These should be assessed and if possible factored into the treatment efficacy when the required dose is being estimated and confirmed.

3.4. Proposal Submission

The format of a proposal for assessment of a disinfestation treatment differs from country to country and should be prepared on the advice of the proposed recipient country. The proposal should be supported by full records of irradiation treatments, including timings, dosimetry results, and any other relevant parameters. It is important that skilled technical advocates are available to support proposal submission to ensure that there are no misunderstandings. It may also be advantageous to enlist the assistance of importers and retailers in the recipient country to counter the effects of producer lobbyists seeking to avoid competition.

Because mortality from low-dose irradiation often occurs late in the life cycle of a pest, any proposed treatment should be supported by “quality control” or “pest management” programmes that ensure that any pest infestations are below the level of detection at any inspection [4]. This is usually feasible because domestic markets will not normally tolerate infested produce for pests such as fruit fly. However for scale insects on fruit and thrips and mites on cut flowers this would not apply. An additional supporting facet would be a means of determining whether the pest had been irradiated (e.g. phenol oxidase test for fruit flies).

The following tables of data should be included with a submission:

- Development times for stages of the pest in culture medium used for naked insect tests, range of times and/or mean or modal times ± S.E, or, development times for

stages of the pest in each variety of the fruit or flower proposed for export, range of times and/or mean or modal times ± S.E.

- Survivors of each stage of each pest species in each cultivar at five evenly spaced doses resulting in 5-100% survival.

- Regression analyses using Finney's Probit Analysis or Wadley's Problem analysis of responses of each stage of each pest species in each cultivar. These should include representative probit mortalities with 95% fiducial limits, regression line parameters, parallelism test results and if applicable, comparisons of regression lines. They should meet all of the assumptions intrinsic to the analysis model. Fruit size range should be given (size or weight).

- Alternatively, ANOVA of response data at one or more doses comparing response of stages, with means separation values.

- Results of tests to predict the dose needed for the required quarantine security e.g. 99.5%, 99.99% or 99.9968% on the most tolerant stage in the cultivar in which it is most tolerant.

- Results of large-scale trials: replicate number; date treated; fruit size range; stage of pest; proportion present; number of control fruit; number of treated fruit; number of pupae from controls; number of pupae from treated; number of adults from controls; number of adults from treated; and mortality of treated should all be tabulated.

- Results of fruit injury testing giving the cultivar, maturity, ripeness and where the fruit flowers were grown.

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LIST OF PARTICIPANTS

Akinbingol, B.	Turkish Atomic Energy Authority, Ankara Nuclear Research and Training Centre, Istanbul Yolu, 06983 Ankara, TURKEY E-mail: berna@saray.snaem.gov.tr
Bansiddhi, K.	Entomology and Zoology Division, Department of Agriculture, Jatuchak, Bangkok 10900, THAILAND Tel: +662 579 8541 Fax: +662 940 5396 Email: bansidd@doa.go.th
Castro, D.	Fundacion para el Desarollo Fruticola, Sargento Aldea #305, Buin, CHILE Tel: +56-2-821-5995 Fax: +56-2-821-6004 Email: central@fdf.cl
Follet, P.A.	USDA Agricultural Research Service, Tropical Fruit, Vegetable and Ornamental, Crop Research Laboratory, P.O. Box 4459, Hilo, Hawaii 96720, UNITED STATES OF AMERICA Tel: +808 959-4303 Fax: +808 959-5470 Email: pfollett@pbarc.ars.usda.gov
Hallman, G.J.	U.S. Department of Agriculture, USDA-ARS-CQFIR, 2413 E. Highway 83, Weslaco, Texas 78596, UNITED STATES OF AMERICA Tel: +956 447 6313 Fax: +956 447 6323 E-mail: hallman@pop.tamu.edu
Heather, N.W.	57 Ruthven StreetCorinda, Q 4075 AUSTRALIA Tel.: +61 7 3379 6582 Fax: +61 7 5460 1283 E-mail: nheather@gil.com.au

Ignatowicz, St.
Agricultural University of Warsaw,
Applied Entomology Department,
ul. Nowoursynowska 166,
02-766 Warsaw,
POLAND
Tel./ Fax: +4822-843 4942
E-mail: ignatowiczst@yahoo.com

Lustre, A.
Food Development Centre, National Food Authority,
Food Development Center,
FTI Complex, Taguig, Metro Manila,
PHILIPPINES
Tel: +632 838-4014, 4015
Fax: +632 838-4692, 4016
E-mail: nolustre@mnl.seque1.net

Mansour, M.
Syrian Atomic Energy Commission,
Agriculture Department,
P.O. Box 6091,
Damascus, SYRIAN ARAB REPUBLIC
Tel: +963 11 6111 926/7
Fax: +963-11-611 2289
Email: Mansour83@excite.com

Meixu, G.
Acadamy of Agricultural Sciences,
Institute for the Application of Atomic Energy,
P.O. Box 5109,
2 Yuanmingyuan West Road,
Haidian District, Beijing 100094,
CHINA
Tel: +86-10-62896296,
Fax: +86 10-62896314
E-mail: gaomx@mailcity.com

Moy, J.H.
College on Tropical Agriculture & Resource Management,
Unviersity of Hawaii at Manoa,
Molecular Bioscience and Biosystems Engineering,
3050 Maile Way, Honolulu,
Hawaii 96822,
UNITED STATES OF AMERICA
Tel: +808 956-3853
Fax: +808 956-8663
E-mail:jmoy@hawaii.edu;
jhmoy@Worldnet.att.net

Sulaiman, V

Malaysian Agricultural Research and Development,
Horticulture Research Centre,
P.O. Box 12301, G.P.O.,
50774 Kuala Lumpur,
MALAYSIA
Tel: +603 943-7111
Fax: +603 948-3664
E-mail: midah@mardi.my

Zolfagharieh, H.R.

Atomic Energy Organization of Iran,
Nuclear Research Centre for Agriculture and Medicine,
P.O. Box 31585-4395,
Karadj, ISLAMIC REPUBLIC OF IRAN
Tel: +98-261-411108
Fax: +98-261-411105 & 6
Email: hzolfagharieh@seai.neda.net.ir

Arthur, V.

Center for Nuclear Energy in Agriculture (CENA),
Avenue Centenário, 303,
Caixa Postal 96,
13400-970 – Piracicaba, SP,
BRAZIL
Tel.: +55 (0)19 3429 4600
Fax: +55 (0)19 3429 4610
Email: arthur@cena.usp.br

Hayashi, T.

National Food Research Institute,
Ministry of Agriculture Forestry and Fisheries,
Kannondai, Tsukuba,
Ibaraki 305-8642,
JAPAN
Tel.: +81 298 38 7991
Fax: +81 298 38 7996
Email: toruha@nfri.affrc.go.jp