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1 Irradiation as a phytosanitary treatment for insects and mites on agricultural commodities

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Abstract

Trade in agricultural commodities is growing at a rapid rate. At the same time that agricultural trade is increasing, the probability of introducing exotic insects into areas where they may become pests will increase. Quarantine or phytosanitary treatments eliminate, sterilize or kill regulatory pests in exported commodities to prevent their introduction and establishment into new areas. Irradiation is a versatile technology to disinfest fresh and durable agricultural commodities of quarantine pests. Irradiation is broadly effective against insects and mites, and generally does not significantly reduce commodity

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quality at the doses used to control insect pests. Research methodology specific to developing irradiation treatments to control insects is presented, and several research issues including probit 9, generic treatments, and varietal testing are discussed. The recent publication of an international standard on the use of irradiation as a phytosanitary measure, and the establishment of generic doses for tephritid fruit flies and other insect groups, will promote wider acceptance and application of the technology.

Introduction

Quarantine or phytosanitary treatments eliminate, sterilize or kill regulatory pests in exported commodities to prevent their introduction and establishment into new areas. Irradiation is a versatile quarantine treatment technology to disinfest fresh and durable agricultural commodities of regulated pests. Irradiation is broadly effective against insects and mites, cost competitive with other disinfestation methods (such as fumigation, heat and cold) and fast. Irradiation generally does not significantly reduce commodity quality at the doses used to control insect pests, and may even extend shelf-life. Additionally, irradiation can be applied to the commodity after packaging.

A quarantine pest is a plant pest of potential economic importance to an area that is not yet present there, or present but not widely distributed and being officially controlled. Unlike other disinfestation techniques, irradiation does not need to kill the pest immediately to provide quarantine security, and therefore live (but sterile) insects may occur with the exported commodity making inspection for the target pests redundant as a confirmation of treatment application and efficacy. This places an added level of importance on the certification procedures for irradiation facilities and proper documentation accompanying each shipment confirming treatment at approved doses. It also places a responsibility on researchers to ensure that the minimum absorbed dose approved for each quarantine pest has an adequate margin of safety.

Ionizing radiation breaks chemical bonds within DNA and other molecules, thereby disrupting normal cellular function in the insect (Ducoff 1972, Koval 1994). Many tissues and functions of the insect may be disrupted by exposure to irradiation (Vinson et al. 1969; Nation and Burditt 1994). Insects and other living organisms are able to repair molecular damage done by small amounts of ionizing energy, but large amounts are fatal and this is the basis for using irradiation to disinfest commodities of insects. Insect life stages vary in their susceptibility to radiation damage and this may be related to the degree of reproductive activity in cells and to the level of cell differentiation (Bergionie and Tribondeau 1959). Radiation induced injury in an early stage of development may disrupt development later on when a particular tissue becomes important to the function or survival of the insect.

The history of quarantine uses of irradiation and the relative tolerance of various arthropod groups have been reviewed by Rigney (1989), Heather (1992), Burditt (1994), and Hallman (1998, 2001). In this review we provide an update and synthesis of previous information, and discuss current trends in the use of irradiation as a phytosanitary treatment, with an emphasis on research methodology and the regulatory framework.

Developing irradiation quarantine treatments

Methodology

The goal of irradiation as a phytosanitary treatment is to provide quarantine security for any regulated pests residing in or on the exported commodity. This is most often accomplished by preventing development to the reproductive stage or sterilizing the reproductive stage of the insect. If multiple species on a commodity are regulated pests, irradiation studies begin by comparing the tolerance of the quarantine pests, then, in-depth studies focus on the most tolerant stage of the most tolerant species, to arrive at a single dose providing quarantine security for the commodity. Typically the most advanced developmental stage of the insect occurring in the commodity is the most tolerant when the goal is preventing adult emergence or reproduction. The most advanced stage may be the larva (or nymph), pupa, or adult. When larval development is completed in the host but the insect pupates outside the host, irradiation is applied to prevent adult emergence. In the case of tephritid fruit flies, preventing adult emergence is the desired response required for regulatory purposes because it prevents the emergence of adult flies that could be trapped and trigger regulatory actions, despite being sterile. When the insect pupates in the host, preventing adult emergence may be difficult so adult sterility is the goal.

Often adults occur with the commodity. When the adult stage can occur in the commodity and is the most tolerant stage, the measure of treatment efficacy is the level of sterility. For sexually reproducing species, sterilizing one sex may be sufficient to prevent reproduction but both sexes must be sterilized if mating status is unknown as is usually the case. Males are often but not always more tolerant than females. Reciprocal crosses between irradiated and control males and females at several sub-sterilizing doses are useful to determine the more tolerant sex (Follett and Lower 2000). In large-scale confirmatory tests, males and females should be mated before treatment and females should have begun ovipositing. After irradiation treatment, surviving males and females are combined and allowed to mate and reproduce to determine the success of the dose. Adult females irradiated at a sterilizing dose will often oviposit (particularly if they were gravid when irradiated) but eggs will not hatch or hatching neonates do not develop. With asexual species

the female is the focus of all tests. In rare cases irradiated insects will recover so it is important to continue tests until all insects have died. Many insect species have life history attributes that complicate testing methods. For example, diaspidid scale insects are sessile (attached to the plant) and long-lived, and so experiments must use host material (e.g. pumpkin) that does not deteriorate after irradiation treatment and before the insects die. Some species require live host material to survive. The long-lived semi-sessile coccid scale, green scale (*Coccus viridis*) only survives on live host material such as gardenia, coffee and hibiscus, which complicates testing since irradiation treatment causes rapid plant deterioration (Hara et al. 2002). Diapausing and non-diapausing strains of insects may have different tolerances to radiation, and may require different bioassay methods (Hallman 2003).

To determine the most tolerant stage for a species, all stages are treated with a range of irradiation doses. Ideally five doses should be selected and five replicates of at least 30-50 insects should be used. In some cases a single diagnostic dose is used to separate tolerance among stages or species. The ideal diagnostic dose causes only moderate mortality in the stage or species predicted to be most tolerant. This improves the chances that statistical tests can be used to separate mean responses among groups. Tests should be designed with the biology of the insect in mind, and insects should always be tested in the commodity of interest if possible. For example, pupae may be inherently more tolerant of irradiation than larvae but because they only occur at the surface of the fruit they may be easier to sterilize than larvae that feed at the center of the fruit where hypoxic conditions exist. If artificial inoculation is used, insects should be placed where they occur naturally or allowed time to redistribute to preferred feeding sites in the commodity. Dosimeters should be placed where the insects occur to accurately measure absorbed doses. Once dose response tests are completed, large-scale tests are conducted with the most tolerant life stage at a dose predicted to cause 100% mortality. The dose providing quarantine security is often higher than that predicted from dose response tests to give 100% mortality. Insects are irradiated in the commodity after inoculation with a known number of insects or in naturally infested host material. For internal feeding insects naturally infesting the commodity, pupae or adult insects are reared from untreated controls to calculate the number of viable insects that were treated. Untreated control insects are always included in tests with irradiated insects so that mortality can be adjusted for natural variation and to guard against changes in experimental conditions over the course of testing. While control mortality ≤20% is desirable, higher mortality may be normal when using wild insects and naturally infested commodities.

As mentioned, the actual dose to achieve quarantine security at a given level of precision may exceed the dose predicted from small-scale dose response tests. For example, the dose predicted to prevent emergence of adult

melon flies treated in papaya from dose response data was 90 Gy (0 survivors in 900 tested insects) (Follett and Armstrong 2004); however, subsequent large-scale testing at 120 Gy resulted in 1 survivor out of 50,000 treated third instars and several partially emerged pupae. Increasing the dose for large-scale testing to 150 Gy resulted in 0 survivors in 96,700 treated insects and no partial pupal emergence (Follett and Armstrong 2004). This demonstrates the need for large-scale testing to verify a dose.

Probit analysis is the standard method to evaluate dose response data, but other models (e.g. logit) should be used if they provide a better fit to the data (Robertson and Preisler 1992). These analyses are used to compare radiotolerance among life stages or species, and to help identify a target dose for large-scale testing. Covariance analysis is an alternative to compare response among stages or between species. Covariance analysis requires the slopes of the regression lines fitted to each group to be parallel, so the test of parallelism (nonsignificant stage or species by dose interaction effect) is tested before comparing stage or species effects (e.g. Follett 2004).

Accurate dosimetry is critical to the success of insect irradiation studies. The objective in research is to minimize the dose uniformity ratio (DUR) (also called the maximum:minimum ratio), thus reducing variation in dose response tests. This allows the researcher to more accurately pinpoint an efficacious dose without excessive overkill. The maximum dose measured during large-scale testing becomes the minimum dose for a treatment (Heather 2004). Dose rate decreases with the square of the distance from the source (e.g. if distance from source is doubled, dose rate decreases by a factor of 4). Small scale research irradiators such as the Gammacell 220 types (MDS Nordion, Canada) have a small radiation chamber volume and hence all locations in the product during irradiation are a short distance from the source and DURs can be minimized (typically <1.2:1). It is generally accepted that large-scale commercial irradiators are not useful for conducting dose response research because of high DURs, sometimes in the range of 3:1. High DURs are the result of product volume and density, not the size of the irradiator. When using commercial irradiators for research applications, DURs can be minimized by presenting product of minimal depth (e.g. individual fruits) and irradiating the product in a forward then reverse orientation. For example, Follett and Armstrong (2004) irradiated fruit fly larvae in papayas at a commercial x-ray facility using an electron linear accelerator (5 MeV, model TB-5/15, SureBeam Corp., San Diego, California). To minimize the DUR, infested fruit were placed upright in plastic tubs in a single row facing the beam. Dose mapping demonstrated that doses were sometimes lower near the sides and floor of the metal carrier, so the tubs with fruit were elevated by placement on a cardboard box and positioned in the exact center of the carrier. Each carrier passes in front of the beam in a forward then reverse orientation. DURs in this study were consistently <1.2 (Follett and Armstrong 2004).

Table 1. Range of doses predicted to control various pest groups.

Pest group	Required response	Dose range (Gy)
Hemiptera	Sterilize adult or prevent generation turnover	50-250
Thrips	Sterilize actively reproducing adult	150-350
Tephritid fruit flies	Prevent adult emergence from larva	50-150
Bruchid seed weevils	Sterilize actively reproducing adult	70-300
Curculionid weevils	Sterilize actively reproducing adult	80-150
Scarab beetles	Sterilize actively reproducing adult	50-150
Stored product beetles	Sterilize actively reproducing adult	50-250
Stored product moths	Sterilize actively reproducing adult	100-600
Lepidopteran borers	Prevent adult emergence from larva	100-250
	Sterilize adult from late pupa	200-400
Mites	Sterilize actively reproducing adult	200-400
Nematodes	Sterilize actively reproducing adult	approx. 4,000

Modified from FAO (2003) "Guidelines for the use of irradiation as a phytosanitary measure."

Animal groups vary in their tolerance to irradiation (Table 1). Among insects, Diptera (flies) Coleoptera (beetles), Hemiptera (true bugs) tend to be less radiotolerant than Lepidoptera (moths and butterflies), although there is considerable variation among the species that have been tested within these groups. Estimates for Hemiptera (scales, mealybugs, aphids and whiteflies) and Thysanoptera (thrips) are based on a small number of studies. Two of the most radiotolerant insects are the Indianmeal moth, *Plodia interpunctella*, and the Angoumois grain moth, *Sitrotroga cerealella*, both stored products pests (Ahmed 2001, Ignatowicz 2004). Several species of mites have been tested and they appear to be relatively tolerant of ionizing radiation. Nematodes are highly tolerant. Few studies have conducted the large-scale tests needed to confirm the efficacy of an irradiation dose predicted to give 100% mortality. Table 2 provides a list of quarantine insect pests that have been rigorously tested; much of this information is recent and will be used to update and revise approved irradiation treatment doses for specific pests. Most insects are sterilized at doses below 300 Gy.

Probit 9 efficacy and alternatives

Postharvest commodity treatments for pests requiring a high degree of quarantine security are commonly referred to as probit 9 treatments. A response at the probit 9 level results in 99.9968% response. The USDA has used 99.9968% efficacy as the basis for approving many quarantine treatments against tephritid fruit flies. Probit 9 or 99.9968% mortality is often incorrectly interpreted to mean that 3 survivors are allowed in 100,000 treated insects or 32 survivors in 1 million treated insects (Baker 1939) without regard to the precision associated with this level of survivorship. To achieve probit 9 mortality at the 95% confidence level, 93,613 insects must be tested with no survivors. Quantitative methods have been developed to calculate the number of test insects and confidence limits for other levels of precision and treatment

Table 2. Insects subjected to large-scale confirmatory testing to establish treatment efficacy.

Species	Common name	Target dose	Stage	# Tested	Reference
Anastrepha ludens	Mexican fruit fly	100	L	101,794	Bustos et al. 2004
		69	L	95,000	Hallman & Martinez 2001
A. obliqua	West Indies fruit fly	100	L	100,400	Bustos et al. 2004
A. serpentina	sapote fruit fly	100	L	105,252	Bustos et al. 2004
A. striata	guava fruit fly	100	L	13,094	Toledo et. al. 2003
A. suspensa		50	L	100,000	Gould & von Windeguth 1991
Bactrocera dorsalis	oriental fruit fly	250	L	620,000	Seo et al. 1973
		150	L	173,000	Komson et al. 1992
		125	L	55,743	Follett & Armstrong 2004
B. cucurbitae	melon fly	210	L	169,903	Seo et al. 1973
		150	L	93,666	Follett & Armstrong 2004
B. jarvisi	Jarvis' fruit fly	101	L	153,814	Heather et al. 1991
B. latifrons	solanaceous fruit fly	150	L	157,111	T. Phillips (unpublished)
B. tryoni	Queensland fruit fly	75	L	24,700	Rigney and Wills 1985
		101	L	138,635	Heather et al. 1991
Ceratitis capitata	Mediterranean fruit fly	250	L	110,800	Seo et al. 1973
		218	L	70,400	Seo et al. 1973
		150	L	100,854	Bustos et al. 2004
		100	L	31,920	Follett & Armstrong 2004
Rhagoletis pomonella	apple maggot	57	L	22,360	Hallman 2004
Conotrachelus nenuphar	plum curculio	92	A	25,000	Hallman 2003
Cylas formicarius elegantulus	sweetpotato weevil	150	A	62,600	Follett (in press)
		165	A	30,655	Hallman 2001
Euscepes postfasciatus	West Indian sweetpotato weevil	150	A	50,000	Follett (in press)
Omphisa anastomosalis	sweetpotato vine borer	150	P	12,000	Follett (in press)
Cydia pomonella	codling moth	200	L	132,000	Mansour 2003
Grapholita molesta	oriental fruit moth	232	L	58,779	Hallman 2004
Brevipalpus chilensis	false spider mite	300	A	8,042	Castro et al. 2004
Cryptophlebia illepida	koa seedworm	250	L	11,256	Follett and Lower 2000

Stage: L , larva; P, pupa; A, adult. Source: Follett & Griffin In press.

efficacy, with and without survivors (Couey and Chew 1986). A probit 9 treatment usually provides adequate quarantine security (but see Mangan et al. 1997; Powell 2003), and developing the treatment frequently proves to be the quickest and most easily accepted method for overcoming phytosanitary restrictions. Other countries (Japan, Australia, New Zealand) accept quarantine treatment efficacy at 99.99% (at the 95% confidence level) which is obtained by treating 29,956 insects with no survivors (Couey and Chew 1986). Japan and New Zealand require three replicates of 10,000 test insects with no survivors (Sproul 1976). The number of insects tested may need to be adjusted (increased) to account for control mortality (Follett and Neven In press).

For insects that are difficult to obtain or rear in the laboratory, lower numbers may be acceptable in certain cases. For example, an irradiation treatment of 300 Gy was accepted for the mango seed weevil, *Sternochetus mangiferae* (Federal Register 2002), a monophagous pest of mangos, based on evidence for its limited potential impact in the U.S. (Follett and Gabbard 2000), and cumulative data from several studies with a few thousand insects showing prevention of adult emergence at a target dose of 300 Gy and sterilization at lower doses (Heather and Corcoran 1992; Seo et al. 1974; Follett 2002). When low numbers of insects are used, the number tested without survivors can be used to calculate the level of quarantine security. When dose response or small-scale tests are used to predict an irradiation dose to control the pest, increasing the dose by 20-25% adds a margin of safety.

Landolt et al. (1984) pointed out that the probit 9 standard may be too stringent for commodities that are rarely infested or poor hosts. The *alternative treatment efficacy* approach measures risk as the probability of a mating pair or reproductive individual surviving in a shipment. The main quantitative argument for deviating from probit 9 treatment efficacy is low infestation rate of the commodity, but many other biological and non-biological factors affect risk (Vail et al. 1993, Whyte et al. 1994, Follett and McQuate 2001). An advantage to using the alternative treatment efficacy approach is that fewer insects may be needed during development of quarantine treatments (Follett and McQuate 2001). The alternative treatment efficacy approach fits with the systems approach where multiple procedures are used to cumulatively provide quarantine security (Jang and Moffitt 1996). For example, irradiation of avocados within the range of doses providing probit 9 kill of tephritid fruit flies and other pests (100-400 Gy) causes discoloration to the fruit flesh. In Hawaii, oriental fruit fly is the main quarantine pest of avocados, although the avocado fruit on the tree is a poor host for fruit flies. Whereas 120 Gy is required to give probit 9 efficacy (prevent adult emergence) for oriental fruit fly, irradiation treatment at a dose of 80 Gy provides >99% efficacy (Follett and Armstrong 2004), and potentially could be combined with poor host status, inspection, field control and other mitigation procedures to give a high level of quarantine security.

Maximum pest limit is another approach to quarantine security that focuses on survival rather than mortality and is closely related to the alternative treatment efficacy approach (Baker et al. 1990, Mangan et al. 1997). It is defined as the maximum number of insects that can be present in a consignment imported during a specified time at a specified location (Baker et al. 1990). A minimum sample size for inspection is determined from an estimate of the level of pest infestation, the efficacy of the postharvest treatment, and the maximum lot size assembled per time period at a location. This level of inspection is predicted to detect infestation levels greater than the maximum level of permissible infestation with a certain probability and confidence limits (Baker et al. 1990).

Varietal testing

When the pest infests more than one host cultivar or variety, disinfestation studies should theoretically be carried out on the variety in which the pest is most tolerant to irradiation. For a given absorbed dose, pest response to irradiation in the host may vary depending on the milieu surrounding the pest. Oxygen concentration is known to modify sensitivity to irradiation and conditions producing hypoxia can increase radiation tolerance (Alpen 1998). Fruit flies have higher radiotolerance when treated in a nitrogen atmosphere compared with ambient air (Fisher 1997), and when treated in fruit compared with diet (Follett and Armstrong 2004). Radiation damage and mortality was less in codling moth larvae treated in 0.25% O_2 compared with 3% O_2 (Batchelor 1989). Varieties of a commodity with higher water content may have lower available oxygen, and insects infesting these varieties might show higher radiotolerance. Variety was shown to have a dramatic effect on egg hatch and larval development during irradiation studies with Mediterranean fruit fly in nectarines (8 varieties) and plums (4 varieties) (Kaneshiro et al 1983), and a link with fruit moisture content was suspected but not measured. In the absence of comparative tests among varieties, the variety at greatest risk of infestation or the variety which makes up the greatest proportion of trade is used.

Generic treatments

A "generic" quarantine treatment is one that provides quarantine security for a broad group of pests. From a regulatory standpoint, "generic" can also refer to a treatment for a pest on all commodities it infests. A generic treatment for a group of insects could be applied at many taxonomic levels, e.g. to all Diptera (flies), or to flies in the family Tephritidae (fruit flies), or to tephritid fruit flies in the genus *Bactrocera*. Irradiation is the ideal technology for developing generic treatments because it is effective against most insects and mites at dose levels that do not affect the quality of most commodities. Before

a generic treatment can be recommended, information is needed on effective irradiation doses for a wide range of insects within the taxon.

Initially, development of the generic dose concept has focused on tephritid fruit flies. The International Consultative Group on Food Irradiation (ICGFI) was the first group to formalize a recommendation for generic irradiation treatments (ICGFI, 1991). In 1986, based on irradiation data for several 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. Adoption of the 150 Gy dose for fruit flies was stymied by research suggesting three tephritid fruit fly species in Hawaii required higher irradiation doses to prevent adult emergence from infested fruit (Seo et al. 1973). Based on the data presented by Seo et al. (1973), USDA-Animal Plant Health Inspection Service (APHIS) approved irradiation doses of 210, 225 and 250 Gy for melon fly, Mediterranean fruit fly, and oriental fruit fly, respectively, for exporting fruits and vegetables from Hawaii (Federal Register 1997). The majority of economically important tephritid fruit flies come from four genera—*Anastrepha, Bactrocera*, *Ceratitis*, and *Rhagoletis*, and irradiation studies have been conducted with species in each of these genera. Although results from various irradiation studies with fruit flies have not always been consistent (reviewed by Burditt 1994, 1996; Rigney 1989; Hallman and Loaharanu 2002), the preponderance of evidence suggested these genera could be controlled by doses at or below 150 Gy. Recently, Follett and Armstrong (2004) demonstrated that irradiation doses of 100, 125 and 150 Gy controlled *C. capitata*, *B. dorsalis*, and *B. cucurbitae*, respectively, which supported lowering the dose for Hawaii's fruit flies and acceptance of the proposed 150 Gy generic dose for tephritids. A proposed rule is due out from USDA-APHIS in the near future recommending a generic dose of 150 Gy for all tephritid fruit flies. This will be the first use of a global phytosanitary treatment for any pest group or treatment type.

The generic dose concept has been applied on a limited scale to irradiation treatment for fruits exported from Hawaii to the U.S. mainland. In 2001, the USDA-APHIS convened a meeting to establish treatment protocols for a new commercial irradiation facility (Hawaii Pride LLC) 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 taxa (Follett and Armstrong 2004). This was the first time USDA-APHIS recommended a generic irradiation dose for any group of insects albeit on a limited scale and only for certain Hawaii fruits.

New Zealand is preparing a rule to allow import of tropical fruits from Australia using generic irradiation treatments of 150 Gy for fruit flies, 250 Gy for other insects, and 300 Gy for mites (Corcoran and Waddell 2003).

Broad application of the generic irradiation concept to other taxa at the family or order level would be beneficial to promote trade in agricultural commodities and provide a treatment alternative for infested consignments arriving in importing countries. An International Database of Insect Disinfestation and Sterilization (IDIDAS 2003) under development by the International Atomic Energy Agency contains information on many Coleoptera (79 species, mainly curculionids) and Lepidoptera (72 species, mainly pyralids and tortricids); however the majority of the studies referenced were not designed for quarantine purposes and lack the necessary large-scale tests. Information for other important regulatory arthropod groups such as Thysanoptera, Hemiptera, and Acari is limited.

The "high dose" approach is a variation on the generic dose concept. With this approach a dose is set in excess of that believed to be required to control the pests associated with the commodity. For example, sweetpotato growers in Hawaii are unable to ship sweetpotatoes to California and the U.S. mainland without a quarantine treatment because of the presence of three regulatory pests, West Indian sweetpotato weevil, *Euscepes postfasciatus* (Coleoptera: Curculionidae), sweetpotato vine borer, *Omphisa anastomosalis* (Lepidoptera: Pyralidae), and sweetpotato weevil, *Cylas formicarius elegantulus* (Coleoptera: Curculionidae). An irradiation treatment of 400 Gy for sweetpotatoes was approved based on data from IDIDAS and the irradiation literature on curculionid and pyralid pests suggesting this dose would be adequate. This provisional irradiation treatment was published as a final rule in the Federal Register on February 18, 2004 (Federal Register 2004). This was the first time USDA-APHIS considered the high-dose approach for controlling a pest complex until research is completed to confirm a lower dose.

Before generic treatments can be recommended for a wider range of insects and on a broader scale, information from coordinated research projects and large-scale tests is needed on effective irradiation doses for key pests and under-represented taxa. The most radiotolerant insect species tested to date is the Angoumois grain moth which successfully reproduced at 500 Gy but not at 600 Gy (Ignatowicz 2004). Theoretically this dose could be set as a generic treatment for all insects; however, a limiting factor for the practical use of a generic treatment at 600 Gy is the 1000 Gy (1 kGy) maximum allowed dose for fresh produce set by the Food and Drug Administration. With typical dose uniformity ratios of 1.5-3.0 at commercial irradiation facilities, treatment to achieve a minimum absorbed dose of 600 Gy without exceeding 1 kGy would be problematic. Also, doses above 600 Gy adversely affect the organoleptic properties of many fresh fruits and vegetables (Kader 1986, Morris and

Jessup 1994). A generic irradiation dose of 400 Gy for arthropods is supported by available data if Lepidoptera pupae and adults and mites are excluded.

Case study: Commercial irradiation in Hawaii

Hawaii has been an active proponent for the use of irradiation as a phytosanitary treatment for fresh produce. Due to the presence of medfly, oriental fruit fly, and melon fly, Hawaii is under a federal quarantine and cannot export host commodities to the U.S. mainland without a quarantine treatment. Research in the state into the practical aspects of using irradiation technology began in the mid-1960s, resulting in formal approval in 1989 for use of irradiation as a quarantine treatment to export Hawaii-grown papaya (Moy and Wong 2002). Beginning in 1995, Hawaii was the first in the world to use irradiation commercially as a quarantine treatment for fresh produce for export, shipping a variety of tropical fruits (mainly papaya, rambutan, litchi and atemoya) and vegetables to Illinois and New Jersey for treatment and subsequent distribution in retail markets in 17 states (Moy and Wong 2002). The important lesson from this program was that wholesalers, retailers and consumers were not adverse to buying irradiated fresh produce if the product is of high quality and available at a fair price. Also, fruit growers, the business community, and regulatory agencies supported application of the technology.

In August 2000, the first commercial x-ray irradiation treatment facility (Hawaii Pride LLC) for fresh produce opened in Hawaii. In its first year the facility treated approximately 4.5 million lbs of fresh tropical fruits for export, primarily papaya but also rambutan, lychee and longan. In 2003, sweetpotato exports started and this crop is now second behind papaya with exports of 3-5 million lbs per annum (Follett in press). Hawaii is the only place irradiating fresh fruits and vegetables for export, with approved treatments for 10 fruits and 5 vegetables (Follett 2004). The minimal loss of quality after irradiation treatment is particularly important for Hawaii's tropical fruits, which are generally sensitive to the fumigation, heat, and cold treatments (e.g. Follett and Sanxter 2003). Hence, the use of irradiation is expected to grow and help open export markets for high quality tropical fruits.

Regulatory aspects of irradiation

The establishment of national regulations for the use the irradiation as a phytosanitary treatment began in 1930 with a failed proposal to use X-ray for treating fruit exported from Formosa (Koidsumi, 1930). Seven decades later, the International Plant Protection Convention (IPPC) adopted an international standard for the use of irradiation as a phytosanitary treatment (IPPC, 2003a). The evolution of irradiation as a phytosanitary treatment from its disappointing start to international success was marked by a long history of national,

regional, and international initiatives and several watershed events (discussed below), including the official acceptance of irradiation as a "safe" treatment and the establishment of a regulatory and policy framework by the U.S. for the implementation of irradiation as a phytosanitary treatment.

Safety

The safety of irradiated food has been an issue of public concern despite extensive research being carried out in many countries without negative results. One of the principal studies was The International Project on Food Irradiation from 1970 to 1982. The Project included extensive feeding studies carried out by an international consortium over a range of commodities irradiated at 10 kGy. None of the studies gave any indication of the presence of radiation-induced carcinogens or other toxic substances (Diehl, 2001).

The data generated by this project and other related investigations were reviewed at several international meetings organized by key agencies of the United Nations, including the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA). This series of meetings culminated in 1980 with the joint FAO-IAEA-WHO Expert Committee on the Wholesomeness of Irradiated Food. This Committee concluded that the irradiation of any commodity up to an overall average dose of 10 kGy presented no toxicological hazard, and that irradiation up to 10 kGy creates no special nutritional or microbiological problems, hence toxicological testing of foods so treated was no longer required (WHO, 1981).

Codex Alimentarius (Codex), the international organization responsible for establishing harmonized standards for food safety, adopted its Codex General Standard for Irradiated Food (CAC/RS 106- 1979) in 1979. Although the standard does not specifically apply to phytosanitary treatments, it was the first international standard for irradiated food, and many phytosanitary treatments are for food commodities. The standard was subsequently revised in 1983 following the recommendations of the joint FAO-IAEA-WHO Expert Committee, and again in 2003 based on additional research indicating that the maximum absorbed dose could exceed 10 kGy when necessary to achieve a legitimate technological purpose (Codex, 2003).

Associated with the General Standard is the Codex Recommended International Code of Practice for the Operation of Irradiation Facilities. This was significant because it represented the first internationally harmonized guidelines on how to measure absorbed dose. It also describes relevant parameters in facilities, dosimetry and process control, good radiation processing practice, and product and inventory control, (Codex, 1984).

The Code includes two annexes: Annex A is related to dosimetry, indicating how to calculate the overall average adsorbed dose and explaining the concept of

limiting dose values, routine dosimetry, and process control. Annex B gives some examples of technological conditions for the irradiation of certain items. Mango is one of the examples. It is noted that mangoes may be irradiated for three objectives: (1) control of insects, (2) to improve quality (extend shelf life), and (3) to reduce microbial load using up to 1 kGy as an average dose.

It is significant that the Code focused on mangoes because the chemical treatment of mangoes became a serious political issue in 1982 after the U.S. Environmental Protection Agency (EPA) announced a ban on the use of ethylene dibromide (EDB) because it was demonstrated to be a carcinogen (Ruckelshaus, 1984). EDB was popular and widely used as a phytosanitary treatment at the time. The ban forced phytosanitary officials to seek alternative treatments for many commodities that were routinely treated for import and export, especially tropical fruits.

Political pressures and growing interest in the commercialization of irradiation for the treatment of food in the United States spurred the Food and Drug Administration (FDA) to open the regulatory door in 1986 by publishing 21 CFR 179.26, "Irradiation in the Production, Processing and Handling of Food". Among other things, this regulation authorized the use of irradiation up to 1 kGy for the disinfestation of arthropod pests in food, the use of up to 8 kGy for the control of microbial pathogens on seeds for sprouting, and up to 30 kGy for the microbial disinfestation of spices. This rule cleared the regulatory path for the USDA to authorize irradiation as a phytosanitary treatment on commodities for consumption.

One of the most significant contemporary challenges to the safety of irradiated food emerged from studies conducted in the early 1970's showing that detectable levels of 2-alkylcyclobutanones were formed when fats were irradiated at high doses. This triggered a series of studies, primarily supported by the European Commission, to determine the health risks associated with these compounds. Some adverse effects were evident from *in vitro* studies of rats that ingested very high concentrations, however the sum of all studies showed a negligible risk to human health from concentrations that might result from irradiated fat-containing foods (European Commission, 2002).

European authorities have historically been among the most reluctant to accept irradiation as a treatment for foods, but also among the most active in supporting research on the safety of irradiation. Concerns are principally focused on health risks to food processing workers, possible long-term effects of consuming irradiated food (especially for children), and fears that food producers and processors will be less motivated to use good manufacturing practice to ensure the wholesomeness of food if they are able to rely on irradiation treatment to produce clean products.

As a result, the regulatory structure for the European Community is based on strict listing and labeling requirements supported by strong enforcement

measures. A very limited list of herbs, spices and seasonings is currently authorized from approved facilities with mandatory labeling requirements. In 2001, the European Commission suggested that this list be considered complete and recommended further research on the effects of consuming irradiated food and identifying alternative treatments rather than expanding the possibilities for irradiation (European Commission, 2001).

A *Motion For a Resolution* from the European Parliament in 2002 supports the recommendations of the Commission and adds that only Belgium, France, Italy, the Netherlands, and the UK allow irradiation of foods other than herbs, spices, and seasonings and, in practice that few foods are irradiated in these countries, demonstrating little technological need. The same document doubts the benefit of irradiation and predicts the misuse of the technology as a substitute for good hygiene. It also considered that irradiation is a dangerous process which should be replaced with safer processes (European Parliament, 2002).

A similar situation occurs with Japan, where nuclear technologies of any kind are perhaps a more sensitive issue than for other countries. Like the Europeans, the Japanese allow and use irradiation for the treatment of food on a very limited and highly restricted basis. Further, they prefer electronic sources (which can be switched on and off) rather than gamma sources (which present a constant risk and greater environmental hazard). To date, the only phytosanitary treatment reported by Japan is for potatoes. A small proportion of Japan's potato production is treated for sprout inhibition (Furuta, 2004).

USDA regulations

The USDA had decided as early as 1966 that 150 Gy was the minimum dose to "prevent adult emergence" of three fruit flies: oriental fruit fly, *Bactrocera dorsalis*, Mediterranean fruit fly *Ceratitis capitata*, and melon fruit fly, *Bactrocera curcubitae* associated with papaya from Hawaii (Balock, et al, 1966). In 1989, soon after FDA's regulations went into effect, the Animal and Plant Health Inspection Service (APHIS), the USDA Agency responsible for regulations dealing with protecting animal and plant health, published the first rule to allow the use of irradiation as a phytosanitary treatment. The rule specified a treatment of 150 Gy in order to ship fresh papaya from Hawaii to the mainland, Guam, Puerto Rico and the Virgin Islands (Hawaii was later changed to 250 Gy).

Despite being limited to a specific commodity, origin, and domestic program, (and despite the fact that no fruit were immediately shipped due to the lack of a treatment facility in Hawaii), this minor domestic regulation had major global impacts as a result of the regulatory and policy implications it represented for the phytosanitary community. By publication of this rule, the United States made clear its acceptance of irradiation as both a safe and

effective phytosanitary treatment and, for the first time, APHIS approved a treatment that dealt with a complex of pests (fruit flies) rather than a single pest. At the same time, APHIS recognized the legitimacy of a non-mortality treatment (the required response was "inability to fly") and the possibility of detecting and accepting 'live' quarantine pests in treated shipments (USDA-APHIS, 1989).

Regulatory interest in irradiation was peaked again in 1992 when the fumigant methyl bromide (MB) was listed under the Montreal Protocol as one of the substances that causes depletion of the ozone layer. The Montreal Protocol is an international treaty for the regulation of ozone depleting substances in the atmosphere (EPA, 1993). At the Meeting of the Parties to the Montreal Protocol held September 1997 in Montreal, Canada, it was agreed that the production of MB should be phased-out by a certain percentage each year beginning in 1999. Developed countries were expected to phase-out completely by 2005 and developing countries by 2015 (EPA, 1996).

Although the Montreal Protocol makes an exception for the use of MB as a quarantine treatment, the overall reduction in production of the fumigant over time has caused cost increases and reduced the availability of the compound with the net effect of making it increasingly less practical. The effect is not as immediate as was the ban on EDB, but the repercussions are just as significant because MB is also popular and widely used as a phytosanitary treatment for both food and non-food items (e.g., cut flowers, wood products).

After 1995, rapidly increasing global trade pressures and the possible loss of methyl bromide as a fumigant for regulatory pest treatments made it imperative for practical treatment options to be explored. Unfortunately, the perception of public reluctance to accept irradiation and the relatively high initial costs associated with changing to irradiation as a preferred treatment technology made it less desirable than lower-cost alternatives. At the same time, technological advances, greater experience, and a growing body of research indicated that irradiation had increasingly greater potential as a treatment, or as an alternative treatment, for many quarantine pest problems.

It is in this light that APHIS decided in 1996 to expand its regulatory framework addressing irradiation treatment, develop comprehensive policy statements, and begin encouraging international harmonization while also updating its own treatments and approving new ones. In a Policy Notice of 1996 titled "The Application of Irradiation to Phytosanitary Problems", APHIS listed key positions and procedures, defined terms, offered research protocols, and proposed generic doses for nine fruit fly pests (USDA-APHIS 1996).

In response to a petition from Hawaii, APHIS further expanded its authorization in 1997 to add the possibility to treat fresh papaya, lychees and carambolas from Hawaii at 250 Gy. Following this, APHIS also approved the irradiation of sweetpotato and other commodities from Hawaii. Fruits and

vegetables from Hawaii that are currently authorized for irradiation treatment include abiu, atemoya, bell pepper, carambola, litchi, longan, eggplant, mango, papaya, pineapple (other than smooth Cayenne), rambutan, sapodilla, Italian squash, sweetpotato and tomato (7 CFR 318.13-4f).

Consistent with its Policy Notice, APHIS supplemented its authorizations for exports from Hawaii with regulations to also allow foreign imports by publishing a rule on Irradiation as a Phytosanitary Treatment for Imported Fresh Fruits and Vegetables (7 CFR 319.305). This regulation sets out specific standards for irradiation treatment to provide protection against 11 species of fruit flies and the mango seed weevil. Included also in this regulation are provisions that require the exporting country to establish Framework Equivalency Work Plans with APHIS demonstrating that the exporting country accepts irradiated commodities for import.

Current plans are to continue expanding regulatory authorizations for the use of irradiation as a phytosanitary treatment based on additional research and experience (Pers. Comm, 2005). Data currently under review offers possibilities for significant refinement of existing treatments and would make some new treatments available, including doses of 150 Gy for all tephritid fruit flies, 300 Gy for the false red spider mite (*Brevipalpus chilensis*), 200 Gy for codling moth (*Cydia pomonella*), 250 Gy for koa seedworm (*Cryptophlebia illepida*), 250 Gy for litchi fruit moth (*Cryptophlebia ombrodelta*), 200 Gy for oriental fruit moth (*Grapholita molesta*), 92 Gy for plum curculio (*Conotrachelus nenaphur*), and 150 or 165 Gy for sweetpotato weevil (*Cylas formicarius elegantulus*).

Regional and international harmonization

The North American Plant Protection Organization (NAPPO), the regional organization responsible for setting phytosanitary standards recognized under the North American Free Trade Agreement (NAFTA), formally recognized the effectiveness of irradiation as a broad-spectrum quarantine treatment for fresh fruits and vegetables in 1989. In addition to NAPPO, other regional plant protection organizations that operate within the framework of the IPPC, including the European and Mediterranean Plant Protection Organization (EPPO), the Asia and the Pacific Plant Protection Commission (APPPC), the Comite de Sanidad Vegetal del Cono Sur (COSAVE), the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), endorsed irradiation as a quarantine treatment for fresh horticultural products at the Technical Consultation of Regional Plant Protection Organizations held in San Salvador in 1992, (FAO, 1992).

At the NAPPO Annual Meeting in 1994, a roundtable discussion was organized on "The Application of Irradiation to Phytosanitary Problems". NAPPO delegates from Canada, Mexico and the U.S. provided enough

encouragement for the NAPPO Executive Committee to agree on an initiative to elaborate a regional standard. The policies put forward by APHIS in 1996 provided the framework for the development of "Guidelines for the Use of Irradiation as a Phytosanitary Treatment" that were adopted as a NAPPO standard in (NAPPO, 1997). This marked a significant step forward in international harmonization and became the springboard for creation of the International Standard for Phytosanitary Measures (ISPM) No. 18 *Guidelines for the use of irradiation as a phytosanitary measure*, adopted by the IPPC in 2003.

Since 1993, the IPPC has prepared international standards for phytosanitary measures designed to promote international harmonization and facilitate safe trade by avoiding the use of unjustified measures as barriers. Standards adopted by the IPPC must be observed by members of the World Trade Organization according to the Agreement on the Application of Sanitary and Phytosanitary Measures (the WTO-SPS Agreement). Governments must provide a technical justification (generally a risk assessment) for measures that are inconsistent with international standard or for measures put in place in the absence of a standard (WTO, 1994).

The Interim Commission on Phytosanitary Measures (ICPM), governing body of the IPPC considered the global application of irradiation as a phytosanitary measure at its Third Session in 2001. The ICPM agreed that, with financial support provided by IAEA, an expert working group would be established to elaborate an international standard for irradiation as a phytosanitary treatment (IPPC, 2001). The experts prepared a draft standard using the NAPPO standard and other information that had been developed by this time. The draft standard was adopted by the ICPM as International Standard for Phytosanitary Measures No. 18 in April 2003 (IPPC, 2003b). The IPPC standard describes specific procedures for the application of ionizing radiation as a phytosanitary treatment for regulated pests or articles. The document is organized like other IPPC standards, with sections including an introduction, scope, references, definitions and abbreviations, and an outline of requirements preceding the general and technical requirements. In addition, the standard includes two annexes and two appendices. Following is a brief summary of the content:

Authority. The National Plant Protection Organization is responsible for phytosanitary aspects of the evaluation, adoption and use of irradiation as a phytosanitary measure.

Treatment objective. The objective is to prevent the introduction or spread of regulated pests. This may be realized by achieving a specific response in the targeted pest(s) such as mortality, non-emergence of adults, sterility or inactivation. Live target insects may be found after treatment, therefore plant quarantine officials must have confidence in the research that

supports efficacy of the treatment as well as the integrity of the treatment process itself. The importing country should specifically define the required treatment efficacy, consisting of a precise description of required response and the statistical level of response required.

Treatment. The types of radiation sources authorized for treatment are the same as those authorized by Codex. Treatment procedures should ensure that the minimum absorbed dose (Dmin) is fully attained throughout the commodity to provide the prescribed level of efficacy. Irradiation can be applied as an integral part of packing operations or to bulk unpackaged commodities (such as grain moving over a belt). The treatment may also be done at a centralized location such as the port of embarkation or import. Treated commodities should be certified and released only after dosimetry measurements confirm that the Dmin was met. This section corresponds to Annex 1 which lists the doses for specific approved treatments (to be completed). Appendix 1 provides some scientific information on absorbed dose ranges for certain pest groups. Appendix 2 provides guidance on undertaking research to develop irradiation treatments for regulated pests.

Dosimetry. Dosimetry ensures that the required Dmin was delivered to all parts of the consignment. The selection of a dosimetry system should be such that the dosimeter response covers the entire range of doses likely to be received by the product. In addition, the dosimetry system should be calibrated in accordance with international standards or appropriate national standards. Dose mapping and routine dosimetry are integral to the dosimetry system.

Approval of facilities. Facilities should be approved by relevant nuclear regulatory authorities where appropriate, and are also subject to approval by the national plant protection organization in the country where the facility is located prior to applying phytosanitary treatments. Annex 2 provides a check-list for facility approval and certification of irradiated commodities for international trade.

Phytosanitary system integrity. Confidence in the adequacy of an irradiation treatment is primarily based on assurance that the treatment is effective against the pest (s) and the treatment process is carried out with maximum integrity, including phytosanitary security measures at the treatment facility, labeling and separation of treated/untreated materials, and procedures for verification.

Documentation. The national plant protection organization of the country where the facility is located is responsible for monitoring recordkeeping and documentation by the treatment facility, and ensuring that records are available to concerned parties. As in the case of any phytosanitary treatment, trace-back capability is essential.

Inspection and phytosanitary certification. This section integrates information from other standards and parts of standards on export inspection,

phytosanitary certification, import inspection, verification methods for treatment efficacy in export and import inspection, and administration and documentation.

Trade

The establishment of the NAPPO standard in 1997 opened new possibilities for the use of irradiation in trade between Mexico and the United States. Mexico has great potential because of the high volume of fruit and vegetable exports requiring phytosanitary treatments. Mexico also has trained personnel and significant experience with irradiation treatments. What may be more important is that Mexico already has a regulatory framework in place for sanitary and phytosanitary treatments that allow food to be irradiated for consumption and for importation (Verdejo, 1997).

In 1998, a meeting was organized in Mexico to evaluate the capability of the country to initiate such trade. Although it was recognized that Mexico had substantial potential for the export of irradiated fruits, especially mango, the producers opted instead to continue with treatments, such as hot water dip, that required a much lower initial investment in equipment and had no controversial implications for consumers. This attitude is changing as Mexico is currently engaged in constructing new treatment facilities and pursuing necessary agreements with APHIS for the export of irradiated foods (Pers. comm., 2004). The United States, however, has not only opened the door for shipments of irradiated commodities from Mexico, but from all countries. Several countries, including Brazil, Colombia, and Thailand are pursuing Framework Equivalency Work Plans with APHIS in order to initiate bilateral trade in products irradiated for phytosanitary purposes (Pers. Comm., 2005).

Based on the information available after 1980 and recognizing that the United States was embracing irradiation as a viable alternative treatment technology, many countries began to also consider legislation or regulations for irradiated food. Approximately 40 countries currently have regulations pertaining to irradiation as a treatment for food products and are treating or accepting treatment for at least one irradiated commodity. Nine countries have authorized the use of irradiation for insect disinfestation of fresh fruits or vegetables (Follett and Griffin, In press). Although a large number of countries have approved irradiation as a treatment for food, few have large-scale commercial operations. There are several reasons for this, but the problems are partly related to regulatory barriers and partly related to the lack of facilities and markets. Also, ensuring adequate throughput can be a substantial challenge given the seasonality of many agricultural products.

The situation is slightly less complicated with non-food treatments. Commodities such as wood products, cut flowers, and bird seed that may also require phytosanitary treatments are not subject to the same degree of

regulation associated with food products. As a result, regulatory frameworks for these treatments do not extend in scope to health and safety concerns but rather emphasize the efficacy of the treatment and the integrity of the treatment process and facility.

The evolution of regulatory frameworks for the adoption and implementation of irradiation as a phytosanitary treatment has been marked by numerous successes around the world. In the past, regulatory uncertainties have heightened anxiety among investors and producers who were already concerned about potential problems with public acceptance despite extensive information about the safety and effectiveness of irradiation. Today, the world has an international standard as a global reference point for the use of irradiation as a phytosanitary treatment, and the United States has put in place a regulatory framework demonstrating full acceptance of the technology. The uncertainties associated with potential regulatory barriers are substantially reduced and the path to realizing the full potential of irradiation as a phytosanitary treatment is opening much wider.

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