Phytosanitary Irradiation of *Liriomyza trifolii* (Diptera: Agromyzidae)

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ABSTRACT Agromyzid leafminers are economic and quarantine pests of a variety of vegetables, flowers, and ornamental foliage. Methyl bromide fumigation is often used as a phytosanitary treatment when quarantined agromyzids are found in shipped commodities; alternative treatments are sought. Ionizing radiation is a viable alternative that is increasing in use worldwide. A dose of 400 Gy is accepted by USDA-APHIS for all insects (except Lepidoptera pupae and adults) on all commodities. Efforts to lower this dose and make it acceptable to other countries involve determining radiotolerance of families of major quarantine pests. Agromyzidae is one such family for which no useful information on radiotolerance exists. This research sought to determine the dose required to control a major agromyzid pest, *Liriomyza trifolii* (Burgess) and was performed on *L. trifolii* collected in Weslaco, TX, reared on *Phaseolus vulgaris* L. and *Capsicum annuum* L. and irradiated in the late puparial stage. The measure of efficacy was prevention of F_1 mine formation. Puparia collected from *Gossypium hirsutum* L. and reared on *P. vulgaris* were more radiotolerant than those collected and reared on *C. annuum*. A dose of 214 Gy may prevent F_1 mine formation of *L. trifolii*. This research used a variation of probit analysis where the direct response of the treated individual is not measured, but the response of the F_1 generation is. This type of analysis is useful in phytosanitary irradiation research where the measure of efficacy often involves a response of the F_1 generation.

KEY WORDS quarantine treatment, phytosanitary treatment, American serpentine leafminer, ionizing radiation

Leafminers of the family Agromyzidae feed on the foliage of a wide variety of cultivated vegetables and flowers (Kaspi and Parrella 2003). They may be quarantine pests on any marketed commodity that contains foliage or other parts of the plant that may be infested, especially cut flowers and foliage (EPPO 2008). Methyl bromide fumigation is often used to disinfest cut flowers of agromyzids. Alternatives to the fumigant are sought, as it is a stratospheric ozone-depleting substance (Heather and Hallman 2008).

Ionizing radiation is a relatively new commercially-used phytosanitary treatment that is increasing in use worldwide (Hallman 2011). The concept of generic phytosanitary treatment doses that can be used for groups of regulated pests, hosts, or both has been applied very broadly with phytosanitary irradiation (PI). Currently, generic doses of 150 and 400 Gy are approved by USDA-APHIS and used commercially for Tephritidae and Insecta (except pupae and adults of Lepidoptera), respectively, on all regulated articles (APHIS 2006). The 400-Gy dose can probably be reduced, economizing resources and time while reducing the risk of radiation damage to fresh produce (Hallman 2011). To substantiate a reduction in the 400-Gy dose and make it more broadly accepted by other countries, representatives of key regulatory pest taxa should be evaluated for their susceptibility to radiation. An international cooperative research project under the auspices of the Joint FAO/IAEA Programme on Nuclear Techniques in Food and Agriculture is developing further generic treatments for inclusion in the International Standard for Phytosanitary Treatment (ISPM) No. 28, Phytosanitary Treatments for Regulated Pests (IAEA 2009, FAO 2009). Currently, the only pest-generic PI treatment in ISPM No. 28 is 150 Gy for Tephritidae.

Agromyzidae is one key family of regulated pests that has not been adequately evaluated for PI. Yathom et al. (1990) found that at least 750 Gy was required to prevent adult emergence from irradiated middle-aged puparia of *Liriomyza trifolii* (Burgess). Although *L. trifolii* normally leaves the host to pupariate in the soil, puparia often may remain adhered to plants, especially pubescent foliage, meaning that the entire puparial stage may occur on transported plant material. Furthermore, emerging larvae in packed plant

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Table 1. Resulting F_1 mine formation in *Phaseolus vulgaris* leaves after *Liriomyza trifolii* late puparia reared on foliage of *P. vulgaris* and *Capsicum annuum* were irradiated

Dose (Gy)[a]	No. puparia		No. mines		Expected no. mines		No. mines prevented		% mines prevented	
	P. vulgaris	*C. annuum*	*P. vulgaris*	*C. annuum*	*P. vulgaris*	*C. annuum*	*P. vulgaris*	*C. annuum*	*P. vulgaris*	*C. annuum*
0	60	60	2973	3660	2973	3660	0	0	0	0
75	100	100	55	33	4955	6100	4865	6067	98.2	99.5
100	399	100	16	6	19,770	6100	19,755	6094	99.92	99.90
120	974	200	2	1	48,262	12,200	48,260	12,199	99.996	99.992
150	105	200	0	0	5203	12,200	5203	12,200	100	100

[a] Each replicate consisted of 20–100 puparia and data were pooled for analysis.

material would most likely pupariate in the packaging, although cold storage conditions may prevent it from developing to the late puparial stage. Nonetheless, for phytosanitary purposes all stages from the egg through the late puparium should be considered as occurring on shipped host material. Hallman et al. (2010) reviewed the literature and found that insects increase in radiotolerance as they mature, and *L. trifolii* is no exception (Yathom et al. 1990). Thus, the most developed stage that could be transported with fresh commodity would be the most radiotolerant one that should be controlled by PI.

Hallman (1998) suggested that the measure of efficacy for insects that may occur in the pupal or puparial stages be prevention of reproduction because high doses not tolerated by fresh commodities would be required to prevent adult emergence from late pupae or puparia. There are no useful data on PI of agromyzids; however, the sterile insect technique (SIT) literature offers guidance. Although the objectives of SIT differ from PI, sometimes research outcomes from one discipline are applicable to the other. Agromyzids used for SIT research are irradiated in the late puparium, the same stage of interest for PI. Kaspi and Parrella (2003) found that 170 Gy absorbed by female *L. trifolii* mated to nonirradiated males resulted in a mean of <0.1 mine per female. Reproductive success when both sexes are irradiated may be less than when only one of the two sexes is irradiated (Heather and Hallman 2008).

The objective of this research was to develop methodology to estimate the PI dose required to provide quarantine security against an agromyzid quarantine pest. *Liriomyza trifolii* was used because it is a major economic and quarantine pest of vegetable and ornamental crops in many countries (Kaspi and Parrella 2003) and endemic around Weslaco, TX.

Materials and Methods

Agromyzid. *Liriomyza trifolii* (sex ratio ≈50:50) was field collected from cotton, *Gossypium hirsutum* L., and bell pepper, *Capsicum annuum* L., foliage in the Weslaco, TX area in April 2009 and reared, respectively, on beans, *Phaseolus vulgaris* L. ('Nash'), and bell pepper, *Capsicum annuum* L. ('California Wonder 300 TMR'), in a quarantine greenhouse to prevent feral infestation by leafminers. Rearing conditions were ≈26°C, ≈70% RH, and a photoperiod of 12:12 (L:D) h. Puparia were collected from plants or the bottom of the cage and placed with noninfested plants in 0.6-m^3 nylon (60 mesh/cm) cages (BD2120 F Insect Tent, MegaView Science Co. Ltd., Taichung, Taiwan). Voucher specimens were kept at the ARS-Weslaco insect collection in Building 200. Puparia are within 48 h of emergence when the eyes turn from red to black; puparia beginning from this stage until before adult emergence were used in all of the trials. Immediately before irradiation any emerging adults were removed from vials.

Irradiation System. The radiation source was ^{137}Cs (Husman model 521A, Isomedix, Inc., Whippany, NJ) located at the USDA-APHIS Mexican Fruit Fly Rearing Facility in Mission, TX. The unit delivered a gamma ray dose rate of ≈33 Gy/min during the time of this research.

Reference standard dosimetry was done in 1996 with the Fricke system. Routine dosimetry was done with radiochromic film (1-cm^2, Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) in paper envelopes placed in the tubes with *L. trifolii* puparia and read 24 h after exposure at 600 nm with a spectrophotometer (Genesys model 4001, Spectronic Instruments, Rochester, NY). The optical density of this exposed film becomes stable after 24 h (IAEA 2004).

Estimate of Dose to Prevent Mine Formation. *Liriomyza trifolii* were reared on *P. vulgaris* and *C. annuum* to the late puparial stage and subjected to irradiation in glass vials (97 mm long by 11.5 mm in diameter) positioned in the center of the irradiation chamber. The total number of puparia irradiated for each host at each dose is given in Table 1. After irradiation the puparia were placed in open petri dishes in the bottom of the nylon cages with plants so that adults could emerge and infest the plants.

The measure of efficacy was prevention of the formation of F_1 mines of any length. This measure provides a simple and rapid evaluation with minimal F_1 development. PI treatments that are not designed to completely prevent the F_1 generation should stop development early in the F_1 to reduce the risk of the live quarantine pests being found in importing countries. Such treatments also provide an added level of security for PI treatments that are often applied to insects

Table 2. Probit regression (Gompertz distribution) parameter estimates for prevention of F_1 mine formation from irradiated late *Liriomyza trifolii* puparia reared on foliage of *Phaseolus vulgaris* and *Capsicum annuum*

Host[a]	Slope	Pearson χ^2	Probability that data fit model	Estimated dose to prevent 99% of mines[b]
P. vulgaris	4.60 ± 0.28	0.11	0.95	80.3 (78.6–82.0)[b]
C. annuum	0.012 ± 0.0018	0.58	0.58	65.2 (56.0–71.0)[b]

df = 2.
[a] For *P. vulgaris* dose was converted to log 10 values for better fit.
[b] Values in parentheses are 95% fiducial limits.

and commodities for which they have not specifically been tested, and for which there is no independent confirmation of efficacy (Hallman et al. 2010). The total number of F_1 mines was counted, and tests were terminated after all of the parent generation adult *L. trifolii* had died and any remaining eggs had sufficient time to hatch.

Analysis of dose–response data usually requires a binary response from each treated individual. In this case it was not known which *L. trifolii* reproduced, only how many F_1 mines resulted. The data were converted to number of expected mines by multiplying the number of females by the mean number of mines produced per nonirradiated female. The number of mines prevented was obtained by subtracting the number of mines observed from the number of mines expected. The number of mines prevented was then analyzed by probit analysis, SAS version 9.2 (SAS Institute, Cary, NC).

Efficacy Testing. Because the level of efficacy required of a phytosanitary treatment (≥99.99%) is too high for confident prediction via statistical models, the treatment must usually be verified through large scale testing of thousands of organisms. In some cases up to 100,000 individuals are tested before a treatment is approved (Heather and Hallman 2008). Efficacy testing was started at 150 Gy and the dose was raised by 50 Gy when it failed until 3,000 late puparia were irradiated with a single dose with no F_1 mines resulting. This amount normally would be insufficient to support a phytosanitary treatment (although some have been successfully supported with less), but this study is part of an international effort to provide a generic PI dose for Agromyzidae. Other researchers will compare multiple species of agromyzids and do large-scale efficacy testing on those that are found to be most radiotolerant (IAEA 2009).

Adult emergence was counted for control and irradiated puparia and analyzed with analysis of variance (ANOVA), with dose and host being fixed factors.

Results

Dosimetry. In the narrow field occupied by the small vial with *L. trifolii* puparia, the dose varied by −1.5 to +7%. Therefore, at the largest target dose used, 200 Gy, the maximum dose was 214 Gy.

Estimate of Dose to Prevent Mine Formation. The total number of mines resulting per dose, the number of expected mines, and the number of mines prevented are given in Table 1. Results for both hosts seem very similar: 120 Gy provided 99.996 and 99.992%, respectively, prevention of mine formation on *P. vulgaris* and *C. annuum*, whereas 150 Gy prevented mines completely. Results of probit analysis are presented in Table 2. The data for both hosts fit probit analysis to a high degree. Both the intercepts (χ^2 = 225.9, $P < 0.001$) and slopes (χ^2 = 13.3, P = 0.0003) were significantly different between the two hosts. The estimated mean dose to prevent 99% of mine formation for *P. vulgaris* (80 Gy) was higher than for *C. annuum* (65 Gy), and 95% fiducial limits did not overlap.

Efficacy Testing. At a target dose of 150 Gy, 1,675 late puparia in total, reared on *P. vulgaris*, were irradiated before one F_1 mine was found. After 2,880 late puparia were reared on *C. annuum* and irradiated no F_1 mines were found. The target dose was raised to 200 Gy and 3,271 late puparia in total (2,510 from *P. vulgaris* and 761 from *C. annuum*) were irradiated with no F_1 mines found.

Irradiation at 150 and 200 Gy reduced adult emergence by 11–18% compared with the nonirradiated control (Table 3). There was no significant difference between the two host plants or interaction between host plant and dose (interaction: F = 0.55, df = 2, 71, probability = 0.79; host plant: F = 0.17, df = 1, 71, probability = 0.70; dose: F = 15.4, df = 2, 71, probability = 0.002).

Discussion

It generally has been assumed that plant host does not affect efficacy of PI, although Hallman et al. (2010) noted that there are few direct comparisons of hosts for measures of efficacy used in PI. *Liriomyza trifolii* reared on *P. vulgaris* was apparently more tolerant than those reared on *C. annuum* for the measure of efficacy used in this research: prevention of F_1 mine formation. That observation was made not only in the

Table 3. ANOVA of percentage adult emergence among nonirradiated and irradiated late puparia reared on foliage of *Phaseolus vulgaris* and *Capsicum annuum*

Dose (Gy)	*P. vulgaris*	*C. annuum*
0	78.8 ± 2.0 a	80.6 ± 5.2 a
150	70.3 ± 5.3 b	66.9 ± 2.8 b
200	68.2 ± 3.9 b	66.1 ± 5.1 b

Means followed by the same letter in columns and rows are not significantly different at the 95% confidence limit (Tukey's Test).

estimate of dose for 99% prevention of F_1 mine formation but also in the efficacy testing where one F_1 mine was found at 150 Gy for *P. vulgaris* but none were found from a larger number of puparia reared on *C. annuum.*

The two populations of *L. trifolii* were collected from two different hosts, and it is, of course, possible that there are heritable differences among them. Hallman et al. (2010) concluded that variations among populations for radioresistance are probably insignificant, although they suggested that a few more studies may be warranted because those done do not represent a broad range of taxa.

Although the levels of control in Table 1 do not look very different between puparia reared on the two host plants given the same dose of radiation, it is worth noting that seemingly minor differences at levels of control near 100% can be significant and result in large differences in dose required to achieve the same level of control. It is recommended that researchers should use multiple hosts to affirm that a treatment is efficacious across hosts when conducting PI research on polyphagous pests.

A dose of 214 Gy (the maximum dose measured in efficacy testing when the target dose was 200 Gy) may provide quarantine security for *L. trifolii.* A larger number of individuals should be tested to confirm this dose. But the objective of the cooperative research project (IAEA 2009) is to develop a dose that is efficacious for all quarantine pest agromyzids. That work will be carried out by additional researchers in the project who will compare other agromyzid species to develop a generic PI treatment for that taxon.

This research uses a successful variation of probit analysis for phytosanitary treatment research where the direct response of the treated individual is not measured, but the response of the F_1 generation is subjected to probit analysis. This type of analysis is useful in PI research where very often and unlike all other commercially applied treatments, the measure of efficacy involves measuring a response of the F_1 generation. Although probit analysis is very often used to analyze phytosanitary treatment data when a response of the treated generation is evaluated (Heather and Hallman 2008) it is not generally used to examine F_1 responses. Most dose–response analyses of F_1 data use less appropriate statistical analyses, such as ANOVA, which should not be used for continuous data and which lacks predictive capabilities. Studies that also successfully used probit analysis on F_1 data are eclosion of *Ostrinia nubilalis* (Hübner), *Plodia interpunctella* (Hübner), and *Sitotroga cerealella* (Olivier) eggs laid from parents irradiated as late pupae or adults (Hallman and Phillips 2008, Hallman and Hellmich 2009).

If results for other agromyzid species are similar to *L. trifolii,* it is likely that a generic dose for this group and many other insects that are commonly found on cut flowers would be 250 Gy (Hallman 2011). When irradiation is applied on a commercial scale the maximum dose absorbed by a load can be 1.2–2.5 times the minimum dose applied, so that when 250 Gy is sought some of the load could receive several hundred gray. Tanabe and Dohino (1995) found that half of 17 species of cut flowers tolerated 600 Gy (the highest dose used) whereas one-fourth of the species did not tolerate 200 Gy, the lowest dose delivered. Sangwanangkul et al. (2008) evaluated several techniques to reduce the damage of radiation to cut flowers and foliage.

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