Phytosanitary irradiation against leafminers (Diptera: Agromyzidae) and radiotolerance of shelled peas, *Pisum sativum* (Fabales: Fabaceae)

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Abstract

The objectives of this research were to develop a phytosanitary irradiation treatment against 3 species of agromyzid leafminers (Diptera: Agromyzidae), and to study the effects of gamma radiation on the physical, chemical, and sensorial properties of shelled peas, *Pisum sativum* L. (Fabales: Fabaceae). Late pupae (pharate adults) of the 3 species were treated with Co-60 gamma radiation at 0, 80, 100, 120, 150, and 180 Gy. The measurement of efficacy used was the prevention of mine formation in leaves by F_1 generation offspring of the leaf miners. *Liriomyza sativa* Blanchard, *L. trifolii* (Burgess) and *L. huidobrensis* (Blanchard) were found to have similar responses to the irradiation doses that prevented formation of leaf mines by F_1 offspring; thus no leaf mines were formed by these 3 species in the 150 Gy and 180 Gy treatments. Confirmatory testing successfully undertaken on all 3 species using a grand total of > 30,000 pharate adults corroborated that irradiation with 150 Gy completely prevented the formation of leaf mines. The radiotolerance of shelled peas was studied to ensure that the irradiation treatment does not significantly affect the composition, palatability and flavor of the produce. Pea samples were irradiated at 0.25, 0.5 and 1 kGy and tested for vitamin C, total carotenoids, protein secondary structure, color, and sensory properties. These properties of the peas were significantly but not greatly affected by irradiation with any of the doses tested.

Key Words: quarantine treatment; *Liriomyza*; puparium; palatability; vitamin C; total carotenoids

Resumen

Los objetivos de esta investigación fueron para desarrollar un tratamiento de radiación fitosanitaria contra 3 especies de minadores agromizidos (Diptera: Agromyzidae), y para estudiar los efectos de la radiación gamma sobre las propiedades físicas, químicas y sensoriales de guisantes pelados, *Pisum sativum* L. (Fabales: Fabaceae). Las pupas mas viejas (adultos pharate) de las 3 especies fueron tratadas con radiación gamma Co-60 a 0, 80, 100, 120, 150 y 180 Gy. La medida de la eficacia utilizada fue la prevención de la formación de galerias en las hojas por la descendencia de la generación F1 de los minados de las hojas. Se encontró que *Liriomyza sativa* Blanchard, *L. trifolii* (Burgess) *y L. huidobrensis* (Blanchard) tienen respuestas similares a las dosis de irradiación que impidieron la formación de las galerias en la hoja por la descendencia F1; por lo tanto no hay galerias en las hojas formadas por estos 3 especies en el 150 Gy y 180 Gy tratamientos. Las pruebas de confirmación hechas con éxito en las 3 especies utilizando un total de > 30,000 pupas bien desarrolladas corroboró que la radiación con 150 Gy impidió completamente la formación de las minas de las hojas. Se estudió la radiotolerancia de guisantes sin cáscara para asegurar que el tratamiento de radiación no afecta significativamente a la composición, la palatabilidad y el sabor de los productos. Se irradiaron muestras de guisantes a 0.25, 0.5 y 1 kGy y fueron evaluadas para la vitamina C, carotenoides totales, la estructura secundaria de proteína, color, y propiedades sensoriales. Estas propiedades de los guisantes fueron afectadas significativamente pero no en demasía por la irradiación a cualquiera de las dosis probadas.

Palabras Clave: tratamiento de cuarentena; *Liriomyza*; finales pupas; palatabilidad; vitamina C, carotenoids

Leaf mining flies of the genus *Liriomyza* (Diptera: Agromyzidae) are serious problems as polyphagous pests of ornamental and vegetable crops. Feeding punctures and leaf mines are usually the first and most obvious signs of the presence of *Liriomyza* spp. Four species, *L. bryoniae* (Kaltenbach), *L. huidobrensis* (Blanchard), *L. sativa* Blanchard and *L. trifolii* (Burgess), are listed in the European Union (EU) Plant Health Directive 2000/29 (EPP0 2005). *Liriomyza bryoniae* is indigenous to Europe and is established in the UK as an important pest of tomatoes; however, it is regulated within the EU. The other 3 are all species that originated in the New World. Trade in agricultural products carries a risk of inadvertently transporting quarantine pests to countries or regions where they do not occur. Methyl bromide fumigation is often used as a phytosanitary treatment when quarantined agromyzids are found in shipped commodities; phytosanitary irradiation (PI) is a viable alternative. Currently a generic dose 400 Gy for all insects except pupal and adult lepidopterans can be used to satisfy phytosanitary requirements for importation of host commodities of agromyzids to the United States of America. No dose for agromyzids is accepted by any other plant protection organization. The 400 Gy dose accepted by the USA could probably be reduced, economizing resources and time, while reducing the risk of radiation damage to fresh produce. Some cut flowers will suffer damage at that dose. For example, *Gerbera* spp.

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(Asterales: Asteraceae) daisies and spray carnations (*Dianthus* spp.; Caryophyllales: Caryophyllaceae) only tolerate up to ~500 Gy (Ozyardimci, unpublished data), a dose that would be exceeded by commercial application of a minimum of 400 Gy where in order to obtain the minimum dose in the entire load, some of the load would receive considerably greater radiation.

Hallman et al. (2011) suggest that a generic dose for agromyzids and other insects found on cut flowers could be ~250 Gy. There are no other useful data on PI of agromyzids; however sterile insect technique (SIT) research on agromyzids irradiated in the late puparium gives data of use to PI, although the objectives of SIT differ from PI (Hallman et al. 2010). Kaspi & Parella (2002) found that a high level of sterility was achieved with a dose of 155 Gy for both sexes when *L. trifolii* late pupae (pharate adults) were irradiated for control of *L. trifolii* infesting greenhouse crops. Kaspi & Parella (2003) further determined the optimum sterilization of *L. trifolii* adults by irradiating pharate adults was achieved with the dose of ~170 Gy. In another study where the measurement of efficacy was prevention of mine formation by F_1 offspring of irradiated *L. trifolii,* 150 Gy resulted in 1 mine being formed when a total of 1,675 pharate adults were irradiated (Hallman et al. 2011). It was suggested that 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*.

The measurement of efficacy used was the prevention of mine formation in leaves by F_1 generation larvae. PI is unique among phytosanitary treatments in that acute mortality is not the usual measure of efficacy because doses for 100% acute mortality would be higher than most fresh commodities can tolerate. PI is effective at arresting insect development and preventing reproduction using doses that do not significantly alter the quality of most fresh commodities, and those measurements of efficacy are sufficient for PI (Hallman et al. 2013).

The purpose of the research was to develop a PI treatment for Agromyzidae species of quarantine significance in international trade. Also, the effects of irradiation on some chemical, physical, and sensorial properties of fresh shelled peas, *Pisum sativum* L. (Fabales: Fabaceae), were evaluated. This research may contribute to generic doses for all leafminers and larger pest groupings. Agromyzidae is one such group for which information about radiotolerance is insufficient.

Materials and Methods

COLLECTION AND REARING

Liriomyza sativa, L. trifolii, and *L. huidobrensis,* were collected from greenhouses in the Aegean Region in different seasons of the year and transferred to the Radiation Entomology Laboratory of Sarayköy Nuclear Research and Training Center in Ankara, Turkey. They were reared on common bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae), foliage in an insectary at 27 ± 1 °C, 70 ± 5% RH, and a photoperiod of 14:10 h L:D. A different species was studied in the insectary each year.

When the eggs hatched, the larvae tunneled within the leaf tissue leaving disfiguring mines. The pupae on the leaves and those that had dropped off were collected and held until the eyes of the pharate adults inside turned black, which indicated that they were within 48 h of adult emergence. They were then placed into polyethylene (PE) cylinder containers (H: 8 cm, Ø: 3 cm) for irradiation.

IRRADIATION TREATMENT AND DOSIMETRY

Irradiation treatments were conducted in a Co-60 gamma-cell irradiator (PX-γ-30 Issledovatelj model, Tenex mark, class 1, Russia). The activity was 1.12 kCi on 1 Dec 2011 in the Sarayköy Nuclear Research and Training Center of the Turkish Atomic Energy Agency, which supplied ionizing radiation at a dose rate of 0.64 kGy/h at the sample site. The source consisted of 24 encapsulated cobalt-60 source pencils each with a diameter of 11 mm and length of 81 mm. The gamma irradiator exposure chamber was a cylinder with a 24-cm height and a 15-cm radius, and the product to be irradiated was lowered into the exposure area. Fricke and alanine dosimetry systems, respectively, were used for calibration and measuring the dose of the gamma-cell. The dosimeters have been certified each year by the National Physical Laboratory, England, United Kingdom.

The objective of the experiment was to find the minimum dose applied to pharate adults within the puparium that prevents the formation of leaf mines by F_1 generation larvae. Because radiotolerance in insects is directly related to degree of development, the most tolerant stage for phytosanitary purposes would be the most developed that could occur on the shipped commodity. The measure of efficacy for insects that may occur in the pupal stages is the prevention of reproduction, because the high doses that cause acute mortality of late pupal stages are not tolerated by fresh commodities (Hallman et al. 2010). In the case of agromyzids that stage would be pharate adults 1-2 d before adult emergence, and these were used in all experiments. When the irradiation doses used resulted in surviving F_1 leafminers, the dose was raised in increments until no mines were produced. Pharate adults were treated with Co-60 gamma radiation at 0, 80, 100, 120, 150, and 180 Gy.

Harwell Gammachrome YR Perspex dosimeters (range: 0.1–3 kGy) and alanine dosimeters in pellet form were used as routine dosimeters on the samples for doses >100 Gy. The container with pharate adults was placed in the center of the cell platform of the gamma source. The routine Perspex dosimeters were placed at the bottom, left, and right of the dispenser of the pupal containers, and the alanine tablets were included within the samples of the pupa. Three to 4 h after irradiation, the Perspex dosimeters were routinely read in the spectrophotometer at 530 nm.

Each alanine dosimeter was measured 3 times, and the mean was used for reporting the dosimetry results. The overall uncertainty of the dose measurements was calculated to be ~3% assuming a coverage factor of 2, which corresponds to a 95% confidence limit. Free radical Electron Paramagnetic Resonance (EPR) measurements were carried out using a Bruker e-scan X-band dosimeter reader spectrometer. ISO/ASTM E1607 (ASTM 1999) was the measurement standard used. The least squares method was used to fit 3rd order polynomials for dose measurements. Dose measurements were performed and calculated automatically by using a Bruker e-scan X-band EPR spectrometer and transferred to an Excel spreadsheet.

After irradiation, the emerged adults were collected and transferred in well isolated, separate mating cages (35 × 27 × 27 cm) with abundantly leafed bean plants in pots. The mating cages were covered with chiffon netting. Diluted honey with water on a cotton pad was used as a food source. The cages were placed in the laboratory at 27 ± 1 °C, 70 ± 5% RH and a natural photoperiod light-darkness regime. The plants had not been previously exposed to oviposition by errant flies. The bean plants had been grown in a climatic test chamber (Binder) without any insect contact. To demonstrate reproduction or failure thereof after irradiation the adults that had emerged from the irradiated late pupa were kept alive under favorable conditions for reproduction until they eventually died.

QUALITY OF IRRADIATED SHELLED PEAS

Shelled peas were obtained from local markets in Ankara and packaged in polyethylene bags. The bags were divided into 4 groups, one

of which was chosen as a control (non-irradiated), the other 3 groups were irradiated at 0.25, 0.50 and 1.0 kGy at ambient temperature using the ^{60}Co gamma irradiator at a radiation rate of 0.702 kGy/h.

Measurement of Total Carotenoids

The total carotenoids content was measured according to Alasalvar et al. (2005). Irradiated and non-irradiated pea samples were stored at 4 ± 1 °C and analyzed soon after irradiation. All the analyses were done in triplicate. Pea seed samples (0.5 g) were homogenized in 25 mL of acetone containing dimethyl sulfoxide (10%) in an ice bath for total carotene content. All manipulations were carried out under a yellow fluorescent lighting (Thorn Lighting Ltd., United Kingdom) because carotenoids are highly sensitive to light, heat and air. The homogenate was filtered (Whatman No. 4, GE Healthcare) and washed until the residue was colorless. Finally, the filtrate was brought to 100 mL with the extraction solvent, and the absorbance was measured at 471 and 477 nm against an acetone blank using a spectrophotometer UV/VIS 6505 (Jenway, United Kingdom). The total carotenoids were calculated according to the following equation.

$$\textit{Total caroteniods}\ (\%) = \frac{Abs_{max.}}{250} \times \frac{25\ mL\ acetone \times dilution}{sample\ weight} \times 100$$

Color Measurements

The color of the samples was measured using a spectrophotometer (Minolta, CM 3600 d) based on 3 color coordinates: lightness (L*), redness (a*, red ± green), and yellowness (b*, yellow ± blue). In addition, Chroma (C*), is measure of chromaticity, which denotes the purity or saturation of color (Voss 1992), hue angle (h*) expresses the color nuance, and total color difference (TCD) was calculated using the following equations, where L_0, a_0, and b_0 are the control samples (non-irradiated) for pea seeds. TCD indicates the magnitude of color difference between irradiated and control samples. Differences in perceivable color can be analytically classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3) and small difference (TCD < 1.5) (Patras et al. 2011). It should be noted that combinations of color parameters may be more effective to evaluate the overall color changes of irradiated pea seeds than individual *L**, *a**, and *b** parameters. The instrument was calibrated using the black and white tiles provided. Three replicate measurements were performed and results were averaged.

$$\textit{Chroma} = \sqrt{a^2 + b^2}$$

$$\textit{Hue angel} = tan^{-1}\left|\frac{b}{a}\right|$$

In accordance with the methodology of the International Commission on Illumination (CIE) these parameters can be used to define the difference (delta E) in the color of the peas from a color standard by the following equation:

$$\Delta E = \left[(L* - L_0)^2 + (a* - a_o)^2 + (b* - b_0)^2\right]^{1/2}$$

ATR-FTIR Analysis of Protein Structure

The variation of the protein structure was determined by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) (Muyonga et al. 2004). The pea seed samples were dried at 50 °C overnight. These samples were placed on top of the ATR crystal consisting of zinc selenide covered with diamond. The spectra were collected in the 4500–650 cm^{-1} range at a resolution of 4 cm^{-1} with 64 scans.

Analysis of Vitamin C

All chemicals were HPLC grade, and they were supplied by Merck Chemicals Ltd. (Dorset, England, United Kingdom). Standard chemicals for Vitamin C (L-ascorbic acid) were purchased from Sigma-Aldrich (Taufkirchen, Germany). The method described by Abushita et al. (1997) was used for the extractions of vitamin C. Some modifications were made for ascorbic acid extraction that used accelerated solvent extraction (ASE) (Hossain et al. 2011). Samples were stored at −18 °C before extraction. All extractions were made just before the injection. Extracts were kept cold and protected against daylight during the sample preparation steps and analysis. Sample preparation was done from each sample in duplicate and each extract was injected in triplicate.

In the extraction of vitamin C with accelerated solvent extraction (Dionex ASE 350 model), 3 g of frozen sample was weighed into stainless steel 5 mL ASE cell, cellulose filters (Dionex, 27 mm diam) were put on upper and base parts of the extraction vessel to filter any impurities coming from the solvent. After putting the sample into the vessel, ~ 3 g of silica wool was placed into vessel for filling in order to completely fill the vessel. Finally the extraction vessel was placed into the instrument carousel. Analytical grade, freshly prepared, cold stored (4 °C) metaphosphoric acid solution (3% w/v) was used as an extraction solvent. Extraction was carried out at ambient temperature under a pressure of 9.652.650–10.342.125 Pa and 2 cycles out of 5 min static time were achieved. Purge time with inert N_2 gas was 90 sec. After collecting a known volume of extract, it was filtered to remove the suspended fruit debris through 0.45 µm pore sized hydrophilic membrane filtered into an amber-colored vial for HPLC analysis.

HPLC Analysis

An HPLC system (Waters 2695 Separations Module and Waters 2996 Photodiode Array Detector Waters Corp., Milford, Massachusetts, USA) was used for the analysis. The detector recorded the signals between 190 and 400 nm. Vitamin C peak was extracted and quantified at 243.8 nm. Injections (10 mL) were made by auto sampler onto analytical column (EC 150/4.6 Nucleosil, 100-3 C18 Nautilus Macherey-Nagel GmbH&Co. KG, Düren, Germany). Gradient elution with a flow rate of 0.7 mL/min was applied and 1% (w/v) KH_2PO_4 solution and acetonitrile was used as mobile phase. Eluting conditions expressed as proportion of 1% KH_2PO_4: 0.01-3 min: 99%,10 min: 70%, 15 min: 60%, 18-20 min: 99%. Standard L-ascorbic acid (vitamin C) solutions were prepared in 3% MPA and injected onto HPLC column under the same chromatographic conditions as samples. Quantifications of the analysis were achieved by means of a computing integrator that operated in conjunction with the external standard. Therefore, each standard of analyses was injected onto HPLC system with the same chromatographic conditions as samples and obtained values were used for plotting the standard calibration curves needed for analysis.

SENSORY EVALUATION

Sensory evaluation of control and the irradiated samples were carried out immediately after irradiation (Anonymous 1994). Control and irradiated samples were boiled for 20 min and then served on white, paperboard plates. Each sample plate was identified by a 3 digit random number. Eight trained judges (4 women and 4 men) were selected from Sarayköy Nuclear Research and Training Center. The judges were asked to evaluate the samples for color (1 = very light; 9 = quite dark), brightness (1 = very dull; 9 = very bright), browning (1 = no browning; 9 = too much), hardness (1 = very soft; 9 = very hard), aroma (1 = no aroma ;9 = to much), stale taste (1 = no stale taste; 9 = too much), sweetness (1 = no sweet; 9 = so sweet), and bitterness (1 =not bitter;

9 = extremely bitter) using the 9-point hedonic scale. The judges were also asked to rank the samples using overall acceptability (1 = dislike extremely; 9 = like extremely) as a parameter.

STATISTICAL ANALYSIS

Statistical analysis were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using IBM SPPS software version 21.00. The effect of a given dose was considered significantly different when $P \leq 0.05$.

Results

EFFICACY AGAINST LEAFMINERS

Liriomyza sativa, L. trifolii, and *L. huidobrensis* were similarly affected by the various doses. Doses ≥ 150 Gy prevented the formation of leaf mines by the F_1 offspring of all 3 species (Table 1). The controls laid normal numbers of eggs and produced offspring that developed normally. Abundant oviposition and feeding punctures were observed on the leaf surfaces in all of the treatments.

Dosimetry and parameters of the confirmatory tests that demonstrated that irradiation of pharate adults of *Liriomyza sativa, L. trifolii,* and *L. huidobrensis* with 150 Gy prevented the formation of leaf mines by their F_1 offspring are shown in Table 2. The dose uniformity ratio was very close to 1. The experiments were carried out using large samples of all 3 species for a total of > 30,000 insects. The numbers of emerged adults from irradiated pharate adults and the reproduction of these adults were observed in repeated tests with cumulative totals of 10,583 for *L. sativa,* 10,280 for *L. trifolii,* and 10,419 for *L. huidobrensis*. The dose of 150 Gy was identified as appropriate for phytosanitary treatment of leafminers, because this dose prevented the F_1 progeny of these 3 species from creating any leaf mines.

QUALITY OF IRRADIATED PEAS

The doses measured in the irradiated peas were 0.27 ± 0.01, 0.56 ± 0.01 and 1.01 ± 0.03 kGy, while the target doses were 0.25, 0.55 and 1.0 kGy, respectively.

Table 3 presents the total vitamin C and the total carotenoid contents, and the sensory evaluations of the pea samples. Vitamin C in the control sample was 12.34 mg/100 g. As can be seen from Table 3, the vitamin C contents in 0.25, 0.50, and 1.0 kGy irradiated samples were 12.17, 11.05, and 10.94 mg/100g, respectively. Differences between control and irradiated samples were significant in terms of vitamin C content. At the maximum radiation dose of 1.0 kGy allowed by the US Food and Drug Administration for insect disinfestation, an 11.34% reduction in the concentration of vitamin C was observed. Mean vitamin C content in pea seed is between 10–50 mg/100 g of edible portion (Cemeroglu et al. 2001). In comparison with the control samples, the vitamin C contents decreased with increasing irradiation doses.

Table 3 also presents the total carotenoid content of the control and irradiated pea samples. The total carotenoid content in the control samples was 2.6%. In case of the irradiated pea samples, the total carotenoid contents were 2.3, 2.3, and 2.2% in the 0.25, 0.50, and 1.0 kGy samples, respectively. Thus, the profile of the total carotenoids in pea samples was very stable to irradiation and showed no significant change after irradiation when analyzed by one-way ANOVA. Hajare et al. (2006, 2007) also observed similar results for profile of carotenoids in carrot, cucumber, green gram, and garden pea sprouts.

The results for sensory evaluations of pea seed samples are shown in Table 3. There were no significant differences in browning, hardness, aroma, stale taste, sweetness, and bitterness of the irradiated samples and the control. Irradiation dose level significantly affected color, brightness, and the overall acceptability of the pea seeds. However, radiation doses of 0.50 and 1.0 kGy did not negatively affect the overall acceptability of irradiated pea seeds. Pea seeds irradiated at 0.50 and 1.0 kGy were more acceptable than those in the 0.25 kGy treatment.

Table 4 summarizes the color values for the control and irradiated peas. The L* values indicate that there was no significant effect on the lightness of irradiated samples due to γ-irradiation. Negative a* values indicate the greenness of the samples. Irradiation dose significantly affected a* values. Radiation treatment did not have significant effects on yellowness (b* value) and chroma values. Total color difference (TCD) values were slightly (< 1.5) but significantly elevated after irradiation compared with control samples.

Table 1. Effects of doses *γ-irradiation* on adult emergence and on the formation of leaf mines in peas by F_1 offspring of *Liriomyza sativa, L. trifolii* and *L. huidobrensis.*

	Doses (Gy)	No. of irradiated pharate adults	Adult emergence (%)	F_1 offspring & their leaf mines
	Control	4,000	91.4	many
L. sativa	80	500	88	many
	100	500	84.4	many
	120	1,000	83.7	moderate
	150	1,000	76.5	none
	180	200	87.5	none
	Control	4,000	92.6	many
L. trifolii	80	500	87.6	many
	100	1,000	75.5	many
	120	1,000	74.8	moderate
	150	1,000	73.2	none
	180	400	79	none
	Control	4,000	96.35	many
L. huidobrensis	80	500	88.4	many
	100	500	85	many
	120	1,000	84.8	moderate
	150	1,000	83.5	none
	180	500	79.6	none

Table 2. Dosimetry and parameters of the confirmatory tests that demonstrated that irradiation of pharate adults of *Liriomyza sativa, L. trifolii,* and *L. huidobrensis* with 150 Gy prevented the formation of leaf mines by their F_1 offspring.

Test replicates	Number of irradiated pharate adults	Measured dose (Gy)	Confidence level (95%)	Dose uniformity ratio (Dmax/Dmin)
1	1,000	153.3 ± 5.7	6.5	1.06
2	1,500	154.6 ± 4.04	4.57	1.04
3	1,600	164.0 ± 10.3	11.7	1.11
4	1,600	153.4 ± 4.0	4.52	1.05
5	1,600	154.0 ± 3.46	3.91	1.03
6	1,600	164.3 ± 7.5	8.49	1
7	1,683	165.3 ± 0.57	0.65	1
Total number of irradiated *L. sativa* for confirmatory test = 10,583				
1	1,000	153.3 ± 5.7	6.5	1.06
2	1,500	160.0 ± 0	—	1
3	1,500	165.3 ± 0.57	0.65	1
4	1,500	150.0 ± 2.64	2.99	1.03
5	1,600	156.0 ± 1.73	1.95	1.01
6	1,600	153.6 ± 2.88	3.28	1.03
7	1,580	158.6 ± 1.15	1.30	1.01
Total number of irradiated *L. trifolii* for confirmatory test = 10,280				
1	1,300	145.6 ± 2.08	2.35	1.02
2	1,500	165.0 **± 2**.58	2.53	1.03
3	1,500	174.6 ± 0.57	0.65	1.05
4	1,500	143.6 ± 1.15	1.30	1.01
5	1,500	157.3 ± 3.05	3.45	1.03
6	1,500	162.0 ± 4.0	4.52	1.05
7	1,619	154.6 ± 4.04	4.57	1.04
Total number of irradiated *L. huidobrensis* for confirmatory test = 10,419				

Analysis of the amide I region revealed that the non-irradiated control contained 30.5 % α-helices and 46.6 % β-sheets, whereas samples irradiated with 0.25, 0.50 and 1.0 kGy, respectively, contained 26.2 % α-helices, 47.5 % β-sheets; 26.9 % α-helices, 46.8 % β-sheets; and 27.6 % α-helices, 47.2 % β-sheets (Table 5). Radiation doses of 0.25, 0.50 and 1.0 kGy reduced the ratio of α- helices to β-sheets by 16.6 %, 12.1 % and 10.6 %, respectively, in pea proteins. Thus the secondary structure of proteins in the pea samples showed significant dose-dependent changes after irradiation when analyzed by one-way ANOVA, yet these changes were not very great.

Discussion

Doses to prevent adult emergence from pharate adults in puparia are generally too high to be used as a phytosanitary treatment. Approximately a 450 Gy dose was necessary to prevent adult emergence from pharate adults of *L. sativae* and *L. trifolii* (Table 6). It is generally not possible to prevent adult emergence from late pupae (or puparia in the case of cyclorrhaphus Diptera including agromyzids) of any insect at a dose tolerated by fresh commodities, so the most appropriate measure of efficacy for these stages is prevention of reproduction

Table 3. Vitamin C and total carotenoid contents and sensory attributes of shelled pea samples analyzed immediately after γ-irradiation with various doses ranging up to 1 kGy.

	Dose (kGy)[a]			
	0	0.25	0.50	1.0
Vitamin C (mg/100 g)	12.3 ± 0.0c	12.1 ± 1.0bc	11.0 ± 0.3ab	10.9 ± 0.4a
Total carotenoids (%)	2.5 ± 0.3a	2.3 ± 0.5a	2.2 ± 0.2a	2.2 ± 0.2a
Sensory attributes[b]				
Color	6.5 ± 0.6b	3.7 ± 0.9a	8.3 ± 0.3b	7.1 ± 0.6b
Brightness	6.1 ± 0.1ab	4.7 ± 1.0a	8.3 ± 0.2c	7.2 ± 0.4bc
Browning	3.6 ± 1.0a	5.0 ± 1.2a	2.1 ± 0.7a	4.0 ± 1.1a
Hardness	4.3 ± 0.8a	3.7 ± 0.9a	4.5 ± 0.8a	4.2 ± 0.7a
Aroma	6.6 ± 0.4a	6.0 ± 0.6a	7.5 ± 0.4a	7.3 ± 0.3a
Stale taste	2.1 ± 0.6a	1.6 ± 1.4a	1.7 ± 0.7a	1.8 ± 0.7a
Sweetness	5.3 ± 0.7a	6.3 ± 0.5a	6.2 ± 0.8a	6.8 ± 0.5a
Bitterness	1.8 ± 0.5a	1.7 ± 0.5a	1.5 ± 0.5a	1.5 ± 0.5a
Overall acceptability	6.2 ± 0.5ab	4.8 ± 0.7a	7.7 ± 0.4b	7.7 ± 0.3b

[a]Means in each row not followed by the same letter are significantly different ($P \leq 0.05$).
[b]Attributes were measured on a 9-point hedonic scale, where 1 = no effect and 9 = very great effect.

Table 4. Changes in color parameters in samples of fresh shelled peas γ-irradiated with various doses ranging up to 1 kGy.

	Dose (kGy)[a]			
	0.0	0.25	0.50	1.0
L	55.4 ± 0.4a	55.5 ± 0.0a	55.9 ± 0.5a	56.0 ± 0.1a
a	-17.7 ± 0.1c	-17.2 ± 0.1a	-17.6 ± 0.0bc	-17.4 ± 0.0b
b	31.9 ± 6.1a	24.9 ± 5.6a	28.5 ± 0.4a	28.7 ± 0.1a
C	33.6 ± 0.3a	33.1 ± 0.1a	33.5 ± 0.4a	33.6 ± 0.1a
h	121.8 ± 0.1b	121.3 ± 0.0a	121.7 ± 0.2b	121.3 ± 0.1a
TCD	0.0 ± 0.0a	0.7 ± 0.3b	0.4 ± 0.1b	0.7 ± 0.2b

[a]Means in each row not followed by the same letter are significantly different ($P \leq 0.05$)

(Hallman et al. 2010). In this research the measurement of efficacy was the prevention of mine formation in leaves by F_1 progeny. Hallman et al. (2013) emphasized that the measurement of efficacy of phytosanitary treatments must be specifically defined; it is insufficient to use general terms such as "mortality" and "sterility" without defining precisely at what threshold efficacy is no longer achieved. They explained the measure of efficacy for lepidopteran eggs and larvae could be any of a progression of thresholds ranging from highest to lowest dose, i.e., acute mortality, prevention of development to the next metamorphic stage, complete prevention of adult emergence from pupa, prevention of emergence of normal-looking adults from pupa, prevention of oviposition by surviving females, prevention F_1 egg hatch. Moreover, they indicated that ideally measures of efficacy for PI should prevent significant F_1 development to provide a margin of security because there is no independent verification of efficacy for PI such as exists for all other commercial phytosanitary treatments, i.e., dead insects soon after treatment.

Determination of vitamin C content requires that vitamin C must be extracted and purified before introduction to HPLC. After 2 cycles of extraction with accelerated solvent extraction and purification with $NaSO_4$, the extract was immediately injected into HPLC to prevent the decomposition of L-ascorbic acid to dehydroascorbic acid. To differentiate and quantify the L-ascorbic acid and dehydroascorbic acid, a standard solution of both acids were injected separately. But, each peak was observed at the same retention time in approximately 2.5 min and they overlapped. In the calculation of total vitamin C content, L-ascorbic acid and dehydroascorbic acid were evaluated together (Llyod et al. 1987). Therefore, the peak at 2.5 min was assumed to represent the total vitamin C content containing both acid moeities.

ATR-FTIR was used to study the structure of pea seed protein. The main protein secondary structures are constructed either of α-helices or β-sheets. The ratio of α- helix to β-sheet provides basic information about the quality of a protein. The percentage of these 2 structures in a protein's secondary structures influences its nutritive value, quality and digestive behavior. A high percentage of the β-sheet structure may in part allow only little access to gastrointestinal digestive enzymes, which results in a low protein value (Yu 2007). Also, in the amide I region, a single major peak at 1,636.36 cm^{-1}, which corresponds to β-sheet structure (Withana-Gamage et al. 2010; Amonsou et al. 2012), was identified in the pea seed proteins. In a study that examined the effect of heat, roasting reduced the percentage of α- helices from 47.1% to 36.1%, increased the percentage of β-sheets from 37.2 % to 49.8 % and reduced the α- helix to β-sheet ratio (from 0.7 to 0.3) in flaxseed, which indicated a negative effect of the roasting on protein values, utilization and bioavailability (Yu 2007).

In conclusion, irradiation with doses of up to 1.0 kGy applied to shelled peas had only very limited effects on the content of vitamin C, total carotenoids, protein secondary structures, and sensory properties of shelled pea.

Table 5. Effects of γ-irradiation on secondary structure of proteins in shelled peas.

	Dose (kGy)[a]			
	0	0.25	0.50	1.0
α- helix (%)	30.5 ± 4.1b	26.2 ± 2.7a	26.9 ± 2.1ab	27.6 ± 3.4ab
β-sheet (%)	46.6 ± 0.5a	47.5 ± 0.5b	46.8 ± 0.4a	47.2 ± 0.6ab
α- helix/β-sheet	0.6 ± 0.0b	0.5 ± 0.0a	0.5 ± 0.0ab	0.5 ± 0.0ab

[a]Means not followed by the same letter are significantly different ($P \leq 0.05$)

Table 6. Percentage of adult emergence from late pupae of *Liriomyza sativa* and *L. trifolii* that were irradiated with various doses of gamma radiation.

Species	Doses (Gy)	No. of irradiated late pupae	Adult emergence (%)
L. sativa	Control	100	100
	180	100	100
	260	100	30
	350	100	14
	360	100	8
	368	100	7
	410	100	8
	460	100	1 (defect)
L. trifolii	Control	100	96
	250	100	38
	330	100	15
	350	100	10
	360	100	18
	400	100	7
	450	100	0
	500	100	0

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