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Irradiation of 'Valencia' Citrus Fruit as a Postharvest Quarantine Treatment for Mediterranean Fruit Flies (Diptera: Tephritidae)

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Abstract: **The objective of this research was to evaluate the effect of gamma irradiation on fruit fly (*Ceratitiscapitata*) eggs and larvae (1st, 2nd and 3rd instars) in 'Valencia' oranges, and evaluate the effect of the irradiation on the chemical composition of the fruits. The fruits were artificially infested with the immature stages of the fruit fly and treated with 0, 10, 20, 30, 40, 50, 100, 150 and 200 Gy Cobalt-60 doses. The treatment with gamma irradiation can be recommended for quarantine treatment of all immature stages of *C. capitata* in 'Valencia' oranges if applied at the doses of 72.88 Gy. Larvae of 3rd instar are more radio resistent when compared to eggs and larval of 1st and 2nd instar. The doses of gamma radiation used do not affect the chemical proprieties of 'Valencia' orange fruits.**

Keywords: **Ionizing Radiation, Quarantine Treatment, Disinfestation, *Ceratitis Capitata*, *Citrus Sinensis*.**

1. Introduction

Brazil is the largest producer fruits in the world, the citrus industry is today the most competitive sectors and higher growth potential of agrobusiness. Brazil holds about 40% of the world production of oranges and 60% of the production of orange juice, brazilian orange juice exports totaled 1.47 billion dollars, which represents 80% share of the global market [24]. Even these small fruit exports could be stopped due to the presence of the Mediterranean fruit fly and other fruit flies, as well as the orange fruit borer, a serious lepidopterous pest of citrus.

Fruit flies of the family Tephritidae have been considered the most important group of quarantined insects for their ability to render unmarketable significant portions of fruit harvests and for the extra burden placed on fruit production by detection, monitoring, and control programs which must be in place often despite the pests not actually being present to any significant degree [15]. Another problem related to the presence of these agricultural pests arises from commercial and phytosanitary restrictions to export fresh fruits to countries in which these insects do not occur [6]. In order to maintain or even increase exporting orange, the model of agricultural production will be completely refurbished so as to allow both the exporters and importers are aware of phytosanitary problems of determined region, beyond the control tactics employed as a way to track the production in order to ensure the sanity, quality and food security to human health [31] [10].

The principal control tactics grounded in Integrated Pest Management (IPM) and preventing the entrance and dissemination of an undesirable insect in certain country is the quarantine treatment, responsible for ensuring international transactions of fresh fruits [15] [10] [32]. Traditional treatments, which most commonly involved chemical fumigants, such as ethylene dibromide and methyl bromide, and both hot (43–48°C) and cold (0–3°C) temperatures, work by killing essentially 100% of all stages of quarantined pests which might be present in the commodity [15].

In this system, among the methodologies used for disinfestations of post-harvest fruits, the physical methods when compared to chemicals have the advantage of eliminating insects and pathogens without leaving residues. These include the controlled atmosphere, heat treatment (cold, steam and hot air) and ionizing radiation [29] [11] [22] [18] [30] [32] [12].

The use of ionizing radiation, important because it does not affect the quality of most commodities, addition to being efficient, fast, safe, and low cost [23] [9] [10], constitutes like a tool oriented for the post-harvest treatment of oranges infested with fruit flies larvae, principally to prevent the complete development of the insect and interfere negatively in the reproduction of adults, which prevents the spread of this pest to others regions [8] [20] [32]. Ionizing radiation at an absorbed minimum dose of 250 Gy is being used as a quarantine treatment for eight fruit hosts of four species of Tephritidae that are shipped from Hawaii to the mainland United States. A dose of 150 Gy is used to disinfest guavas, *Psidiumguaja* L., of Caribbean fruit fly, *Anastrephasuspensa* (Loew), before shipment from Florida to California or Texas. Irradiation will probably be expanded for use against a variety of tephritid species world- wide [15]. The US Animal and Plant Health Inspection Service has recently proposed quarantine treatment doses against 11 species of tephritids, including 150 Gy against Mexican fruit fly (APHIS, 2000).

The advantages of radiation over other treatments is the can be applied in a matter of minutes; other treatments require hours to days, it can be applied to commodities even after they are packed; only cold treatment can be applied to packed commodities, unlike fumigation, there is no residue, a wide variety of fruits tolerate doses required for quarantine security, probably more than any other treatment, unlike heat treatments, efficacy is not affected by fruit size, irradiation quarantine treatment facilities can be used for a variety of other purposes [16].

Consequently, development of postharvest treatments that are effective against a wide range of fruits species without causing unacceptable damage to fruit quality are needed. The use of ionizing radiation as a quarantine post-harvest treatment for fresh fruit commodities was authorized in 1984 by the Food and Drug Administration of the United States (FDA 1984). Dosages to control a number of fruit ßy species have been determined, and the effects on the quality of many different types of fruit have been assessed [13] [14].

However, the Brazilian researches are very scarce and principally oriented to hydrothermal treatment [2] [17]. Thus, the objective of this research was to evaluate the effect of gamma irradiation on fruit fly (*Ceratitiscapitata*) eggs and larvae (1st and 2nd and 3rd instars) in 'Valencia' oranges, and evaluate the effect of the irradiation on the chemical composition of the fruits

2. Material and Methods

The research was conducted at the Laboratory of Biology and Rearing of Insects (LBRI), Department of Plant Protection, Faculty of Agrarian and Veterinarian Sciences (FCAV/UNESP), Jaboticabal, São Paulo, Brazil, and at the Center of Nuclear Energy in Agriculture (CNEA-USP), Piracicaba, São Paulo, Brazil. The bioassays were conducted in a climate-controlled room maintained at 25 ± 1 °C and 70 ± 5% RH, with a 14: 10 photoperiod.

Fruit Flies:

Pupae to start creating *C. capitata*, were obtained from the Laboratory of CENA Radio entomologia and conducted the Laboratory of Insect Biology and Creation, which were separated from the vermiculite, counted and transferred to Petri dishes lined with filter paper, and placed in breeding cages for adult emergence. Upon emerging, the adults were fed with distilled water and a solution of water and 20% honey, they were offered in isolation cotton balls. Every day was the substitution of the solution of honey and water.

Two days after emergence, the cages were transferred and maintained on plastic trays containing 1.5L of distilled water, with the front facing a lamp, which served as an attraction to females. On this side of the cage, they made the females ovi position on organzafabric, the eggs fell into the water in the tray. The water containing the eggs was collected for Becker, which was expected about on eminute for the eggs decantassem, excess water was removed with the aid of a hypodermicsyringe. The collected eggs were transferred to Petri dishes with artificial diet for larval development.

Three days after transfer to diet eggs, larvae occurred at emergence that remained in these plates for about 12 days. After this period, the plates were transferred to uncapped and rectangular plastic pots, taking its cover a coated organzafabric opening. The interior of this pots containing a2 cmlayer of fine, sieved and sterilized vermiculite, simulating the ground, where the larvae were bound to empupar. After one week, the vermiculite was sieved to collect the pupae, which were transferred to Petri dishes with filter paper and placed inside the cages.

Obtaining fruit and implementation of treatments:

To perform the experiment we used oranges from 'Valência' variety, provided by Fundecitrus (Fund for Citrus Plant Protection) and the company 'Fiorin Fruits', collected in the region of Araraquara, SP, and Vista Alegre do Alto, SP, respectively. The fruits were carried at the laboratory, selected and standardized as to size (≅ 7 cm), shape and color.

We use eggs and larvae of 1st, 2nd and 3rd instars, obtained from rearing stock. The artificial infestation with eggs was performed with a leaker by making an orifice of 0.5 cm depth in the fruit and then 100 eggs were transferred into the fruit with a hypodermic syringe. The larvae were separated in two groups, one with larvae of 1st and 2nd instars, considered 'new larvae', and another with larvae of 3rd instar, considered 'old larvae'. For larvae infestation we use the same methodology proposed for eggs infestation, in which the orifice made in the fruit was approximately 2 cm depth, transferring into the fruit 25 larvae, performed with a stylet.

To close the orifices we made caps with a top number leaker than that used to do the orifices, placed in the fruits and sealed with adhesive tapes overlapping in a cross shape. The fruits utilized in bioassays were left to rest for 4 h, and then followed to the treatments.

Irradiation of infested fruit:

The infested oranges were conducted at the CENA, where they were performed for irradiation with doses of Cobalt-60: 0, 10, 20, 30, 40, 50, 100, 150 and 200 Gy. For this, we used a source of Cobalt-60 type Gammabeam-650, from the company 'Atomic Energy of Canada Ltda.', with dose rate of 482 Gy/h and diameter of 18 cm. After the irradiation process, the oranges were carried at the LBRI and maintained in climate-controlled room (25 ± 1 °C and 70 ± 5% RH, with a 14: 10 photoperiod), placed in plastic pots with approximately 2 cm depth of vermiculite.

The efficiency of the treatments was evaluated through the eggs inviability and larvae mortality of 1st, 2nd and 3rd instars, performed according to the number of pupae obtained, final inviability and mortality (calculated as function of the total of viability of eggs and mortality of the larvae instars), and the sterility effects in adults from the treatments, through the percentage of viable eggs. The research was conducted in a completely randomized design with nine treatments and ten replicates, one orange per replicate. The means were compared by Tukey test at 5% probability. We also estimated the lethal doses for the different stages of the insect [16].

Quality of irradiated citrus:

The effect of gamma irradiation on chemical properties of the fruit was analyzed through the evaluations of the variations: ascorbic acid, pH, soluble solids, titratable acidity, and ratio (relation between soluble solids / total acidity). For this, three fruits were subjected to each dose of ionizing radiation, with subsequent extraction and chemical analysis of the juice. The juice of each fruit was one replicate.

3. Results and Discussion

Gamma radiation produces several effects on immature insects. Lower doses generally allow the insect to complete adulthood but causes sterility and deformation of wings. With the increase of dose, the process becomes more deleterious; eggs and larvae develop normally, pupae form, but adults emerge. Increasing even if the dose is lethal process, or the insect skilled in their immature stadium. Such doses vary according to the species and also to the stage of the insect in which radiation is applied.

The effect of gamma radiation on eggs of *C. capitata* caused hight inviability, with values above 90%. The lowest dose responsible for causing 100% of eggs inavailability was 100 Gy (Table 1). Egg eclosion was reduced gradually as the irradiation dose increased. Bustos et al. [4], observed the same result for *A. ludens*, *A. obliqua* and *C capitata* in mangoes. These results are similar to those reported by Mahmoud and Barta [19], in which gamma radiation doses above 90 Gy were able to suppress completely the viability *Bactrocerazonata* Sauders (Diptera: Tephritidae) eggs. The eggs inviability of *C. capitata* presents in guava fruits (*Psidiumguajava* L.) was greater with increasing dose of ionizing radiation used, with suppression above 85% at a dose of 100 Gy [5].

The larvae treatment of 1st and 2nd instars with gamma radiation caused mortality below 50%, ranging between 9.6 to 45.20%. Dória et al. [5] also reported low mortality of 1st and 2nd instars of *C. capitata* when exposed to gamma radiation up to 250 Gy. These results show that the general dose of 150 Gy proposed for control all species of fruit flies [14] should not be generalized, because different factors are responsible by the mortality variation in relation to gamma radiation doses.

Larvae of 3rd instar exposed to gamma radiation doses showed mortality ranging from 16.4 to 40% (Table 1). Similar results were observed by Fésus et al. [8], in which the lowest dose of gamma radiation responsible to control larvae of 3rd instar of *C. capitata* in orange was above 200 Gy. However, Adamo et al. [1] reported that the lowest dose of 200 Gy was able to prevent the adult emergence of *C. capitata*. In other fruit species, the quarantine treatment of third instar larvae of *C. capitata* with ionizing radiation showed variation from 40 to 225 Gy, responsible to prevent the adult emergence [28] [20] [26] [4] [9] [32].

The deleterious effect on larvae inside the fruits was not directly proportional to increasing dose. This result may have relation with the capacity of larvae locomotion, which become deeper in fruit and increase the volume of water to be overtaken by ionizing radiation, which has extremely difficult to overtaken the water, and therefore the largest fruit mass to be traversed until target (immature stages) correspond to less directly efficiency of ionizing radiation [3].

The final inviability of *C. capitata* eggs showed no statistically significant results, in which only the gamma radiation of 10 Gy did not provide 100% of viable eggs. Therefore, the necessary dose to preclude the adult emergence was 20 Gy (Table 2). These results agree with those found by Faria [7], citing dose of 20 Gy as necessary to prevent the adult emergence of *C. capitata* in papayas infested in lab. Rigney and Wills [27] also estimated doses similar to those observed in this experiment, which used ionizing radiation at a dose of 25 Gy in oranges infested with *Dacustryoni* Frog gatt (Diptera: Tephritidae) eggs, causing 100% of inviability.

In relation to the treatment with gamma radiation on larvae of 1st, 2nd and 3rdinstars of *C. capitata*, we found that the final mortality did not present significant results in doses of 30, 40, 50, 100, 150 and 200 Gy, with 100% of larvae mortality for doses above 50 Gy (Table 2).

To confirm the efficiency of this method, we counted the normal adult emergence as well as deformed morphologically, in which the normal adults were transferred, in couples, to small rearing cages, with the purpose to obtain eggs to subsequently perform the viability test. In relation of 1st and 2nd larvae, although the doses of 30 and 40 Gy permit the adult emergence, the eggs obtained from females originated of the treatments were not viable, whereas for 3rd instar larvae required 50 Gy to impossibility the adult emergence (Table 3). A direct relationship between the irradiation dose and the percentage of malformed pupa and the prevalence of adult emergence was observed by Bustos et al. [4].

The lethal doses of gamma radiation estimated to cause 99% of mortality for eggs, larvae of 1st and 2ndinstars and larvae of 3rd instar were 11.58; 34.19 and 72.88 Gy, respectively (Table 4). These results are similar to those of Balock et al. [3], who treat the eggs of *C. capitata* with ionizing radiation and estimated the LD_{95} at 14.5 Gy to prevent adult emergence. Faria [7] and Raga et al. [26] estimated the dose for Probit 9 at 30.14 Gy for papayas and 24.67 Gy for mangoes to impossibility the adult emergence of *C. capitata*. Larvae of 3rd instar were more radio resistent, a fact confirmed by [20], in infested oranges with larvae of 3rd instar, estimating the dose for Probit 9 at 67.3 Gy to prevent the adult emergence of *C. capitata*.

Hallman and Worley [15] estimated the dose for Probit 9 at 80.1 Gy to impossibility the adult emergence of *C.*

capitata in 'grapefruit', similar results when compared to this research, principally because this fruit is larger than oranges and require higher doses. Bustos et al. [4] observed that *C. capitata* was more tolerant than the others species of fruit flies tested, when exposed to ionizing radiation, estimating the dose for Probit 9 at 112.7 Gy to prevent the adult emergence of *C. capitata*.

The quality of chemical properties of the fruits exposed to gamma radiation was similar to those untreated, in which the doses did not cause any chemical change. These results are similar to those reported by Patil et al. [25], who found no significant differences in any chemical properties of 'grapefruit' when exposed to ionizing radiation up to 700 Gy. Moy [21] also verified that the chemical quality of oranges was not affected when exposed to radiation up of 300 Gy.

4. Conclusion

The treatment with gamma radiation can be recommended to quarantine purposes of all immature stages of *Ceratitiscapitata* in oranges of 'Valência' variety, since treated with the lowest dose of 72.88 Gy.

Larvae of 3rd instar are more radio resistent when compared to eggs and larvae of 1st and 2ndinstars of *C. capitata*.

The doses of gamma radiation used do not affect the chemical properties of oranges of 'Valência' variety.

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Table 1.Effect of Gamma Radiation on Different Stages of *Ceratitis Capitata*in Orange Fruits of 'Valência' Variety.

Doses (Gy)	Eggs inviability	Larvae mortality (%)	
		1st and 2nd	3rd instar
0	67.60 c[1]	13.60 b	5.20 b
10	93.60 b	13.60 b	28.80 a
20	97.20 ab	9.60 b	16.40 ab
30	99.60 ab	12.40 b	31.20 a
40	98.00 ab	10.00 b	29.60 a
50	99.60 ab	18.40 b	40.00 a
100	100.00 a	24.00 ab	32.00 a
150	100.00 a	12.80 b	30.80 a
200	100.00 a	45.20 a	38.80 a
Test F	20.79**	53.09**	38.78**
CV	8.86	5.13	4.97
dms	12.73	22.31	24.77

[1]Means followed by the same letter do not differ at 5% of probability by Tukey test.

Table 2.Final Inviability and Mortality*in Different Stages of *Ceratitis Capitata* Irradiated in Oranges of 'Valência' Variety.

Doses (Gy)	Inviability	Mortality (%)	
	E.T.	L.T.1-2	L.T. 3
0	88.40 b[1]	21.60 c	12.80 d
10	97.60 a	20.40 c	59.20 c
20	100.00 a	67.60 b	74.00 b
30	100.00 a	98.80 a	92.00 a
40	100.00 a	99.20 a	92.80 a
50	100.00 a	100.00 a	100.00
100	100.00 a	100.00 a	100.00
150	100.00 a	100.00 a	100.00
200	100.00 a	100.00 a	100.00
Test F	21.63**	356.42**	205.60**
CV (%)	2.66	7.31	7.97
dms	3.73	8.19	9.22

[1]Means followed by the same letter do not differ at 5% of probability by Tukey test.
E.T – Eggs treatment; L.T.1-2 – Larvae treatment of 1st and 2nd instars; L.T.3 – Larvae treatment of 3rd instar.
* measured by the number of adults emerged.

Table 3.Adult Emergence (%) ("Normal", "Deformed") From Different Stages of *Ceratitis Capitata* Irradiated in Orange Fruits of 'Valência' Variety and Reproductive Viability of This Specie.

Doses (Gy)	Adults (%)								
	E.T.			L.T.1-2			L.T. 3		
	N	D	E.V.	N	D	E.V.	N	D	E.V.
0	35.8 a[1]	0.0	92.0 a	88.4 a	2.3	92.0 a	90.7 a	1.3	87.0 a
10	25.0 b	12.5	60.0 b	83.8 a	8.3	75.0 b	53.9 b	3.4	70.0 b
20	0.0 c	0.0	0.0 c	29.2 b	6.6	56.0 c	28.7 c	2.4	51.0 c
30	0.0 c	0.0	0.0 c	1.4 c	0.0	0.0 d	8.7 d	2.9	10.0 d
40	0.0 c	0.0	0.0 c	0.9 c	0.0	0.0 d	5.7 d	4.5	1.2 e
50	0.0 c	0.0	0.0 c	0.0 c	0.0	0.0 d	0.0 e	0.0	0.0 f
100	0.0 c	0.0	0.0 c	0.0 c	0.0	0.0 d	0.0 e	0.0	0.0 f
150	0.0 c	0.0	0.0 c	0.0 c	0.0	0.0 d	0.0 e	0.0	0.0 f
200	0.0 c	0.0	0.0 c	0.0 c	0.0	0.0 d	0.0 e	0.0	0.0 f
Test F	35.72		46.87	64.11		34.40	55.43		23.76
CV	28.98		33.45	36.74		29.21	41.34		24.87
dms	7.34		4.49	6.81		5.74	2.67		5.78

[1]Means followed by the same letter do not differ at 5% of probability by Tukey test.
E.T – Eggs treatment; L.T.1-2 – Larvae treatment of 1st and 2nd instars; L.T.3 – Larvae treatment of 3rd instar.
N – Normal; D - Deformed; E.V. – Eggs viability.

Table 4.Lethal Dose of Gamma Radiation Estimated for the Different Stages of *Ceratitis Capitata* in Orange Fruits of 'Valência' Variety.

Lethal Dose (Gy)	Stages		
	Egg	Larvae of 1st and 2nd instars	Larvae of 3rd instar
LD_{50}	9.23	18.69	10.59
LD_{90}	10.46	26.07	30.65
LD_{95}	10.84	28.65	41.43
LD_{99}	11.58	34.19	72.88
x^2	0.00^{ns}	10.77*	21.38**

x^2 (ns) the data adjust perfectly to this analysis.
x^2 (*) e (**) the data adjust moderately to this analysis.