

# Postharvest phytosanitary irradiation disinfestation of *Planococcus citri* and *P. ficus* (Hemiptera: Pseudococcidae)

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## Abstract

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is widespread and occurs on all continents. It can infest a range of ornamental plant and fruit species and is directly and indirectly damaging to foliage and fruits. An insectary was established in Citrusdal, South Africa to rear the citrus mealybug on butternut squash (*Cucurbita moschata* Duchesne; Cucurbitales: Cucurbitaceae). *Planococcus citri* was reared to the required stages, viz., eggs, 1st–3rd instars, as well as pre-ovipositing and ovipositing females on butternut squash fruits, and treated *in situ* with ionizing irradiation ranging from 50–400 Gy. Eggs treated with 100 Gy hatched, but all *P. citri* died as 1st instars. When 1st instars were treated with  $\geq 100$  Gy, all nymphs died as 1st or 2nd instars. Second and 3rd instars treated with 100 Gy developed into reproductive females that produced sterile eggs. At 150 Gy only a small number of the nymphs developed into mature females, but no eggs were produced. At 150 Gy pre-ovipositing females produced sterile eggs. When ovipositing females were irradiated with 150 Gy, all eggs produced by them were killed and additional egg production was prevented. Efficacy of the 150 Gy dose as a phytosanitary treatment was subsequently confirmed for ovipositing females by treating a total of 70,440 individuals with the result that none of their progeny developed past the 1st instar. The efficacy of 150 Gy as an effective phytosanitary treatment was also confirmed for the vine mealybug *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae) on 10,000 pre-ovipositing and ovipositing females, none of whose progeny developed past the 1st instar.

Key Words: ionizing radiation; citrus mealybug; vine mealybug

## Resumen

La cochinilla de los cítricos, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), está muy extendida y se presenta en todos los continentes. Esta plaga puede infestar una gama de especies de plantas ornamentales y de frutas y dañar directamente e indirectamente al follaje y frutos. Se estableció un insectario en Citrusdal, Sudáfrica para criar a la cochinilla de los cítricos sobre calabaza (*Cucurbita moschata* Duchesne; Cucurbitales: Cucurbitaceae). Se crió los estadios requeridos de *Planococcus citri*, incluyendo huevos, ninfas del 1-3 estadio (instar), así como hembras antes y durante la oviposición sobre frutos de ayote (butternut), y tratadas *in situ* con radiación ionizante de 50 hasta 400 Gy. Los huevos tratados con una dosis de 100 Gy esclosionaron, pero todos las ninfas de *P. citri* murieron en el primer estadio. Cuando se trataron con un mínimo de 100 Gy, todas las ninfas del primer estadio murieron como instares del primero o segundo estadio. Ninfas de segundo y tercer estadio tratadas con 100 Gy se desarrollaron en hembras reproductivas que produjeron huevos estériles. A 150 Gy solamente se produjo un pequeño número de las ninfas que desarrollaron en las hembras maduras, pero no produjeron huevos. A 150 Gy, las hembras en pre-oviposición produjeron huevos estériles. Todos los huevos producidos por las hembras durante la oviposición fueron matados con 150 Gy y se evitó la producción de huevos adicionales. La eficacia de la dosis de 150 Gy como un tratamiento fitosanitario se confirmó posteriormente para la hembras en oviposición por tratar un total de 70,440 individuos con el resultado de que ninguno de su progenie se desarrollara más allá del primer estadio. La eficacia de 150 Gy como un tratamiento fitosanitario eficaz también fue confirmada a la cochinilla harinosa de la vid, *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae), en 10,000 hembras en pre-oviposición y oviposición, en el que ninguno de sus descendientes desarrollaron más allá del primer estadio.

Palabras Clave: radiación ionizante; cochinilla de los cítricos; cochinilla de la vid

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The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is widely distributed (Wakgari & Giliomee 2003). It is a general pest of ornamental plants and can be of economic concern in deciduous fruit species, avocado, date palm, and many other host plant species. It is also one of at least 7 pseudococcid species occurring in southern African citrus orchards (Wakgari & Giliomee 2003). The damage caused by *P. citri* is diverse and includes the disruption of biological control by encouraging ants in trees through the secretion of honeydew, foliar damage by malformation of leaves and inhibition of

photosynthesis due to sooty mold growth, abscission of fruitlets and malformation of fruit especially around the calyx area. The accumulation of citrus mealybugs in the blossom ends of navel oranges is equally detrimental as thereby the incidence of *Alternaria* decay is thereby promoted (Hattingh et al. 1995). *Planococcus citri* is not necessarily a cryptic pest in the field as it can occur in large, easily noticeable populations. However, it tends to congregate, multiply and best survive in secluded spots on the host plant. If that occurs, for example, under the calyx of citrus fruits or in bunches of grape berries, the citrus mealybug

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can be problematic in exportable produce, resulting in rejections from export (Hattingh et al. 1998), or at best, delays to have the insects identified to avoid confusion with mealybug species that may be of phytosanitary concern, such as the oleander mealybug *Paracoccus burnerae* (Brain) (Hemiptera: Pseudococcidae) (Wakgari & Giliomee 2003).

The study reported here was conducted to determine the lowest effective dose of ionizing radiation that would prevent immature and adult stages of *P. citri* from producing viable  $F_1$  generation progeny. For comparison, a second species, the vine mealybug, *P. ficus* Signoret (Hemiptera: Pseudococcidae), was added later to the study.

## Materials and Methods

### REARING

A consignment of *P. citri* was obtained from Vital Bugs, Letsitele, South Africa. A rearing unit was established in the laboratories of Citrus Research International in Citrusdal, South Africa. The insect identification was verified by a morphological study and confirmed with DNA sequencing by Pieterse (Plant Protection Research Institute, Agricultural Research Council, National Department of Agriculture, Stellenbosch, South Africa). The mealybugs were reared on butternut squash (*Cucurbita moschata* Duchesne; Cucurbitales: Cucurbitaceae) of similar size and shape. Rearing and experimentation were conducted at 26 °C.

Five to 6 mealybug infested butternut squash fruits were placed on their sides into a stainless steel wire frame tray, 410 mm × 290 mm × 80 mm, to keep them from rolling around. The tray with butternut fruits was then placed into a plastic tray, 530 mm × 430 mm × 85 mm. The inside periphery of the tray was lined with a sticky barrier to confine crawlers. This set-up was replicated as many times as necessary to assemble the required number of insects. The parental generation of *P. citri* was reared, treated and evaluated on the same butternut fruits to reduce the chances of injury. The population was well synchronized, e.g., more than 90% of females commenced oviposition within 24 h of each other. Fresh butternut fruits were used when necessary to study migration and development of the  $F_1$  generation. The mealybug culture was maintained by placing fresh butternut fruits on top of infested stock when the eggs eclosed. The 1st instars migrated to the fresh butternut fruits. The newly-infested butternut fruits were removed 24 h later and incubated. When required for treatment, butternut fruits were similarly infested and incubated until the mealybugs had developed to the required developmental stage.

### DOSIMETRY

#### Dose Rate Study

When the research commenced a dose rate study was conducted to ensure that absorbed irradiation doses corresponded with target doses. A cobalt-60 point source, panoramic irradiator at Xsit (Pty) Ltd in Citrusdal, Western Cape, South Africa, was used. Individual butternut fruits were placed upright on PVC rings (60 mm × 20 mm × 4 mm) on up to 8 secondary turntables (0.3 m in diam) that were located on a primary turntable, 1.37 m in diam. The turntables rotated at 15 and 2 rpm, respectively.

The mealybugs were exposed on the surface of the butternut fruits and were considered to be low density. A close-fitting PVC irradiation chamber (200 mm × 120 mm × 4 mm) that provided sufficient dose build-up to ensure charged particle equilibrium was consequently placed over each butternut fruit during treatment.

The initial output factor for dose absorbed from the irradiator was conducted using Fricke dosimeters, applying the standard G-val-

ue for cobalt-60 of  $\text{Fe}^{3+} = 15.5/100$  eV. Optical density readings were performed using a Hewlett Packard diode array spectrophotometer. For this instrument a molar extinction coefficient of 2 081 L/Mole/cm at 304 nm was determined using spectroscopic grade  $\text{Fe}^{3+}$  solutions. This instrument specific constant relates optical density readings in a quartz glass flow cell with an optical path of 10 mm to  $[\text{Fe}^{3+}]$  concentrations, and is used to calculate dose absorbed values in Gy (J/kg). Different Fricke solutions read within 1% when irradiated in a cobalt-60 reference field. The dosimeter response was checked in a cobalt-60 reference radiation field of 300 mm × 300 mm with 2 mL Fricke solutions placed in a Perspex container producing 6 mm build-up and 50 mm backscatter. The output factor for the reference field was determined using a Farmer tissue equivalent ionization chamber calibrated in a standard field of the South African National Metrology Laboratory.

Mapping was conducted at the top, middle and bottom of the butternut fruits and the dose uniformity ratio from top to bottom was 1.094. No reference position was used; the dosimetry was conducted for various positions in the irradiation set-up and the mean was calculated.

Repeated readings taken on the same day at each position showed differences to be less than 2%. The radiation set-up was calibrated using a range of doses that included the radiation levels to be used. Uncertainties in the dose rate from repeated dose readings were less than 4%. The 95% confidence interval of a reading at any position was 4%.

Due to the dose uniformity ratio of 1.094 the actual doses applied in the various experiments have to be regarded as means, varying 4.7% above and below those specified. Dose rates were updated before each irradiation treatment using the decay half-life of cobalt-60 at 63.26 months. All treatments for calibration and experiments were applied at 15 °C.

#### Dosimetry Comparison Scheme

An independent analysis of treatment dose accuracy for the butternut fruit set-up was conducted during the course of the study (Parker, Insect Pest Control Laboratory, IAEA Seibersdorf, personal communication). Two sets of 3 Gafchromic dosimeters were read following exposure to a target dose of 200 Gy. The mean was calculated for each exposure and the confidence intervals determined. A deviation of 0.4% and 3.2% with a 95% confidence interval of 4.3%, respectively, was measured between the absorbed dose and the target dose for the 2 dosimeter sets.

Exposure times for doses administered during each mealybug experiment were calculated from the dose rate determined in the dose rate study and corrected for the decay of cobalt-60. As the irradiation set-up remained identical throughout this work no dosimeters were employed on each irradiation day. The close agreement between the respective doses measured in the dose rate study and the dosimetry comparison scheme confirmed the accuracy of doses applied in the study.

#### RADIOTOLERANCE OF *PLANOCOCCUS CITRI*

The study was conducted to establish the most radiotolerant stage of mealybug. The lowest dose capable of preventing this particular stage from producing  $F_1$  generation 1st instars or alternatively, causing total mortality of this instar would be validated in a conclusive large scale assessment to establish a treatment suitable for phytosanitary compliance. Fundamentally, it was accepted that any individual treatment less than 100% effective for the above prerequisites would be rejected. Relative differences between treatment doses assisted in the

preliminary selection of treatments required for successive studies on more developed stages.

Butternut fruits infested with a particular developmental stage were selected for each experiment from the rearing unit for uniformity of infestation and were allocated at random to the different treatments.

### Eggs

Ten adjoining sectors, each 45 mm × 40 mm, were marked on each of 5 non-infested butternut fruits per treatment. The sector margins were treated with Tangle-Trap<sup>®</sup> adhesive (Contech Enterprises, Victoria, British Columbia, Canada) to prevent insects from migrating between sectors. An ovisac containing a mean of 350 eggs was placed in the center of each sector. The butternut fruits with eggs were treated with 50 and 100 Gy, respectively, and incubated at 26 °C for 6 wk. The sectors on each butternut were then scored for the F<sub>1</sub> generation life stages that had developed from the eggs (parental generation) in each treatment with the aid of a stereomicroscope.

### First and 2nd to 3rd Instars

The numbers of 1st and 2nd to 3rd instars infesting each butternut were too large to reasonably count. Therefore, the mean number of adult F<sub>1</sub> mealybugs eventually produced per untreated control butternut was regarded as representative of the mean numbers of insects per butternut fruit in all treatments of the particular experiment.

### Pre-Ovipositing Females

Females were regarded as pre-ovipositing when inspections at 12 h intervals revealed the first secretion of wax filaments indicating the impending production of ovisacs. These females commenced oviposition within 24–48 h. Two similar experiments were conducted using 5 infested butternut fruits per treatment. All adult females in the untreated controls were counted and the mean total of insects per butternut fruit was considered representative of the populations in the treatments. Doses of 50–400 Gy in 50 Gy increments were applied per treatment and then the butternut fruits were incubated.

In the first experiment the treated females' reproductive capability was assessed 33 d post-treatment by examining the total surface area of each treated butternut fruit with a stereomicroscope for individuals of the F<sub>1</sub> generation. In the second experiment ovisacs of the treated females were examined 35 and 45 d post-treatment and, respectively, 5 and 10 females with ovisacs were selected at random per butternut fruit on each date. Each ovisac, containing 100–300 eggs was teased apart with a dissection needle and examined for 15–20 seconds for live nymphs. An ovisac with one or more live crawlers was considered to be indicative of a too low treatment dose.

### Ovipositing Females

The most developed stage of insects is often the least sensitive to radiation (Hallman et al. 2010; Hofmeyr et al. 2016). Based on the presence of ovisacs with and without eggs, only a few minutes' difference could practically separate pre-ovipositing from ovipositing females. Nonetheless, ovipositing females were assumed to be the most-developed stage and thus potentially the least sensitive to radiation. A small confirmatory experiment was conducted to study the tolerance of ovipositing females to 150 Gy. Two infested butternut fruits were used in each of an untreated control and a single 150 Gy treatment dose. On the treatment date all females with ovisacs containing eggs

were counted per butternut. The butternut fruits were then treated, incubated and examined for live progeny 21 d later.

### CONFIRMATORY TESTING OF 150 GY AS A PHYTOSANITARY TREATMENT AGAINST *P. CITRI*

The study was conducted at probit-8.7 level, i.e., no survivors from 30,000 treated insects. Similar primary and secondary experiments were conducted consecutively, differing only in the number of infested butternut fruits used per treatment. The population of adult females was well synchronized and consisted mainly of ovipositing females. The remainder were pre-ovipositing females within 24–48 h of oviposition. A single treatment dose of 150 Gy was used.

### Untreated Controls

In the primary experiment 5 randomly allocated butternut fruits were used in each of 2 batches. In the secondary experiment 2 batches of 4 butternut fruits each were used. All mealybugs were counted destructively in the first batch. The second batch was used to verify the infestation capability of the F<sub>1</sub> generation. This was accomplished by placing 8 fresh, non-infested butternut fruits on the infested butternut fruits for 24 h for infestation by 1st instars. The newly infested butternut fruits were incubated for ~30 d to allow development to F<sub>1</sub> generation females.

### Ionizing Radiation Treatments

In the primary experiment 20 butternut fruits were treated with 150 Gy and then randomly divided into 2 batches of 10 butternut fruits each. In the secondary experiment 2 batches of 5 similarly treated butternut fruits were used. The first batch was incubated and examined for nymph development at weekly intervals for a period of 45 d. The second batch was used to determine the infestation potential of the F<sub>1</sub> generation progeny. This was accomplished by placing 8 fresh, non-infested butternut fruits on the treated butternut fruits for infestation by F<sub>1</sub> 1st instars. The newly infested butternut fruits were removed 7 d later and incubated for another 38 d to monitor nymph development. Under untreated conditions this was time enough for the development of 1.5 generations.

### CONFIRMATORY TESTING OF 150 GY AS A PHYTOSANITARY TREATMENT AGAINST *P. FICUS*

*Planococcus ficus* was included as an additional species subsequent to the study on *P. citri* and a single 150 Gy dose was assessed. Infested butternut fruits were obtained from Williams (Entomology Department, ARC-Infruitec Institute for Deciduous Fruit, Vines and Wine, Agricultural Research Council, Stellenbosch). Sixteen butternut fruits infested with pre-ovipositing and ovipositing females were used in each of 2 similar consecutive experiments. In both experiments 8 butternut fruits were randomly allocated to each of an untreated control and a 150 Gy treatment. A single destructive assessment was conducted 30 d post-treatment in the experiments to count the number of treated females. The assessments enabled close examination of the detached females and the butternut fruits for the presence of a possible progeny. The numbers of females removed were considered to be representative of the population numbers on the untreated butternut fruits. The progeny on the control butternut fruits consisted of F<sub>1</sub> 3rd instars and young, pre-ovipositing females and was counted at the same time to establish the reproductive potential of the insects.

## Results

### RADIOTOLERANCE OF *P. CITRI* EGGS

In the untreated control up to 80 ovipositing females developed per sector from the eggs; in 92% of the sectors the females produced nymphs in large numbers (Table 1). In the 50 Gy treatment not more than 20 females developed per sector, and most were sterile producing either no ovisacs or ovisacs without eggs. In this treatment, fertile females producing nymphs developed in only 22% of the sectors. In the 100 Gy treatment, a small number of eggs hatched but all 1st instars died.

### RADIOTOLERANCE OF *P. CITRI* 1ST INSTARS

Due to overpopulation on the butternut fruits many thousands of 1st instars dropped off to die in the tray bottoms. A mean of 825 adult females developed per butternut fruit in the untreated control, eventually yielding viable  $F_1$  generation 2nd and 3rd instars before the treatment was terminated. First instars treated with 50 Gy developed into a mean of 293 fertile females per butternut fruit yielding 2nd and 3rd instars before treatment termination. In the 100 Gy treatment a few treated 1st instars molted but died as 2nd instar. At 150–400 Gy all treated 1st instars died without developing.

### RADIOTOLERANCE OF *P. CITRI* 2ND AND 3RD INSTARS

Each butternut was infested with thousands of nymphs consisting predominantly of 2nd instars, as well as a small number of 3rd instars. Again, most nymphs dropped off the overpopulated butternut fruits to die. In the untreated control a mean of 351 nymphs per butternut fruit developed into fertile females. Approximately the same number of females developed from nymphs treated with 50 Gy. Their reproductive capability was not totally suppressed and small numbers of  $F_1$  generation nymphs were produced. Development of the 2nd and 3rd instars was severely affected by the 100 Gy treatment. Only a few ovipositing females developed and produced sterile eggs from which no nymphs hatched. At 150 Gy a small number of females developed but no eggs were produced, and at 200–400 Gy no females developed from the treated nymphs.

### RADIOTOLERANCE OF *P. CITRI* PRE-OVIPOSITING FEMALES

In the first experiment a mean of 3,721 pre-ovipositing females were counted per butternut fruit in the control treatment. A few females had commenced secretion of ovisacs, although oviposition had not yet commenced. Within 24–48 h they developed into ovipositing females producing ovisacs with eggs. Thousands of nymphs were produced that developed into  $F_1$  pre-ovipositing females 33 d later. Compared to the control many fewer ovipositing females developed in the 50 and 100 Gy treatments, but they were fertile, producing much re-

duced numbers of progeny. Ovipositing females also developed in the 150–400 Gy treatments, but no eggs eclosed.

In the second experiment a mean of 1,359 pre-ovipositing females were counted per butternut fruit in the control treatment. In 2 assessments 35 and 45 d post-treatment, the untreated females had produced fertile progeny consisting of reproductive  $F_1$  females and large numbers of  $F_2$  2nd and 3rd instars, respectively. The treated females produced fertile eggs at 50 and 100 Gy, but they were sterile at 150–400 Gy.

### RADIOTOLERANCE OF *P. CITRI* OVIPOSITING FEMALES

A total of 869 untreated ovipositing females per butternut fruit produced 2,767  $F_1$  3rd instars and young females 21 d later. Just prior to application of the 150 Gy treatment to 2,718 ovipositing females, very small numbers of 1st instars were noticed. In the assessment 21 d post-treatment only the dried-out remains of these nymphs were observed. All eggs had started to shrivel indicating that they had been killed by the treatment. No freshly deposited eggs were noticed and no live nymphs were present in the ovisacs or on the butternut fruits.

The experiment was not designed to explore definitive differences in radiotolerance between pre-ovipositing and ovipositing females. Rather, confirmation was needed, and obtained, that the 150 Gy dose, which effectively sterilized pre-ovipositing females, was equally detrimental to ovipositing females.

A dose of 100 Gy was sufficient to prevent the eggs and 1st to 3rd instars of *P. citri* from producing a viable  $F_1$  generation. An increase to 150 Gy was necessary to sterilize pre-ovipositing and ovipositing females and this dose was consequently selected for validation in larger scale experiments.

### CONFIRMATORY TESTING OF 150 GY AS A PHYTOSANITARY TREATMENT AGAINST *P. CITRI*

The butternut fruits in the first control batch were infested with more mealybugs than anticipated and a mean of 4,361 individuals were counted per butternut fruit (Table 2). Of these, 3,522 (80.8%) were confirmed to be ovipositing females, 621 (14.2%) pre-ovipositing females and 218 (5.0%) immatures. The number of progeny from parent females in the second control batch was not recorded. However, they were allowed to develop until  $F_2$  generation 1st instars were detected to indicate the reproductive potential of the insects used in the study.

The mean number of adult mealybugs recorded per butternut fruit in the untreated control (4,143) was used to determine the number treated, namely 82,860 pre-ovipositing and ovipositing females. Of these, 70,440 were calculated to be ovipositing (Table 2). Treatment was consequently postponed for 48 h to enable pre-ovipositing females to commence oviposition. The number of these females was not added to the total of treated ovipositing females. On the treatment date the delay had resulted in a small number of hatched eggs yielding 1st instars. In the first batch of butternut fruits all 1st instars died in situ without further development. In the second batch a small number of the nymphs migrated to the fresh butternut fruits where they died without further development. No additional  $F_1$  generation progeny was observed when the experiment was concluded 45 d post-treatment.

Initially we intended to divide the probit study into 3 consecutive experiments involving approximately 10,000 insects each. However, the number of mealybugs required in the study had already been exceeded by a factor of more than 2.7 in the primary experiment. These results were thus confirmed with a smaller secondary experiment only.

A mean of 354 mealybugs was counted on each butternut fruit. Of these, 309 (87.3%) were ovipositing females, 27 (7.6%) pre-ovipositing

**Table 1.** The development of *Planococcus citri* from eggs treated with 50 or 100 Gy of ionizing radiation 6 weeks earlier.

Dose (Gy)	Number of sectors ( $n = 50$ ) with the following life stages present:			
	No $F_1$ adult ♀♀	$F_1$ adult ♀♀ without eggs	$F_1$ adult ♀♀ with dead eggs	$F_1$ adult ♀♀ with live 1st instars
0	0	4	0	46
50	21	8	10	11
100	50	Treated eggs eclosed and all 1st instars died		



**Table 2.** Validation of 150 Gy ionizing radiation to totally suppress the reproduction of pre-ovipositing and ovipositing *Planococcus citri* and *Planococcus ficus* females.

<i>Planococcus citri</i>				
Assessment	Experiment 1		Experiment 2	
	0 Gy	150 Gy	0 Gy	150 Gy
No. of ovipositing females	> 70,440	> 70,440	3,090	3,090
Reproductive capability	F <sub>2</sub> progeny produced	No F <sub>1</sub> progeny	F <sub>2</sub> progeny produced	No F <sub>1</sub> progeny

<i>Planococcus ficus</i>				
Assessment	Experiment 1		Experiment 2	
	0 Gy	150 Gy	0 Gy	150 Gy
No. of mature females	3,275	3,275	7,018	7,018
No. of F <sub>1</sub> progeny	5,411	0	14,219	0

females and 18 (5.1%) immatures. As before, the reproductive potential of the non-irradiated females was indicated by large numbers of 1st instars that infested and completed their development to the F<sub>2</sub> generation on fresh butternut fruits (Table 2).

A calculated total of 3,090 reproductive females were treated. Similar to the primary experiment a small number of F<sub>1</sub> 1st instars were already present at the time of treatment. All died in both batches of butternut fruits without further development (Table 2). No 1st instars hatched subsequent to treatment.

CONFIRMATORY TESTING OF 150 GY AS A PHYTOSANITARY TREATMENT AGAINST *P. FICUS*

A combined total of 10,293 predominantly ovipositing, as well as pre-ovipositing, *P. ficus* females were treated with 150 Gy in the 2 experiments. Similar to *P. citri*, no live progeny that had survived treatment past the 1st instar of the F<sub>1</sub> generation was detected 30 d post-treatment (Table 2). During this post-treatment period the progeny on the untreated butternut fruits had developed into mixed numbers of 3rd instars and young females. The efficacy of 150 Gy to suppress development of ovipositing *P. ficus* females was thus validated.

Discussion

Doses ranging from 50–400 Gy were applied to eggs, nymphs and adults of *P. citri*. Doses capable of totally preventing either further development of the treated stage or production of a viable off-spring past the F<sub>1</sub> generation 1st instar, ranged from 100 Gy for eggs and 1st–3rd instars, to 150 Gy for pre-ovipositing and ovipositing females. Large-scale efficacy testing of 150 Gy on ovipositing females, the stage regarded to be the most tolerant to ionizing radiation, was conducted on 82,860 individuals. The results were confirmed in smaller experiments on *P. ficus* and validated the effectiveness of 150 Gy to prevent reproduction of these mealybug species past the F<sub>1</sub> 1st instar.

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