Development of phytosanitary irradiation against *Aceria litchii* (Trombidiformes: Eriophyidae) on lychee

Valter Arthur[1,2,*], *and André R. Machi*[1,2]

Abstract

The lychee erinose mite, *Aceria litchii* (Keifer) (Trombidiformes: Eriophyidae), is the most important pest of lychee (*Litchi chinensis* Sonn. (Sapindales: Sapindaceae) in parts of China, India, Southeast Asia, South Africa and Brazil. This study sought to develop the basis for phytosanitary irradiation of lychee to provide quarantine security against this pest. New methodology had to be devised for this purpose because the adult, the largest life stage—about 200 µ long—cannot be seen without magnification, and because this species does not survive more than a few d even on detached young lychee leaves, or under other artificial conditions. Initially we adapted a method devised by Azevedo et al. (2013) for keeping the adults alive long enough to evaluate the lethal effects of candidate acaricides for at 48 h post treatment. We collected infested leaves from a lychee orchard and irradiated then with doses increasing by increments of 200 Gy in the range 0–2,000 Gy. Each infested leaf had 30 to 40 adult mites. Each of 3 replicates involved ~816 adult mites and ~2,450 adult mites per treatment. Because of the presence of predators hidden within the erinea, we collected 30 adult mites per replicate immediately after irradiation, and placed them in a 14-cm-diam petri dish with a new young lychee leaf and moist cotton. We covered each petri dish with parafilm® to prevent escape of mites and loss humidity. At 24, 36, and 48 h post irradiation, we counted the numbers of live and dead mites. At 24 h post irradiation mortality occurred only in 1,800 and 2,000 Gy treatments, and it was only 1.7% in both treatments. At 36 h mortality had increased to 11.1 and 24.4% in the 1,600 and 2,000 Gy treatments, respectively. At 48 h statistically significant mortality occurred with all doses in the 200–2,000 Gy range; and it was 73.3% and 100.0% in the 1,800 and 2,000 Gy treatments, respectively. Since 2,000 Gy is unacceptable for phytosanitary irradiation, a dose had to be identified that prevents reproduction, i.e., a F_1 generation. To prolong the survival of irradiated mites for at least 13 d, which appears to be the generation time of *Aceria* spp., we cut infested fragments of leaf blades, examined the under a microscope to remove adults and immature forms of predators mites, placed them in petri dishes, irradiated them with doses increasing by increments of 100 Gy in the range 0–500 Gy, and glued each irradiated leaf fragment onto a newly flushed leaf of a potted lychee tree in a screened greenhouse. Each treatment had 4 replications, and each consisted of ~10 adult mites for a total of ~40 adult mites per treatment in the first trial and 18 adults per replicate for a total of ~72 mites in the second trial. By 72 h post irradiation the mortality rate was considerably greater with all doses in the range 200–500 Gy than at 48 h in the earlier experiment, and the percent mortality with 100 Gy was significantly greater than in the control (0 Gy). Therefore, 72 h post irradiation seems to be the minimum time required for the lethal symptoms of irradiation to develop within the dose-range that is relevant to phytosanitary irradiation of fresh plant materials. In both the first and the second trials, moderate symptoms of erinose developed during 18 d post irradiation in the treatments with 0–300 Gy, but no erinose symptoms developed in the 400 and 500 Gy treatments. All symptomatic leaves displayed the patchy growth of erinea, i.e., abnormal felt-like hairs on the abaxial leaf epidermis. In addition by d 18 some galls had been formed on the upper sides of some of the leaves with erinea. These data show that irradiation of *A. litchii* with ≥ 400 Gy prevented it from reproducing, which is a critically important criterion of phytosanitary irradiation. Additional experiments are needed in the 300–400 Gy range to find the minimum dose required for phytosanitary irradiation.

Key Words: lychee erinose mite; acute mortality; erinose symptoms; erinea; lethal symptom development; detached leaves; prolonged survival; prevention of reproduction

Resumen

El ácaro erinoso de lichi, *Acería litchii* (Keifer) (Trombidiformes: Eriophyidae), es la plaga más importante de litchi (*Litchi chinensis* Sonn.; Sapindales: Sapindaceae) en algunas partes de China, India, el sudeste de Asia, África del Sur y Brasil. Este estudio se trató de desarrollar una base para la irradiación fitosanitaria de lichi para garantizar la seguridad cuarentenaria contra esta plaga. Una nueva metodología tuvo que ser creada para este fin porque el adulto, el estadio de la vida de mayor tamaño — alrededor de 200 µ de largo — no puede ser visto sin aumento, y porque esta especie solo sobrevive unos pocos dias en las hojas jóvenes cortadas de lichi o bajo otras condiciones artificiales. Inicialmente hemos adaptado un método creado por Azevedo et al. (2013) para mantener a los adultos con vida por el tiempo suficiente para evaluar los efectos letales de acaricidas sobre los candidatos para 48 horas pos-tratamiento. Recolectamos hojas infestadas de un huerto lichi y que luego fueron irradiadas con dosis que aumentaron en incrementos de 200 Gy en un rango de 0-2.000 Gy. Cada hoja infestada tenía de 30 a 40 ácaros adultos. Cada uno de las 3 réplicas involucraron ~ 816 ácaros adultos y 2.450 ácaros adultos por tratamiento. Debido a la presencia de depredadores ocultos dentro de la erinea, se recolectaron 30 ácaros adultos por réplica inmediatamente después de la irradiación, y los colocó en un plato Petri de 14 cm de diámetro, con una nueva hoja jóven de lichi y algodón húmedo. Hemos cubierto cada plato Petri con parafilm para evitar el escape de los ácaros y la perdida de humedad. A los 24, 36 y 48 horas después de la irradiación, se contó el número de ácaros vivos y muertos. A las 24 horas, la mortalidad pos-irradiación se produjo sólamente en los tratamientos de 1.800 y 2.000 Gy y fue sólo del 1,7% en ambos tratamientos. A las 36 horas la mortalidad había aumentado hasta el 11,1 y el

[1]University of São Paulo, Center for Nuclear Energy in Agriculture (CENA), Department of Environmental and Radiobiology. Piracicaba -SP – Brazil
[2]University of São Paulo, Department of Environmental and Radiobiology, Institute of Nuclear Energy Research (IPEN). Lineu Prestes Avenue, 2242. University City – zip code: 05508-000 - São Paulo – SP, Brazil
*Corresponding author; E-mail: arthur@cena.usp.br

24,4% en los tratamientos de 1.600 y 2.000 Gy, respectivamente. A las 48 horas, una mortalidad estadísticamente significativa ocurrió con todas las dosis en el rango de 200-2.000 Gy; y fue del 73,3% y 100,0% en los tratamientos de 1.800 y 2.000 Gy, respectivamente. Como la dosis de 2000 Gy no es aceptable para la irradiación fitosanitaria, una dosis que pueda impidir la reproducción a la generación F_1 tuvo que ser identificada. Para prolongar la sobrevivencia de los ácaros irradiados por lo menos 13 días, lo que parece ser el tiempo de generación de *Acería* spp., cortamos fragmentos de hojas infestadas examinados bajo un microscopio para eliminar las formas inmaduras del ácaro y los depredadores, que fueron puestas en platos Petri e irradiados con dosis cada vez mayor en incrementos de 100 Gy en el rango de 0-500 Gy y luego pegaron con goma cada fragmento de la hoja irradiado sobre una hoja joven de un árbol de lichi en sembrado en una maceta en un invernadero con tela. Cada tratamiento tuvo 4 repeticiones, y cada uno consistía en ~ 10 ácaros adultos para un total de ~ 40 ácaros adultos por tratamiento en el primer ensayo y 18 adultos por réplica para un total de ~ 72 ácaros en el segundo ensayo. A las 72 horas pos-irradiación, la tasa de mortalidad fue considerablemente mayor en todas las dosis en el rango de 200 a 500 Gy que a las 48 horas en el experimento anterior, y el porcentaje de mortalidad con 100 Gy fue significativamente mayor que en el control (0 Gy). Por lo tanto, las 72 horas después de la irradiación parece ser el tiempo mínimo requerido para los síntomas letales de la irradiación para desarrollar dentro del rango de dosis que es relevante para la irradiación fitosanitaria de los materiales vegetales frescos. En los ensayos tanto de la primera y la segunda, síntomas moderados de erinium se desarrollaron durante 18 días pos- irradiación en los tratamientos que applicaron 0-300 Gy, pero no se desarrollaron síntomas de erinium en los tratamientos de 400 y 500 Gy. Todas las hojas sintomáticas muestran el crecimiento irregular de erinia, pelos anormales como fieltro en la epidermis abaxial de la hoja. Además a los 18 dias, algunas agallas se habían formadas en los lados superiores de algunas de las hojas con erinea. Estos datos muestran que la irradiación de *A. litchii* con ≥ 400 Gy prevenió la reprodución del ácaro, que es un criterio de importancia crítica de la irradiación fitosanitaria. Se necesitan experimentos adicionales en el rango de 300-400 Gy para encontrar la dosis mínima requerida para la irradiación fitosanitaria.

Palabras Clave: lichi erinose ácaros; mortalidad aguda; síntomas erinosas; erinea; desarrollo de los síntomas letales; hojas desprendidas; supervivencia prolongada; la prevención de la reproducción

Resumo

O ácaro da erinose da lichia, *Aceria litchii* (Keifer) (Trombidiformes: Eriophyidae), é a praga mais importante da lichia (Litchi chinensis Sonn (Sapindales: Sapindaceae) em partes da China, India, Sudeste Asiático, África do Sul e Brasil. Este trabalho teve como objetivo desenvolver uma metodologia para o tratamento fitossanitário da lichia visando garantir a segurança quarentenária contra esta praga. Essa metodologia foi realizada, porque os ácaros na fase adulta possuem o maior período de vida, eles medem aproximadamente 200 μ, e não podem ser observados em um microscópio comum ou seja sem ampliação, essa espécie só sobrevive alguns dias, mesmo em folhas de lichia jovens quando retirada das plantas, ou mesmo sob condições artificiais. A metodologia utilizada foi adaptada do trabalho de Azevedo et al. (2013) que conseguiram manter os ácaros adultos vivos por tempo suficiente para avaliar os efeitos letais dos potenciais de acaricidas nos ácaros em 48 horas após o tratamento. Para o desenvolvimento do experimento foram coletadas folhas de lichia infestadas com ácaros e posteriormente foram irradiadas com doses crescentes de 200 a 2.000 Gy. Cada folha coletada estava infestada com aproximadamente 30 a 40 ácaros adultos. Cada uma das 3 repetições nos tratamentos tinha aproximadamente 816 ácaros adultos e no total 2.450 por tratamento. Devido à presença de predadores escondidos dentro dos erineos das folhas, foram coletados 30 ácaros adultos por repetição após a irradiação e colocados em uma placa de petri de 14 cm de diâmetro com uma folha nova de lichia circundada com algodão umedecido, em seguida cada placa de Petri foi fechada com parafilm® para impedir a fuga dos ácaros e evitar a perda de umidade. Após 24, 36 e 48 horas da irradiação, foram feitas as contagens do número de ácaros vivos e mortos. Para 24 horas após a irradiação a mortalidade ocorreu apenas nos tratamentos de 1.800 e 2.000 Gy sendo de apenas 1,7% em ambos os tratamentos. Já para 36 horas, a mortalidade aumentou para 11,1 e 24,4% nos tratamentos de 1.600 e 2.000 Gy, respectivamente. Para 48 horas os resultados de mortalidade apresentou diferença estatística significativa para todas as doses radiação de 200 a 2.000 Gy; sendo de 73,3% e 100,0% nos tratamentos de 1.800 e 2.000 Gy, respectivamente. Como 2.000 Gy é uma dose de radiação inaceitável como tratamento fitossanitária, uma dose menor foi determinada para impedir a reprodução dos ácaros na geração F_1 e que também pudesse prolongar a sobrevivência dos ácaros irradiados por pelo menos 13 dias, por ser o tempo aproximado de cada geração do acaro. Posteriormente, as amostras foliares infestados com os ácaros foram cortados e examinados sob um microscópio para remover as possíveis formas imaturas e adultas de predadores, em seguida os as amostras foram colocados em placas de petri e irradiadas com doses crescentes de 100 a 500 Gy, em seguida cada amostra de folha irradiada foi colada em uma folha selecionada de uma planta de lichia cultivada em um vaso dentro de uma estufa telada. Cada tratamento constou de 4 repetições, e cada uma com aproximadamente 10 ácaros adultos num total de aproximadamente 40 ácaros adultos por tratamento no primeiro teste, já para o segundo teste constou com 18 ácaros adultos por repetição num total de aproximadamente 72 ácaros. Após 72 horas da irradiação, a taxa de mortalidade foi significativamente maior em todas as doses de 200 a 500 Gy, em relação aos resultados obtidos em 48 horas no experimento anterior. A porcentagem de mortalidade na dose de 100 Gy foi significativamente maior do que na dose controle (0 Gy). Portanto, 72 horas após a irradiação parece ser o tempo mínimo necessário para o desenvolvimento dos sintomas letais da irradiação dentro da faixa de dose aplicada que é relevante para a irradiação fitossanitária de frutas e vegetais frescos. No primeiro e segundo ensaio, os sintomas moderados de erinose foram observados durante 18 dias após a irradiação nos tratamentos de 0 a 300 Gy, já nos tratamentos com 400 e 500 Gy não apresentaram sintomas de desenvolvimento de erinose. Todas as folhas exibiram sintomas de crescimento irregular do erineo, isto é, pelos anormais na epiderme inferior das folhas. Em aproximadamente 18 dias, já havia a formação de galhas sobre os lados superiores de algumas das folhas com o erineo. Estes resultados mostram que a irradiação em *A. litchii* com doses ≥ 400 Gy impediu que os ácaros se reproduzissem, o que é um critério importante da irradiação como tratamento fitossanitário. Experimentos adicionais são necessários nos intervalos de 300-400 Gy para se determinar a dose mínima necessária para a utilização da irradiação como tratamento fitossanitário.

Palavras Chave: ácaro da erinose; mortalidade aguda; sintomas de erinose; desenvolvimento de sintoma letal; folhas destacadas; prolongação da sobrevivência; prevenção de reprodução.

Lychee (*Litchi chinensis* Sonn.; Sapindales: Sapindaceae) is native to southern China where it has been cultivated for about 1,000 yr. China and India are the leading producers of this fruit, and there is substantial production in various countries of Southeast Asia and in South Africa. Commercial litchi orchards have been initiated in Brazil since 1970 (Fornazier et al. 2014), but the planted area is still modest and largely restricted to the State of São Paulo (Martins et al. 2001). In the 2007/2008 season the state of São Paulohad 1,615 ha of lychee (São Paulo 2008). The profitability of lychee production is outstanding, and if lychee production could be increased Brazil, it would provide a substantial additional income to tropical fruit producers (Centrais de Abastecimento de Campinas–CEASA 2009).

In China, India, Thailand and Vietnam, the lychee erinose mite, *Aceria litchii* (Keifer) (Trombidiformes: Eriophyidae) is the major problem in lychee production (Siddiqui 2002). Also, severe damage to lychee occurs in Brazil (Azevedo et al. 2014). The adult female mite—about 200 µ long—is the largest life stage, and magnification of at least 20X is needed to observe them. Lychee trees with symptoms of this pest were first noticed in an orchard in Sao Paulo State in 2003 (Mineiro & Raga 2003) and the presence of the mite was confirmed in 2010 (Raga et al. 2010). In Dec 2012, this pest was discovered in Espírito Santo State (Fornazier et al. 2014). Lychee erinose mites cause hairy, blister-like galls on the upper side of the leaves, thickening, wrinkling and distorting them, and brown, felt-like wool on the underside (DAF-QLD-AU 2012). The mites feed on the 'felt' on the underside of the leaves. This "felt" consists of patches of erinea formed from trichomes that develop abnormally in response to the feeding of these mites. In addition to the development of erinea on the underside of the leaf, small blisters or galls develop on the upper side of the leaf, and the galls can cover the entire leaf and cause curling. These diseased leaves lack the ability to engage efficiently in photosynthesis, which debilitates the tree. In severe instances, flowers are destroyed and fruit-setting is largely prevented so that yields are very small (FAO 2002). Moreover, much of the fruit can be damaged cosmetically.

The life history of the lychee erinose mite was summarized by DAF-QLD-AU (2012). Adult mites migrate from older infested leaves to infest young leaves on which they lay eggs which hatch in 3–4 d. The life cycle is completed in 13 d under favorable conditions. The lifespan of the adult stage has not been reported, but the citrus bud mite, *Aceria sheldoni* Ewing, lives approximately 14 d (Sternlicht & Goldenberg 1971). Adults and immature forms are found within the erinea on the lower surface of the leaf. As trees produce new leaf and flower flushes, the mites migrate to these young organs where they establish new patches of erinea in which to feed, shelter and multiply. According to Manson & Oldfield (1996), generally in the Eriophyidae from the egg, a larva hatches and later molts into a nymph, which molts into an adult. Generally, eriophyid males are smaller than females, and although the 2 sexes are similar in appearance, the males lack a genital cover flap and their genitalia differ from those of females (Lindquist 1971). Manson & Oldfield (1996) noted that female eriophyids always appear to be more numerous than males.

Although the dispersal of *A. litchii* has not been reported in detail, probably it is similar to that of *Aceria guerreronis* Keifer, a coconut mite. Howard (2006) noted that *A. guerreronis* probably disperses from one palm to another on air currents or by phoresy (e.g., riding on insects or birds that visit palm flowers). Where coconut palms are dense, coconut mites can crawl from palm to palm.

Eriophyid mite species tend to have only 1 or a few species of host plants. *Aceria litchii* has been reported only from lychee. This extreme host specificity should provide an advantage in restricting the spread of this important pest and possibly eradicating it in areas of significant lychee production.

Because imported infested commodities are a major pathway whereby invasive pest species enter an area in which they were previously absent, such species are a legitimate trade barrier, which can be overcome by phytosanitary measures such as irradiation. Phytosanitary irradiation (PI) has become increasingly adopted since the International Plant Protection Convention (IPPC) issued "Guidelines for the Use of Irradiation as a Phytosanitary Measure" (ISPM No. 18; IPPC 2003). The effectiveness of irradiation against treated pest species is not determined on the basis of acute mortality, but on the complete arrest of insect development and prevention of reproduction at doses that do not significantly alter the quality of the fresh commodity. Examples include non-emergence of adults when larvae are irradiated and the inability of the irradiated pest to reproduce. For example, irradiated mated females may lay eggs that hatch, but the F_1 neonates die and not reach the 2nd instar. Hallman et al. (2010) recommended that PI doses applied against adults be based on measurements of efficacy that do not allow much development of the F_1 life stages, i.e., preferably not further than the first instar.

Implicit in the requirements of effective phytosanitary irradiation is the need to culture the target species, and to sustain the development of individuals used in experiments so that the normal lifespan and reproductive capacity of untreated control insects are not curtailed. For if the control insects do not perform well under experimental conditions, it is difficult to have confidence that a treatment deemed effective under experimental conditions will be similarly effective under operational conditions. This requirement is not easily met in laboratory experiments in the case of *A. litchii*, because it needs to be established on very young leaves that remain attached to the lychee tree. In developing the methodology to evaluate potential acaricides against *A. litchii*, Azevedo et al. (2013) stated that the first challenge in the research was to develop methodology to maintain *A. litchii* alive in the laboratory at least for a few d.

Research on a phytosanitary irradiation method requires the availability of large numbers of normally-responding organisms. However, currently A. *litchii* mites for each experiment must be obtained from infested lychee trees on which predators are found, and the trees may not produce new leaf flushes on a regular basis. Determining the effects of irradiation of adults on the production and development of F_1 generation eggs, larvae and nymphs is difficult as the various life stages are difficult to observe and count within the erinea. Observations and manipulations on these tiniest of arthropods cannot be carried out without the aid of at least 20X magnification.

Azevedo et al. (2013, 2014) developed several useful methods for determining the effects of acaricides on the mortality of *A. litchii*, and for determining the densities of infestations in the field; and their reports on these studies are of considerable value in the present research.

The methodology successfully developed by Azevedo et al. (2013) to evaluate the effects of acaricides against this pest in the laboratory involved bringing infested lychee leaves from the field, placing each leaf into an open petri dish for 24 h at 25 °C, 60–70% RH and 12:12 h L:D photoperiod. This procedure promoted the movement of mites to the surface of the erinea, as did the light used to illuminate the leaf from below the microscope stage. Adult mites were then transferred with a fine brush into 2.5 cm-diam plastic petri dishes at 50 mites per dish. The bottom of each petri dish had been covered with a layer of a solidified paste made of a mixture of gypsum and activated charcoal that was kept moist to prevent the mites from desiccating; this layer allowed most of the mites to survive for 72 h. The acaricide was applied by spraying the open petri dishes in a Potter tower, and the number killed was determined some h later.

The methodology devised by Azevedo et al. (2014) to determine densities of *A. litchii* in the field involved modifications of the method of Coolen & D'Herde (1972) that is sometimes used to estimate the densities in the field of root-infesting nematodes. The adapted method involved grinding an infested sample of lychee leaves in a blender with a quantity of distilled water and passing the shredded material through a set of sieves, which removed debris and retained smaller particles and the mites. This method was used to elucidate the population dynamics of *A. litchii* in lychee orchards (Azevedo et al. 2014).

Our methods were informed by the work of Azevedo et al. (2013, 2014). The over-arching goal of this work was to lay the foundation for the eventual development of a phytosanitary irradiation treatment against *A. litchii*. Objective 1 was to determine the irradiation dose–

acute mortality response relationship of *A. litchii* adults taken from a lychee orchard, irradiated, and maintained in the laboratory for a few d on detached lychee leaves in a moist environment. Objective 2 was to determine the irradiation dose–acute mortality response relationship of irradiated *A. litchii* that were transferred—immediately after the irradiation—in the erinea on leaf fragments onto newly flushed leaves of potted lychee trees in a greenhouse. The irradiated leaf fragments were glued onto the abaxial surfaces of the leaves of the potted tree. Objective 3 was to determine the relationship of the dose—used to irradiate the mites in the erinea on leaf fragments—to the development of erinose symptoms on young leaves of potted lychee trees in a greenhouse. These leaf fragments—immediately after irradiation—were glued onto the abaxial surfaces of the newly flushed leaves of the potted lychee tree.

Material and Methods

DOSE–ACUTE MORTALITY RESPONSE OF ADULTS ON DETACHED LYCHEE LEAVES

This experiment was conducted to accomplish objective 1, i.e., determine the irradiation dose–acute mortality response relationship of *A. litchii* adults taken from a lychee orchard, irradiated, and maintained in the laboratory for a few d in a moist environment.

Sample Preparation

Lychee branches with leaves infested with *A. litchi* mites were collected at Farm Haddad, Palmital City, São Paulo State, Brazil from an orchard where acaricides have never been sprayed. The infested foliage was placed into a refrigerated box (Easy Patch 5L, São Paulo-SP, Brazil) and transported to the Laboratory of Radiobiology and Environment, Center for Nuclear Energy in Agriculture (CENA / USP), Piracicaba, Sao Paulo, Brazil, where the experiment was performed. Infested lychee leaves were separated from the branches and placed in 40 × 27 × 11 cm plastic trays. The number of mites per leaf was determined by counting the number of adult mites in the erinea of 7 leaves of each 70-leaf sample, i.e., 10% of the leaves in each replicate.

Irradiation Process

Each treatment was replicated 3 times with 70 leaves per replicate. The mites were put in plastic bags (19 × 15 × 11 cm) to prevent loss of humidity and a total 210 leaves per treatment were irradiated—during the same d that they had been collected—with 0 (control), 200, 400, 600, 800, 1,200, 1,400, 1,600, 1,800 and 2,000 Gy at 0.289 kGy/h in a Cobalt-60 Gammacell-220 irradiator. Thus, 3 plastic bags—arranged in a stack for each treatment—were centralized inside the irradiator in order not to disrupt the uniformity of the radiation. Six dosimeters were positioned as follows: 1 on top of the stack, 1 at the bottom of the stack and 4 equally-spaced at lateral positions. Each treatment consisted of ~2,450 adult mites per treatment divided in 3 replicates each with ~816 adult mites per replicate.

For the dosimetric characterization of the amount of gamma radiation actually absorbed in all treatments, we used radiochromic film (Gammachrome with a dose range of 0.1–3 kGy). The readings were made with a spectrophotometer (Genesys 20). A certificate of dosimetry was issued by the Institute for Energy and Nuclear Research–IPEN. The traceability of measurement of doses was maintained by comparison with the international service assurance dose offered by the International Atomic Energy Agency in Vienna, Austria (Khoury et al. 2016).

After irradiation, the adult mites were sampled, which involved counting them in the erinea with the aid of a stereoscopic microscope. Thus it was possible to isolate samples of mites from any predators that in some instances were hidden in the erinea and in irregularities of the surfaces of the leaves. We collected 30 adult mites per each of 3 replicates (90 mites per treatment) and transferred each sample with a paint brush into a separate a 14 cm-diam-petri dish provisioned with a newly flushed lychee leaf and moist cotton. We covered each petri dish with parafilm® to prevent the escape of the mites, and the loss of humidity. At 24, 36, and 48 h after irradiation, we counted the number of live and dead mites with the aid of a manual counter. The above methods of handling the mites were based in part on Azevedo et al. (2013, 2014).

DOSE–ACUTE MORTALITY RESPONSE RELATIONSHIP OF ADULTS TRANSFERRED ON LEAF FRAGMENTS IMMEDIATELY AFTER IRRADIATION ONTO YOUNG LEAVES OF POTTED LYCHEE TREES

Petri dishes in which leaf fragments with erinea and adult mites were centralized in a stack inside of the above mentioned Cobalt-60 Gammacell-220 irradiator in order not to disrupt the uniformity of the radiation. Six dosimeters were positioned as follows: 1 on top of the stack, 1 at the bottom, and 4 equally-spaced at lateral positions.

Infested lychee leaves were examined under a microscope, and the light beneath the stage caused the mobile forms of the mite and the predators to move to the upper surfaces of the erinea. With a fine brush, we meticulously removed mite eggs, and mite immatures, and predators. From these examined leaves, leaf fragments with erinea were cut, and the leaf fragments were placed in petri dishes with 30 adult mites per petri dish. These samples were irradiated in stacks of petri dishes with 0 (control), 100, 200, 300, 400 and 500 Gy. Immediately after irradiation these leaf fragments were attached by entomological glue (Stickem®, PROMIP, Limeira –SP, Brazil) to newly flushed leaves of uninfested lychee plants growing in pots inside a screened greenhouse. Each inoculated leaf and the leaf fragment glued to it were examined daily by use of a hand lens and a battery-operated portable light to induce mobile life forms to move to the surface of the erinea. All predators discovered during leaf examination were removed. Mortality was evaluated at 72 h by counting the number of live and dead mites on the inoculated leaves and leaf fragments with a stereoscopic microscope. In the first trial each treatment was replicated 4 times with ~10 mites per replicate for a total of ~40 mites per treatment. However, in a second trial, we used ~18 adults per replicate for a total of ~72 adults. The number of live and dead mites was recorded and the percentage mortality of mites on the leaf fragments and leaves was determined.

DOSE–DEVELOPMENT OF ERINOSE SYMPTOMS RELATIONSHIP

Since the induction of erinea by A. litchii is critically important to its survival and reproduction, the failure of *A. litchii* adults irradiated with a certain dose to induce erinea on the abaxial surfaces of young lychee leaves by be proof that this dose prevented the production of an F_1 population of progeny—provided that a F_1 population was produced in the non-irradiated control. This dose would be a candidate for the appropriate phytosanitary irradiation dose. Therefore, objective 3 of this study was to determine the relationship of the radiation dose to the development of erinose symptoms on young leaves of potted lychee trees in a greenhouse; these young leaves, shortly after they had flushed, were inoculated with leaf fragments with erinea and mites by gluing these leaf fragments—immediately after irradiation—onto the young leaves of the potted tree. The inoculated leaves and leaf fragments were checked daily with the aid of a 20X hand lens and a portable electric torch. Symptoms were recording at 18 d post irradiation.

The development of the symptoms of erinose was found to occur over a period of ~2 wk. To be reasonably certain that no symptoms would develop in the treatments with the largest doses of irradiation, we waited until d 18 to score the infested leaves for symptom development. This assessment involved the use of a 20X hand lens and a portable electric torch for the examination of the abaxial surface for erinea and the adaxial surface for gall formation.

Each treatment was replicated 4 times. In the first trial ~10 mites were used per replicate for a total of ~40 mites per treatment. The number live and dead mites were recorded and the percentage mortality of mites on the leaves was determined. In the second replicate ~18 adult mites were used for a total of ~72 mites per treatment.

STATISTICAL ANALYSIS

The data were analyzed by ANOVA applied to the completely randomized experimental design of each experiment using the Statistical Analysis System (SAS) version 9.0 ® (SAS Institute 2002). Averages were separated by Tukey's HSD test at 5% probability.

Results

DOSE–ACUTE MORTALITY RESPONSE OF ADULTS ON DETACHED LYCHEE LEAVES

The average density collected on infested leaves of lychee trees in the Haddad Farm orchard was in the range of 30–40 adult mites per leaf. The uncertainty associated with the absorbed dose measured in each plastic bag was ± 1.6%. The variation of measured doses was of ± 1.5% in the Gammacell-220 source.

Mortality of adult *A. litchii* mites at 24 h after irradiation (Table 1) occurred only in the 1,800 and 2,000 Gy treatments and it was 2.2% at both doses. However, at 36 h after irradiation (Table 1), some mortality had occurred in all of the treatments. Mortality in the 1,800 and 2,000 Gy treatments had risen to 14.4 and 24.4%, respectively. At 48 h after irradiation (Table 1) mortality had continued to increase in all treatments but most notably and significantly in those with the large doses; mean mortality was 73.3 and 100% in the 1,800 and 2,000 Gy treatments, respectively. The mean mortality of each of the treatments in the 200–2,000 Gy range differed statistically from the control treatment (Table 1).

DOSE–ACUTE MORTALITY RESPONSE RELATIONSHIP OF IRRADIATED ADULTS ON INFESTED LEAF FRAGMENTS TRANSFERRED ONTO YOUNG LEAVES OF POTTED LYCHEE TREES

The uncertainty associated with the absorbed dose measured in each stack of petri dishes was ± 1.6%. The variation of measured doses was of ± 1.5% in the Gammacell-220 source.

Table 2 shows the number of live and dead mites in the 0 (control) 100, 200, 300, 400 and 500 Gy treatments. The control mortality at 72 h was 12.5%, which suggests that not all adults were able to cope with the transfer from a degenerating leaf fragment to an attached leaf, whereas the control mortality at 48 h in petri dishes was only 4.4% (Table 1). We found that at 72 h most of the adults still had not departed from the leaf fragments, and this seemed most recognizable at the 4 largest doses. Nonetheless the results on attached leaves show that percent mortality was directly related to dose at 72 h (3 d) after gamma irradiation and it reached about 95% with 500 Gy (Table 2). In contrast at 48 h, the percent mortality in the dose range of doses 0–600 Gy still did not differ significantly from the control (Table 1).

In the second trial with irradiated with erinose leaf fragments glued to newly flushed leaves of potted lychee trees (Table 2), the control mortality was slightly greater than in the first trial. In the 200–500 Gy treatments of Trial #2, the percent mortality was considerably less than at each corresponding dose in Trial #1.

DOSE–DEVELOPMENT OF ERINOSE SYMPTOMS RELATIONSHIP

Table 2 shows that in both Trial #1 and Trial #2 during the 18 d post irradiation, moderate symptoms of erinose had developed in the treatments involving 0–300 Gy, but no erinose symptoms had developed in the 400 and 500 Gy treatments. All symptomatic leaves displayed the patchy growth of erinea, i.e., abnormal felt-like hairs on the abaxial leaf epidermis. In addition by d 18 some galls had been formed on the upper sides of some of the leaves with erinea. Within the 0–300 Gy range of treatments, no differential response to the magnitude of the dose was captured by our scoring system.

Table 1. Acute mortality induced by gamma irradiation of adult *Aceria litchi* mites on young lychee detached leaves in petri dishes with high humidity at 24, 36 and 48 h after irradiation. The sample size was 30 and the number of replicates was 3.

	No. of dead mites (mean ± SD)*	Percentage (± SD) of dead mites	No. of dead mites (mean ± SD)*	Percentage (± SD) of dead mites	No. of dead mites (mean ± SD)*	Percentage (± SD) of dead mites
Dose (Gy)	24 h post irradiation		36 h post irradiation		48 h post irradiation	
0	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 0.1a	3.3 ± 0.1a	1.3 ± 0.1a	4.4 ± 0.1a
200	0.0 ± 0.0a	0.0 ± 0.0a	1.6 ± 0.2a	5.5 ± 0.2b	2.3 ± 0.2a	7.7 ± 0.1b
400	0.0 ± 0.0a	0.0 ± 0.0a	1.6 ± 0.2a	5.5 ± 0.2b	2.6 ± 0.1a	8.8 ± 0.1c
600	0.0 ± 0.0a	0.0 ± 0.0a	2.0 ± 0.2b	6.6 ± 0.1b	2.6 ± 0.1a	8.8 ± 0.2c
800	0.0 ± 0.0a	0.0 ± 0.0a	2.0 ± 0.2b	6.6 ± 0.2b	3.0 ± 0.1b	10.0 ± 0.3d
1,000	0.0 ± 0.0a	0.0 ± 0.0a	2.7 ± 0.1c	8.8 ± 0.3c	3.3 ± 0.2b	11.1 ± 0.3d
1,200	0.0 ± 0.0a	0.0 ± 0.0a	3.0 ± 0.2c	10.0 ± 0.3d	3.3 ± 0.1b	11.1 ± 0.3d
1,400	0.0 ± 0.0a	0.0 ± 0.0a	3.0 ± 0.2c	10.0 ± 0.3d	4.7 ± 0.2c	15.7 ± 0.3e
1.600	0.0 ± 0.0a	0.0 ± 0.0a	3.1 ± 0.2c	11.1 ± 0.3d	7.0 ± 0.2d	23.3 ± 0.3f
1.800	0.6 ± 0.1b	2.2 ± 1.0b	4.3 ± 0.2d	14.4 ± 0.3e	22.0 ± 0.2e	73.3 ± 0.4g
2,000	0.6 ± 0.1b	2.2 ± 1.0b	7.3 ± 0.2e	24.4 ± 0.3f	30.0 ± 0.3f	100.0 ± 0.4h
F	3.0	6.1	5.2	10.0	7.3	15.9
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

*Means followed by the same letter do not differs significantly based on the Tukey HSD test at $P \leq 5\%$.

Table 2. Relationship of irradiation dose to acute mortality at 72 h post irradiation of *Aceria litchii*, and determination of irradiation dose needed to prevent irradiated *A. litchii* from inducing symptoms of erinose on newly flushed attached leaves of lychee. Immediately after irradiation, the leaf fragments with erinea and adult mites were glued onto newly flushed leaves of potted lychee trees in a screened greenhouse. Acute mortality and erinose symptoms were recorded at 72 h and 18 d post-irradiation, respectively. Treatments were replicated 4 times. Trial #1 had ~40 mites per treatment, and Trial #2 had ~72 adults per treatment.

Dose (Gy)	Adults per treatment	Mean no. alive	Mean no. dead	% Mortality	Erinose symptoms on untreated leaves at 18 d post irradiation
		Readings taken 72 h post irradiation			
Trial #1					
0 (Control)	40	8.7 ± 1.9e*	1.2 ± 1.1a*	12.5 ± 1.1f	++
100	37	7.2 ± 1.6d	2.0 ± 1.2b	21.6 ± 1.4e	++
200	41	5.7 ± 1.4c	4.5 ± 1.5c	43.4 ± 2.0d	++
300	43	1.2 ± 1.1b	8.5 ± 1.9d	79.0 ± 2.8c	++
400	40	0.2 ± 0.5a	9.0 ± 1.9d	90.0 ± 3.5b	–
500	41	0.2 ± 0.1a	9.7 ± 2.0d	94.6 ± 3.8a	–
F		67.3	69.7	63.4	
P		< 0.001	< 0.001	< 0.001	
Trial #2					
0 (Control)	73	15.7 ± 1.8d	2.5 ± 1.1a*	13.7 ± 1.3f	++
100	69	13.5 ± 1.7c	3.7 ± 1.2b	21.8 ± 1.4e	++
200	62	10.5 ± 1.0b	5.0 ± 1.8c	32.1 ± 1.6d	++
300	71	11.5 ± 0.3a	6.0 ± 2.0d	34.6 ± 1.6c	++
400	74	7.0 ± 0.2a	11.5 ± 2.0d	59.4 ± 2.0b	–
500	81	6.0 ± 0.0a	14.2 ± 2.1e	69.0 ± 2.2a	–
F		71.1	70.8	67.3	
P		< 0.001	< 0.001	< 0.001	

*Means followed by the same letter do not differ significantly based on Tukey HSD test at $P = 5\%$.

Scale used to score the magnitude of symptoms of erinose +, weakly developed; ++, moderately developed; +++, strongly developed.

Thus, ++ indicates that a moderate number of patches showing erinose symptoms had developed, i.e., erinea on the abaxial leaf surfaces, and galls on the adaxial surfaces of some leaves, galls.

Discussion

We have devoted much research effort to laying the foundation of a generic phytosanitary irradiation dose for mites that belong to the Order Trombidiformes. In conducting such research on the eriophyid *A. litchii*, the challenge was to establish handling and rearing techniques that would assure the survival of the adults for a sufficient number of d not only to determine acute mortality caused by irradiation, but identify doses that prevent metamorphosis of eggs to successive instars or stages and/or prevent reproduction. The failure of *A. litchii* adults irradiated with a certain dose to induce erinea on the abaxial surfaces of young lychee leaves is proof that this dose prevented the production of an F_1 population of progeny—provided that a F_1 population was produced in the non-irradiated control.

Lethal effects on mites of irradiation, especially at relatively moderate doses, are expressed slowly. For example, Arthur & Mineiro (2009) found that a dose of 600 Gy was sufficient to kill all *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae) mites only at 11 d post-irradiation.

Because of the difficulty of assuring even short term survival of eriophyids under artificial conditions, in our first experiment we included relatively large doses that might assure the killing of most of the *A. litchii* adults within a few d. We attempted unsuccessfully to establish laboratory colonies of *A. litchii* on young freshly detached lychee leaves, but we failed to obtain establishment or even to substantially increase adult survival. It is not known how long adults live in the field, but it seems likely to be several weeks. In the field, adult mites readily migrate to young leaves. We attempted to simulate a suitable environment by maintaining high humidity in the petri dishes by means of moist cotton and a Parafilm® cover. However, the adult mites did not establish on detached young leaves.

Within the brief period (48 h) when *A. litchii* can survive on detached leaves, the irradiation of *A. litchii* adults with 1,800 Gy caused 73.3% mortality and 2,000 Gy caused 100% mortality at 48 h in comparison to the control mortality of 4.4%. About 2,450 adult mites were irradiated at each dose in this study; hence these data are fairly reliable.

Generally, the doses needed to kill arthropod pests within 1 or 2 d of irradiation are too large to be tolerated by harvested fruits and vegetables. However, smaller doses (e.g., 50–350 Gy)—which are tolerated by most fresh commodities—can prevent development or reproduction of arthropod pests and prevent them from causing infestations.

In this study we found that symptoms of erinose disease presented on very young lychee leaves when naturally occurring *A. litchii* cultures irradiated on infested leaf fragments with 300 Gy were transferred to uninfested new leaves on potted lychee plants, but not when they were irradiated with 400 Gy. Therefore, the phytosanitary dose for *A. litchii* is > 300 Gy but < 400 Gy. A generic phytosanitary dose of 350 Gy for mite species was postulated by Hallman (2012). It is noteworthy that New Zealand has adopted a generic treatment of 400 Gy against mites of the family Tetranychidae, and a generic treatment of 500 Gy against all other families of mites (MPI 2015).

Acknowledgments

This work was part of the FAO/IAEA Coordinated Research Project D62008 on Development of Generic Irradiation Doses for Quarantine Treatments. We thank the International Atomic Energy Agency (IAEA) for financial support through Research Contract N. 15.201/R0. Also, Dr. Waldemar Klassen assisted in the revision of the manuscript.

References Cited

Alam MZ, Wadud MA. 1963. On the biology of litchi mite, *Aceria litchi* Keifer (Eriophyidae, Acarina) in East Pakistan. Pakistan Journal of Science 15: 232-240.

Arthur V, Mineiro JLC. 2009. Irradiation as a phytosanitary treatment for mites of the species *Tyrophagus putrescentiae* (Acari: Acaridae). International Nuclear Atlantic Conference Rio de Janeiro, RJ. Brazil, September 27 to October 2, 2009.

Azevedo LH, de Moraes GJ, Yamamoto PT, Z. Zanardi OZ. 2013. Development of a methodology and evaluation of pesticides against *Aceria litchii* and its predator *Phytoseius intermedius* (Acari: Eriophyidae, Phytoseiidae). Journal of Economic Entomology 106(4): 2183-2189.

Azevedo LH DE, Maeda EY, Inomoto M M, Moraes GJ DE 2014. A method to estimate the population level of *Aceria litchii* (Prostigmata: Eriophyidae) and a study of the population dynamics of this species and its predators on litchi trees in southern Brazil. Journal Economic Entomology 107: 361-367.

Bhuiya AD, Majumder MZR, Nahar G, Shahjahan RM, Khan M.1999. Irradiation as a quarantine treatment of cut flowers, ginger and turmeric against mites, thrips and nematodes. IAEA TECDOC No. 1082, pp. 57-65.

Centrais de Abastecimento de Campinas – CEASA. 2009. Informações técnico-econômicas: boletim informativo diário de preços. Boletim 551. Campinas. 4 pp.

Coolen WA, D´Herde CJ. 1972. A method for the quantitative extraction of nematodes from plant tissue. State Agriculture Research Center, Ghent, Belgium. 77 pp.

DAF-QLD-AU [Department of Agriculture and Fisheries, Queensland Government, Australia] 2012. Lychee erinose mite. https://www.daf.qld.gov.au/plants/fruit-and-vegetables/a-z-list-of-horticultural-insect-pests/lychee-erinose-mite . Accessed 29-VI-2016.

FAO [Food and Agriculture Organization]. 2002. Expert consultation on lychee production in the Asia-Pacific Region. FAO, Rome, Italy. 88 pp.

Fornazier MJ, Martins DCM, Fornazier DL, Azevedo HL, Zanuncio Jr JS, Zanuncio JC. 2014. Range expansion of the litchi erinose mite, *Aceria litchii* (Acari: Eriophyidae) in Brazil. Florida Entomologist 97(2): 846-848.

Hallman GJ. 2012. Generic phytosanitary irradiation treatments. Radiation Physics and Chemistry 81: 861-866.

Hallman GJ, Levang-Brilz NM, Zettler JL, Winborne IC. 2010. Factors affecting ionizing radiation phytosanitary treatments, and implications for research and generic treatments. Journal of Economic Entomology 103: 1950-1963.

Howard FW. 2006. A coconut mite, *Aceria guerreronis* Keifer (Arachnida: Acari: Eriophyidae). Featured Creatures, EENY-398. University of Florida.

Ignatowicz S. 1997. Post radiation mortality of the mold mite *Tyrophagus putrescentiae* (Acarida: Caridae) infesting agricultural commodities and used packagings. Annals of the Warsaw Agriculture University, Horticulture and Landscape Architecture 18: 3-11.

IPPC [International Plant Protection Convention]. 2003. ISPM #18, Guidelines for the use of irradiation as a phytosanitary measure. IPPC, FAO, Rome.

Khoury HJ, Mehta K, de Barros VS, Guzzo PI, and Parker AG. 2016. Dose assurance service for low energy x ray irradiators using an alanine-EPR transfer dosimetry system. Florida Entomologist 99(special issue 2): 14-17.

Laviolette P. 1963. Action des rayons gama du Cobalt 60 sur la mortalité et fertilité des adultes d'un charanço nduriz. Bulletin Biological. 97: 305-333.

Lester PJ, Petry RJ. 1995. Gamma irradiation for after harvest disinfestations of diapausing two spotted spider mite (Acari: Tetranychidae). Journal of economic entomology 88: 1361-1364.

Lindquist EE, Sabelis MW, Bruin J. 1996. Eriophyoid mites their biology, natural enemies and control. World Crop Pests, Elsevier. 822 pp.

Machi AR, Arthur V. 2016. Oxygen atmosphere potentiates radiation effects on *Brevipalpus yothseri* (Trombidiformes: Tenuipalpidae). Florida Entomologist 99(special issue 2): 18-23.

Machi AR, Arthur V, Sarriés GA, and De Stefano Piedade SM. 2016 Effect of gamma irradiation of gravid *Tetranychus desertorum*, *Tetranychus urticae* and *Oligonychus ilicis* (Trombidiformes: Tetranychidae) females on the viabilities and durations of F1 life stages. Florida Entomologist 99(special issue 2): 186-190.

Majumder MZR, Bhuiya AD, Chowdhury N. 1996. Effects of radiation on mortality, fecundity and sterility of *Oligonychus biharensis* (Hirst) infesting common flower plants in Bangladesh. Bangladesh Journal Zoology, 24: 25-32.

Manson DCM, Oldfield GN. 1996. Chapter 1.4.1. Life forms, deuterogyny, diapause and seasonal development, pp. 173–184 *In* Lindquist EE, Sabelis, MW, Bruin J. [eds.], Eryophyoid Mites – Their Biology, Natural Enemies and Control. Elsevier Science. B.V.

Martins ABG, Bastos DC, Scallopi Júnior EJ. 2001. Lichieira (*Litchi chinensis* Sonn). Jaboticabal: Sociedade Brasileira de Fruticultura. 48 pp.

Mineiro JLC, Arthur V. 2003. Efeitos da radiação gama do Cobalto-60 em adultos de *Tetranychus urticae* Koch (Acari: Tetranychidae) *In* Encontro cientifico dos pós-graduandos no CENA/USP, 9, Piracicaba. Resumos. Piracicaba: CENA/USP, 1 CD-ROM.

Mineiro JLC, Raga A. 2003. Ocorrência de ácaros (Arachnida: Acari) em plantas de lichia (*Litchi chinensis* Sonn.) (Sapindaceae) no Estado de São Paulo, pp. 1-4 *In* Reunião Anual do Instituto Biológico 16, São Paulo, Brasil, Anais. Instituto Biológico.

MPI [Ministry for Primary Industries]. 2015. Standard 152.02: Importation and Clearance of Fresh Fruits and Vegetables into New Zealand. Ministry for Primary Industries, Wellington.

Nicholas RC, Wiant UE. 1969. Radiation of important grain infesting pests. Order of death curves and survival values for the various metamorphic forms. Food Technology 13: 58-82.

Raga A, Mineiro JLdeC, Sato ME, Moraes GJde, Flechtmann CHW. 2010. Primeiro relato de *Aceria litchii* (Keifer) (Prostigmata: Eriophyidae) em plantas de lichia no Brasil. Revista Brasileira de Fruticultura 32: 628-629.

São Paulo (Estado), Secretaria de Agricultura e Abastecimento. 2008. Coordenadoria de assistência Técnica Integral. Instituto de Economia Agrícola. Levantamento censitário de unidades de produção agrícola do Estado de São Paulo - LUPA 2007/2008. São Paulo: SAA/CATI/IEA http://www.cati.sp.gov.br/projetolupa (last accessed 7 set 2014).

Seth R, Zarin M, Khan Z, Seth, RK. 2016. Phytosanitary irradiation against *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae). Florida Entomologist 99(special issue 2): 102-113.

Siddiqui SBMAB. 2002. Lychee production in Bangladesh, pp. 28-40 *In* Papademetriou MK, Dent FJ [eds.], Lychee production in the Asia-Pacific Region. Bangkok, FAO.

Sternlicht M, Goldenberg S. 1971. Fertilisation, sex ratio and postembryonic stages of the citrus bud mite *Aceria sheldoni* (Ewing) (Acarina, Eriophyidae). Bulletin of Entomological Research 60(3): 391-397.