Milbemectin and etoxazol acaricide resistant and susceptible strains of *Tetranychus urticae* (Trombidiformes: Tetranychidae) are equally radiosusceptible and unable to reproduce when irradiated with 400 Gy

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Abstract

The twospotted spider mite, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), is considered one of the most important phytophagous mites causing considerable damage in several agricultural crops. The aim of this study was to evaluate the susceptibility to gamma irradiation of strains of *T. urticae* resistant (R) and susceptible (S) to the acaricides, milbemectin and etoxazol. The R and S strains for milbemectin and etoxazol were irradiated with 200 and 400 Gy of gamma radiation in a Gammacell 220 source to evaluate the effects of gamma radiation on the growth rate of the mites. A dose of 400 Gy totally eliminated populations of both R and S strains of *T. urticae* within 10 d. A dose of 200 Gy was not sufficient to totally eliminate both *T. urticae* strains, but it significantly reduced egg viability of both strains. In the most likely measure of efficacy for phytosanitary irradiation of mites—i.e., prevention of F_1 egg hatch when parent adults are irradiated—no differences were found in response to irradiation among the 4 strains.

Key Words: phytophagous mites; phytosanitary irradiation; phytosanitation; irradiation

Resumen

La árañita roja de dos manchas, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), es considerado uno de los ácaros fitófagos más importantes que causan daños considerables en varios cultivos agrícolas. El objetivo de este estudio fue evaluar la susceptibilidad a la irradiación gamma de las cepas de *T. urticae* resistentes (R) y susceptibles (S) a los acaricidas, milbemectina y etoxazol. Se irradiaron las cepas R y S para milbemectina y etoxazol con 200 y 400 Gy de radiación gamma en una fuente Gammacell 220 para evaluar los efectos de la radiación gamma sobre la tasa de crecimiento de los ácaros. Una dosis de 400 Gy eliminó totalmente las poblaciones de ambas cepas resistentes y susceptibles de *T. urticae* a los acaricidas un periodo de 10 días. Una dosis de 200 Gy no fue suficiente para eliminar totalmente las mismas poblaciones de *T. urticae*, pero redujo significativamente la viabilidad de los huevos de ambas cepas resistentes y susceptibles. En la medida más probable de la eficacia de la irradiación fitosanitaria de los ácaros — la prevención de la eclosión de los huevos de F_1 cuando los adultos fueron irradiados — no se encontraron diferencias en la respuesta a la irradiación entre las 4 cepas.

Palabras Clave: ácaros fitófagos; irradiación fitosanitaria; fitosanidad; irradiación

Resumo

O ácaro-rajado, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), é considerado um dos ácaros fitófagos mais importantes causando danos consideráveis em diversas culturas agrícolas. O objetivo deste estudo foi avaliar a susceptibilidade a irradiação gama em linhagens de *T. urticae* resistente (R) e susceptível (S) à acaricidas, milbemectina e etoxazol. As linhagens R e S para milbemectina e etoxazol foram irradiadas com doses de 200 e 400 Gy de radiação gama, em uma fonte de cobalto-60 Gammacell 220, para avaliar os efeitos da radiação gama sobre a taxa de crescimento dos ácaros. Uma dose de 400 Gy foi suficiente para eliminar totalmente as populações de ambas as linhagens S e R de *T. urticae* em um período de 10 dias. Já a dose de 200 Gy não foi suficiente para eliminar totalmente as populações de ambas as linhagens de *T. urticae*, mas reduziu significativamente a viabilidade dos ovos. A mais provável medida de eficácia em termos de irradiação fitossanitária de ácaros é a prevenção da eclosão de ovos na F-1 quando a geração paterna foi irradiada. Não foram encontradas diferenças em resposta a irradiação entre as 4 linhagens.

Palavras Chave: ácaros fitófagos; a irradiação fitossanitário; fitossanidade; a irradiação

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Brazil is one of the largest producers of ornamental plants in the world (Anefalos & Guilhoto 2003; Landgraf & Paiva 2009). It is estimated that this sector accounts for the generation of 3.7 direct jobs per hectare (Junqueira & Peetz 2008). The Netherlands and the USA were the most important trading partners of the Brazilian floriculture industry among 38 destination countries in 2007, accounting for 78.4% of Brazilian exports in this sector (Kiyuna et al. 2008).

However, this large trade in live plants may pose risks to importing countries due to the introduction of non-indigenous pests, which may result in potential economic losses. According to Childers & Rodrigues (2005), 11 mite families were identified in 12 plant shipments arriving via air cargo from Guatemala, Honduras, and Costa Rica at Miami International Airport, Florida in 2003. The intensive use of acaricides for the control of pest mites such as the twospotted spider mite *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), has caused the development of acaricide resistance in populations of these mites (Sato et al. 1994).

Methods of eliminating postharvest pests in flowers were discussed by several authors (Seaton & Joyce 1992; Hansen & Hara 1994; Hara et al. 1996). The current methods of disinfestation are acaricides and fumigation with methyl bromide; however, fumigation with methyl bromide is being eliminated due to environmental concerns. Under the Montreal Protocol, this effective fumigant was banned in 2005 in developed countries in 2015 and in developing countries, although exemptions for phytosanitary disinfestation and for other critically important uses still exist. Gamma irradiation of ornamental plants can be a useful tool for the control of *T. urticae*. However, some species of cut ornamental plants may be damaged with irradiation at the doses used against mites.

The advantages of irradiation include the absence of undesirable residues and the short treatment time compared to other treatments. Radiation doses needed for immediate mortality of mites are not recommended because they may cause phytotoxicity to the horticultural produce. Hence, lower doses that are tolerated by the produce should be considered.

The objective of this study was to evaluate the effect of gamma radiation on strains of *T. urticae* resistant (R) and susceptible (S) to milbemectin and etoxazol at doses suitable for operational phytosanitary use against mites on postharvest ornamental plants.

Materials and Methods

STRAINS OF MITES

Mite strains with high resistance to etoxazol and milbemectin were collected from commercial rose (*Rosa* spp.; Rosales: Rosaceae) fields in 2009 and 2010 in the municipalities of Atibaia and Holambra, State of São Paulo (SP). The susceptible strain was collected from an organic raspberry (*Rubus* spp.; Rosales: Rosaceae) field in Campos do Jordão County (SP) in 2010. The strains were reared continuously on Brazilian broad bean plants, *Canavalia ensiformis* (L.) DC (Fabales: Fabaceae), under laboratory conditions of 25 ± 1 °C, 70 ± 5% RH, and a 14:10 h L:D photoperiod.

IRRADIATION TESTS

The irradiation tests were performed at the Laboratory of Radiobiology and Environment, Center for Nuclear Energy in Agriculture, University of São Paulo (CENA/USP). Mites were irradiated with a cobalt-60 source (Gammacell-220, MDS Nordion, Kanata, Ontario, Canada), at a dose rate of 0.424 kGy/h. The dosimetry was done with radiochromic film (Gammachrome with a dose range of 0.1–3 kGy). The readings were made with a spectrophotometer (Genesys 20). The certificate of dosimetry was provided by the Institute for Energy and Nuclear Research – IPEN, University of Sao Paulo. The traceability of measurement of dose is maintained by comparison with the international service assurance dose offered by the International Atomic Energy Agency in Vienna, Austria (see Khoury et al. 2016, this issue)

GROWTH RATES OF ACARICIDE RESISTANT AND SUSCEPTIBLE STRAINS EXPOSED TO RADIATION

Twenty females each of the 3 strains were irradiated with 200 and 400 Gy and kept on bean leaf disc arenas at 25 ± 5 °C and a 14:10 h L:D photoperiod. The total number of mites (eggs, protonymphs, deutonymphs and adults) was counted on the 10th day after gamma irradiation to estimate the instantaneous growth rate for the different strains of *T. urticae*.

The eggs were observed for an additional period of 6 days to verify their viability. Only viable eggs were considered for estimating growth rates. The instantaneous growth rate (r_i) was calculated using the EXCEL 2010® program in the following equation:

$$r_i = \ln(N_f/N_o)/\Delta T$$

where Nf is the final number of animals, N_o is the initial number of animals, and ΔT is the number of d the experiment was run. The solution of the equation for r_i yields either the rate of population increase or decline. A positive value of r_i indicate a growing population, $r_i = 0$ indicates a static population, and a negative r_i value indicate a population in decline, possibly toward extinction (Saxena & Bhatia 1981).

The number of eggs per female per d and the instantaneous rate of increase (r_i) for each strain (R and S) of *T. urticae* were compared using ANOVA and Tukey's test to analyze the effects of different gamma radiation doses, and the *t*-test ($P < 0.05$) was used to compare the effect of gamma radiation on different strains of *T. urticae* with software Statistical Analysis System (SAS) version 9.0® (SAS Institute 2002). The experiment was replicated 20 times and the sex ratio of progeny developing from eggs of the irradiated females was assessed.

HATCHABILITY OF EGGS OF ACARICIDE RESISTANT AND SUSCEPTIBLE STRAINS EXPOSED TO RADIATION

Forty adult females of each strain were placed on a bean leaf disc (4 cm diam) on water-soaked cotton in a Petri dish (9 cm diam). Adults were removed after 12 h whey they had oviposited ~400 eggs. Eggs were irradiated at 200 and 400 Gy and observed during 15 d to determine the percent hatch compared with the control.

Results

GROWTH RATES OF ACARICIDE RESISTANT AND SUSCEPTIBLE STRAINS EXPOSED TO RADIATION

Irradiation of *T. urticae* populations with 400 Gy resulted in 100% mortality of eggs and active stages and totally eliminated the populations of acaricide resistant and susceptible *T. urticae* strains within 10 d (Table 1). Irradiation with 200 Gy was not sufficient to totally eliminate the mite populations, but it significantly reduced the egg viability of both strains ($P < 0.05$, df = 4, $F = 67.8$; Table 2).

The instantaneous growth rates for all of the strains (Table 2) irradiated at both doses were greatly reduced in comparison with the non-irradiated control ($r_i = 0.447$). The experiment also showed that the gamma radiation doses significantly reduced the oviposition rates.

Table 1. Mean number of eggs per female per d and the instantaneous rate of increase (r_i) per d of acaricide resistant and susceptible strains of *Tetranychus urticae* irradiated with either 200 or 400 Gy. The acaricides were milbemectin and etoxazol.

Strain[b]	Mites	No. of eggs laid	Mean (± SE) eggs/female/d[a]	r_i/d
		Irradiation dose = 200 Gy		
M-R	20	850	4.25 ± 0.55a	0.124 ± 0.04
M-S	20	873	4.36 ± 0.44a	0.081 ± 0.02
E-R	20	722	3.61 ± 0.65a	0.088 ± 0.04
E-S	20	879	4.39 ± 0.28a	0.104 ± 0.03
		Irradiation dose = 400 Gy[b]		
M-R	20	629	3.14 ± 0.39b	−1[c]
M-S	20	536	2.68 ± 0.52b	−1
E-R	20	685	3.42 ± 0.42a	−1
E-S	20	586	2.93 ± 0.65b	−1
		Non-irradiated control		
M-R	20	1.026	15.47 ± 0.52a	0.409 ± 0.009
M-S	20	969	13.95 ± 0.38a	0.447 ± 0.004
E-R	20	857	11.4 ± 0.97a	0.435 ± 0.012
E-S	20	1.115	10.42 ± 0.86a	0.438 ± 0.01

[a]Values followed by the same lower case letter in the same column and dose do not differ significantly (Tukey's test, $P < 0.05$).
[b]M-R = milbemectin resistant strain; E-R = etoxazol resistant strain; S = susceptible strain.
[c] −1 final number of mites was zero, because none of the eggs oviposited by females irradiated with 400 Gy hatched.

The smallest number of eggs laid per d was observed for the milbemectin susceptible *T. urticae* strain exposed to 400 Gy. The least effect of this dose on eggs laid per d was observed for the etoxazol R strain, and the latter was significantly less affected than the milbemectin R and S strains. In the most likely measure of efficacy for phytosanitary irradiation of mites—i.e., prevention of F_1 egg hatch when parent adults are irradiated—no differences were found in response to irradiation among the 4 strains (Table 2).

Discussion

Several authors have shown that insecticide resistant and susceptible strains of stored grain insects showed no significant differences in susceptibility to gamma radiation (Brower 1973; Bhatia & Sethi 1978; Saxena & Bhatia 1981; Misra & Bhatia 1998).

However, Erdman (1966) showed that DDT-resistant strains of *Tribolium castaneum* (Herbst) were more radiosensitive than the wild-type strains, and Nakakita (1985) found that a phosphine-resistant strain of *T. castaneum* was more tolerant to radiation than a susceptible one.

Some eggs laid by female mites irradiated with 200 Gy developed to the adult stage, though egg viability was significantly reduced as observed in Table 2. However irradiation with 200 Gy did not achieve total sterility in either the resistant or the susceptible strains, as observed in *Phyllocoptruta oleivora* (Ashmead) (Hu 2004).

Eggs irradiated with 400 Gy were not viable during the period observed. In the control more eggs were produced in relation to the irradiated treatments. These results indicate that eggs of both strains were sensitive to gamma irradiation and these findings are in accordance with observations on *P. oleivora* by Hu (2004).

A dose of 400 Gy prevented population growth of all strains of *T. urticae* within 10 d, while 200 Gy was not sufficient to totally eliminate the mite population, but reduced egg viability by ~95%. The susceptible and resistant mite strains did not show differences in radio-resistance at 200 and 400 Gy.

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Table 2. Mean (± SEM) numbers of F_1 immature stages from the parent generation of adult females of *Tetranychus urticae* strains irradiated at 200 Gy[a]. The acaricides were milbemectin and etoxazol.

Strain	No. of mites	Eggs laid	Larvae	Protonymph	Deutonymph	F_1 Adults	
						Males	Females
Milbemectin R	20	850	42.5 ± 0.23a	30 ± 1.5a	20 ± 1.4a	2	3
Milbemectin S	20	873	43.6 ± 1.21a	22 ± 1.1b	16 ± 1.1a	1	3
Etoxazol R	20	722	36.1 ± 1.18a	36 ± 1.1a	18 ± 1.1a	3	4
Etoxazol S	20	879	43.9 ± 1.60a	25 ± 1.2b	11 ± 1.0b	2	3

[a]Means followed by same letter in a column are not significantly different (Tukey's test, $P < 0.05$).
[b]R = resistant, S = susceptible.

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