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USE OF RADIATION TO STERILIZE TWO-SPOTTED SPIDER MITE (ACARI: TETRANYCHIDAE) EGGS USED AS A FOOD SOURCE FOR PREDATORY MITES

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ABSTRACT

One-, 2- and 3-day-old two-spotted spider mite eggs were treated with increasing doses of gamma radiation ranging from 0-280 Gy. Percent egg hatch decreased as radiation increased for each age group; however, older eggs required higher doses of radiation to prevent egg hatch than did younger eggs. Based on the regression lines for 1-, 2- and 3-day-old eggs, the best estimates of the doses of radiation that would prevent 100% of the eggs from hatching were 43.6 Gy, 55.1 Gy and in excess of 280 Gy, respectively. In general, irradiating spider mite eggs had no significant effect on their acceptability as prey by females of the predatory mite *Neoseiulus californicus* McGregor, except for 1-day-old eggs treated at 240 Gy. Female *N. californicus* consumed 50-75% fewer of these eggs than they did eggs of other treatments, in both no-choice and choice experiments.

Key Words: *Tetranychus urticae*, *Neoseiulus californicus,* biological control, rearing

RESUMEN

Huevecillos de *Tetranychus urticae* de uno, dos y tres dias de edad fueron tratados con dosis de radiación gamma entre 0 y 280 Gy. En general, el porcentaje de eclosión de los huevecillos tratados disminuyó proporcionalmente al aumento en la dosis de radiación en huevecillos de todas edades, sin embargo, los huevecillos de mayor edad requirieron mayores dosis para prevenir eclosión. Basados en las líneas de regresión obtenidas en estos experimentos, las dosis requeridas para prevenir el 100% de eclosión en huevecillos de *T. urticae* son de 43.6 Gy, 55.1 Gy y mas de 280 Gy, para huevecillos de uno, dos y tres dias de edad, respectivamente. En general, la irradiación de huevecillos no tuvo un efecto significativo en cuanto a su aceptabilidad como alimento para hembras de *Neoseiulus californicus* McGregor, exceptuando en huevecillos de un dia de edad tratados con 240 Gy. Las hembras de *N. californicus* consumieron 50-75% menos de este tratamiento, tanto en experimentos donde tuvieron opción de escoger, como en experimentos donde no tuvieron opción de escoger entre varios tratamientos como alimento.

Translation provided by author.

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an extremely polyphagous pest that has been reported from more than 900 host species and is described as a serious pest of at least 30 economically important agricultural and ornamental plants, including corn, cotton, cucumber, beans, tomato, eggplant, peppers and roses (Helle & Sabelis 1985a, b; Navajas 1998). Unfortunately, chemical control of this pest can be compromised because of resistance (Gould et al. 1982; Croft et al. 1984; Cranshaw & Sclar 2001). As a result, a more integrated approach utilizing biological control with predatory mites is increasingly being recommended (Hamlen & Lindquist 1981; Osborne et al. 1985; Grafton-Cardwell et al. 1997; Nicetic et al. 2001).

Biological control of spider mites has centered on the use of predatory mites in the family Phytoseiidae (Helle & Sabelis 1985a, b; Schausberger & Croft 1999). *Phytoseiulus persimilis* Athias-Henriot is the most studied species and is the primary species used to control spider mites in greenhouses (Osborne et al. 1985; UF/IFAS 2002).

Another promising predatory mite is *Neoseiulus* (=*Amblyseius*) *californicus* (Chant) (Castagnoli & Simoni 1999; Roy et al. 1999). Although less specialized than *P. persimilis*, it has been shown to provide excellent control of spider mites over a wide range of climatic and management conditions (Oatman et al. 1977; Pickett & Gilstrap 1986; McMurtry & Croft 1997). In the United States there are five species of predatory mites that are available commercially and used in biological control programs: *Galendromus* (=*Metaseiulus*) *occidentalis* (Nesbitt), *Mesoseilulus* (=*Phytoseiulus*) *longipes* (Evans), *N. californicus*, *N. fallicus* (Garman), and *P. persimilis* (UF/IFAS 2002).

Castagnoli and Simoni (1999) showed that the long-term feeding history of *N. californicus* affects its functional and numerical responses when exposed to different densities of two-spotted spider mite eggs and protonymphs. In general, *N. californicus* that were wild collected or routinely reared on spider mites performed better than those reared on pollen or dust mites (*Dermatophagoides* spp.). Because of studies such as this, commercial shipments of predatory mites usually contain spider mite eggs as a food source (Osborne et al. 1985; Penn 1999). The eggs are easy to handle and help insure that the predatory mites are in good condition when they arrive.

A problem with the use of live host material in commercial shipments of predatory mites (or other natural enemies) is the risk of introducing new pest species or strains, including chemically resistant strains, along with the natural enemy (Penn 1999). One solution to this concern that has been discussed but never tested is the use of radiation to reproductively sterilize host material prior to shipment.

Although radiation has been used extensively for many years to sterilize insects used in sterile insect release programs (IAEA 2000), there is limited published literature on the radiation biology of spider mites. Henneberry (1964) irradiated two-spotted spider mite adults and examined the effects on male and female fertility and their progeny. Feldmann (1975) studied the induction of heritable sterility factors such as translocations and inversions in the two-spotted spider mite at different doses of gamma radiation and confirmed the holokinetic nature of their chromosomes. Nothing is known about the effect of radiation on spider mite eggs.

In an effort to determine the potential for using irradiated spider mite eggs as a food source for predatory mites, studies were initiated with the following objectives: (1) determine the dose of gamma radiation that would prevent specific age groups of *T. urticae* eggs from hatching; and (2) assess the impact of egg age and exposure to gamma radiation on acceptability as a food source by the predatory mite, *N californicus*.

MATERIALS AND METHODS

Experimental Material and Site

Lima bean (*Phaseolus lunatus* L.) leaves and two-spotted spider mites were obtained from the North Florida Research and Education Centers at Monticello and Quincy, FL. Bean plants were started from seeds sown in Perlite® planting medium every 2-3 days and kept in a greenhouse until they reached the four-true leaf stage. They were then transferred to a humid cement-block room, where they were infested with spider mites and kept for 7-10 days before being discarded and replaced with fresh plants.

Irradiation of spider mite eggs was conducted at the USDA-ARS Crop Pest Management and Research Unit in Tifton, Georgia, using a Cobalt^{60} Gammacell 220 Irradiator® with a dose rate of approximately 20.06 Gy/min.

The experiments were carried out in the USDA-ARS Laboratory at the Florida A&M University Center for Biological Control, Tallahassee, FL. All experiments were set-up at ambient room temperature and relative humidity (approximately 21-24°C and 60-65% RH). Controlled conditions after experimental set-up were maintained using a Forma Scientific Growth Chamber® with a photoperiod of 12:12 (L:D), temperature of 28 ± 1°C, and relative humidity of 58%. Under these conditions, normal egg hatch began four days after oviposition.

Neoseiulus californicus were purchased from IPM Laboratories of New York, placed in 15 cm plastic petri dishes with fresh bean leaves, and fed spider mites *ad libitum* until needed for tests.

Dose Response

Approximately 25 gravid female two-spotted spider mites were transferred to freshly excised, young, bean leaves devoid of spider mites and other arthropods and allowed to oviposit for 18-24 h. The leaves were prevented from drying out by placing them on moist filter paper in covered 7.0 cm plastic petri dishes and kept in a growth chamber as specified above. After 24 h, the petri dishes containing the bean leaves were placed under a dissecting microscope and all motile stages of spider mites removed, leaving only newly oviposited (0-24 h old) eggs. The bean leaves were then cut into pieces containing 25 eggs and each piece placed separately in the center of a new 7.0 cm petri dish containing moist filter paper.

Petri dishes containing 1-day-old (0-24 h) eggs were transported by car (approximately 2 h) in a small cooler to Tifton, GA, and irradiated. Petri dishes containing eggs that were to be irradiated at 2- (24-48 h) and 3-days (48-72 h) of age were kept in the growth chamber for an additional 1 and 2 days, respectively, before transport to Tifton. Once irradiated, petri dishes and eggs were immediately driven back to Tallahassee, FL, and placed in a growth chamber as before. Petri dishes were checked daily for egg hatch. Newly eclosed larvae were removed, and the number of larvae and remaining eggs recorded. Egg hatch in each petri dish was monitored for a period of 2 weeks from the time egg hatch began. Filter paper in the petri dishes was moistened as needed throughout the course of the experiment.

Spider mite eggs were treated with increasing doses of radiation until none of the eggs hatched. The 1-day-old eggs were treated with the following doses of radiation during June 2001: 0, 10, 20, 30, 40, and 50 Gy. Two-day-old eggs were exposed to 0-60 Gy at intervals of 10 Gy from June to September 2001, and 3-day-old eggs were exposed to 0 to 140 Gy at intervals of 10 Gy and 160 to 280 Gy at intervals of 20 Gy from June to November 2001. Four replicate petri dishes of 25 eggs were used at each dose. Dose response for this experiment was analyzed with polynomial regression analysis (Damon & Harvey 1987; SAS Institute 1994). Before analysis, proportion data were transformed with an arcsine square-root transformation. Backtransformed data are presented in regression equations.

Predation Study, No-Choice Test

Fresh spider mite eggs were obtained as previously described by placing 25-30 gravid females on clean bean leaves and allowing them to oviposit for 18-24 h. The bean leaves were cut into 2 cm square pieces containing 25 eggs. One- and 3-day-old eggs were then irradiated at 0, 40, 140 or 240 Gy. Following irradiation, the filter paper in a given petri dish was moistened to the point of standing water and a few drops of detergent were added to prevent mites from leaving the leaf disc. A single adult female *N. californicus*, which had been starved for 24 h, was added to each arena. Each female was allowed to feed for 24 h, after which the number of eggs eaten was recorded. Twenty replicates of both 1- and 3-day-old eggs at each treatment dose were conducted. The data were analyzed by ANOVA, and means separated using Duncan's Multiple Range Test (SAS Institute 1994).

Predation Study, Choice Test

Two-spotted spider mite eggs were obtained and treated as above, except that bean leaves were cut into pieces 2 cm × 1 cm containing 10 eggs. Following irradiation (0, 40, 140 and 240 Gy), one leaf strip containing 10 1-day-old eggs was fitted next to a leaf strip containing 10 3-day-old eggs that had been treated with the same dose of radiation. Petri dishes were prepared as before, and a single adult female *N. californicus* that had been starved for 24 h was added to the center of each 2 cm × 2 cm arena. Each female was allowed to feed for 24 h, and the numbers of eggs eaten were recorded. Twenty replicates at each treatment dose were conducted. Analysis of the data was done by determining the preference of 1-day-old eggs over 3-day-old eggs (=number of 1-day-old eggs consumed minus the number of 3-day-old eggs consumed) for each pairing. Data were analyzed by paired difference t-test (SAS Institute 1994).

RESULTS

Dose Response

Within each age group percent egg hatch decreased as the dose of radiation increased (Fig. 1). Egg hatch for the three age groups showed significant curvilinear responses to radiation. For each age group, there appeared to be a threshold beyond which the magnitude of the radiation effect decreased as dose increased. Significant differences in the slopes of the regressions indicate that

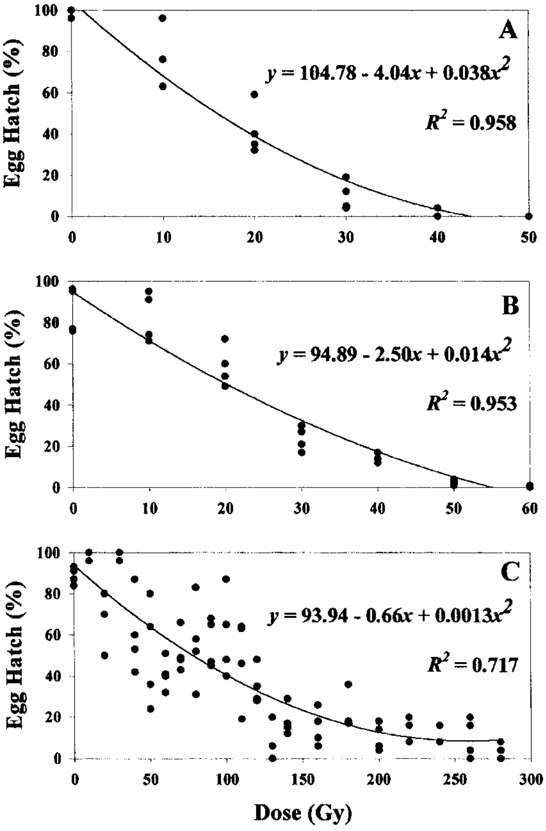

Fig. 1. Eclosion dose response curves for two-spotted spider mite eggs that were irradiated when they were (A) 1-, (B) 2-, or (C) 3-days-old with increasing doses of gamma radiation. Note different scales on x-axes.

one-day-old eggs were more sensitive to radiation ($y = 104.78 - 4.04x + 0.038x^2$, $R^2 = 0.958$, df = 2, 21, $n = 24$, $P = < 0.0001$) than were 2-day-old eggs ($y = 94.98 - 2.50x + 0.014x^2$, $R^2 = 0.953$, df = 2, 25, $n = 28$, $P < 0.0001$), and that 3-day-old eggs were the most resistant to radiation ($y = 93.94 - 0.66x + 0.0013x^2$, $R^2 = 0.717$, df = 2, 85, $n = 88$, $P < 0.0001$). Irradiation of 1-day-old eggs at 40 Gy resulted in a 1.0 ± 1.0% egg hatch, and at 50 Gy no eggs hatched. Irradiation of 2-day-old eggs at 40 Gy resulted in 14.0 ± 1.0% egg hatch; 50 Gy resulted in 3.0 ± 1.0% egg hatch, and at 60 Gy 0.3 ± 0.3% of the eggs hatched. Irradiation of 3-day-old eggs at 60 Gy resulted in 41.0 ± 4.0% egg hatch. Even at 280 Gy, 5.0 ± 1.9% of the eggs hatched, although none of the hatching individuals survived past the second larval instar.

Predation Study, No-Choice Test

At 40 and 240 Gy female *N. californicus* consumed a greater number of 3-day-old spider mite eggs than 1-day-old eggs (Table 1). Within an age class, irradiation of the eggs did not affect the number of eggs eaten except at the highest dose, 240 Gy, for the 1-day-old eggs.

Predation Study, Choice Test

When *N. californicus* females were given a choice between 1- and 3-day-old spider mite eggs that had been exposed to either 0, 40, 140, or 240 Gy of gamma radiation, the paired t-test indicated that there was no preference for 1- or 3-day-old eggs, except at 240 Gy, where 3-day-old eggs were preferred over 1-day-old eggs (Table 2).

DISCUSSION

The results show that it is possible to use gamma radiation to prevent two-spotted spider mite eggs from hatching. The results also showed that irradiated eggs are still acceptable as a food source to the predatory mite *N. californicus*. As such, irradiated spider mite eggs could be used to provision shipments of predatory mites to eliminate concerns that the shipments are contaminated with reproductively viable pest material.

TABLE 1. MEAN (±SD) NUMBER OF SPIDER MITE EGGS CONSUMED PER ADULT FEMALE *N. CALIFORNICUS* IN 24 H IN A NO-CHOICE TEST, WHEN PROVIDED WITH 25 1- OR 3-DAY-OLD EGGS THAT HAD BEEN EXPOSED TO 0, 40, 140 OR 240 GY OF GAMMA RADIATION.

	Mean no. eggs eaten	
Dose (Gy)	1-Day-Old	3-Day-Old
0	7.7 ± 3.95 A, a[1]	9.0 ± 4.27 A, ab
40	6.3 ± 2.14 A, a	10.8 ± 4.05 B, a
140	7.6 ± 3.91 A, a	10.0 ± 5.38 A, ab
240	2.4 ± 2.34 A, b	6.7 ± 3.01 B, b

[1] Means followed by different capital letters within a row or lower case letters within a column are significantly different at $P < 0.05$ using Duncan's Multiple Range Test.

TABLE 2. MEAN (±SD) NUMBER OF SPIDER MITE EGGS CONSUMED PER ADULT FEMALE *N. CALIFORNICUS* IN 24 H IN A CHOICE TEST, WHEN PROVIDED WITH 10 1-DAY-OLD AND 10 3-DAY-OLD EGGS THAT HAD BEEN EXPOSED TO 0, 40, 140 OR 240 GY OF GAMMA RADIATION.

	Mean no. eggs eaten	
Dose (Gy)	1-Day-Old	3-Day-Old
0	6.00 ± 2.17 a[1]	5.60 ± 2.52 a
40	4.05 ± 2.41 a	3.75 ± 1.50 a
140	3.55 ± 2.68 a	4.55 ± 2.45 a
240	2.40 ± 2.03 a	6.00 ± 3.07 b

[1] Means followed by different letters within a row are significantly different at $P > 0.05$ using a paired difference *t*-test.

Gamma radiation treatment had a negative effect on egg hatch overall, with egg hatch decreasing as dose increased. One- and 2-day-old eggs were much more sensitive to radiation than were 3-day-old eggs. Based on the regression lines for 1- and 2-day-old eggs, the best estimates of the doses of radiation that would prevent 100% of the eggs from hatching are 43.6 Gy and 55.1 Gy, respectively. Although 280 Gy, the highest radiation level tested against 3-day-old two-spotted spider mite eggs, did not prevent 100% of the eggs from hatching, none of the hatching individuals survived past the second instar.

Not only were older eggs much less sensitive to radiation, but the variation in the dose response (i.e., the percentage of eggs hatching at a given dose) increased with egg age. This can be seen in the R^2 values for the regression lines, which were 0.958, 0.953 and 0.717 for 1-, 2- and 3-day-old eggs, respectively. Why older eggs were less sensitive to radiation and showed a greater variation in dose response is not known. The egg stage in the two-spotted spider mite only lasts about 4 days at 28°C, so many developmental and physiological changes are occurring during this time. Radiation dose response experiments that controlled egg age more precisely (e.g., used eggs that were laid over 1 h intervals rather than 24 h intervals) would likely show less variation. If such experiments were coupled with studies on the developmental changes occurring in two-spotted spider mite eggs, particularly between 2 and 3 days of age, they might provide insights as to why older eggs are more radio-resistant.

In general, irradiating two-spotted spider mite eggs had no significant effect on their acceptability as prey by female *N. californicus*, except for 1-day-old eggs treated at 240 Gy. Female *N. californicus* consumed 50-75% fewer of these eggs than they did eggs of other treatments, in both no-choice and choice experiments. Research has shown that phytoseiid mites determine prey acceptance primarily by contact chemoreception (Dicke et al. 1988; Vet & Dicke 1992). Our research did not attempt to determine what factors are assessed by *N. californicus* to determine prey (egg) acceptability or what biochemical or physiological effects radiation was having on spider mite eggs, other than reducing egg hatch. However, it should be noted that 240 Gy was 5-6 times the dose of radiation needed to prevent egg hatch of 1-day-old eggs. A similar drop in acceptability might have been seen with 3-day-old eggs if a dose of radiation that was 5-6 times their lethal dose had been used (280 Gy × 5 = 1,400 Gy). If this were true, one explanation for these results might be that female *N. californicus* are able to assess egg viability and prefer healthy live prey to dead prey; thus, eggs that were irradiated at a dose well in excess of the dose that would prevent them from hatching were less preferred.

The fact that irradiated spider mite eggs were still acceptable as prey to *N. californicus* was not unexpected. Non-viable lepidopteran eggs resulting from irradiated parents have been shown to be acceptable as developmental hosts for trichogrammatid egg parasitoids (Cossentine et al. 1996; Carpenter et al. 2003). Not only could irradiated spider mite eggs be used as a risk-free, high quality food source in shipments of predatory mites, but they could also be used to maintain predatory mite colonies that are free of spider mites and yet avoid the quality problems associated with rearing them on non-host foods such as pollen. Future studies should attempt to rear *N. californicus* over multiple generations on irradiated spider mite eggs and compare demographic effects (e.g., longevity, reproductive potential, etc.) with those when using other food sources or host stages.

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