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Acceptance and suitability of ultraviolet-irradiated *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) eggs for *Trichogramma chilonis* Ishii (Hym., Trichogrammatidae)

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Abstract: *Helicoverpa armigera* (Hbn.) eggs were irradiated with a 30 W ultraviolet (UV)-lamp from a distance of 30 cm. Egg mortality increased with duration of exposure to the UV-light source; exposure for 48 min was sufficient to kill 98% of the eggs. Survival of the egg parasitoid *Trichogramma chilonis* Ishii was lower on eggs irradiated for 60 and 90 min than on untreated control eggs. Clutch size and sex ratio of the parasitoid progeny were similar in irradiated and control eggs. The lower suitability of irradiated host eggs was not recognized by female wasps as they accepted irradiated and control eggs equally in a choice test. The higher parasitoid mortality in irradiated eggs may be a result of morphological changes in the host egg chorion. Supporting this hypothesis, it was observed that the duration of drilling by the parasitoid was significantly shorter on irradiated eggs than on control eggs.

1 Introduction

Irradiation with ultraviolet (UV)-light is an effective method of killing lepidopteran eggs (Brenier, 1965; Calderon and Navarro, 1971; Voegelé et al., 1974; Maninder and Varma, 1980; Goldstein et al., 1983; Şengonca and Schade, 1991). UV-killed host eggs are preferred for rearing *Trichogramma* (Hym., Trichogrammatidae) egg parasitoids as many of its lepidopteran hosts are cannibalistic. If the host eggs are not killed, larvae hatching from unparasitized eggs may feed on parasitized eggs, decreasing the number of parasitoids produced. This is especially important in *Trichogramma* mass production units and on egg cards prepared for field releases. Irradiated host eggs have also been used in many laboratory studies because the risk of larvae emerging from unparasitized eggs and damaging the experiment is eliminated (e.g. Chassain and Bouletreau, 1987; Wäckers et al., 1987; Wajnberg, 1993). Irradiation of host eggs has not been shown to adversely affect parasitoid development (e. g. Goldstein et al., 1983; Voegelé et al., 1974).

Using UV-irradiation, host development can be arrested at an early stage, which is preferred for oviposition and is most suitable for *Trichogramma* spp. development in many host species (Pak, 1986). Şengonca and Schade (1991) found higher parasitization in irradiated eggs than in untreated eggs in the field. The difference was most likely due to the fact that untreated eggs continued to develop and were therefore acceptable for *Trichogramma* oviposition for a shorter period. For this reason the authors recommended using irradiated host eggs to enhance the parasitoid population in the field.

The recommended duration of exposure to UV-light varies with the strength of the light source and the distance between the light and the eggs. It is likely that the minimum exposure time needed to kill all host eggs is exceeded in many laboratories. In addition, the acceptability of irradiated eggs compared to live eggs has not been tested. We studied the acceptance of *Helicoverpa armigera* eggs for *T. chilonis* in a choice test comparing duration of irradiation and whether irradiated host eggs were as suitable for parasitoid development as untreated control eggs.

2 Materials and methods

2.1 Insect rearing

H. armigera was reared at ambient temperature of 22 ± 2°C and 70 ± 10% RH on a chickpea based diet (Armes et al., 1992). Eggs were collected daily and were thus $\leqslant$ 24 h old when used in the experiments. Average egg volume was 0.42 mm^3 (assuming globular shape; diameter: 0.48 mm, height: 0.45 mm; $n = 20$). *T. chilonis* was reared at 26 ± 2°C, 50 ± 10% RH under constant 16:8 light:dark photoperiod on eggs of *Corcyra cephalonica* Stainton (Lep., Pyralidae). This strain was collected in 1980 on *H. armigera* eggs from cotton and tomato plants near Hyderabad (India). Since then it has been reared on the factitious host *C. corcyra*. The female parasitoids used in these experiments were 1–2 d old, mated, unexperienced and had been fed with honey agar.

2.2 UV-irradiation of *H. armigera* eggs

H. armigera eggs were attached to filter papers with a wet brush and irradiated with UV-light (General Electric, G30T8, 30 W) at a distance of 30 cm for 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 60 or 90 min. Eggs were irradiated in batches of 50 with 50 eggs from the same moths used as an untreated control. Irradiated and control eggs were kept in an incubator (26 ± 2°C, 50 ± 10% RH, 16:8 light:dark) for four days after which the number of unhatched eggs was recorded. Only

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replicates in which more than 35 eggs (70%) in the untreated control hatched were used in the analysis. Thus, each treatment (plus control) was replicated 10–13 times. Higher proportions of unhatched eggs were sometimes observed due to a high number of infertile eggs. The occurrence of infertile eggs is common in *H. armigera* cultures because of a lower mating efficiency in the laboratory (ARMES and COOTER, 1991; RILEY et al., 1992). The proportion of unhatched eggs will be referred to as mortality.

2.3 Acceptance of UV-irradiated eggs (Experiment 1)

Fertile *H. armigera* eggs (identified by the horizontal brown ring which develops after ≈ 12 h) were exposed to UV-light for 60 min, 90 min or left untreated. Sixteen eggs, half irradiated and half untreated, were arranged alternately in a 4 × 4 rectangular grid on filter paper and offered to a single *T. chilonis* female. The distance between eggs was 2–3 mm and therefore within the 'reactive distance' as *Trichogramma* spp. can recognize host eggs by chemical and visual cues from a maximum of 4 mm (LAING, 1937; GLAS et al., 1981; PAK et al., 1991; BRUINS et al., 1994; WAJNBERG, 1994). The experiments were conducted at 26 ± 2°C and 60–70% RH. The behaviour of the parasitoid was observed under a stereo microscope for 30 min starting with the first host contact (= touching the host with antennae). Illumination was provided by a cold glassfiber light source. Each contact of the parasitoid with a host and the number of hosts accepted (= insertion of ovipositor) were recorded. Parasitized eggs were not replaced during each test. As a measure of preference the acceptance/contact (a/c) ratio was calculated separately for irradiated and control eggs. The total number of eggs accepted was divided by the total number of unparasitized eggs contacted. This method was used earlier to measure host-species and host-age preference as well as host-discrimination (BRAND et al., 1984; VAN DIJKEN et al., 1986; PAK et al., 1986, 1990; WÄCKERS et al., 1987; VAN BERGEIJK et al., 1989; LIU and HE, 1991; BJORKSTEN and HOFFMANN, 1995). Parasitized eggs were kept singly in gelatine capsules in the *Trichogramma* rearing incubator until the progeny emerged. The number of progeny which hatched from an host egg will be referred to as 'clutch size' as it has been shown that larval and pupal mortality of the parasitoids is generally low (KLOMP and TEERINK, 1967; SUZUKI et al., 1984; PAK et al., 1985). The sex ratio of the progeny was also recorded. Each combination of irradiated and control eggs was repeated 22 times with different female parasitoids.

To confirm that host acceptance occurred when the ovipositor was inserted and that eggs were actually laid, 16 eggs (irradiated for 90 min or untreated) were arranged as described above and offered to a single female for 30 min in a no-choice situation. Twelve wasps were tested on each treatment. Eggs in which oviposition insertion was observed were crushed in a drop of water between a microscope slide and a coverslip and examined for parasitoid eggs under a microscope.

2.4 Parasitization behaviour (Experiment 2)

Ten fertile *H. armigera* eggs were attached to filter paper and irradiated with UV-light for 60 min, 90 min or kept untreated. The distance between single eggs was ≈ 10 mm to ensure that females could not perceive adjacent eggs. The experiments were conducted at 26 ± 2°C and 60–70% RH. *T. chilonis* females were released individually on a filter paper containing one of the three treatment groups (no-choice situation). For each wasp the oviposition sequence on the first egg accepted was observed under a stereo microscope. Illumination was provided by a cold glassfiber light source. Three stages in the parasitism behaviour were identified and their duration recorded using the computer software package 'The Observer®' (NOLDUS INFORMATION TECHNOLOGY, 1993): (1) drumming (beginning with the initial contact of the host egg by the parasitoid antennae until the end of tapping); (2) drilling (beginning with placement of the tip of the ovipositor on the egg surface and ending with the insertion of the ovipositor, recognized by raising of the abdomen); and (3) oviposition (ending with withdrawal of the ovipositor). The classification of different stages of the *Trichogramma* oviposition behaviour followed the description by KLOMP et al. (1980) and SUZUKI et al. (1984). The number of additional insertions of the ovipositor into the same egg and the total time spent on the egg were also recorded. A total of 32 parasitoids were observed on eggs of each treatment group; four parasitoids per treatment per day. Parasitized eggs were kept singly in gelatine capsules in the *Trichogramma* rearing incubator until the progeny hatched. Clutch size and sex ratio of the progeny were recorded.

2.5 Statistical analysis

To compare the a/c ratios between irradiated and control eggs in Experiment 1, a 2 × 2 contingency-table for the distribution of acceptance and contacts was constructed and differences in a/c ratios were then evaluated using a χ^2-test. In Experiment 2, the mean differences in the duration of the different stages of parasitism behaviour as well as in the total time spent per host were analysed using Student's t-test. For both experiments, comparison of percentage emergence, sex ratio (% females) and clutch size between the three treatments was made using Tukey test after normalizing percentage data with arcsin $\sqrt{}$ transformation (SYSTAT, INC., 1990).

3 Results

3.1 UV-irradiation of *H. armigera* eggs

The mortality of *H. armigera* eggs after UV-irradiation was dose-dependant and is described by the equation: y = 98.32–86.94 × 0.89^x ($r^2 = 0.97$) (fig.), where y is the mortality (%) and x the duration of the UV-irradiation. Mortality of 50% of the eggs was observed after only 5 min exposure to UV-light, while 98% of the eggs were killed after 48 min exposure.

3.2 Acceptance of UV-irradiated eggs

H. armigera eggs irradiated for 60 or 90 min were as frequently accepted by *T. chilonis* females as control

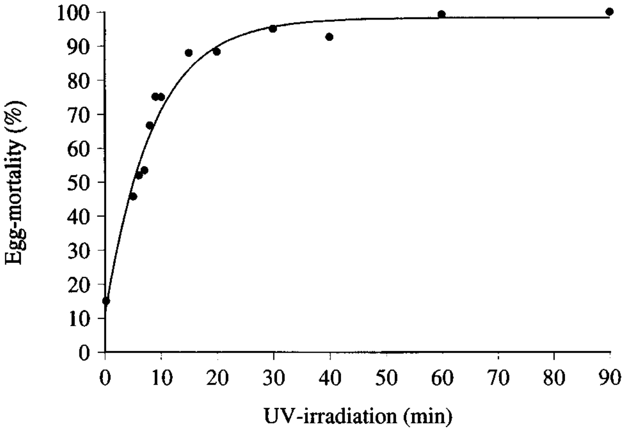

Fig. 1. *Mortality of* Helicoverpa armigera *eggs after UV-irradiation*

Table 1. *Acceptance of UV-irradiated and untreated* Helicoverpa armigera *eggs by* Trichogramma chilonis *under choice conditions (n = 22)*

UV-irradiation (min)	Eggs accepted	Number of contacts	a/c ratio	X^2	P (0.05)
0	130	168	0.77	0.68	n.s.
60	125	170	0.74		
0	138	173	0.80	3.67	n.s.
90	119	168	0.71		

eggs (table 1). Data pooled over all treatment groups gave an a/c ratio of 0.75 for unparasitized eggs. Eggs were rarely rejected after probing with the ovipositor. In the no-choice test, only three out of 92 (3.3%) untreated eggs in which the ovipositor was inserted did not contain a parasitoid egg. Similarly, out of 87 irradiated eggs only three (3.4%) were rejected after internal examination.

3.3 Parasitization behaviour

The duration of the different stages of the parasitization behaviour on the first egg accepted as well as the total time spent on the host were compared between eggs irradiated for 60 or 90 min and the control (table 2). Drilling time was shorter on irradiated eggs than on control eggs. There was no difference in drilling duration between the two irradiation treatments. No significant differences were found in the durations of drumming, oviposition or total time spent per host among any of the three treatments.

The mean handling time (including drumming, drilling and oviposition) required almost 50% of the total time spent per host. More than 60% of the handling time required was utilized for oviposition.

On eggs of all treatment groups, the parasitoids fed on droplets of yolk exuding from the oviposition puncture. Only four out of 96 females left the host without host feeding. Of the parasitoids, 36% inserted their ovipositor at least once more, often using the same hole. These insertions were of shorter duration than the first insertion but were also followed by host feeding. With an average (± SE) of 1.7 ± 0.23 insertions into each control egg, 1.6 ± 0.16 in eggs irradiated for 60 min and 1.7 ± 0.25 in eggs irradiated for 90 min, no significant differences ($P > 0.05$; Student's *t*-test) in the number of insertions of the ovipositor were found between the treatment groups.

3.4 Suitability of UV-irradiated eggs

In both experiments irradiated eggs were found to be less suitable hosts for *T. chilonis* than control eggs (table 3). In Experiment 1, significantly fewer progeny emerged from eggs irradiated with UV light for 90 min than from control eggs. A similar trend was recorded in Experiment 2, though the difference was not significant. Clutch size and sex ratio of progeny were not affected by irradiation (table 3).

4 Discussion

H. armigera eggs were effectively killed with UV-light and mortality increased with duration of exposure. The effect is most likely due to a destruction of the genetic material of the developing embryo. Eggs irradiated for 90 min, almost double the time necessary to kill 98% of the eggs, were not visibly affected by the treatment and did not shrivel, contrary to findings by SENGONCA and SCHADE (1991) for *Eupoecilia ambiguella* Hbn. (Lep., Tortricidae) eggs. They observed that eggs irradiated for 30 min, 50% longer than the time needed to kill almost all eggs, shrivelled within the first 2 days after treatment.

In the present study, *H. armigera* eggs irradiated longer than needed were less suitable for the development of *T. chilonis*. Parasitoid emergence was lower in eggs exposed for 60 or 90 min than in control eggs. The female parasitoids were not able to recognize this lower suitability as they accepted irradiated eggs and allocated the same clutch size with a similar sex ratio. Contrary to our findings, GOLDSTEIN et al. (1983) reported that *T. nubilale* reared on irradiated eggs of *O. nubilalis* showed no changes in adult emergence. This may be due to a relatively low exposure time or to the

Table 2. *Duration (in seconds) of drumming, drilling and ovipositing stage and the total time spent by* Trichogramma chilonis *on* Helicoverpa armigera *eggs irradiated with UV-light or untreated (n = 32)*

		Untreated	UV-irradiated 60 min	 90 min
Parasitism stage				
Drumming	$\bar{x} \pm$ SE	25.8 ± 1.47*a*	25.0 ± 1.79*a*	25.8 ± 1,73*a*
	range	15.3–37.8	13.4–41.7	11.0–56.7
Drilling	$\bar{x} \pm$ SE	53.1 ± 4.69*a*	39.8 ± 2.70*b*	39.7 ± 2.43*b*
	range	31.3–142.9	24.6–92.4	21.1–84.1
Ovipositing	$\bar{x} \pm$ SE	121.4 ± 4.79*a*	112.2 ± 4.00*a*	116.9 ± 4.43*a*
	range	84.5–204.5	68.8–178.6	80.0–178.4
Total time on host	$\bar{x} \pm$ SE	441.2 ± 34.38*a*	391.9 ± 53.79*a*	390.5 ± 35.46*a*
	range	177.6–1009.4	127.5–1930.9	136.9–931.6

Means within a row followed by the same letter are not significantly different ($P > 0.05$; Student's *t*-test)

Table 3*. Suitability of UV-irradiated or untreated* Helicoverpa armigera *eggs for* Trichogramma chilonis

Experiment	Duration of UV-irradiation (min)	Number of eggs parasitized	Percentage emergence	Sex ratio	Clutch size
I	0	268	96.9*a*	72.9*a*	1.53*a*
	60	125	94.9*ab*	75.1*a*	1.50*a*
	90	119	86.2*b*	63.3*a*	1.56*a*
II	0	32	90.6*a*	69.4*a*	1.79*a*
	60	32	87.5*a*	64.8*a*	1.68*a*
	90	32	78.1*a*	73.9*a*	1.63*a*

Means followed by the same letter within a column of an experiment are not significantly different ($P > 0.05$; Tukey test).

fact that they used a different host. Similar to our results they found no significant changes in other important biological parameters such as fecundity, sex ratio and clutch size, but did report a shorter lifespan when the parasitoids were reared on irradiated eggs. Other authors have not recorded any parameters to evaluate suitability of UV-irradiated eggs.

Rejection of host eggs after probing with the ovipositor could not have caused the observed difference in parasitoid emergence, as the percentage of eggs rejected in this stage of the parasitization behaviour was similar between irradiated and control eggs. Multiple insertions of the ovipositor, followed by host feeding, were also not responsible for the difference, as a similar number of insertions were made in eggs of the three treatment groups. Pak and Oatman (1982) have suggested that multiple insertions of the ovipositor could lead to shrivelling and death of host eggs.

The duration of the drilling stage is affected by the thickness and/or hardness of the chorion (Salt, 1938, 1940). Drilling on irradiated eggs was shorter than on control eggs, and irradiation probably caused morphological changes to the egg chorion. This may have caused physiological changes to the egg and could explain the greater parasitoid mortality. It was reported earlier that the permeability of the chorion is important for the acceptance of host eggs (Quednau, 1955; Fulmek, 1955; Taylor and Stern, 1971). But in our study no difference in the acceptance of irradiated eggs, which are easier to penetrate, and control eggs was observed. The changes to the egg chorion due to irradiation could also explain the observation by Şengonca and Schade (1991) that *E. ambiguella* eggs shrivelled following UV-irradiation. A second possibility to explain the observed differences in parasitoid mortality could be that an egg without a developing embryo is unsuitable for *T. chilonis* development. However, this is unlikely as it was found that *Trichogramma* females kill the host embryo within a few hours by injecting a venom with each oviposition (Strand, 1986).

The non-significant difference in drumming time between irradiated and control eggs was expected as its duration is directly dependent on the egg diameter and drumming is done by the parasitoid to measure the volume of the host egg in order to adjust clutch size (Schmidt and Smith, 1989). The size and shape of the eggs was not visibly affected by irradiation.

The duration of the different oviposition stages measured in this study could not be compared with those reported for other *Trichogramma* spp.-host systems (e.g. Pak and Oatman, 1982; Suzuki et al., 1984; Pak et al., 1986, 1990; Wajnberg, 1989), as the time varies with temperature (Pak and van Heiningen, 1985; Schmidt and Pak, 1991) and also with the parasitoid species or strain and host species used (Pak et al., 1986). However, it was generally found that at least half of the handling time is devoted to oviposition.

Because UV-killed *H. armigera* eggs are as frequently accepted as untreated control eggs, their use is recommended in laboratory experiments where good host acceptance is desirable (e.g. van Dijken et al., 1986; Hassan, 1989; Romeis et al., 1996). Irradiated eggs are also useful for monitoring *Trichogramma* populations in the field and to enhance natural parasitoid populations (Şengonca and Schade, 1991). In mass-rearing programs, host eggs should be irradiated for the minimum time needed as the negative effect of irradiation on parasitoid development depends on the duration of exposure. Laboratories using UV-light to kill host eggs should routinely evaluate the minimum exposure time needed for their specific UV-light source/distance conditions.

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