# Effects of UV-B on Environmental Preference and Egg Parasitization by *Trichogramma* Wasps (Hymenoptera: Trichogrammatidae)[1]

Kyle J. Van Atta, Kristen A. Potter, and H. Arthur Woods[2]

Division of Biological Sciences, University of Montana, Missoula, Montana 59812 USA

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**Abstract** The intensity of ultraviolet (UV) radiation varies widely in space, both across the globe and over small spatial scales (a few millimeters), depending on patterns of light and shade. Gradients of UV radiation can control the movement of organisms on landscapes and how the organisms perform. It is likely that insects are at high risk from UV radiation because of their small size; radiation may penetrate significantly deeper into insect tissues than into larger organisms, thereby disproportionately affecting their performance. We investigated the effects of UV radiation on the behavior and parasitism success of the agriculturally important egg parasitoid wasp, *Trichogramma* spp. We found that *Trichogramma* preferred to move toward higher intensities of UV-B radiation and parasitized more eggs in areas with higher UV-B radiation. However, higher UV-B radiation reduced the number of adult wasps emerging from host eggs. *Trichogramma* reproductive behavior may, therefore, be maladaptive depending on environmental context. These results could be of particular importance in the agricultural release of *Trichogramma,* especially in greenhouse settings, where levels of UV-B radiation are low.

**Key Words** egg parasitoid, *Manduca sexta*, phototaxis, preference-performance, ultraviolet radiation

*Trichogramma* egg parasitoids are one of the most-widely used biocontrol agents in the world because they are easy to rear and effectively destroy many insect pests in the egg stage before they feed on crops (Ksentini et al. 2011). Parasitoids also play important roles in natural systems by influencing plant and herbivore populations (Hairston et al. 1960). Parasitoids can have strong effects on the fitness of their host insects which, in turn, controls plant populations by limiting herbivory. Despite their importance, however, relatively little is known about the reproduction and development of *Trichogramma* (Cônsoli et al. 2010), partly because they are so much smaller than other insects (∼0.5 mm adult body length).

*Trichogramma* and other small insects may be particularly sensitive to ultraviolet (UV) radiation. The ultraviolet spectrum can be divided into three parts: UV-A, UV-B, and UV-C. In comparison, UV-A is the least harmful to organisms and UV-C is the most. However, UV-C is almost entirely blocked by the ozone layer and does

[1]Received 02 February 2015; accepted for publication 10 May 2015.

[2]Corresponding author (email: art.woods@mso.umt.edu), Division of Biological Sciences, University of Montana, 32 Campus Drive HS104, Missoula, MT 59812 USA.

not reach the biosphere (Paul and Gwynn-Jones 2003). UV-B (wavelengths 280–320 nm) reaches the biosphere and has damaging effects on a variety of species including insects. These effects include reduced feeding, deformities, reduced learning, free radical production, genetic defects, immunosuppression, and (in humans) sunburns and skin cancer (Longstreth et al. 1998, Blaustein et al. 2001, Kats et al. 2001, 2012, Mazza et al. 2002). Risks may be particularly high for insects because they are small and some of their life stages are immobile, although exoskeletons may provide some protection (Paul and Gwynn-Jones 2003).

*Trichogramma* reproduce by parasitizing the eggs of other insects, and these host eggs can receive drastically different intensities of UV-B depending on where they are positioned (Potter and Woods 2013). *Trichogramma* offspring have to endure these UV intensities for three full life stages before they eclose from a host egg as adults. Selection, therefore, may have favored behaviors and parasitization strategies that minimize their potential exposure to UV-B. Eggshells of host eggs provide one line of defense during this vulnerable time. The eggshells block a substantial proportion of the incoming UV-B, reducing UV-B damage and shielding the developing wasps (Potter and Woods 2013). Even so, selecting host eggs with lower levels of abiotic danger may be important for females.

Many insect species have photoreceptors that allow them to perceive UV (Romeis et al. 1998, Briscoe and Chittka 2001, Mazza et al. 2002, Lobdell et al. 2005, Foggo et al. 2007), and these photoreceptors probably originated over 350 million yr ago (Paul and Gwynn-Jones 2003). Insects use UV vision for navigation, orientation, host-finding, feeding, and partner selection (Raviv and Antignus 2004, Diaz and Fereres, 2007, Fischer et al. 2011). Fischer et al. (2011) showed that the very small eyes (1/40th the eye size of the common wasp *Vespula vulgaris* L.) of one *Trichogramma* species were also sensitive to UV radiation. *Trichogramma* may, therefore, adjust their behavior and oviposition sites in response to UV to limit damage to their offspring. In many agricultural systems, *Trichogramma* are released in greenhouses with UV-blocking materials, which may affect patterns of behavior and oviposition.

We used *Trichogramma* wasps to examine whether (a) *Trichogramma* prefer or avoid UV-B, and (b) UV-B radiation affects the performance of adults and their offspring. We used *Trichogramma* wasps obtained from a wild population in southeastern Arizona together with eggs of *Manduca sexta* L. We examined the responses of *Trichogramma* to experimental variation in levels of UV-B, both with and without access to host eggs, and also examined the effects of UV-B on the development and eclosion of wasps from host eggs.

## Materials and Methods

Tests were conducted using a newly established colony of *Trichogramma* (Hymenoptera: Trichogrammatidae) emerging from eggs of *M. sexta* collected within a few kilometers of Portal, AZ, USA. At this field site, *Trichogramma* often parasitize host eggs on the tops of leaves (K.A.P. unpubl. data), and the UV-B levels tested here are similar to those that occur in the field (Potter and Woods 2013). Two *Trichogramma* species occur at this site (*Trichogramma deion* Pinto & Oatman and *Trichogramma sathon* Pinto), so both were likely included in our colony

(Potter and Woods 2012). The two species are similar in appearance, development, and metabolic physiology (Potter and Woods, 2012) and can be distinguished only by genotype (Stouthamer et al. 1999). Preliminary preference tests using an established colony of *T. deion* alone yielded results similar to those presented here.

The UV lamp used for these tests was an XX-15L UV bench lamp (UVP, Upland, CA, part #95-0042-03) fitted with a 302-nm Sankyo Denki G15T8E bulb (UVP, part #34-0039-01). All UV-B measurements were taken with an intensity meter (G&R Labs, Santa Clara, CA, model #202) outfitted with a custom-ordered UV-B filter (G&R Labs, 280–320 nm).

**Preference of *Trichogramma* for UV-B.** Groups of five wasps were introduced into a 2.5-cm long clear plastic tube (5 mm outside diameter, 3 mm inside). Wasps of both sexes were loaded into the plastic tube, first by placing them into a freezer for 30 s (which moderately slowed their activity) and then rapidly transferring them into the plastic tube with a fine-bristled paintbrush. Each end of the tube was plugged with a small piece of cotton and connected to an empty Petri dish arena (9 cm diameter), one of which was covered with plastic wrap and the other with Plexiglas. Mean UV-B intensities in the arenas were 0.40 mW/cm$^2$ (plastic wrap side) and 0 mW/cm$^2$ (Plexiglas side). The cotton was removed from the tube at the start of the experiment, and wasps were allowed to move freely between arenas for 2 h. All experiments were conducted in an incubator so that temperature and humidity were stable (30°C, ~70% relative humidity). Besides the UV-B lamp, an artificial white light source also illuminated the inside of the incubator. After 2 h, the numbers of wasps in both arenas were counted. A few wasps were found stuck in small sections of the tape holding the plastic or Plexiglas on the Petri dishes or could not be found after the experiment ended. These wasps were excluded from analysis. This experiment was repeated 21 times.

**Parasitization of host eggs.** This experiment tested how UV-B affects the number of eggs parasitized by *Trichogramma*. Female wasps were placed in Petri dish arenas (5 wasps per arena) each containing 15 *M. sexta* eggs. Host eggs were $<$24 h old and the *M. sexta* embryos had been killed (by freezing for ~2 h) to account for differences between treatments due to UV-B effects on host embryos. The arenas in this experiment had the same type of filters on them as described above, but the arenas were not connected. The mean UV-B intensities were 0.26 mW/cm$^2$ (plastic wrap side) and 0 mW/cm$^2$ (Plexiglas side). The wasps were given access to host eggs for 2 h. The eggs were then held until they could be scored for successful parasitization, which became visible after about 4 d. This experiment was repeated 5 times.

**Egg survival.** Freshly-laid ($<$24 h old) *M. sexta* eggs collected from the wild and from a colony were exposed to the wild *Trichogramma* colony for 24 h. The following day, the eggs were removed and divided equally between two Petri dishes. One dish was covered with plastic wrap and the other with Plexiglas. Both dishes were placed under a UV-B lamp for 4 h daily for 6 d; radiation intensity in each dish was 0.15 mW/cm$^2$ (plastic wrap) or 0 mW/cm$^2$ (Plexiglas). After the UV-B exposure on day 6, eggs were stored individually in 30-mL plastic cups until wasps emerged, a *M. sexta* larva hatched, or the egg and its contents were determined to be dead. The number of emerging wasps was quantified for each egg. Unsuccessfully parasitized eggs were removed from the analysis, leaving a total

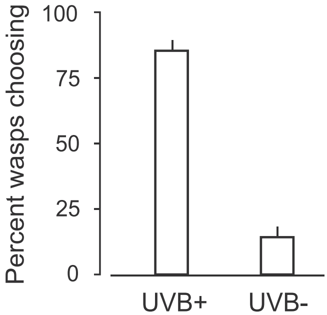


**Fig. 1. Binary choice assays of adult *Trichogramma* response to arenas with and without UV-B exposure expressed in mean (bar = SE) percentage of wasps responding. Differences were statistically significant ($\chi^2$ = 49.5, df = 1, $P < 0.001$).**

of 7 eggs from the plastic wrap arena and 5 eggs from the Plexiglas arena after excluding nonparasitized eggs.

**Statistics.** Data were analyzed using the R statistical package version 2.15.0 (R Development Core Team 2012). To analyze the preference experiment we used a proportions test to compare the mean proportions of wasps choosing high versus low UV-B arenas. Treatment data from the parasitization experiment were analyzed using analysis of variance to compare the fit of generalized linear mixed-effects models with and without UV, and survival data were analyzed using a likelihood ratio test to compare the fit of generalized linear models with and without UV.

## Results

**Preference of *Trichogramma* for UV-B.** Our data suggest that *Trichogramma* strongly prefer UV-B radiation ($\chi^2 = 49.5$, df = 1, $P < 0.001$) (Fig. 1). Ninety-four wasps were given a choice in this experiment. Ten of the 94 were excluded from analysis because they either stuck to sections of tape or disappeared before the end of the experiment. Of the remaining wasps, ~85% (71 of 84) chose the plastic wrap arena (UV-$B^+$) and ~14% (12 of 84) chose the Plexiglas arena (UV-$B^-$). One wasp (~1%) did not move to either end and was still in the center tube at the end of the experiment.

**Parasitization of *M. sexta* eggs by *Trichogramma*.** We observed higher parasitization rates in the plastic wrap arena (UV-$B^+$) than in the Plexiglas arena (UV-$B^-$) ($\chi^2 = 30.36$, df = 1, $P < 0.001$) (Fig. 2). Twenty-three percent (17 of 75) of eggs were parasitized in the plastic wrap arena (UV-$B^+$) and 1.3% (1 of 75) of eggs were parasitized in the Plexiglas arena (UV-$B^-$).

**Egg survival.** UV-B exposure significantly reduced the number of wasps emerging from parasitized host eggs ($\chi^2 = 10.97$, df = 2, $P < 0.01$) (Fig. 3). Five of the 11 eggs in the UV-$B^+$ treatment were successfully parasitized; six unparasitized eggs were subsequently discarded from the analysis. The parasitized eggs in the UV-$B^+$ treatment produced a mean of 8.6 (±0.68) adult wasps per egg (43 total). Seven of the 10 eggs in the UV-$B^-$ treatment were successfully parasitized and the

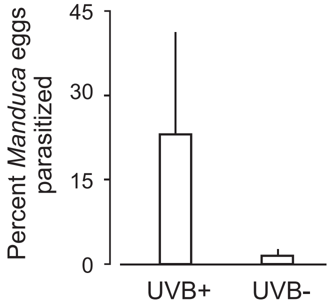


**Fig. 2. Mean (bar = SE) *M. sexta* eggs parasitized by *Trichogramma* in arenas with and without UV-B exposure. Differences were statistically significant ($\chi^2 = 30.36$, df = 1, $P < 0.001$).**

other three were discarded from analysis. The parasitized eggs in the UV-B$^-$ treatment produced a mean of 23.6 ($\pm$3.57) adult wasps (165 total). The origin of the host eggs (wild versus colony) had no significant effect on the number of emerging adult wasps ($P > 0.5$).

## Discussion

When given a choice in binary assays, *Trichogramma* strongly preferred UV-B radiation to no UV-B, a result consistent with data presented by Brower and Cline (1984). In our experiments, the UV-B$^+$ arenas were exposed both to UV-B and to additional light from the fluorescent (non-UV) bulb mounted in the incubator, whereas the UV-B$^-$ arenas were exposed only to white light. Thus, the UV-B$^+$ arenas contained both more UV-B and more total photons that the wasps could sense. The observed preference could reflect (a) that wasps preferred UV-B specifically, or (b) that they oriented toward the arena with the larger total flux of photons. These possibilities would have to be untangled in additional work.

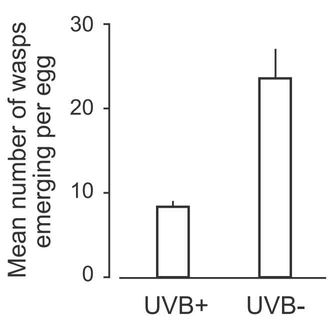


**Fig. 3. Mean (bar = SE) number of *Trichogramma* parasitoids emerging from *M. sexta* eggs maintained in arenas with and without UV-B exposure. Differences were statistically significant ($\chi^2 = 10.97$, df = 2, $P < 0.01$).**

Nevertheless, a preference for UV (or, operationally, or, operationally, sensible photons) may be general among parasitoids, as *Eretmocerus mundus* Mercet, a parasitoid in the same superfamily, exhibited similar behavior (Chiel et al. 2006). Even more broadly, many flying insects prefer higher UV intensities, which typically indicates an affinity for open spaces (Gao et al. 2008). Again, such an effect could arise from preferring UV-B specifically or from preferring higher total light intensities.

Although adult *Trichogramma* oriented toward the UV-$B^+$ arenas, and preferentially parasitized eggs in them, their offspring suffered as a result. From an evolutionary perspective, this behavior seems maladaptive. Additional factors affecting oviposition may explain this strategy. For example, females may choose sites that maximize their own survival; perhaps high-UV microsites are less likely to contain other predaceous insects. Or, perhaps high UV partially sterilizes eggshell surfaces, minimizing the risk of host eggs becoming infected via the wound made by the wasp's ovipositor. Leaf surfaces support a diverse microbiome (Lindow and Brandl 2003), including entomopathogenic bacteria, viruses, fungi, and nematodes. Exposure to ultraviolet radiation can strongly depress the pathogenicity of these agents (David 1969, Gaugler and Boush 1978). If the danger from infection outweighs the danger from high UV, ovipositing in high UV may have a net positive influence on offspring produced. A third possibility is that females mate in high-UV microsites and then simply choose to oviposit nearby (Gripenberg et al. 2010, Refsnider and Janzen 2010, Potter et al. 2012).

Parasitoids, like *Trichogramma*, play important roles in structuring natural populations of herbivores and plants (Hairston et al. 1960, Cronin and Reeve 2005), and they can be inexpensive, effective methods of biocontrol (Nagarkatti et al. 2003). The environmental and sensory factors that affect host finding are, therefore, relevant both to understanding their basic ecology and to using them more efficiently in agricultural settings. Laing (1937) showed that visual stimuli are important to *Trichogramma*. Our research suggests two potential problems with using *Trichogramma* in greenhouses, which generally are constructed with UV-blocking plastics and glass. The first is that local sources of UV radiation (e.g., from ventilation windows) may strongly attract parasitoids, causing them to shift away from the plants and potential insect hosts. The second possibility is that lack of UV may impair the vision of *Trichogramma* during host-finding and make parasitization more difficult. This second issue would arise only if UV were one of the primary wavelengths that wasps used. In addition, in both applied and agricultural contexts, more research is necessary to determine (a) whether or not the preference for high UV affects parasitoid behavior in the field and, if so, what effects on performance it has, and (b) why fewer offspring emerge from eggs exposed to UV-B, e.g., to determine whether females inject fewer eggs in high-UV microsites or whether wasp eggs or larvae are killed by UV-B exposure inside the host egg.

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