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Lethal and sub-lethal effects of UV-B radiation exposure on the collembolan *Folsomia candida* (Willem) in the laboratory

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ABSTRACT

This study assessed the effects of UV-B irradiation on the physiology, life history, DNA, and behavior of the blind non-pigmented soil-dwelling collembolan *Folsomia candida* (Willem). In three sets of controlled laboratory studies, adult *F. candida* were reared in darkness and exposed to light with no UV-B (filtered), low UV-B or high UV-B (weighted irradiance energy of 3.12 mW/m^2 and 22.1 mW/m^2, respectively). Study I investigated UV-B impacts on mortality, egg viability, and egg development time of *F. candida* under continual exposure. Study II assessed the movement patterns, mortality, egg production and fecundity of adult *F. candida* in response to UV-B exposure within choice environments. Study III determined the degree of UV-B induced DNA damage (thymine dimer formation) on *F. candida* and subsequent DNA repair. Continuous irradiation resulted in increased mortality, under high and low UV-B conditions compared to controls. Consistent with other studies on the impact of light on collembola, we found that *F. candida* avoided light when given the option. Though preferring darkness, animals were more likely to venture into lighted regions with no UV-B (filtered) and experienced higher mortality rates than in regions with high or low UV-B. Eggs were laid preferentially in the dark, with fewer total eggs observed in the habitat with the highest UV-B. We further demonstrated that UV-B induced the formation of thymine dimers in a dose dependent manner. DNA repair was not evident in animals that had returned to the dark after a brief intense UV-B irradiation. These findings indicate that UV-B exposure and exposure to longer wavelengths of light has both lethal and sub-lethal effects on *F. candida* that can adversely affect its survival.

Introduction

Anthropogenic-induced thinning of the ozone layer in the upper atmosphere has focused interest on the impacts of UV light exposure on organisms and the ecosystem processes they engage in (Caldwell et al. 1998, 2007). Exposure to elevated levels of UV-B (280–320 nm) has been linked to changes in plant growth rates, the quality of plant tissues, the allocation of photosynthate from shoots to roots and the decomposition of surface plant litter (Klironomos and Allen 1995; Verhoef et al. 2000; King et al. 2003). Enhanced UV-B exposure in the field has resulted in decreased population densities of microbes and the diversity and density of surface-active arthropods, particularly among non-pigmented forms (Verhoef et al. 2000). Ambient levels of UV-B radiation may deter the activities and suppress the densities of soil organisms as well. King et al. (2003) report that mites and Collembola with little or no pigment were found in higher densities in soils that were shielded from UV-B compared to unshielded controls, while heavily pigmented mites showed no response.

The responses of soil organisms to enhanced or reduced UV-B exposure in the field include direct and indirect effects. Indirect effects of UV-B may be expressed as altered trophic interactions between soil organisms and plants, and plant-based detritus. These effects are mediated through UV-B induced changes in plant physiology and chemistry. Direct effects of UV-B on soil organisms include changes in physiology and behavior, as well as cellular damage and mutagenic effects on DNA, e.g., dimer formation. Organisms sensing UV-B radiation in some manner may migrate away from irradiated areas. Direct physiological responses include a combination of lethal and sub-lethal responses such as increased mortality, reduced fecundity or egg viability. Physiological responses of soil organisms and alterations in their life histories to changes in environmental conditions, or exposure to physical and chemical mutagens are well documented, as are behavioral responses toward or away from external stimulation (e.g., light, temperature, chemical).

In this paper we present the results from three laboratory studies designed to assess the effect of UV-B radiation exposure on

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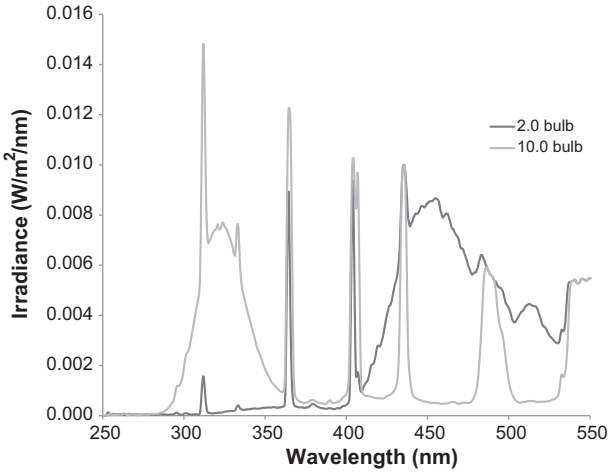

Fig. 1. Wavelength characteristics of the Exo Terra Repti Glo® fluorescent light bulbs (Terra, Mansfield, MA) designated 2.0 (low UV-B) and 10.0 (high UV-B) 26-watt fluorescent UV-B emitting bulbs. The spectra of the 2.0 and 10.0 bulbs were measured using a Stellarnet model EPP2000 spectrometer (Tampa, FL) equipped with a fiber-coupled, quartz diffuser fore optic. The weighted irradiance energy or intensity of radiation of the 2.0 or 'low UV-B' bulbs and 10.0 or 'high UV-B' bulbs was 3.12 mW/m^2 and 22.1 mW/m^2, respectively, estimated using the erythemal action spectrum of McKinlay and Diffey (1987) used to measure the carcinogenic effectiveness on human skin. The erythemal action spectrum is useful in comparing the relative effectiveness of different wavelengths producing a biological response. The lamps used here differ in their spectra at longer wavelengths. The higher energy UV-B portion, which carries the greatest weighting, is assumed to be most effective at eliciting a reaction.

the blind non-pigmented soil dwelling collembolan *Folsomia candida* (Willem). Study I explored the impacts of UV-B irradiation on movement and egg laying patterns of adult *F. candida*. Study II investigated the effects of UV-B on the life history and physiology (e.g., mortality, fecundity, egg viability, gestation period, and instar duration) of *F. candida*. Study III determined the degree of UV-B induced DNA damage (thymine dimer formation) on *F. candida* exposed to different intensities and times of irradiation.

Materials and methods

Test animals

The studies were conducted on the collembolan *Folsomia candida* (Willem). This species is devoid of pigment, appearing white, and does not possess patches of ocelli on their head or on other parts of their bodies. Adult *F. candida* (~1 mm in length) were obtained from laboratory cultures that were initiated from adults collected from the sediments within the caverns of Wind Cave, South Dakota. The identity of the test animals was confirmed using the keys of Christiansen and Bellinger (1980) and by molecular analysis of the 28S rDNA and mt*COI* genes. The test animals are parthenogenic as evidenced by long-term observations of the cultures (Moore et al. 2005). The cultures have been maintained in darkness since their inception and the caverns from which they originated are devoid of light.

Test arenas

The test arenas for the experiments were 2.5-gallon aquaria with the bottoms lined with about 3 cm of media consisting of charcoal:Plaster of Paris:water (1:1:2) *sensu* Snider et al. (1969). The cured media was moistened with sufficient water to wet it without leaving standing water. Bulbs that emitted either low-levels or high-levels of UV-B lighting were fitted to the ceiling lid for each test arena. The bulb spectra and outputs are summarized in Fig. 1. The weighted irradiance energy or intensity of radiation of the 2.0 or 'low UV-B' bulbs and 10.0 or 'high UV-B' bulbs was 3.12 mW/m^2 and 22.1 mW/m^2, respectively. For each experiment, adult *F. candida* were placed in test arenas in the number indicated, and replicate test arenas were placed in a larger container that was supplied with water for humidity, a temperature monitor (HOBO temp®), covered with a lid and wrapped in black backing to block any outside light.

Study I – effects of continuous exposure

To determine the effect of continual irradiation on *F. candida*, adults and eggs were either kept in constant darkness or exposed to constant light with either the 2.0 or 10.0 UV-B bulbs. The test arenas were set up in a manner that maintained the same constant temperature and humidity for all treatments. For adults, in each of 18 trials, 25 adult *F. candida* were placed in a 120 ml glass jar with 0.5 cm of an agar solution at the bottom (agarose from Promega, Madison, WI). The jars were placed inside the test arenas fitted with the different light levels. For eggs, in each of three trials, five eggs were collected the day they were laid and placed in jars as described above. The live or dead status of adults in the trials described above and time to hatching (days that juveniles were present) for the trials with eggs, were tracked and recorded for 2 weeks, twice a day (9 a.m. and 7 p.m.). Corpses and juveniles were recorded and removed when encountered.

Study II – behavioral and life history responses to exposure

For each trial (n = 7–16), 15 adult *F. candida* were placed in test arenas fitted with a 10.0 bulb (n = 16), 2.0 bulb (n = 16), or 0 bulb (UV-B filtered 2.0 bulb, n = 7); five in a continuously illuminated area of the arena, five in a partially shaded area, and five in a dark area. The arenas were separated into three equal areas with black foam board with a 2 cm gap at the bottom so *F. candida* could move freely. The light was blacked out of one and a half of the three areas with black foam board. For controls (UV-B filtered 2.0 bulb), a glass lid was placed between the 2.0 bulb and the arthropods to block the UV-B in the test arenas. Three grains of baker's yeast was added to each area every 2 days, and water added to the surface of the medium for moisture. The location of and the number of eggs laid by the *F. candida* was tracked and recorded for 2 weeks twice a day (9 a.m. and 7 p.m.). Corpses and eggs were recorded and removed when encountered.

Study III – effects of continuous UV-B exposure on DNA

Groups of 20 adult *F. candida* were exposed to UV-B irradiation using the 2.0 and 10.0 bulbs for 4 days. A control group was irradiated using the same set-up for 14 days with the aquarium glass lid set between the bulbs and the animals to filter-out the UV-B. The DNA from the irradiated adults from each trial was purified and tested for the presence of thymine-dimers using the assay described below. The blots were checked for the presence of thymine dimers by chemiluminescence. To determine if DNA damage could be repaired by *F. candida*, groups of collembolans were exposed to 20 min of ultraviolet radiation from a transilluminator and were returned to the dark. DNA was isolated at given intervals and examined for the presence of thymine dimers.

Thymine-dimer assay

DNA damage was determined by the presence of thymine-dimers. Purified DNA from irradiated *F. candida* and Salmon sperm DNA (Trevigen, Gaithersburg, VA) as a positive control was examined by Western blot *sensu* Sinha et al. (2001). Genomic DNA was purified using the DNeasy kit from Qiagen (Valencia,