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Foraging Behavior of Honey Bees (Hymenoptera: Apidae) on *Brassica nigra* and *B. rapa* Grown Under Simulated Ambient and Enhanced UV-B Radiation

SEAN A. COLLINS, JEFFREY K. CONNER,[1] AND GENE E. ROBINSON

Department of Entomology, University of Illinois, 320 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801

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ABSTRACT Two species of mustard, *Brassica nigra* and *B. rapa*, were grown under simulated ambient and enhanced ultraviolet-B (UV-B) radiation and exposed to pollinators, *Apis mellifera* L. Observations were made to determine whether UV-B-induced changes in these plants affected pollinator behavior. Total duration of the foraging trip, number of flowers visited, foraging time per flower, search time per flower, total amount of pollen collected, and pollen collected per flower were measured. There were no significant differences between UV-B treatments in any of the behaviors measured or in any of the pollen measurements. These results suggest that increases in the amount of solar UV-B reaching the earth's surface may not have a negative effect on the relationship between these members of the genus *Brassica* and their honey bee pollinators.

KEY WORDS *Apis mellifera, Brassica nigra, Brassica rapa,* foraging behavior, ultraviolet-B radiation

THERE HAS BEEN a great deal of concern over increasing levels of potentially harmful solar radiation reaching the earth's surface (van der Leun et al. 1991). Most of this concern has centered on ultraviolet-B (UV-B) (λ = 285–320 nm), the part of the UV spectrum that is increasing most with ozone destruction. UV-B can damage organisms in a variety of ways (Giese 1964, Caldwell 1971).

Many studies have documented harmful effects of enhanced levels of UV-B on animals (Bothwell et al. 1994, McCloud and Berenbaum 1994) and plants (reviewed by Teramura 1990, Bornman and Teramura 1993). To date, the only studies on the effects of UV-B on insect–plant interactions have been in the context of herbivory (Bothwell et al. 1994, Hatcher and Paul 1994, McCloud and Berenbaum 1994). The effect that elevated levels of UV-B may have on the symbiotic relationship between plants and their insect pollinators has not been studied previously.

The probability of an insect visiting flowers of a given plant species often enough to be an effective pollinator is determined in large part by the ability of the insect to locate floral resources and acquire a sufficient reward. Elevated levels of UV-B can cause plants to reallocate biomass from reproduction to growth and maintenance or other necessary functions (Teramura 1990). As a result, increased UV-B may reduce the number or size of flowers produced, decrease the quantity or quality of pollen and nectar, or both. All these traits have been shown to affect pollinator visitation (reviewed by Kearns and Inouye 1993). In addition, elevated levels of UV-B may affect the relative abundance or attractiveness of a given plant species in an area because of interspecific differences in tolerance levels. Such alterations in pollinator visitation may ultimately lead to serious consequences for plant reproduction and crop production as well.

We examined the foraging behavior of honey bees on 2 species of *Brassica* that were grown under elevated UV-B radiation. The genus *Brassica* contains many species of plants widely grown as crops (Free 1993). Many members of this genus require pollination for successful reproduction (Free 1993) and honey bees are among the most important pollinators of this genus (Sihag 1986, Williams et al. 1987, Singh and Singh 1992). Therefore, alterations in the foraging behavior of honey bees on *Brassica* caused by UV-B could have wide-ranging effects on both natural and agricultural ecosystems.

Materials and Methods

We used rapid-cycling strains of *Brassica nigra* (black mustard) and *Brassica rapa* (syn. *B. campestris*; field mustard, turnip, rutabaga, oilseed rape, canola) (Williams and Hill 1986); these strains

[1]Department of Ecology, Ethology and Evolution, University of Illinois, Shelford Vivarium, 606 E. Healy Street, Champaign, IL 61820. Current address: Kellogg Biological Station, Michigan State University, 3700 E. Gull Lake Drive, Hickory Corners, MI 49060.

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flower at ~21 d and produce new flowers every 48 h. Seeds were obtained from the Crucifer Research Genetics Cooperative, Madison, WI. Members of the family Brassicaceae have been shown to be sensitive to enhanced levels of UV-B based on measurements of physiological and growth characters (e.g., Wilson and Greenberg 1993, Feldheim and Conner 1996).

Experimental Plants. Seeds of *B. nigra* and *B. rapa* were germinated and grown in a mixture of 1–1–1 soil–peat–perlite in a greenhouse at the University of Illinois Plant Sciences Laboratory in Urbana. They were exposed to UV-B for 5 h/d centered around solar noon from the time they were planted until they were no longer being used in trials (following 5 exposures to the bees). The average daily dose consisted of 6.12 kJ m^{-2} $UV\text{-}B_{BE}$ (6 kJ treatment), which simulates the average daily level of UV-B irradiance at this latitude during mid-summer (Green 1983), or 17.24 m^{-2} $UV\text{-}B_{BE}$ (17 kJ treatment). The UV-B dose was measured from the average height of the flowers on the plants on each bench (i.e., each treatment). UV-B levels were based on the biologically effective dose according to the generalized plant-damage spectrum proposed by Caldwell (1971). For a more detailed description of the experimental plants see Feldheim and Conner (1996).

Plants were grown under these conditions to ensure that the only serious stress that they were exposed to was enhanced UV-B. In this way we could determine the effects of UV-B alone on these plants and whether these effects would result in behavioral changes for foraging honey bees.

Pollinators. A single average-sized colony of honey bees (≈20,000 individuals) was housed in a plastic screen mesh cage 2.13 by 2.44 by 5.48 m in a stand of trees outside the University of Illinois Shelford Vivarium in Champaign. The colony was provided with water, a 25% solution of sucrose, and ground pollen ad libitum. The pollen was removed from the flight cage while foraging behavior was being observed. The colony was inspected every 10 d to ensure that the queen was laying eggs and the colony was otherwise functioning normally. Absence of a queen or other abnormal colony conditions can affect foraging behavior (Free 1967).

Observation Techniques. Following their daily UV-B treatment, plants were transported from the greenhouse to the flight cage for observations of foraging behavior. Observations were conducted from 26 July to 27 August 1994, starting at 1400 hours (CDT). Each of the 4 groups of plants (2 treatment groups, 2 species each) used on a day were exposed to bees for ≈30 min. Pilot studies indicated that this period was optimal for observing a sufficient number of bees without totally depleting the flowers of pollen. One flat of 14–18 plants from 1 of the 4 groups was placed ≈1 m from the entrance of the colony just before the start of observations. The daily order of exposure for each of the 4 treatment groups was randomized to eliminate the possibility of an order effect.

Observations were conducted only on bees foraging exclusively for pollen. Pilot studies allowed us to discriminate between pollen- and nectar-foraging honey bees on the basis of their behavior while on a flower. Each bee was observed for the duration of its foraging trip. Following the return of each bee to the colony, an attempt was made to capture it to measure its pollen load. However, because the entrance of the hive was not modified in any way, some observed bees entered the hive before they could be captured. Pollen loads from each captured bee were removed and weighed to ±0.1 mg. Four aspects of foraging behavior were measured, as follows: (1) total trip duration, the total amount of time a bee spent away from the colony, starting from the time it visited its first flower and ending when it returned to the colony entrance; (2) flowers visited per trip, the number of flowers a forager visited and attempted to collect pollen from during a foraging trip; (3) foraging time per flower, calculated by dividing the total amount of time that a bee spent actually foraging on the flowers it visited (foraging time) by the number of flowers visited by that bee; this measure allowed us to determine the average amount of time foraging bees required to collect pollen from individual flowers while on a foraging trip and (4) search time per flower, the amount of time that a bee spent flying between flowers (total trip duration minus foraging time) divided by the number of flowers visited. These data allowed us to determine the amount of time that a bee spent searching for the next adequate flower to forage upon while it was on a foraging trip. These 4 variables were related by the following equation:

$$T = N \times \left(\frac{\text{search time}}{\text{flower}} + \frac{\text{foraging time}}{\text{flower}} \right)$$

where T = the total trip duration and N = the total number of flowers visited.

Data Analysis. Two-way analyses of variance (ANOVAs) were performed with each of the above behavioral and pollen measures as dependent variables and UV-B treatment and plant species as independent variables. Multivariate analysis of variance (MANOVA) also was performed, with all of the behavioral measures as dependent variables and the UV-B treatment and plant species as independent variables. Two-way ANOVAs were done using Statview IV for Macintosh (Abacus Concepts 1992), and MANOVAs were done using JMP (SAS Institute 1994).

Results and Discussion

In total, 258 foraging honey bees were observed; 101 of those were captured for pollen analyses. Two-way ANOVAs indicated no effect of species or treatment, or species treatment interaction ($P >$

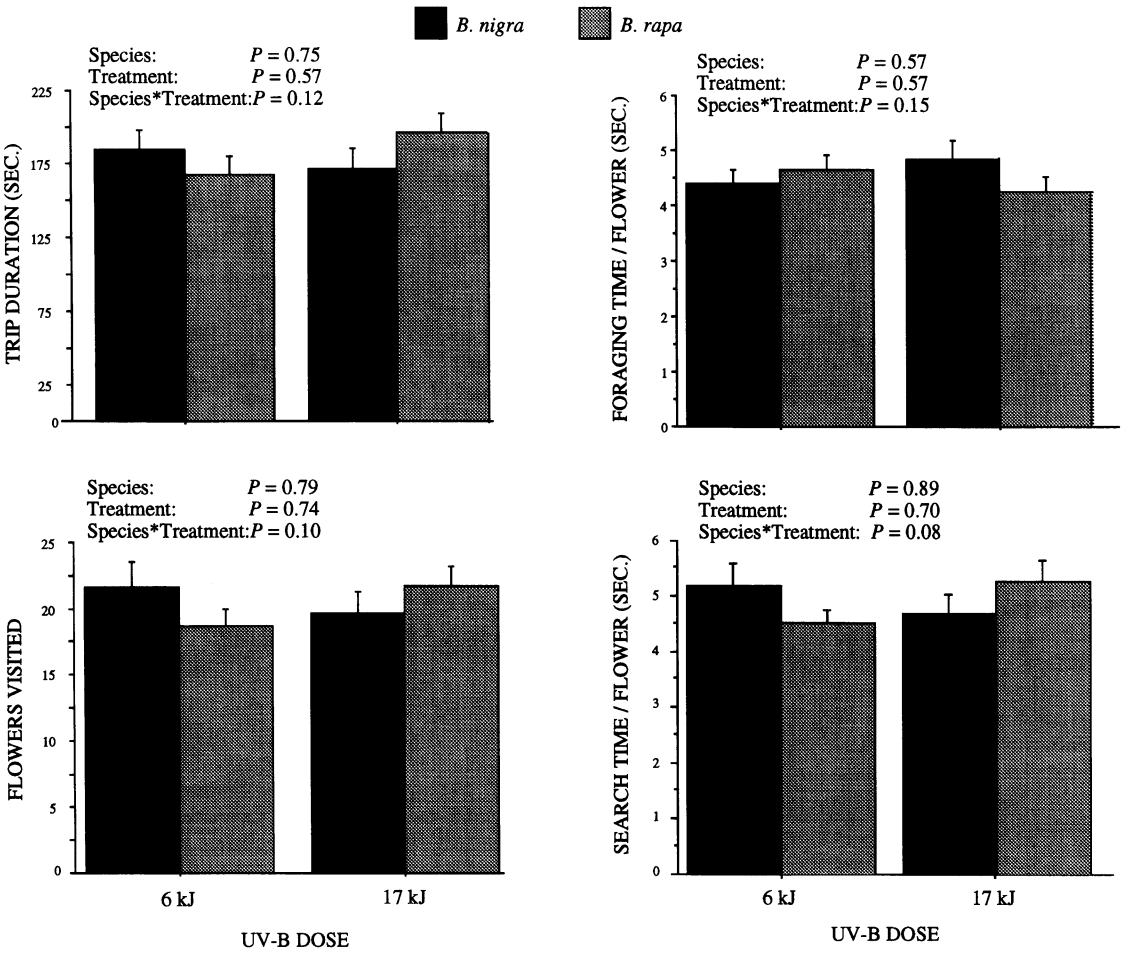

Fig. 1. Effects of elevated UV-B on pollinator behavior. Means and standard errors for each of the behavioral measures are shown. Results of 2-way ANOVAs are above each graph. n = 66 for *B. rapa* and 63 for *B. nigra*.

0.05) for the direct behavioral measurements (Fig. 1) or pollen measurements (Fig. 2). MANOVAs also revealed no significant effects (Table 1). There was a trend toward a species treatment interaction effect in all the behavioral measures. These results indicate that, under our conditions, growing plants under elevated levels of UV-B did not cause significant differences in foraging behavior among the honey bees observed in this study. Although plant characteristics were not measured in the current study, Feldheim and Conner (1996) found reductions in flower production but no change in nectar or pollen production using identical protocols.

Table 1. Results of MANOVA for the behavioral measures shown in Fig. 1

Parameter	*F*	*P*
Whole model	0.9788	0.4759
Species	0.7003	0.6237
Treatment	0.4222	0.8331
Species × Treatment	1.7931	0.1147

Ultraviolet-B-induced differences in pollinator behavior were not observed in this study. As always, there exists the possibility that different experimental conditions could lead to different results. Because the colony used in this study was maintained inside a flight cage, it is possible that the observed forager behavior was not natural. However, the time spent foraging per flower by bees in this study ranged from 4.2 to 4.6 s per flower (Fig. 1), which is very similar to previous field observations of Langridge and Goodman (1975, 1982) and Morse (1986).

The plants grown for this study received a rather high dose of UV-B but otherwise were exposed to a much less stressful environment than if they had been grown outdoors. Plants in nature often experience a combination of stresses simultaneously. Some plants have been shown to become less sensitive to UV-B when other stresses are present (Teramura et al. 1984, Balakumar et al. 1993). This suggests that studies incorporating a combination of UV-B and additional plant stresses would not be

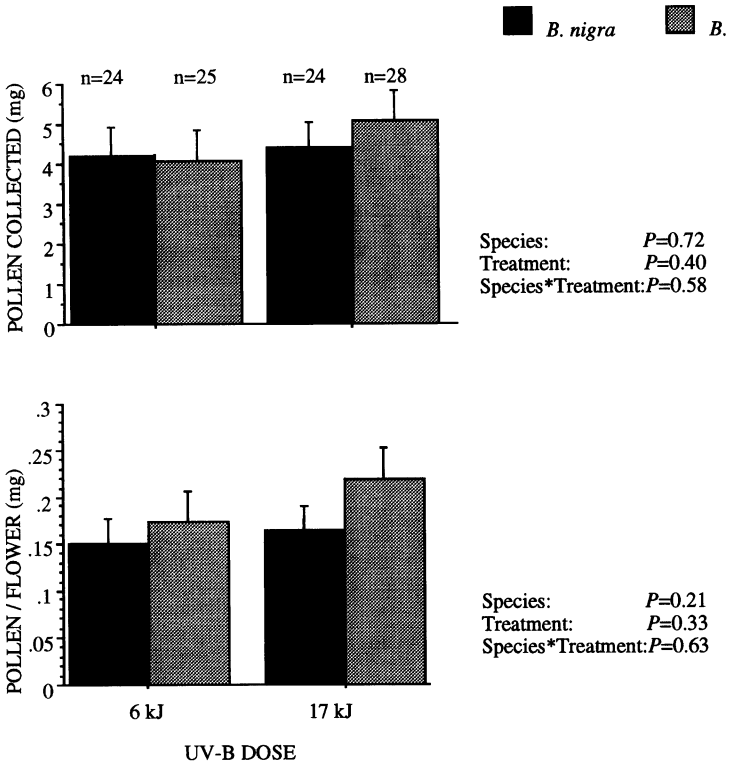

Fig. 2. Effects of elevated UV-B on amount of pollen collected (see Fig. 1 for details).

likely to find differences in pollinator foraging behavior.

Because there are interspecific, interstrain, and intercultivar differences in tolerances for elevated levels of UV-B in plants (e.g., Biggs et al. 1981, Teramura and Murali 1986, D'Surney et al. 1993), the results of the current study cannot be generalized to other species without caution. It is necessary to conduct similar studies using different species of plants and pollinators, as well as complementary field studies, to test further the generality of our results. The findings of this 1st study do suggest, however, that elevated levels of UV-B alone have no effect on the foraging behavior of honey bees on rapid-cycling strains of *B. nigra* and *B. rapa*.

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