Journal of Chemical Ecology, Vol. 20, No. 3, 1994

STRATOSPHERIC OZONE DEPLETION AND PLANT-INSECT INTERACTIONS: EFFECTS OF UVB RADIATION ON FOLIAGE QUALITY OF *Citrus jambhiri* FOR *Trichoplusia ni*

ERIC S. McCLOUD* and MAY R. BERENBAUM

Department of Entomology
University of Illinois
Urbana, Illinois 61801

(Received June 17, 1993; accepted October 18, 1993)

Abstract—Projected decreases in stratospheric ozone may result in increases in shortwave ultraviolet (UVB) irradiation at the earth's surface. Furanocoumarins, phototoxic compounds found in *Citrus jambhiri* foliage, increase in concentration when these plants are grown under enhanced UVB. Survivorship schedules of *Trichoplusia ni* (Lepidoptera: Noctuidae) caterpillars reared on plants in the presence and absence of enhanced UVB regimes differ significantly; larvae develop more slowly in early life when reared on plants exposed to increased UVB. This same developmental pattern is observed when *T. ni* larvae are reared on artificial diets amended with ecologically appropriate amounts of furanocoumarins. Thus, anthropogenically derived changes in stratospheric ozone and concomitant changes in UV light quality at the earth's surface may influence ecological interactions between insects and their host plants by altering secondary metabolism and hence foliage quality for herbivores.

Key Words—*Citrus jambhiri*, *Trichoplusia ni*, Lepidoptera, Noctuidae, bergapten, furanocoumarins, phototoxins, plant-herbivore interactions, psoralen, ultraviolet-B radiation.

INTRODUCTION

Current evidence suggests that anthropogenically derived atmospheric inputs have caused, and will continue to cause, decreases in stratospheric ozone levels

*To whom correspondence should be addressed.

(International Ozone Trends Panel, 1988). The result of these decreases will be an increase in ultraviolet-B (UVB; 280–315 nm) radiation at the earth's surface (Blumthaler and Ambach, 1990, 1991). Increases in UVB have been shown to impair plant growth (Teramura, 1990), alter competitive interactions between plants (Barnes et al., 1988), and change levels of allelochemicals in plant tissues (McCloud et al., 1992; Larson et al., 1990; and see reviews in Caldwell et al., 1989; Berenbaum, 1988). While a recent study (Quaite et al., 1992) suggests that the direct effects of enhanced UVB on plant DNA may be less harmful than expected, effects on DNA may not be readily generalized to whole plants. Indeed, when whole plant effects are considered, the impact of UVB radiation varies widely both within and between species; there are both resistant and susceptible cultivars in crops such as soybeans and there are differences in UV sensitivity between plant species (Teramura, 1990). Effects of UVB radiation on plant growth have often proven difficult to demonstrate, yet alterations of secondary metabolism are well documented (Caldwell et al., 1989). Despite these findings, there is a remarkable paucity of studies investigating the effects of increased UVB on interactions between plants and their consumers (Panagopolous, 1992; Orth et al., 1990; Yazawa et al., 1992). Collectively, plants and their insect consumers represent approximately 50% of biotic diversity on the planet (Strong et al., 1984). Thus, effects of increased UVB radiation present the possibility for profound alterations of terrestrial ecosystems through effects on interactions between producers and consumers.

Citrus jambhiri (rough lemon) is a rootstock plant used in commercial citriculture. Rootstock selection can have a profound effect on the properties of the scion; also, large numbers of rootstock seedlings must be propagated for grafting. We chose citrus for our model system since few studies have examined the effects of increased UVB irradiation on tropical or semitropical plants. Because natural levels of UVB radiation are higher in the tropics, tropical plants may be less susceptible to UV damage, although, since UVB radiation is less variable in the tropics than in temperate regions (Bowman and Kreuger, 1985), these plants may nonetheless be susceptible to radiation levels at or above the contemporary extremes.

Another reason for selecting *Citrus jambhiri* was that we were interested in examining the effects of UVB not on growth and primary metabolism but rather on secondary metabolism and foliage quality for insect herbivores. Several classes of allelochemicals are ubiquitous in the genus *Citrus*: among these are monoterpene essential oils, the oxygenated triterpene limonoids, coumarins, and furanocoumarins (Waterman and Grundon, 1983; Murray et al., 1982). Furanocoumarins, phototoxic compounds found in the foliage of *C. jambhiri* and other rutaceous plants, are highly toxic to generalist insect herbivores and have been implicated in pathogen resistance in *Citrus* as well as other plants (Martin et al., 1966a,b; Pirone et al., 1960). *Trichoplusia ni* (Lepidoptera: Noctuidae),

the cabbage looper, is a generalist lepidopteran that includes *Citrus* among its hosts (Eichlin and Cunningham, 1978; Tietz, 1972). The susceptibility of *T. ni* to many types of phototoxic and nonphototoxic allelochemicals is documented (Lee, 1991; Ahmad and Pardini, 1990; Bosio et al., 1990, Lee and Berenbaum, 1989; Larson et al., 1988; Wadleigh and Yu, 1988; Prabhaker, 1986; Altieri et al., 1984); so far, limonoids have not been active against *T. ni* (Altieri et al., 1984), while furanocoumarins have proven highly toxic to this generalist herbivore. In *C. jambhiri* leaves, the major furanocoumarins that we have been able to detect are bergapten and psoralen (McCloud et al., 1992).

Here, we present the results of the first study to test directly the consequences of UVB enhancement on plant–insect interactions in a way that approximates stratospheric ozone depletion. In addition, we provide evidence that radiation in the UVB waveband can activate plant-derived photosensitizing agents, resulting in phototoxicity to insects. Although previous work has demonstrated that ultraviolet-A (UVA) radiation can potentiate furanocoumarins (Berenbaum, 1991; Trumble et al., 1991), this study represents the first evidence that UVB radiation applied in a fashion directly relevant to stratospheric ozone depletion can do so.

METHODS AND MATERIALS

We separated the components of this plant–insect interaction by exposing *C. jambhiri* to elevated UVB radiation in the absence of herbivores, by rearing *T. ni* on artificial diets amended with ecologically relevant concentrations of furanocoumarins, and, finally, by rearing *T. ni* to pupation on plants receiving increased UVB irradiation.

Plant Culture and UVB Irradiation. We examined furanocoumarin changes in *C. jambhiri* in the presence of increased UVB irradiation by growing rooted cuttings in the greenhouse under banks of fluorescent UVB bulbs. Cuttings taken from three mature *C. jambhiri* trees were rooted and allowed to grow six weeks before exposure to UVB. Ten cuttings of similar size and degree of apical dominance were selected from each of the three parental groups to yield three clones. Each clone of 10 cuttings was then randomly divided in half such that 15 potted cuttings (five individuals from each of three clones) were assigned to treatment and control groups.

To expose plants to UVB irradiation, we employed the standard irradiation protocols used in studied of simulated stratospheric ozone depletion (e.g., Middleton and Teramura, 1993; Mirecki and Teramura, 1984). Two banks of Westinghouse FS-72 fluorescent UV bulbs were suspended over each half of a 1.67 $\times$ 4.3 m greenhouse bench. Each rack was composed of 14 bulbs placed on 20-cm centers. Half of the lamps (one side of the bench) were wrapped with

type S Mylar film (Gar-Ron Plastics, Baltimore, Maryland) to block UVB. This film does not transmit UVB wavelengths (280–320 nm); its cutoff is near 315 nm. The remaining lamps were wrapped with cellulose acetate (Folex, Inc., Palmyra, New Jersey) film, which has a lower cutoff and allows UVB to pass. UVB bulbs were illuminated for 8 h/day, centered in the 14-hr photoperiod of the experiment. The plants were arranged in a 3 × 5 array under each bench half. Plants were periodically randomized within a bench half and bench sides were periodically switched to avoid position effects.

UVB dose was measured as biologically effective radiation normalized at 300 nm (BE_{300}, see Mirecki and Teramura, 1984; Warner and Caldwell, 1983) and monitored frequently with a UVB radiometer (SED-240, International Light Inc., Newburyport, Massachusetts) calibrated with a portable spectroradiometer (Optronics OL752, Optronics Labs Inc., Orlando, Florida). BE_{300} is a measure of the integrated fluence over the UVB waveband weighted according to Caldwell's generalized plant action spectrum (Caldwell, 1971). We used a moderate (6.4 kJ/day, BE_{300}) daily dose of UVB for our treatment. This dose simulates a very modest increase in UVB flux during the early season leaf flushing period for most citrus-growing regions in the United States. For instance, at 30°N latitude, the UVB flux predicted by a standard radiative transfer model (Green et al., 1980) ranges from 1.4 kJ/day BE_{300} on January 1 to 5.5 kJ/day BE_{300} on April 1. According to this model, UVB flux levels can increase to 9.1 kJ/day at this latitude; however, the model may tend to predict higher flux levels than are actually measured. Booker et al. (1992) found that, in some cases, the model predicted flux levels that were 30% higher than measured levels. Thus, our experimental conditions represent a very conservative increase in UVB fluence, which may more realistically simulate the UVB environment of citrus in the near term.

Since photosynthetic photon flux density can interact with enhanced UVB, aggravating its effects when photosynthetically active radiation levels are low (Caldwell, 1971; Mirecki and Teramura, 1984), we supplemented light levels with an additional bank of metal halide lamps. Eight high intensity discharge metal halide lamps were situated approximately 1.5 m over the banks of fluorescent UVB bulbs to supplement light entering the greenhouse. The metal halide lamps were evenly spaced in a rectangular array over the bench upon which the plants were grown.

The potted cuttings, which were undergoing a vigorous growth flush, grew for 10 days under the light racks before harvest and chemical analysis. At harvest, leaves were divided into those that completed or began expansion during the experimental period (young leaves) and those that were fully expanded and hardened before the experimental period began (old leaves). A randomly selected sample of young leaves and old leaves from each plant was removed and immediately frozen on dry ice for furanocoumarin and soluble protein analysis.

Furanocoumarin and Soluble Protein Assay. Fresh-frozen leaf material was weighed and ground in a mortar under liquid N_2. The leaf powder was then suspended in cold Tris buffer (50 mM, pH 8) and vortexed; an aliquot was taken for soluble protein measurement. Aliquots were centrifuged to remove particulates (14,000*g*, 10 min) and the supernatants were precipitated with an equal volume of cold 10% trichloroacetic acid. The precipitates were than redissolved in 0.1 M NaOH for protein analysis by the Lowry method (Lowry et al., 1951). The remaining leaf suspension was extracted with EtOAc and furanocoumarins were measured by HPLC of the extracts (McCloud et al., 1992).

Artificial Diet Experiments. C. jambhiri foliage contains amounts of furanocoumarins that are relatively low in comparison to other furanocoumarin-containing species (Berenbaum, 1991; McCloud et al., 1992). In order to determine if such small amounts of these potent phototoxins might affect the growth and development of *T. ni*, we reared caterpillars on artificial diets to which furanocoumarins had been added. Furanocoumarin toxicity was assayed in two separate experiments: in the first we exposed the caterpillars to levels of UVB that simulate ambient midsummer radiation in temperate latitudes; in the second, the range of furanocoumarin levels assayed was expanded and supplemental UVB was not provided. Comparison of these two experiments provides an estimate of UVB-potentiated phototoxicity of furanocoumarins. In the experiment in which larvae received supplemental UVB irradiation, caterpillars were irradiated for 2 hr each day under Westinghouse FS-40 bulbs wrapped in cellulose acetate as above. Here, the dose was 3.2 kJ/day BE_{300}. During irradiation, visible light was supplied with fluorescent (Phillips Cool White) bulbs. Fluence levels (photosynthetically active radiation) were typically 250 μmol/m^2/sec.

Artificial diets were prepared according to a standard method modified from Waldbauer et al. (1984). Bergapten, psoralen, or a 1 : 1 mixture of the two were dissolved in a small volume of acetone and added to artificial diet while the diet was still liquid. Furanocoumarin concentrations used in the experiment in which caterpillars received UVB irradiation were 100 and 10 μg/g. In the second experiment, the larvae received furanocoumarin concentrations ranging from 10 to 160 μg/g. Due to limitations in space and chemical availability, some combinations were omitted (see results). Control diets had acetone added alone. Diets were mixed thoroughly, dispensed into 1.5-oz plastic creamer cups, and allowed to solidify. Solid diet were held for two days to allow the acetone to evaporate before neonate *T. ni* were introduced. Caterpillars that had hatched within an 8-hr period were considered a cohort and were placed singly into diet cups. The cups were covered and held in an insectary at 30°C for the duration of larval development.

T. ni on C. jambhiri. Cohorts of *T. ni* larvae were reared from egg hatch to pupation on plants growing under enhanced UVB. Three neonate *T. ni* were introduced onto each of 60 rooted *C. jambhiri* cuttings. Half of the plants were

randomly assigned to receive UVB treatment and half served as controls. Plants were periodically rotated as before, and the cuttings had been growing under control or enhanced UVB light conditions for two weeks before the caterpillars were placed on them. UV fluence levels were monitored and adjusted as in previous experiments. Larval development was tracked until pupation, at which point pupae were brought to the laboratory and held until adult eclosion. Larval development was assessed by censusing infested plants daily and noting the instar of each caterpillar on each date.

RESULTS

Exposure to elevated levels of UVB irradiation significantly increased foliar furanocoumarins (Table 1). Results from the furanocoumarin assays were analyzed as a balanced completely crossed three-way analysis of variance with plant clone, UVB treatment, and leaf age as fixed effects. Because of variability from clone to clone, we included clonal identity in our experimental design in order to increase our ability to see effects due to UVB irradiation. However, clonal origin did not account for a significant portion of the variance in furanocoumarin concentration in this experiment. Furanocoumarin induction was greatest in young leaves, in which we observed an appropriate 2.5-fold rise in psoralen levels, while the concentration of bergapten was slightly less than doubled (Table 1). Soluble leaf protein content was not significantly altered by UVB treatment (Table 1).

TABLE 1. MEAN CONCENTRATIONS (±SE) OF FURANOCOUMARINS AND SOLUBLE PROTEIN IN LEAF TISSUE OF *C. jambhiri*[a]

			P values		
	UV_+	UV_-	UVB	Leaf age	UVB × leaf age
Psoralen					
Young leaves	49.3(9.4)	20.8(3.8)			
Old leaves	13.0(3.1)	8.2(2.0)	0.006	0.0001	0.0506
Bergapten					
Young leaves	29.1(4.6)	16.0(1.9)			
Old leaves	27.8(7.1)	13.5(1.9)	0.01	0.876	0.736
Protein					
Young leaves	9.7(2.4)	7.6(1.1)			
Old leaves	10.3(0.9)	8.7(1.0)	0.132	0.707	0.680

[a] Furanocoumarin amounts are $\mu g\ g^{-1}$, protein amounts are mg/g. *P* values from ANOVA with UV treatment, leaf age, and plant clone (not shown) as main effects.

Larvae reared from egg hatch to pupation in the presence of supplemental UVB suffered complete mortality when their diets contained psoralen and bergapten (Table 2). The same total furanocoumarin amount (100 μg/g) was less toxic when the added furanocoumarin was bergapten alone. However, bergapten did lower survivorship relative to controls at this concentration (Table 2). The low bergapten concentration (10 μg/g), did not. Mean development time of caterpillars on unamended diet was not significantly different from that of caterpillars surviving to pupation on amended diets, not did pupal weights of survivors on the control and furanocoumarin-containing diets differ significantly.

An expanded range of test concentrations of furanocoumarins allowed us to determine the minimum concentrations necessary to affect caterpillar development in the absence of supplemental UVB. Early larval development was significantly retarded by a dose of 80 μg/g of either bergapten or psoralen in artificial diet (Table 3). Caterpillars feeding on diets at this concentration grew to less than half the size of control larvae after five days. In contrast, the bergapten concentration required to extend the larval period relative to controls was twice this amount, 160 μg/g (Table 3). While psoralen is far more toxic to a variety of organisms than bergapten in the presence of UV light (Pathak and Fitzpatrick, 1959; Tuveson et al., 1986), its "dark" toxicity in this experiment was similar to that of bergapten (Table 3). Caterpillars reared from diets containing concentrations of furanocoumarins less than 160 μg/g suffered developmental rate depression in early life but completed development at the same time as controls.

TABLE 2. PERFORMANCE OF *T. ni* EXPOSED TO SIMULATED AMBIENT UVB ON ARTIFICIAL DIETS CONTAINING FURANOCOUMARINS[a]

	50 μg/g each psoralen and bergapten (N = 35)	100 μg/g bergapten (N = 35)	10 μg/g bergapten (N = 35)	Control (N = 36)	P
% 5th instar survivorship	0	54.3	77.1	88.9	0.001
% survivorship to pupation	0	25.7	54.3	52.8	0.001
Days to pupation		12.4	12.7	11.9	0.176
Mean pupal weight		148.6	148.9	148.5	0.999

[a] P values for days to pupation and mean pupal weight (mg) from one-way analysis of variance. P values for survivorship to pupation and 5th instar survivorship from G test for 4 × 2 contingency tables. Fifth instar survivorship and survivorship to pupation of caterpillars receiving 100 μg/g bergapten in diet are significantly different from control (G = 11.05, P = 0.0012, fifth-instar survivorship; G = 5.535, P = 0.0197, survivorship to pupation). Caterpillars were reared under 3.2 kJ/day (UVB, BE_{300}).

TABLE 3. DAYS TO PUPATION (±SE) AND WEIGHTS (mg ± SE) AT FIVE DAYS OF LARVAL LIFE OF *T. ni* (15 CATERPILLARS PER TREATMENT COMBINATION) REARED ON ARTIFICIAL DIETS WITH ADDED FURANOCOUMARINS.[a]

	Weight at 5 days			Days to pupuation		
Dose	Psoralen	Bergapten	Both	Psoralen	Bergapten	Both
10	—	—	35.6 (4.4)ab	—	—	9.35 (0.17)d
20	28.2 (4.0)abcde	30.1 (5.2)abcd	40.3 (4.3)a	9.40 (0.16)cd	9.73 (0.21)bcd	9.47 (0.11)cd
40	25.2 (3.3)bcdef	22.6 (2.5)abcdef	31.8 (4.1)abc	9.71 (0.11)bcd	9.75 (0.11)bcd	9.43 (0.15)bcd
80	15.3 (1.6)cdef	14.1 (0.8)def	18.1 (2.5)cdef	10.61 (0.33)abc	10.35 (0.28)bcd	10.18 (0.24)abcd
100	12.8 (3.2)ef	17.8 (2.4)cdef	—	11.28 (0.63)a	9.67 (0.34)bcd	—
160	9.0 (1.2)f	12.35 (1.7)ef	13.2 (1.2)def	11.38 (0.31)a	10.71 (0.38)ab	10.53 (0.30)abcd
Control		35.8 (4.2)ab			9.46 (0.15)cd	

[a]Doses are μg/g of psoralen, bergapten, or a 1:1 mixture of both added to diet. Means sharing the same letter(s) within each half of the table are not significantly different at $P = 0.05$, Bonferroni's corrected means comparison test.

Change in foliar furanocoumarin levels following exposure to UVB and developmental delay in young larvae reared from furanocoumarin-containing diets provide a context in which to evaluate larval development when the insects are reared on UVB-irradiated plants. We used survivorship analysis (SAS Institute, 1989) to compare mean life expectancies between the two groups. This analysis treats plants as experimental units and compares the distributions of the times elapsed until the loss of all caterpillars from a plant. Survivorship of larvae reared on plants grown under enhanced UVB was significantly lower than that of larvae on plants without supplemental UVB (log rank chi-square = 4.36, $df = 1$, $P = 0.036$, Figure 1). Unexpectedly, the pupal weights of survivors from the UVB-treated plants were greater (163 ± 5 mg, SE, $N = 49$, no UVB; 178 ± 5 mg, SE, $N = 35$, UVB treated; $P = 0.0315$) than pupal weights of survivors from the treated plants.

Development on UVB-irradiated plants paralleled the pattern observed when low concentrations of furanocoumarins were fed to the caterpillars in artificial diets. Caterpillars feeding on plants under enhanced UVB had depressed devel-

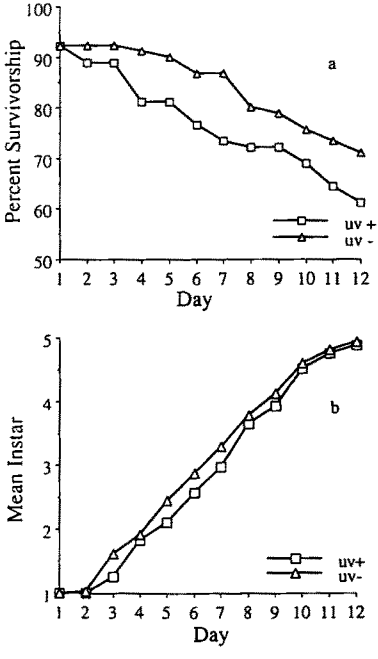

FIG. 1. Survivorship schedules (a) and growth (b) of *Trichoplusia ni* reared on *Citrus jambhiri* plants with and without supplemental UVB radiation (12.1 kJ/day UVB BE_{300}). Caterpillars began to pupate on day 12. $N = 90$ caterpillars in each group.

opment through the first three larval stadia. These larvae reach third instar about 0.5 days after controls (5.76 ± 0.12 days, SE, $N = 66$, UVB-treated; 6.3 ± 0.12 days, SE, $N = 75$, no UVB; $P = 0.0015$, t test). Moreover, we used a repeated-measures analysis of variance on the mean larval instar on each plant to examine the developmental curves (Figure 1b) over the first nine days of larval life. We found that caterpillars feeding on plants under enhanced UVB exhibited a significantly depressed developmental rate through this period ($F_{1,54} = 11.78$, $P = 0.0012$). However, over the entire period of larval and pupal development, surviving caterpillars in both control and treatment groups developed at the same rate. The total preadult period did not differ between the two groups (22.9 ± 0.41 days, SE, $N = 38$, UVB-treated; 22.3 ± 0.20 days, SE, $N = 43$, no UVB; $P = 0.561$, Mann-Whitney U test).

DISCUSSION

Our findings suggest that the combination of furanocoumarins and exposure to UVB radiation is more toxic to *T. ni* than furanocoumarins alone (cf. Tables 1 and 3). Thus, this study provides, to our knowledge, the first evidence for potentiation of phototoxins by UVB wavelengths in a eukaryotic organism. In comparison to controls, caterpillars feeding on diet containing bergapten at a concentration of 100 μg/g fresh weight with exposure to UVB radiation suffered greater mortality and had an extended larval period. This furanocoumarin concentration was not sufficient to extend the developmental period of caterpillars not exposed to UVB. Moreover, inclusion of psoralen with bergapten into the diet of caterpillars that were exposed to UVB radiation resulted in complete mortality in this experiment.

While it is appropriate to compare the relative differences between control and treated caterpillars in the two artificial diet experiments, we cannot address the issue of direct effects of UVB radiation on *T. ni* in the absence of phototoxins. In nature, and in our greenhouse study, *T. ni* live and feed on the undersides of leaves (Jones and Granett, 1982). Since they spend their larval lives in shade, the total UVB exposure of the *T. ni* is therefore likely to be low. Nevertheless, diffuse and backscatter radiation may impinge on caterpillars feeding at the edge of the canopy (Iqbal, 1983). Our artificial experiment conducted without supplemental UVB, then, represents a conservative estimate of the detrimental effects of furanocoumarins in a nutritionally rich artificial diet.

We observed the same distinctive effect, that is, prolongation of early larval development with subsequent recovery in later life, in larvae reared on whole plants under enhanced UVB and larvae reared on artificial diets to which furanocoumarins had been added (Figure 1, Table 3). Delayed early larval development was reproduced by similar furanocoumarin concentrations in our artificial

diet experiments. However, the concentration required to produce this effect in artificial diets was 1.6 to 6.1 times higher than foliar levels measured in these studies. This difference may be due to the fact that artificial diets tend to improve performance relative to natural substrates, and both nutrient deficiency and allelochemical levels can combine to affect larval performance in an additive fashion (Lindroth, 1990; Reese and Field, 1986). Moreover, in other work we have documented foliar furanocoumarin levels in *C. jambhiri* well within the effective range suggested by the artificial diet experiments (McCloud et al., 1992).

Extrapolation of results from the greenhouse to the field can be risky; nevertheless, we suggest that our findings are sufficient for such inference. Young *C. jambhiri* foliage is preferred by *T. ni* (McCloud, unpublished data), presumably because it is less tough and has a higher water content than mature foliage. This preferred foliage, which contains the highest furanocoumarin concentrations in *C. jambhiri*, displays the greatest furanocoumarin induction in response to increased UVB. Under field conditions, insects such as *T. ni* typically suffer exponentially decreasing survivorship in which the probability of death is constant in each instar; percent survivorship may be even lower in earlier developmental stages (Price, 1984). In either case, extension of the early period of larval life could lead to increased mortality when caterpillars are forced to contend with less than ideal conditions in the field (Price et al., 1980). Recent work has shown that extension of the early larval period as a result of host toxicity can interact synergistically with parasitoids to increase larval mortality (Johnson and Gould, 1992). Survivorship under laboratory conditions more nearly parallels physiological curves, and the ecological effects of increased vulnerability in early larval stages can, therefore, be masked. In our artificial diet experiment in which supplemental UVB was not provided, we did not observe markedly enhanced mortality in any caterpillars except those receiving the highest levels of dietary furanocoumarins. Greenhouse conditions may be considered to be intermediate between those in the laboratory and those in the field, especially when larvae are not caged on plants. While differential survivorship may have contributed to the apparent developmental rebound in the greenhouse experiment, both this experiment and the artificial diet experiment without enhanced UVB suggest that low levels of dietary furanocoumarins can have greater effects on the early growth and development of *T. ni* than on later growth.

Another factor that may contribute to the developmental rebound of mature caterpillars may be ontogenetic changes in enzymatic detoxification systems of *T. ni*. Ahmad (1992) noted that activity levels of the antioxidant enzymes superoxide dismutase, catalase, and glutathione reductase increase over the period between third and fifth instar in *T. ni*. This generalist is capable of only low levels of cytochrome P-450-mediated metabolism of furanocoumarins (Ivie et al., 1983; Lee and Berenbaum, 1990). Lee and Berenbaum (1990) provided

evidence for the hypothesis that animals that metabolize photosensitizers slowly depend on antioxidant enzymes for metabolic defense against these compounds. An ontogenetic increase in ability to cope with oxidative stress, then, may have contributed to the patterns we observed.

Given the documented allelochemical changes of plants exposed to increased UVB irradiation, it is reasonable to expect stratospheric ozone depletion to have repercussions for a wide variety of plant–insect interactions, including many interactions between insects and crop plants. In this study, in contrast with other studies of anthropogenically induced alterations in plant quality for herbivores (Fajer et al., 1989; Heinrichs, 1988), we have shown that enhanced UVB can potentially increase, rather than decrease, plant resistance to herbivores. Results from our study may not be easily extrapolated to other systems; nevertheless, our results clearly suggest the potential for field-level effects. As a semitropical tree, *C. jambhiri* experiences high ambient levels of UVB and is presumably more resistant to its damaging effects than are temperate species. Additionally, *C. jambhiri* contains UVB-photoactivated secondary metabolites; such compounds are probably not widely distributed among plants. Effects of elevated UVB are likely to be idiosyncratic and possibly unique to each herbivore–host association, rendering the forecasting of future ecological and economic impacts of elevated UVB a challenging prospect. More studies on a broad diversity of systems will be necessary to develop a general predictive paradigm for this impending global change.

Acknowledgments—We thank Evan DeLucia and Arthur Zangerl for reviewing earlier versions of the manuscript. Terry Harrison, Cheryl Frankfater, Joe Lompart, and V. Lyle Trumbull provided expert and unflagging assistance in the greenhouse and laboratory. This work was supported by United States Department of Agriculture grant AGR89-37280-4897.

REFERENCES

AHMAD, S. 1992. Biochemical defence of pro-oxidant plant allelochemicals by herbivorous insects. *Biochem. Syst. Ecol.* 20:269–296.

AHMAD, S., and PARDINI, R.S. 1990. Antioxidant defense of the cabbage looper, *Trichoplusia ni*: Enzymatic responses to the superoxide-generating flavonoid, quercitin, and photodynamic furanocoumarin, xanthotoxin. *Photochem. Photobiol.* 51:305–311.

ALTIERI, M.A., LIPPMANN, M., SCHMIDT, L.L., and KUBO, I. 1984. Antifeedant effects of nomilin on *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera:Noctuidae) and *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) under laboratory and lathehouse conditions. *Protection Ecol.* 6:91–94.

BARNES, P.W., JORDAN, P.W., GOLD, W.G., FLINT, S.D., and CALDWELL, M.M. 1988. Competition, morphology, and canopy structure in wheat (*Triticum aestivum* L.) and wild oat (*Avena fatua* L.) exposed to enhanced ultraviolet-B radiation. *Funct. Ecol.* 2:319–330.

BERENBAUM, M.R. 1988. Effects of electromagnetic radiation on insect–plant interactions, pp. 167–186, *in* E.A. Heinrichs (ed.). Plant Stress–Insect Interactions. John Wiley & Sons, New York.

BERENBAUM, M.R. 1991. Coumarins, pp. 221–249, *in* G.A. Rosenthal and M.R. Berenbaum (eds.).

Herbivores, Their Interactions with Secondary Plant Metabolites, 2nd ed. Academic Press, San Diego, California.

BLUMTHALER, M., and AMBACH, W. 1990. Indication of increasing solar ultraviolet-B radiation flux in alpine regions. *Science*. 248:206–208.

BLUMTHALER, M., and AMBACH, W. 1991. Spectral measurements of global and diffuse solar ultraviolet-B radiant exposure and ozone variations. *Photochem. Photobiol.* 54:429–432.

BOOKER, F.L., FISCUS, E.L., PHILBECK, R.B., HEAGLE, A.S., MILLER, J.E., and HECK, W.W. 1992. A supplemental ultraviolet-B radiation system for open-top field chambers. *J. Environ. Qual.* 21:56–61.

BOSIO, C.F., MCCREA, K.D., NITAO, J.K., and ABRAHAMSON, W.G. 1990. Defense chemistry of *Solidago altissima*: Effects on the generalist herbivore *Trichoplusia ni* (Lepidoptera: Noctuidae) *Environ. Entomol.* 19:465–468.

BOWMAN, K.P., and KRUEGER, A.J. 1985. A global climatology of total ozone from the Nimbus 7 total ozone mapping spectrometer. *J. Geophys. Res.* 90:7967–7976.

CALDWELL, M.M. 1971. Effects of solar UV irradiation on the growth and development of higher plants, pp. 131–177, *in* A.C. Giese (ed.). Photophysiology VI. Academic Press, New York.

CALDWELL, M., TERRAMURA, A.H., and TEVINI, M. 1989. The changing solar ultraviolet climate and the ecological consequences for higher plants. *Trends Ecol. Evol.* 4:363–367.

EICHLIN, T.D., and CUNNINGHAM, H.B. 1978. The Plusiinae (Lepidoptera: Noctuidae) of America North of Mexico, Emphasizing Genitalic and Larval Morphology. USDA Technical Bulletin 1567.

FAJER, E.D., BOWERS, M.D., and BAZZAZ, F.A. 1989. The effects of enriched carbon dioxide atmospheres on plant/insect herbivore interactions. *Science*. 243:1198–1200.

GREEN, A.E.S., CROSS, K.R., and SMITH, L.A. 1980. Improved analytical characterization of ultraviolet skylight. *Photochem. Photobiol.* 31:59–65.

HEINRICHS, E.A. 1988. Plant Stress-Insect Interactions. John Wiley & Sons, New York.

INTERNATIONAL OZONE TRENDS PANEL. 1988. Report of the International Ozone Trends Panel 1988 Report 18. World Meteorological Organization, Geneva, Switzerland.

IQBAL, M. 1983. An Introduction to Solar Radiation. Academic Press, New York.

IVIE, G., BULL, D., BEIER, R., PRYOR, N., and OERTIL, E. 1983. Metabolic detoxification: Mechanism of insect resistance to plant psoralens. *Science* 221:374–376.

JOHNSON, M.T., and GOULD, F. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *Heliothis virescens* (Lepidoptera: Noctuidae) in tobacco. *Environ. Entomol.* 21:586–598.

JONES, D., and GRANETT, J. 1982. Feeding site preferences of seven lepidopteran pests of celery. *J. Econ. Entomol.* 75:449–453.

LARSON, R.A., MARLEY, K.A., TUVESON, R.W., and BERENBAUM, M.R. 1988. β-Carboline alkaloids: Mechanisms of phototoxicity to bacteria and insects. *Photochem. Photobiol.* 48:665–674.

LARSON, R.A., GARRISON, W.J., and CARLSON, R.W. 1990. Differential response of alpine and non-alpine *Aquilegia* species to increased ultraviolet-B radiation. *Plant Cell Environ.* 13:983–987.

LEE, K. 1991. Glutathione *S*-transferase activities in phytophagous insects: Induction and inhibition by plant phototoxins and phenols. *Insect Biochem.* 21:353–361.

LEE, K., and BERENBAUM, M.R. 1989. Action of antioxidant enzymes and cytochrome P-450 monooxygenases in the cabbage looper in response to plant phototoxins. *Arch. Insect Biochem. Physiol.* 10:151–162.

LEE, K., and BERENBAUM, M.R. 1990. Defense of parsnip and webworm against phototoxic furanocoumarins: the role of antioxidant enzymes. *J. Chem. Ecol.* 16:2451–2460.

LINDROTH, R.L., ANSON, B.D., and WIESBROD, A.V. 1990. Effects of protein and juglone on gypsy moths: Growth performance and detoxification enzyme activity. *J. Chem. Ecol.* 16:2533–2547.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., and RANDALL, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

MARTIN, J.T., BAKER, E.A., and BYRDE, R.J.W. 1966a. The fungitoxicities of cuticular and cellular components of citrus lime leaves. *Ann. Appl. Biol.* 57:491–500.

MARTIN, J.T., BAKER, E.A., and BYRDE, R.J.W. 1966b. The fungitoxicities of plant furanocoumarins. *Ann. Appl. Biol.* 57:501–508.

MCCLOUD, E.S., BERENBAUM, M., and TUVESON, R.W. 1992. Furanocoumarin content and phototoxicity of rough lemon (*Citrus jambhiri*) foliage exposed to enhanced ultraviolet-B (UVB) irradiation. *J. Chem. Ecol.* 18:1125–1137.

MIDDLETON, E.M., and TERAMURA, A.H. 1993. Potential errors in the use of cellulose diacetate and mylar filters in UV-B radiation studies. *Photochem. Photobiol.* 57:744–751.

MIRECKI, R.M. and TERAMURA, A.H. 1984. Effect of ultraviolet-B irradiance on soybean V. The dependence of plant sensitivity on the photosynthetic flux density during and after leaf expansion. *Plant Physiol.* 74:475–480.

MURRAY, R.D.H., MENDEZ, J., and BROWN, S.A. 1982. The Natural Coumarins Occurrence, Chemistry, and Biochemistry. John Wiley & Sons, New York.

ORTH, A.B., TERAMURA, A.H., and SISLER, H.D. 1990. Effects of ultraviolet-B radiation on fungal disease development in *Cucumis sativus*. *Am. J. Bot.* 77:1188–1192.

PANAGOPOULOS, I., BORNMAN, J.F., and BJORN, L.O. 1992. Response of sugar beet plants to ultraviolet-B (280–320 nm) radiation and *Cercospora* leaf spot disease. *Physiol. Plant.* 84:140–145.

PATHAK, M., and FITZPATRICK, T.B. 1959. Relationship of molecular configuration to the activity of furocoumarins which increase the cutaneous responses following long wave ultraviolet radiation. *J. Invest. Dermatol. Suppl.* 32:255–262.

PIRONE, P.P., DODGE, B.O., and RICKETT, H.W. 1960. Diseases and Pests of Ornamental Plants. The Ronald Press, New York.

PRABHAKER, N., COUDRIET, D.L., KISHABA, A.N., and MEYERDICK, D.E. 1986. Laboratory evaluation of Neem-seed extract against larvae of the cabbage looper and beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 79:39–41.

PRICE, P.W. 1984. Insect Ecology, 2nd Ed. John Wiley & Sons, New York.

PRICE, P.W., BOUTON, C.E., GROSS, P., MCPHERON, B.A., THOMPSON, J.N., and WEIS, E.A. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11:41–65.

QUAITE, F.E., SUTHERLAND, B.M., and SUTHERLAND, J.C. 1992. Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. *Nature* 358:576–578.

REESE, J.C., and FIELD, M.D. 1986. Defense against insect attack in susceptible plants: Black cutworm (Lepidoptera: Noctuidae) growth on corn seedlings and artificial diet. *Ann. Entomol. Soc. Am.* 79:372–376.

SAS INSTITUTE. 1989. SAS/STAT User's Guide Version 6, 4th Ed. SAS Institute Inc., Cary, North Carolina.

STRONG, D.R., LAWTON, J.H., and SOUTHWOOD, R. 1984. Insects on Plants Community Patterns and Mechanisms. Harvard University Press, Cambridge, Massachusetts.

TERAMURA, A.H. 1990. Implications of stratospheric ozone depletion upon plant production. *HortScience* 25:1557–1560.

TIETZ, H.M. 1972. An Index to Described Life Histories, Early Stages, and Hosts of the Macrolepidoptera of the Continental United States and Canada. A.C. Allyn, Sarasota, Florida.

TRUMBLE, J.T., MOAR, W.J., BREWER, M.J., and W.G. CARSON. 1991. Impact of UV radiation

on activity of linear furanocoumarins and *Bacillus thuringiensis* var. *kurstaki* against *Spodoptera exigua*: Implications for tritrophic interactions. *J. Chem. Ecol.* 17:973–987.

TUVESON, R.W., BERENBAUM, M.R., and HEININGER, E.E. 1986. Inactivation and mutagenisis by phototoxins using *Escherichia coli* strains differing in sensitivity to near- and far-ultraviolet light. *J. Chem. Ecol.* 12:933–948.

WADLEIGH, R.W., and YU, S.J. 1988. Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. *J. Chem. Ecol.* 14:1279–1288.

WALDBAUER, G.P., COHEN, R.W., and FRIEDMAN, S. 1984. An improved procedure for laboratory rearing of the corn earworm, *Heliothis zea* (Lepidoptera: Noctuidae). *Great Lakes Entomol.* 17:113–118.

WATERMAN, P.G., and GRUNDON, M.F. (eds.). 1983. Chemistry and Chemical Taxonomy of the Rutales. Academic Press, London.

WARNER, C.W., and CALDWELL, M.M. 1983. Influence of photo flux density in the 400–700 nm waveband on inhibition of photosynthesis by UV-B (280–320 nm) irradiation in soybean leaves: Separation of indirect and immediate effects. *Photochem. Photobiol.* 38:341–346.

WHITESIDE, J.O., GARNSEY, S.M., and TIMMER, L.W. 1988. Compendium of Citrus Diseases. APS Press, St. Paul, MN.

YAZAWA, M., SHIMIZU, T., and HIRAO, T. 1992. Feeding response of the silkworm, *Bombyx mori*, to UV irradiation of mulberry leaves. *J. Chem. Ecol.* 18:561–569.