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IMPACT OF UV RADIATION ON ACTIVITY OF LINEAR FURANOCOUMARINS AND *Bacillus thuringiensis* var. *kurstaki* AGAINST *Spodoptera exigua*:[1] IMPLICATIONS FOR TRITROPHIC INTERACTIONS

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Abstract—Acidic fogs with a pH of 2.0 and duration of 2 hr did not reduce the efficacy of *Bacillus thuringiensis* var. *kurstaki* (Berliner). Therefore, the impact of UV radiation was investigated on the interactions between (1) levels of the antibacterial linear furanocoumarins psoralen, bergapten, and xanthotoxin in *Apium graveolens* (L.) occurring following a 2.0 pH acidic fog episode, (2) the noctuid *Spodoptera exigua* (Hübner), and (3) a sublethal dosage of the microbial pathogen *B. thuringiensis* var. *kurstaki*. Mean time to pupation in the absence of UV radiation (survival was too low to conduct this analysis for insects exposed to UV) was significantly extended by the addition of either psoralens or *B. thuringiensis*. Larvae developing on diets containing *B. thuringiensis* plus psoralens required nearly 40% longer to pupate than controls, but their effects were additive as the interaction was not significant. Although the mean times to adult emergence were significantly different, time spent in the pupal stage did not vary significantly between treatments, indicating that increases in larval developmental time were responsible for the observed decrease in developmental rate. Mean time to mortality, a weighted average time of death, was not significantly affected by any of the treatments. In a $2 \times 2 \times 2$ factorial analysis, all main effects (linear furanocoumarins. *B. thuringiensis*, UV radiation) reduced survival significantly, as did the three-way interaction. Thus, antagonistic interactions with psoralens that would reduce the effectiveness of *B. thuringiensis* in the field were not observed. When pairs of main effects were nested within the two levels (presence and absence) of the third factor, several two-way inter-

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actions were found. Interestingly, the activity of *B. thuringiensis* and the psoralens, individually or in combination, was enhanced by exposure to UV radiation. Implications of this research are discussed for both natural and agricultural ecosystems.

Key Words—Linear furanocoumarins, *Spodoptera exigua*, Lepidoptera, Noctuidae, UV light, tritrophic interactions, *Bacillus thuringiensis*.

INTRODUCTION

Few reports are available on the in vivo impact of plant dietary constituents on the susceptibility of insects to pathogens (Felton and Dahlman, 1984; Krischik et al., 1988). Some plants, such as *Apium graveolens* (L.) (celery), are known to contain the phototoxic linear furanocoumarins psoralen, bergapten, and xanthotoxin (hereafter referred to collectively as psoralens) (Trumble et al., 1990). These compounds generally exhibit increased toxicity in the presence of UV radiation (Igali et al., 1970; Berenbaum, 1978). In addition, the insecticidal activity of *B. thuringiensis* is rapidly degraded in the presence of UV radiation (Dunkle and Shasha, 1988), presumably due to trytophan destruction (Pozgay et al., 1987). To our knowledge, no reports document investigation of such tritrophic interactions in the presence of UV radiation.

Antibacterial substances from plants have been implicated in the reduced insecticidal activity of *B. thuringiensis* (Kushner and Harvey, 1962; Maksymiuk, 1970; Smirnoff, 1972). A primary mode of action of the psoralens is to alkylate DNA (Scott et al., 1976), producing profound mutagenic, carcinogenic, and sometimes lethal effects on bacterial cells (Fowlks et al., 1958; Ashwood-Smith et al., 1986). A second reported activity is as an insect antifeedant (Yajima and Munakata, 1979; Muckenstrum et al., 1981). Thus, the psoralens could theoretically degrade or inhibit (through feeding reduction) the insecticidal activity of microbial pathogens such as *Bacillus thuringiensis* (Berliner), which are under consideration for control of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) in commercial celery production (Trumble, 1990).

Increased production of psoralens in *A. graveolens* can be induced by a variety of environmental stresses (Berenbaum, 1981). Beier and Oertli (1983) demonstrated that his phytoalexin response could be initiated by general elicitors including copper sulfate, UV light, and cold temperatures. Mechanical damage occurring during harvesting and storage also have been shown to increase concentrations from about 2 μg/g to 95 μg/g (Chaudhary et al., 1985). In addition, Berenbaum (1981) and Zangerl and Berenbaum (1987) demonstrated that distribution of psoralens in wild parsnip was significantly correlated with increasing nitrogen content. Not surprisingly, the high-nitrogen acidic fogs of the type and duration occurring in the Los Angeles Basin are known to gen-

erate exceptionally high levels of linear furanocoumarins in celery (Dercks et al., 1989).

Therefore, a multifactor experiment testing several hypotheses was designed to elucidate the various potential interactions. Initially, we wanted to determine if acidic fogs would directly affect the activity of *B. thuringiensis* against *S. exigua*. A second objective was to test the hypothesis that the enhanced levels of psoralens resulting from acidic fog-induced stress on *A. graveolens* would have an impact on the susceptibility of *S. exigua* to *B. thuringiensis*. A third objective was to investigate these interactions in the presence and absence of UV radiation. In addition, the effects of UV radiation on larval developmental rates and survival were documented.

METHODS AND MATERIALS

Simulated Acidic Fogs. Simulated acidic fogs were prepared by adjusting distilled water to pH 2.0 with reagent grade nitric and sulfuric acid mixed at a 2.5:1 (v/v) ratio. This acid ratio is typical of fogs in California (Waldman et al., 1982). The pH levels also are consistent with fogs occurring coastally near Los Angeles (Hoffman, 1984) in areas where the annual value of field-grown celery exceeds $5 million dollars (U.S.) (Ivey and Johnson, 1986). Additional ionic components known to occur in such fogs were also added (R.T. Musselman, U.C. Statewide Airpollution Resource Center, U.C. Riverside, Riverside, California 92521; personal communication). Control fogs consisted of distilled water and background ions adjusted to pH 6.2–6.3. Fogs were created within a 1-m^3 chamber using a fogging apparatus designed by Musselman et al. (1985), which operated at 7.03 kg/cm^2 and produced droplets averaging 20 μm in diameter. Temperature during treatment averaged 22–26°C. Shade cloth was used to reproduce incident radiation levels consistent with coastal fogs of no more than 300 $\mu E/m^2/sec^{-1}$.

Experiment I—Effects of Acidic Fogs on B. thuringiensis *Activity.* Commercially available *B. thuringiensis* (Dipel 2X, Abbott Labs, Chicago, Illinois, containing the HD-1 isolate of *B. thuringiensis* susbsp. *kurstaki*) was spread evenly at 1-mm thickness in standard, uncovered Petri dishes and exposed to acidic or control fogs for 3 hr. Following treatment, the *B. thuringiensis* was dried by pulling air over the Petri dishes with a vacuum for approximately 12 hr. The resulting material then was incorporated into artificial diets using procedures reported previously (Moar and Trumble, 1987).

Larvae used in all tests were obtained from a laboratory colony established in 1982 from insects collected in Orange County, California, and maintained on artificial diet (Patana, 1969) at 27 ± 1°C with a light–dark period of 16:8 hr. New genetic material was added annually.

Bioassays consisted of eight concentrations of *B. thuringiensis* plus a control for each fog treatment. Concentrations tested were as follows: 25, 50, 100, 200, 300, 400, and 800 μg/ml diet. Thirty neonate larvae were tested at each concentration, and the entire test was repeated twice. Larvae were held on diets in an environmental chamber at 27 ± 1°C and mortality was assessed at day 7.

Statistical Analyses for Experiment I. Bioassay data were analyzed using the Proc Probit procedure (SAS Institute, 1985) after correction for control mortality with Abbott's (1925) formula and then judged for suitability using the overlapping fiducial limits criteria described by Vandekar and Dulmage (1982). Control mortality was less than 10%.

Experiment II—Interactions of UV Radiation, B. thuringiensis, *and Psoralens on Development and Survival of* S. exigua. For diets containing phototoxic furanocoumarins (Aldrich Chemical Company, Milwaukee, Wisconsin), psoralen (14.94 μg/ml diet), bergapten (5-methoxypsoralen; 37.75 μg/ml), and xanthotoxin (8-methoxypsoralen; 82.4 μg/ml) were incorporated at the levels found in celery leaves following a single acidic fog incident (pH 2.0, Dercks et al., 1989). These materials were dissolved in ethanol and adsorbed onto alphacel following removal of the ethanol by vacuum as described by Chan et al. (1978). The amount of alphacel constituted 5% of the entire diet media. The ethanol and alphacel procedure was used for all treatments including the control. Diet media then was added to the alphacel and blended for 5 min. *B. thuringiensis* (Dipel 2X, Abbott Labs) was added last, and blended for 5 min prior to dispensing into 30 ml clear plastic cups. The *B. thuringiensis* was incorporated at a level (25 μg/ml diet) expected to kill 25% of the test population as estimated in Experiment I. *B. thuringiensis* and the psoralens were incorporated individually and in combination in the diets.

Diet cups were covered with Teflon FEP Fluorocarbon film (E.I. DuPont de Nemours & Co., Wilmington, Delaware). This film is much more transparent to ultraviolet light than glass. For treatments exposed to UV radiation, cups with larvae were placed beneath UV-producing fluorescent lamps (40-W Sylvania 350 Blacklight, Inland Lighting Supplies, Riverside, California). UV lamps with a peak intensity at 350 nm were chosen because wavelengths between 300 and 400 nm are believed to be critical for activation of the psoralens (Musajo and Rodighiero, 1962). The lamps were adjusted in height such that the intensity of UV light beneath a layer of the Teflon film was 1.023 mW/cm^2, a value less than the 1.3–1.6 mW/cm^2 range observed in coastal southern California at midday in September (Trumble, personal observation) and more typical of the early morning. All UV measurements were made with a System 371 Optical Power Meter equipped with a model 268 detector head (United Detector Technology, Hawthorne, California).

A 2-hr exposure was chosen on the basis of a study by Griswold and Trum-

ble (1985), which showed the first through third instars to be positively phototactic and the fifth and sixth instars to be negatively phototactic. Thus, due to either thermoregulation or light aversion, larvae are directly exposed to UV radiation at the canopy surface only for approximately 2 hr. Our results may be somewhat conservative for early instars in that test larvae were not exposed to additional low levels of UV that would be encountered beneath foliage. Exposure times for larger larvae should be accurate, as later instars often hide beneath the soil and would not be exposed to UV radiation during the peak intensity periods. Insects not exposed to UV radiation were kept in the same room and protected from UV radiation by wooden barriers. An incandescent bulb maintained photoperiod during the 2-hr exposure. When not being exposed, all insects were held at 27 $\pm$ 1°C in a photoperiod of 16:8 hr light–dark.

Thirty neonate larvae were placed on each treatment (all combinations of psoralens, *B. thuringiensis*, and exposure to UV radiation = eight treatment combinations). This test was replicated three times. Larvae were examined daily for mortality, development to the pupal stage, and emergence of the adults. Examinations were stopped when all insects were dead or had emerged as adults. Mean time to pupation was calculated for each treatment by averaging the number of days required to pupate for those larvae reaching the pupal stage. The mean time to emergence was calculated similarly for those insects successfully emerging as adults.

The mean time of mortality (after Moar and Trumble, 1987) was calculated for each treatment by dividing the number of larvae dying on a given day by the total mortality at the end of the study. This value then was multiplied by the respective day. Values for all days then were summed to produce a weighted average time of death.

Statistical Analyses for Experiment II. An analysis of variance of the 2 $\times$ 2 $\times$ 2 factorial was used to compare the survival variables among the eight treatment combinations following an arcsine square root transformation. Each of the factors (psoralens, *B. thuringiensis*, UV radiation) was treated as a fixed effect, each with two levels (presence or absence). A blocking factor was included as a random effect in the model because the experiment was replicated three times. The error mean square of the 2 $\times$ 2 $\times$ 2 factorial was used to construct the F tests. If a three-way interaction was present, dependency of the two-way combined effects on a third factor was removed; the two-way interaction sums of squares were calculated within each level of the third factor (Steel and Torrie, 1980, Chapter 15).

All four treatment combinations with UV radiation exposure were absent when analyzing mean time to emergence and mean time spent as pupae (all insects died before or during the pupal stage). Three treatment combinations with UV radiation exposure were missing when analyzing the mean time to pupation variable (only the insects from the "no psoralen $\times$ no *B. thuringiensis*

× UV radiation" treatment survived to pupation). The effect of the psoralens and *B. thuringiensis* treatments, in the absence of UV radiation, therefore, was considered with a two-way ANOVA comparing four treatment combinations within a replicated experiment. This analysis allowed comparisons of psoralen and *B. thuringiensis* treatments and their interactions in the absence of UV exposure.

RESULTS AND DISCUSSION

Experiment I—Effects of Acidic Fogs on B. thuringiensis *Activity.* Exposure of *B. thuringiensis* to acidic fogs with a pH of 2.0 produced no significant effects on *S. exigua* as compared to the control treatment by the criteria of Vandekar and Dulmage (1982). In the first repetition, the LC_{50} (95% fiducial limits) values were 185 (144–234) μg formulated material/ml diet and 166 (103–249) μg/ml for the acidic fog-treated *B. thuringiensis* and the control fog-treated *B. thuringiensis*, respectively. Slopes (± SEM) of log dose probit lines were 1.86 (0.211) and 1.86 (0.287), respectively. In the second repetition, conducted several months later, LC_{50} values were 340 (253–461) μg formulated material/ml diet and 384 (306–477) μg/ml for the treated *B. thuringiensis* and the untreated *B. thuringiensis*, respectively. Slopes (± SEM) of log dose probit lines were 1.63 (0.238) and 2.60 (0.390), respectively.

The variability in LC_{50} values between repetitions probably is due to changes occurring in susceptibility over time in colonies due to founder effects and the introduction of new genetic material. Such variability is not uncommon among populations of other noctuid moths (Vandekar and Dulmage, 1982). The LC_{50} values found in this study are similar to those reported previously (Moar et al., 1986, LC_{50} = 196; Moar and Trumble, 1987, LC_{50} = 299). Thus, even in populations with variable susceptibility to *B. thuringiensis*, no differences in treatment effects were detected.

Experiment II—Interactions of UV Radiation, B. thuringiensis *and Psoralens on Development and Survival of* S. exigua. Mean time to pupation in the absence of UV radiation (survival was too low to conduct the analysis for insects exposed to UV) was significantly extended by the addition of either psoralens ($F = 13.11$; $df = 1,6$; $P < 0.01$) or *B. thuringiensis* ($F = 73.55$; $df = 1,6$; $P < 0.01$) (Figure 1). Developmental rate reductions on diets containing *B. thuringiensis* were not unexpected as *Bacillus* species-induced feeding inhibition has been reported previously (Hegazy and Antonious 1987; Salama and Sharaby 1988a). Larvae developing on diets containing *B. thuringiensis* plus psoralens required nearly 40% longer to pupate than controls, but the effects were additive as the interaction was not significant ($F = 0.02$; $df = 1,6$; $P = 0.883$).

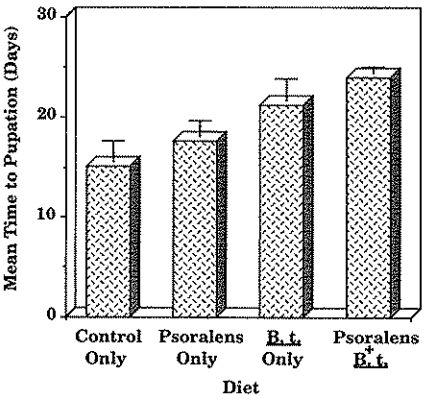

FIG. 1. Mean time to pupation of *S. exigua* in the absence of UV radiation on diets containing psoralens, *B. thuringiensis*, or combinations of both. Thirty neonate larvae were placed on each treatment (all combinations of psoralens, *B. thuringiensis*, and exposure to UV radiation = 8 treatment combinations). This test was replicated three times. Extensions above bars denote standard errors.

This increase in developmental time during the larval stage could have substantial implications for the population dynamics of *S. exigua* in the celery system. Because the majority of the mortality observed in the field occurs in the larval stage (Hogg and Gutierrez, 1980), increased time in this susceptible stage, as opposed to the more protected egg and underground pupal stages (Oatman and Platner, 1972; Tingle et al., 1978), may allow additional opportunity for parasites and predators to reduce populations. Such increases (or decreases) in longevity of larvae previously have been implicated as critical factors in population regulation for other insects (Cardona and Oatman, 1971; Trumble et al., 1987).

The time to emergence of the adult stage in the absence of UV radiation was significantly prolonged by the addition of either psoralens (control mean = 23.64 days; psoralens mean = 26:33; $F = 60.93$; $df = 1,6$; $P < 0.01$) or *B. thuringiensis* (mean = 30.59 days; $F = 431.65$; $df = 1,6$; $P < 0.01$). However, because the mean time spent in the pupal stage (range = 8.5 to 9.3 days) did not vary significantly for either psoralens ($F = 0.03$; $df = 1,6$; $P = 0.88$) or *B. thuringiensis* ($F = 1,63$; $df = 1,6$; $P = 0.25$) as compared to the controls, the increases in developmental time during the larval stage therefore were responsible for the observed decrease in developmental rate. As in the mean time to pupation analysis, there were no significant interactions between psoralens and *B. thuringiensis* for mean time to emergence ($F = 0.16$; $df = 1,6$; $P = 0.70$).

Mean time to mortality, a weighted average time of death, was not signif-

icantly different between treatments ($F = 1.721$; $df = 4,9$; $P = 0.229$). Substantial differences would have suggested either a variable mode or site of action, or the presence of an additional toxic component or interaction. Just such a change in mortality rate was an important factor leading to the discovery of toxic impurities in another insecticidal compound (Umetsu et al., 1981). Because no differences were detected in mean time of mortality for larvae feeding on diets containing psoralens or *B. thuringiensis* does not necessarily suggest that the modes or sites of action may be similar; we simply conclude that this analysis does not clearly separate modes or sites of action.

Larval survival to the pupal stage was variable. In the absence of UV radiation, larval survival on diets containing psoralens > diets with *B. thuringiensis* alone > diets with both psoralens and *B. thuringiensis* (Figure 2A). In

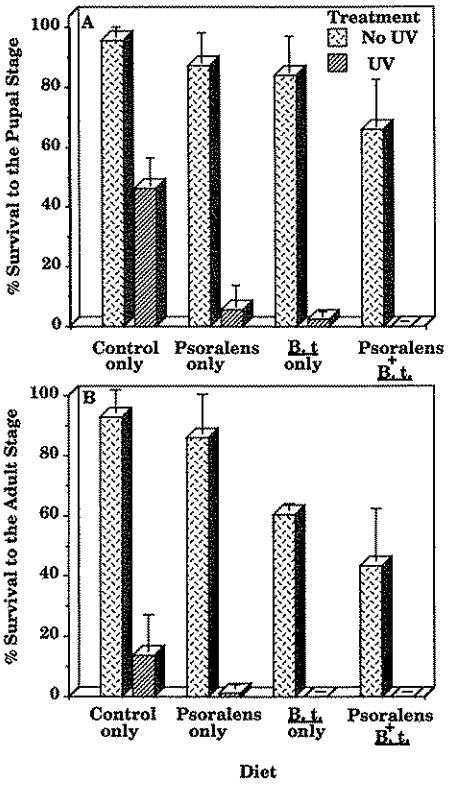

FIG. 2. Percent survival of *S. exigua* to the pupal (A) or adult (B) stages in the presence or absence of UV radiation on diets containing psoralens, *B. thuringiensis*, or combinations of both. See Figure 1 or Methods section for sample sizes. Extensions above bars delineate standard errors.

the presence of UV radiation survival was always reduced, but the same trend was evident.

All main effects (psoralens, *B. thuringiensis*, UV radiation) were significant ($P < 0.01$), with UV radiation being the predominant mortality factor ($F = 239$; $df = 1,14$; $P < 0.01$). Mean square error was 0.0208. Nearly all larvae (95.5%) on control diets that were not exposed to UV light survived to pupate (Figure 2A). Because first to third instars of *S. exigua* prefer to feed on foliage rather than petioles in celery, and the larvae are positively phototactic in the first three stadia (Griswold and Trumble, 1985), the low survival observed in the field (Hogg and Gutierrez, 1980) may be explained in part by the toxic effects triggered by UV light. However, in the aforementioned study all of the phototaxis experiments with early instar larvae were conducted at a single temperature, and larval movements associated with potential thermoregulating activities were not investigated.

The survival of larvae to the pupal stage was reduced significantly by the three-way interaction of exposure to psoralens and *B. thuringiensis* in the presence of UV light ($F = 5.01$; $df = 1,14$; $P < 0.042$) (Figure 2A). Thus, since UV radiation is ubiquitous in field situations, concerns that antagonistic interactions with psoralens would reduce the effectiveness of *B. thuringiensis* are unwarranted.

No two-way interactions were detected ($P > 0.10$) in the $2 \times 2 \times 2$ factorial analysis. However, three-way interactions may mask the two-way combined effects (Steel and Torrie, 1980). Therefore, pairs of main effects were nested within the two levels (presence and absence) of the other main effect to determine if combined two-way effects were important.

Nesting Psoralen and B. thuringiensis *Treatments within UV Radiation Treatment*. The psoralen × *B. thuringiensis* interaction was not significant ($F = 0.14$; $df = 1, 14$; $P = 0.72$) when the diet was not exposed to UV radiation (Figure 3A). When the additional stress of UV exposure was added, the psoralen × *B. thuringiensis* interaction produced a significant decrease in survival rate ($F = 7.79$; $df = 1,14$; $P = 0.015$; Figure 3B). However, this interaction should be interpreted with caution because adding the mortality caused by psoralens and *B. thuringiensis* in combination cannot equal less than 0% survival. Therefore, because the lower bound (0%, Figure 3A) of the mortality measure was reached, the psoralen × *B. thuringiensis* interaction may be constrained by the statistical approach and may not have biological meaning.

Results from similar studies presented in the literature provide no consistent antagonistic trend between potentially defensive plant chemicals and bacteria. Krischik et al. (1988) demonstrated that plant allelochemicals could interfere with microbials such that specialist herbivores feeding on plants or plant parts with high levels of toxins could gain protection from *B. thuringiensis* var. *kurstaki*. Although *S. exigua* is a generalist, the levels of psoralens in our study

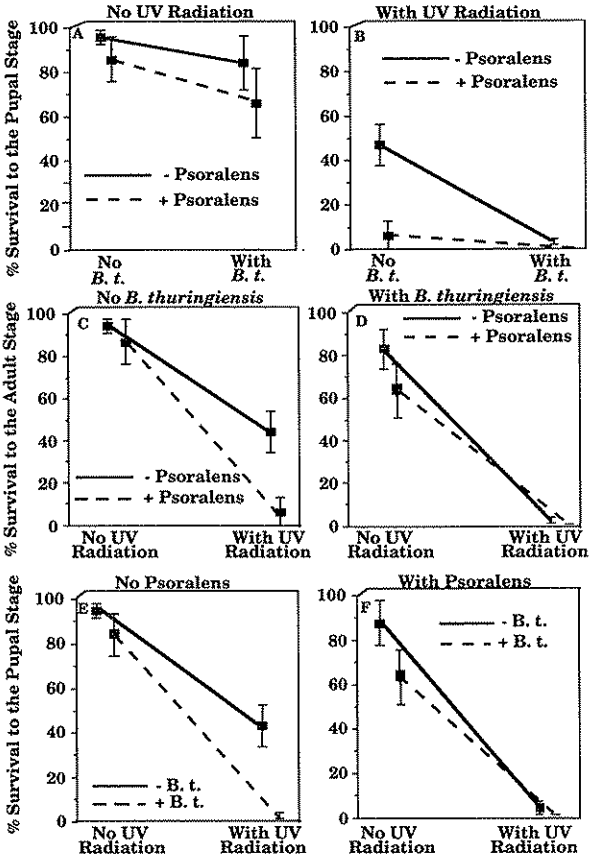

FIG. 3. Nested interaction plots for a 2 × 2 × 2 factorial with the main factors psoralens, *B. thuringiensis* (*B.t.*), and UV radiation. See Figure 1 or Methods section for sample sizes. Two-way interactions were calculated within each level (presence or absence) of the third factor (see text for details). Extensions above bars delineate standard errors. Data points were slightly offset to allow discrimination of standard error bars.

were high, and the percent survival resulting from a combination of *B. thuringiensis* and psoralens was less than observed for either chemical alone (Figure 2A,B). Thus, antagonistic interactions between psoralens and *B. thuringiensis* that would cause the combination of chemicals to become markedly less effective were not indicated. In contrast, our data were not in agreement with the findings of Salama and Sharaby (1988b), where an increase in potency of *B. thuringiensis* was observed in combination with powdered plants known to contain allelochemicals active against another *Spodoptera* species. Similar in vivo

studies with *Manduca sexta* (L.) indicated an enhanced effect of *B. thuringiensis* in the presence of cavanine (Felton and Dahlman, 1984). Unfortunately, direct comparisons are difficult because these studies were conducted in environmental chambers, and the UV radiation exposure levels were not specified.

Nesting Psoralen and UV Radiation Treatments within B. thuringiensis *Treatment.* The psoralen × UV interaction was significant ($F = 5.71$; $df = 1,14$; $P = 0.032$) when larvae were not exposed to *B. thuringiensis* (Figure 3C). Similar results have been summarized by Murray et al. (1982) for a variety of species. In the presence of *B. thuringiensis*, the psoralen × UV interaction was not significant ($F = 0.60$; $df = 1,14$; $P = 0.45$; Figure 3D). Even if photoactivation of the psoralens was occurring in the presence of *B. thuringiensis*, the mortality level could not exceed 100% (the level reached). Under these conditions the interaction would not appear statistically significant.

Nesting B. thuringiensis *and UV Radiation Treatments within Psoralen Treatment.* The *B. thuringiensis* × UV radiation interaction significantly increased mortality beyond a simple additive effect ($F = 7.23$; $df = 1,14$; $P = 0.018$) when the psoralens were not incorporated in the diet (Figure 3E). One potential explanation for this effect is that a partial paralysis resulting from feeding on could serve to enhance the effect of UV light by preventing larvae from moving away from exposed foraging positions. Although this interaction may be enhanced in our study by incorporating the *B. thuringiensis* into the diet (thereby providing some UV radiation protection), the effect would be similar to what can occur if *B. thuringiensis* protein production is incorporated in plants or if commercially available *B. thuringiensis* is applied in the evening.

The *B. thuringiensis* × UV radiation interaction was not significant ($F = 0.23$; $df = 1,14$; $P = 0.64$) when the additional stress of psoralen was added in the diet (Figure 3F). As noted for the previous analysis, survival reaches the lower extreme of the measure (0%), and any potential interaction may be masked by the additional mortality factor of the psoralens.

Survival to Adult Stage. No interactions were detected when measuring survival to the adult stage. UV radiation again triggered the single most important mortality factor, resulting in significant (80–100%) decreases in percent survival as compared to treatments in which larvae were protected from UV exposure ($F = 149$; $df = 1,14$; $P < 0.01$) (Figure 2B). In addition, approximately 65% more mortality occurred during the pupal stage on control diets for those insects exposed to UV light as larvae. Examination of these "pupae" indicated that many had not entirely completed metamorphosis. While this response is typical for nutritionally deprived larvae, no attempt was made to determine the mechanism responsible for the incomplete metamorphosis in our study.

The main effect of *B. thuringiensis* exposure was also significant ($F =$

17.8; $df = 1,14$; $P < 0.01$). This was not unexpected as survival-threatening physical defects and low weights in pupae developing from larvae that have ingested *B. thuringiensis* have been reported previously (Salama and Sharaby, 1988a).

Several conclusions can be drawn from these results. First, acute incidences of acidic fogs as low as pH 2.0 will not reduce the efficacy of *B. thuringiensis* if the bacteria are not physically washed from the leaves. In agricultural systems, spray adjuvants can provide "rain fastness," but the impact of fogs during naturally occurring epizootics has not been quantified. Second, a three-way interaction between psoralens, *B. thuringiensis*, and UV radiation significantly increases mortality, indicating that, in the field, antagonistic interactions with psoralens would not reduce the effectiveness of *B. thuringiensis*. Third, *S. exigua* larvae feeding on celery with high content of psoralens following acidic fog episodes will suffer substantial additional mortality (survival reduced from 50% to 8%, Figure 2A), with the resulting reduction in survival likely to produce substantial economic consequences (Trumble, 1990). Because the psoralens are strong mutagens, and adult fitness (oviposition capacity, longevity, etc.) was not examined, the potential impact on subsequent generations may be greater from psoralens than this study suggests. However, effects on adult populations will be mitigated by migratory movements in the Los Angeles Basin from (1) outside agroecosystems, (2) other geographic locations, or (3) crops that do not contain psoralens providing a consistent supply of fit adults. (Trumble and Baker, 1984). One caveat of this study is that a single concentration of psoralens was used; no effort was made to determine the variability of larval responses with dose.

In addition, the activity of *B. thuringiensis* and the psoralens, individually or in combination, was enhanced by the levels of UV radiation used in this study. While this response was not surprising for the psoralens (Berenbaum, 1978, 1981), which are reportedly activated by UV radiation (Scott et al., 1976), the results for *B. thuringiensis* were unexpected given previous reports that UV radiation rapidly degrades insecticidal activity (Pozgay et al., 1987; Dunkle and Shasha, 1988). We suspect that the enhanced effect of *B. thuringiensis* may have been due to UV radiation causing a general weakening of the larvae, which interferes with their normal immune response.

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