Effects of Air Pollution on Plant-Insect Interactions: Increased Susceptibility of Greenhouse-Grown Soybeans to the Mexican Bean Beetle[1] after Plant Exposure to SO_2[2]

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ABSTRACT

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Foliage from greenhouse-grown soybeans fumigated with 524 $\mu g \cdot m^{-3}$ of SO_2 or from control plants held under the same conditions without fumigation was fed to Mexican bean beetle larvae and adults. The larvae developed faster and grew larger when fed on the fumigated leaves; larval mortality was consistently low and showed no apparent relationship to the type of leaves used as the food source. Adult females showed a significant feeding preference for discs cut from fumigated leaves and were more fecund when fed on the treated foliage than when fed on control leaves. A greater percentage of the females laid eggs, and the viability of the eggs was higher when the females were fed on fumigated leaves. These females produced more eggs per individual, with significant increases in both the number of eggs per mass and the number of masses per female. The longevity of egg-laying females did not differ significantly between the treatments.

Plants and the phytophagous insects exploiting them are in a balance that permits successful but often less than potential reproduction by the insects. Any change in this balance can produce marked ecological and, in the case of cultivated plants, economic consequences. Such changes can be caused by many factors that affect the physiological state of the plant, including stresses such as drought, diseases, or chemical pollutants, and normal developmental processes such as maturation and fruiting. In a recent study (Hughes et al. 1981), we reported that exposure of bean plants, *Phaseolus vulgaris* L., to a low level of SO_2 affected feeding preference and reproduction of the Mexican bean beetle (MBB) on this favored host. The MBB is also an economically important pest of the soybean, but it is only marginally adapted to this host. This study was conducted to determine the effect of the SO_2-plant interaction on the success of the MBB on soybean.

Materials and Methods

A colony of MBB was established from eggs collected on soybeans (cv. Essex) in Delaware. The colony was maintained on stage V1-V2 (Fehr et al. 1971) Essex soybeans (summer and fall) or pinto beans (winter and spring) in a rearing room at 27°C and 40% relative humidity (RH) with a 16-h photophase. The plants were potted in Cornell Mix A (Boodley and Sheldrake 1977) with Osmocote® 9-6-12 and grown in a Purafil®-filtered greenhouse at 24°C. A 16-h photophase was provided by sunlight supplemented with 400-W multivapor, high-intensity discharge lamps (General Electric MV 400/VBU). These lamps provided an irradiance of 20.9 ± 2.2 $nE \cdot cm^{-2} \cdot sec^{-1}$ at the top of the plants on a completely overcast day.

Soybeans for experiments were grown four per 4-in. (ca. 10-cm) pot in Cornell Mix A without Osmocote. The plants were grown in the filtered greenhouse for two weeks under the above conditions but watered with deionized water. The plants were then fertilized and placed in control or fumigation chambers. The chamber conditions, fumigation procedure, and the collection, preparation, and analysis of leaf samples for sulfur were as previously described (Hughes et al. 1981), except that the SO_2 concentration was 524 $\mu g \cdot m^{-3}$ (0.20 ppm). This level, which produced a three- to fourfold increase in foliar sulfur, is just above the current national primary air quality standard for SO_2, but below the threshold for producing visible foliar injury. The test plants were not nodulated, probably due to the presence of calcium nitrate in the potting mix.

Development, survivorship, and growth of larvae, as well as the fecundity of adult females fed control or fumigated leaves, were compared as described by Hughes et al. (1981), using the first (oldest) trifoliolate leaves of stage V2 plants. Since the females in the fecundity test laid relatively few eggs, the test was repeated, using females that had been reared as immatures on pinto beans rather than soybeans. In this second test, all eggs laid were collected and counted until the females died.

To measure adult feeding preference, two 2.5-cm leaf discs from control leaves and two from fumigated leaves were glued with 0.5% agar to moistened filter paper in alternating positions in each of 10 100-mm Petri dishes. One 24-h-old adult female was placed in each dish, and the dishes were held in a controlled-environment chamber at 27°C with a 16-h photophase. After 24 h, the beetles were removed and the feeding damage on each disc was assessed and statistically compared as previously described (Hughes et al. 1981). The test was repeated five times.

To distinguish an actual preference for one tissue

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over the other from an alteration in the rate of feeding, a test was conducted which was identical to the feeding preference test, except that the dishes had only control discs or only fumigated discs (10 dishes of each). Feeding damage was measured and compared statistically as described above.

Results

Results of the development time, growth, and survivorship experiments are summarized in Table 1. Beetles developed significantly faster and grew significantly larger when fed fumigated leaves than control leaves. The difference in rate of development was first apparent at the time of the last larval molt and increased through the prepupal and pupal stages. The difference in growth, measured as weight gain, was statistically significant for each stage from LII through pupa (adults were not weighed). Mortality from LI to adult eclosion was very low and showed no relation to the source of leaves on which the insects were fed.

Females feeding as adults on SO_2-fumigated leaves were more fecund than those fed control leaves. In the first test, using adults reared as immatures on soybeans, only 20% of the females fed on control leaves laid any eggs, whereas 60% of those fed on fumigated leaves oviposited. The preoviposition period was 9 to 10 days for all females, but only those fed fumigated leaves laid more than one egg mass by the time the experiment was terminated (18 days after adult eclosion). Thus, excluding the first eggs laid, the mean number of eggs laid per female was 0 and 59 ± 2 for females fed on control and fumigated leaves, respectively.

This difference in fecundity was also apparent when the test adults had been reared as immatures on pinto beans (Table 2). Females fed on fumigated leaves laid approximately twice as many eggs as those fed on control leaves. The mean longevity of the egg-laying females did not differ between the treatments, but those fed on fumigated foliage deposited ca. 33% more egg masses per individual with an average of ca. 50% more eggs per mass. Those fed on fumigated leaves also tended to have shorter preoviposition times than those fed on control leaves, but the variability was too great and the sample size too small to show a statistical difference. In this test, 50% of the control females and 60% of those fed on fumigated leaves laid eggs. Only two of the five ovipositing control females laid any fertile eggs, and these were only 17% viable. However, five of the six ovipositing females that had been fed fumigated leaves laid eggs with a mean viability of 60%.

The adult females showed a distinct preference for feeding on fumigated leaves over control foliage. The mean percent feeding damage on fumigated and control discs was 33 ± 19% and 21 ± 16%, re-

Table 1.—Summary of development time, growth, and survivorship of MBB larvae on excised trifoliolate leaves from fumigated or control soybeans[a]

Insect Stage	Development time (Avg. no. of days to molt ± SD)		Growth (wt in mg ± SD)		% Survival	
	Control	Fumigated	Control	Fumigated	Control	Fumigated
LI	3.6 (±0.4)	3.4 (±0.2)	0.35 (±0.01)	0.34 (±0.10)	97.8	95.7
LII	6.6 (±0.5)	6.4 (±0.3)	1.68 (±0.40)	2.01 (±.60)**	97.8	95.7
LIII	10.0 (±0.4)	9.9 (±0.5)	4.44 (±0.75)	5.75 (±1.26)**	95.6	89.2
LIV	15.2 (±0.5)	14.6 (±0.4)*	24.10 (±4.22)	32.05 (±6.29)**	95.6	89.2
Prepupa	17.7 (±0.3)	16.9 (±0.5)*			95.6	89.2
Pupa	22.8 (±0.2)	21.8 (±0.4)**	27.23 (±2.67)	31.74 (±4.40)**	89.1	89.2
Foliar S (% dry wt)	0.15 (±0.02)	0.64 (±0.10)	0.13 (±0.2)	0.74 (±0.09)	0.15 (±.02)	0.64 (±.10)

[a] $n = 46$ larvae per age group per treatment for development time and survival rate studies and 30 to 40 larvae per age group for each treatment of the growth study. Means followed by asterisks differ significantly at $P < 0.05$ (*) or $P < 0.01$ (**).

Table 2.—Fecundity of MBB females fed on excised trifoliolate leaves of stage V2 soybeans fumigated for 1 week with 524 $\mu g \cdot m^{-3}$ of SO_2 or non-fumigated plants

Determination	Source of foliage[a]	
	Control ($\bar{x}$ ± SD)	Fumigated ($\bar{x}$ ± SD)
Preoviposition Time (Days)	10.4 ± 1.4 (5)	9.5 ± 2.1 (6) ns
No. of eggs/♀	75.0 ±33.0 (5)	135.0 ±52.0 (6) $P < 0.05$
No. of eggs/Mass	31.0 ±17.0 (12)	43.0 ±15.0 (19) $P < 0.05$
No. of masses/♀	2.2 ± 0.8 (5)	3.2 ± 1.2 (6) $P < 0.001$
Time (days) Between Egg Masses	4.5 ± 1.7 (8)	5.0 ± 0.8 (14) ns
Longevity (days) of		
Ovipositing Females	24.0 ± 4.0 (5)	25.0 ± 6.0 (6) ns
All Females	15.0 ±10.0 (10)	18.0 ± 11.0 (10) ns
Foliar S (% dry wt)	0.18 ± 0.03 (26)	0.53 ± 0.08 (28)

[a] Numbers in parentheses indicate sample size. ns, Nonsignificant.

spectively ($n = 50$). When expressed as a percentage of the total area consumed (rather than of the total area possible), an average of 62% of the feeding was on fumigated discs and 38% was on control discs. This preference for fumigated tissue was highly significant statistically ($P < 0.001$, Wilcoxon matched pairs signed-rank test). When given no choice (i.e., only discs from control leaves or discs from fumigated leaves), the amount of feeding damage was the same on the control tissue as on the fumigated tissue (22 ± 16% on control discs and 22 ± 6% on fumigated discs).

Discussion

The beetles clearly fared better on foliage that had been exposed to SO_2 than on untreated leaves. The larvae developed faster and grew larger, whereas female fecundity was increased in terms of the number of females laying eggs, the number of eggs laid per female, the percent fertility of the eggs, and possibly the age at which egg laying began. All of these effects would tend to create higher population levels of the MBB.

To be of economic importance, this pollutant-plant-insect interaction must occur in the field. The growth, development, and fecundity data from our control insects compare well with past reports for MBB on various cultivars grown in the field (Bernhardt and Shepard 1978, McAvoy and Smith 1979) and grown in the greenhouse during the normal season (Barney and Rock 1975, Kitayama et al. 1979). These reports support our interpretation that the growth, development time, and fecundity observed on SO_2-fumigated foliage represent differences over what would be observed on untreated, field-grown plants.

The MEXSIM computer simulation model (Waddill et al. 1976) was used to estimate the impact of these SO_2-induced changes on MBB populations. The initialized population, the life cycle, and the sampling data used by Waddill et al. were not changed in the model, but all temperatures were changed to 27°C and the predation submodel was excluded. When predictions were compared, using the egg-laying rate and mortality values for either females fed control leaves or those fed fumigated leaves, the model showed substantially higher insect populations after only one generation as a result of SO_2-induced effects. Forty-one days after simulation began (i.e., on Julian day 264 of the model), the "SO_2" population had 3.5 times more adults and 5.5 times more larvae per ha then the "control" population; 10 days later, this difference had increased to 5 times more adults and 17 times more larvae per ha. Although the density-dependent effects of predation would tend to reduce these differences, the predictions suggest that even much smaller changes in egg-laying rate and egg mortality could have significant impact on field populations.

The observed effects on adult longevity and fecundity parallel those reported by Lockwood et al. (1979) and Kitayama et al. (1979) for females fed on two phenological stages of soybeans. In their studies, more of the females fed on reproductive plants (especially stages R7-R8) oviposited, and these ovipositing females laid more eggs per female as compared with females fed on vegetative plants (stage V2). Longevity was not affected by the plant stage, and in choice experiments the beetles preferred leaves from the reproductive plants over those from the vegetative plants. Therefore, the females reacted to the SO_2-fumigated plants in a manner comparable to the way in which they react to mature plants. These similarities suggest the possibility that SO_2 acts in part by inducing physiological changes similar to those occurring with natural maturation, which is supported by the observation that levels of SO_2 below the threshold for foliar injury hasten senescence of soybeans in the field (R. Amundson, unpublished data). However, the precise mechanisms by which SO_2 alters host suitability are yet to be elucidated.

Plant exposure to SO_2 may further exacerbate the pest status of MBB by affecting the time at which the beetles begin to reproduce on soybeans in the field. White (1940) reported that the MBB will not live on young soybeans but thrives on the older plants later in the season. Likewise, studies by Lockwood et al. (1979) and Kitayama et al. (1979) suggested that the vegetative soybean plants are not suitable hosts for the MBB, whereas plants in reproductive growth stages are. If exposure to SO_2 makes the young plants suitable for colonization, damage may be more severe to a more susceptible stage of the plant, and rapid build-up of the MBB population may begin earlier.

The results reported herein and those reported earlier (Hughes et al. 1981) demonstrate clearly that subdamaging levels of SO_2 can cause changes in plants that affect insects. Some of the changes that might account for these effects, including an increase in free amino acids and certain sugars, were discussed in our earlier paper. In the examples studied, the changes have favored the insect's success. If the reactions of the MBB to SO_2-affected plants are the same in the field as in these laboratory studies, damage by this insect and subsequent crop losses could be greater in polluted areas; if such a relationship extends to other pests and crops, then the total impact of the pollutant-plant-insect interaction on crop losses or costs of crop protection could be quite significant. Consideration of this interaction could be important to integrated pest management, especially with the present trend towards the use of computer population models for predicting the numbers and timing of pest outbreaks. A series of studies to further define this interaction and determine its importance under field conditions is currently under way.

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