

Increase in feeding stimulants as the primary mechanism by which SO₂ enhances performance of Mexican bean beetle on soybean

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Abstract

Relative consumption rate (RCR) and relative growth rate (RGR) were significantly higher for larvae of the Mexican bean beetle fed leaves from plants fumigated with SO₂. The insects grew faster primarily because they ate faster, rather than as a result of change in nutritional value of affected host tissue. Soluble carbohydrate content of fumigated or glutathione-treated leaves was much higher than that of corresponding control leaves, and concentrations of sucrose, fructose, and glucose, major feeding stimulants for this insect, were increased 40–50% by the treatments; soluble protein was unchanged (fumigated leaves) or significantly lower (glutathione-treated leaves) than controls. Feeding choice assays using filter-paper discs demonstrated that the beetles can discriminate clearly and respond to differences in sugar content of the magnitude produced by exposure to the pollutant. Thus, the primary mechanism by which SO₂ increases performance of Mexican bean beetle on soybean appears to be increase in foliar concentration of stimulatory sugars, which, at least in part, would be a consequence of the pollutant interfering with phloem loading and translocation of sugar from affected leaves.

Introduction

Effects of air pollution on plant-insect relations have been well established in recent years and are of concern both because of the ecological consequences and as they relate to increased loss in crop production. Elucidation of the mechanisms involved is essential to a complete understanding of this interaction and to integrate the impact of this specific stress with that of other environmental conditions or stresses occurring simultaneously and sequentially. The effect of SO₂ on success of Mexican bean beetle (*Epilachna varivestis* Mulsant) feeding on soybean [*Glycine max* (L.) Merr.] has emerged as one

model system for studying the impact of pollutant-induced changes in host plants on herbivorous insects. Detailed laboratory and field studies with this insect have shown that growth, rate of development and reproduction on soybean [*Glycine max* (L.) Merr.] are greatly enhanced by exposure of the plants to SO₂ (Hughes *et al.*, 1982, 1983, 1985). In addition, adult beetles show a strong preference for feeding on SO₂-affected foliage (Hughes *et al.*, 1982). Effects on insect growth were strongly correlated with change in foliar glutathione (GSH) concentration (Chiment *et al.*, 1986), and subsequent studies demonstrated that all effects of fumigation (i.e., increased rate of development and growth of larvae

and increased fecundity and feeding preference of adults) are elicited when foliar GSH is increased artificially in excised leaves (Hughes & Chiment, 1988). We now report the probable primary mechanism by which exposure of the plant to SO₂ is altering performance of this insect.

Materials and methods

Nutritional parameters. To determine whether increased growth of the insect on fumigated foliage is due to change in nutritional quality of the leaves or to increased feeding, the relationship between amount of food consumed and growth was examined by the procedure of Blau *et al.* (1978). MBB larvae were reared through third instar on *Phaseolus vulgaris* L. (Hughes *et al.*, 1981) and then transferred to soybean leaves for 24 h. After this acclimation period, they were transferred to 11.5 × 11.5 × 4.5 cm polyethylene sandwich boxes containing moistened blotting paper and leaflets from fumigated (0.3 ppm SO₂ for 24 h) or non-fumigated control soybean plants for determination of relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion index (ECI) (Waldbauer, 1968). Five larvae were placed in each box with seven boxes per treatment. Total weights of the larvae and of the leaflets were recorded prior to placing them in each box, and weighed leaflets from freshly fumigated or control leaves were provided each day for three days. Readability of the balance used in all weighings was 0.01 mg. Leaf material remaining in the boxes at the end of each period was removed, dried and weighed. Fumigations were performed daily on 21-day-old plants (stage V3-V4, Fehr *et al.*, 1971) in continuous-flow stirred tank reactor chambers as previously described (Hughes *et al.*, 1985). Samples of 6–10 leaflets of both fumigated and control leaves were collected, weighed, and dried each day and the percent dry weight used to estimate the initial dry weight of leaves fed to the insects on that day. Nutritional parameters were calculated based on the fresh weight of the larvae and dry weight of food consumed (Kogan, 1972; Barney & Rock, 1975).

Analysis of carbohydrates and individual sugars. Results from the above test suggested a change in the

ratio of feeding stimulants to deterrents. Since sucrose is a primary feeding stimulant for the MBB, and its hexose components, fructose and glucose, are also known to be active (LaPidus *et al.*, 1963; Nayar & Fraenkel, 1963; Augustine *et al.*, 1964), the effect of fumigation or GSH-treatment on total nonstructural (soluble) carbohydrates (TNC) as well as on the combined amounts of sucrose, fructose, and glucose in leaves was examined. Second trifoliolate leaves from stage V3-V4 soybean were used for analysis. Treatments consisted of (1) excised leaves that were allowed to imbibe 0.33 mM GSH solution for 24 h, (2) leaves from treatment (1) that were placed in deionized water for 24 h following GSH treatment, (3) leaves from plants fumigated with 0.3 ppm SO₂ for 24 h, and (4) leaves from treatment (3) that were excised and placed in deionized water for 24 h. In this way, analyses were made at times equivalent to the beginning and end of the insect feeding period for a given set of leaves. TNC was determined by the anthrone method (Yemm & Willis, 1954; Hansen & Møller, 1975); extractions were made with methanol-chloroform-water (12:5:3) and concentrated sulfuric acid with 2 g anthrone liter⁻¹ was used as the reagent. Samples were heated 12 min and absorption was read at 625 nm with glucose serving as the calibration standard.

Individual sugars were measured by GLC analysis of the trimethylsilyl derivatives (Sweeley *et al.*, 1963) using Tri-Sil Z as recommended by Pierce Chemical Co. (Rockford, IL). Quantitation was accomplished on a Hewlett-Packard Model 5710A gas chromatograph equipped with a Model 33851 integrator; trehalose served as the internal standard. On-column injection was used with a 91 cm × 2 mm I.D. glass column packed with 5% GE SE-30 on Chromosorb WAW 80/100. The nitrogen flow rate was 30 ml/min and the temperature was programmed from 145 to 250 °C at 4 °C/min.

In addition to the carbohydrate analyses, foliar glutathione was determined as described by Chiment *et al.* (1986) and soluble protein was measured by the Bradford method (Bradford, 1976) using D-ribulose-1,5-bisphosphate carboxylase as the standard.

Feeding discrimination assay. The ability of MBB to discriminate and respond to differences in sugar

content of the magnitude produced by fumigation was assessed by a multiple-choice, filter-paper disc assay. Filter-paper discs (1.3 cm dia.) were impregnated with one of three agar solutions: 1) water only (control), 2) 0.02 M solution of sugars (0.5:1:1 fructose:glucose:sucrose), or 3) 0.03 M solution of the sugar mixture. The concentrations represent approximately those of the control and fumigated leaves, respectively, 24 h after treatment. One disc of each treatment was placed in each of 24 60×15 mm Petri dishes; the positions within a dish were assigned randomly. Newly emergent females were held 48 h on moist blotting paper and then placed one per dish. After ca. 24 h, the females were removed and feeding stimulation was assessed as the proportion of disc area with bite marks and feeding ridges.

Since a very close correlation has been observed between foliar concentration of GSH and effects on the insect, a test was also conducted to determine whether GSH could stimulate (or deter) feeding alone or in the presence of sucrose. The same experimental procedure was used as described above for the sugar mixtures but with four treatments per dish: 1) water, 2) 0.05 M sucrose, 3) 0.002 M GSH, or 4) 0.002 M GSH in 0.05 M sucrose. The test contained 20 replicates.

Results

The nutritional parameters are summarized in Table 1. Both RCR and RGR were significantly higher

Table 1. Nutritional parameters¹ for fourth instar Mexican bean beetle larvae on non-fumigated trifoliolate leaves of stage V3-V4 soybean and leaves fumigated with 0.3 ppm SO₂ for 24 h

Plant treatment	n	RGR (mg gained/mg body wt./day)	RCR (mg ingested/mg body wt./day)	ECI (%)
Control	6	0.252 (±0.024)	0.321 (±0.013)	78.2
Fumigated	7	0.280 (±0.021)	0.340 (±0.017)	82.2
P		0.046	0.044	0.093

¹ RGR = Relative growth rate; RCR = Relative consumption rate; ECI = Efficiency of Conversion Index. Parameters were calculated based on fresh weight of larvae and dry weight of food consumed.

for larvae fed fumigated leaves ($P = 0.046$ and $P = 0.044$, respectively, Student's *t*-test). ECI, which is an over-all measure of the insect's ability to utilize the ingested food for growth, tended to be slightly higher on fumigated leaves ($P = 0.093$). If the increased RGR were due to increased nutritional value of the host, then the points for those fed fumigated leaves would have fallen above the regression line for the control larvae (i.e., growth would be greater than expected for the amount consumed) (Blau *et al.*, 1978). However, analyses of the two treatments showed a common intercept ($F_{1,9} = 4.017$) and a significant, common slope ($F_{1,9} = 0.871$) (Fig. 1), indicating that growth of MBB was faster on fumigated foliage primarily because of increased rate of feeding rather than a change in nutritional value of the leaves. Since ECI frequently decreases as RCR increases (e.g., Slansky & Feeny, 1977), our observation of increased ECI on the fumigated leaves might indicate a subtle increase in their nutritional value which is masked by the dominant effect of increased feeding stimulation.

TNC increased dramatically with both fumigation and GSH treatment (Table 2). This increase was about 3-fold greater 24 h after treatment (i.e., the equivalent of the end of the insect feeding period) than immediately following treatment (the equivalent of the beginning of the insect feeding period).

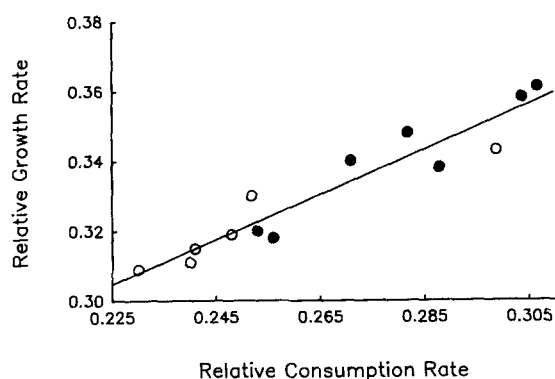


Fig. 1. Relative growth rates [expressed as mg of weight gain (fresh weight) per mg larva (fresh weight) per day] and relative consumption rates [expressed as mg of food eaten (dry weight) per mg of larva (fresh weight) per day] of last instar MBB larvae fed soybean leaves fumigated with 0.3 ppm SO₂ for 24 h (●) or non-fumigated leaves (○). Calculated regression line for combined data is: $y = 0.161 + 0.638x$; $r = 0.951$.

Table 2. Analyses of foliar constituents in second trifoliolate leaves of stage V3-V4 soybean (1) excised and placed in 0.33 mM GSH for 24 h, (2) placed in deionized water for 24 h following the treatment with GSH, (3) fumigated with 0.3 ppm SO₂ for 24 h, or (4) excised and placed in deionized water for 24 h following the fumigation. Data analyzed by Student's t-test

Treatment	Glutathione ¹	Soluble protein ²	TNC ³	Feeding stimulants ⁴
GSH 24 h	7.63	181.0	41.7	22.7
Control	4.15	214.0	33.0	17.0
P	0.000	0.031	0.001	0.043
GSH 24 h + H ₂ O 24 h	4.09	141.8	63.8	35.8
Control	3.11	162.0	34.8	17.9
P	0.001	0.013	0.000	0.000
Fumigated 24 h	4.45	223.6	30.9	14.7
Control	2.12	241.4	27.8	14.9
P	0.000	0.492	0.041	0.871
Fumigated 24 h + H ₂ O 24 h	4.78	243.5	31.0	14.7
Control	3.65	233.0	23.3	10.5
P	0.002	0.087	0.001	0.007

¹ mM free sulfhydryl mg⁻¹ dry weight.

² μg D-ribulose-1,5-bisphosphate carboxylase equivalents mg⁻¹ dry weight.

³ TNC = total nonstructural (soluble) carbohydrates expressed in mg glucose equivalents g⁻¹ dry weight.

⁴ Combined amount of sucrose, glucose and fructose expressed in μg mg⁻¹ dry weight.

The combined amount of primary feeding stimulants (sucrose, fructose, and glucose) increased 40–50% within 24 h after treatment. In parallel with observed effects on the insects, changes in sugar content produced by treatment with GSH were more marked than those produced by fumigation. Soluble proteins were unchanged (fumigated) or significantly lower (GSH) and, therefore, were probably not contributing to the positive effects on the insects. As previously reported for similar fumigations, glutathione concentrations were increased ca. 2-fold by SO₂ treatment.

Results of the discrimination assay showed a very marked ability of the beetles to distinguish between the two levels of feeding stimulants. The mean area with bite marks was 10 and 85% for low and high stimulant treatments, respectively ($P = 0.000$, two-tailed Wilcoxon signed-rank test for paired samples). Expressed as a fraction of the total area 'damaged', an average of 90% was on the high-stimulant discs, 9% on the low-stimulant discs, and less than 1% on water (control) discs.

In the test of the stimulatory/deterrent activity of GSH, there were no bite marks on either the water or the GSH discs in any of the replicates. Therefore, we concluded that GSH was not stimulatory by itself. The mean proportion of disc area with bite marks/feeding ridges for the sucrose and sucrose+GSH treatments were 0.371 and 0.290, respectively, which was not significantly different according to the two-tailed Wilcoxon signed-rank test for paired samples performed on the angular transformation of proportions ($P = 0.456$). Therefore, the GSH neither enhanced nor decreased feeding stimulation in the presence of sucrose.

These results show that MBB grow faster on SO₂-fumigated and GSH-treated soybean foliage primarily because they consume host material at a more rapid rate. They also show that known feeding stimulants are increased by both of these treatments and that the beetles are able to detect and respond to the kind of change produced, so the increased feeding appears to be a response to increased concentration of stimulants. GSH, with which effects

on the insects are closely correlated, does not appear to contribute directly to this stimulation.

Since the ratio of feeding stimulants to deterrents is more important to phytophagous insects than the absolute amounts of the compounds (Dethier, 1982), a decrease in feeding deterrent(s) in SO₂-affected leaves might also be contributing to the observed change in feeding behaviour of MBB. Such a decrease might result from decreased production or interaction with glutathione; these possibilities are under investigation. Sugars other than fructose, glucose, and sucrose also increased upon treatment, and evidence exists that these could contribute to greater stimulation as well (Lippold, 1957; Hughes, unpubl.). In addition to the positive effects on growth rate, increased feeding stimulants and resultant consumption rate by the insects could explain the observed increases in rate of larval development, adult fecundity, and feeding preference on fumigated or GSH-treated leaves.

Elevation of total foliar sugars by exposure of plants to low levels of SO₂ have been reported and shown to be related mainly to sensitivity of phloem loading to the pollutant (Koziol & Jordan, 1978; Noyes, 1980; Lorenc-Plucinska, 1986; Minchin & Gould, 1986; Griffith & Campbell, 1987). Similar effects might be expected with ozone-exposed plants, since short term exposure to low concentrations of this pollutant can also cause an increase in soluble sugar content (Dugger *et al.*, 1966) and foliar GSH (Hughes & Chiment, unpubl. data) in some plants. Indeed, MBB adults have been shown to preferentially feed on O₃-exposed soybean leaves (Endress & Post, 1985), but whether this is due to an increase in stimulants, a decrease in deterrents, or both has yet to be determined.

These results provide new information essential for estimating and predicting the impact of specific pollutant episodes on insect activity. They serve to emphasize the advantage gained by approaching the plant-insect relation from the perspective of the quantitative and qualitative changes in resource allocation by plants in response to their environment and the consequences of this response to the insect. From this viewpoint, one can identify factors affecting synthesis and translocation of sucrose and would expect the impact of fumigation to be greater under

those conditions promoting sucrose production, such as high light intensity, and, conversely, much less under conditions unfavorable to sugar production; this hypothesis is currently being tested. This work also identifies a possible means of predicting the net impact on soybean-MBB relations of multiple stresses occurring simultaneously and sequentially in the field.

Résumé

Augmentation de la phagostimulation, première manifestation de l'accroissement de la consommation de feuilles de soja par Epilachna varivestis provoqué par SO₂

Les taux de consommation relative (RCR) et de croissance relative (RGR) sont significativement supérieurs chez les larves d'*E. varivestis* après fumigation des feuilles de soja par SO₂. L'étude de la relation entre RCR et RGR a révélé que les insectes se sont développés davantage, avant tout parce qu'ils ont consommé plus, et non pas par modification de la valeur nutritive des tissus de l'hôte. La teneur en carbohydrates solubles des feuilles traitées au glutathion ou ayant subi une fumigation était bien supérieure à celle des témoins; les concentrations de sucrose, fructose et glucose, principaux phagostimulants de cet insecte avaient augmenté de 40 à 50%; la teneur en protéine soluble était inchangée (cas de la fumigation) ou significativement réduite (cas des feuilles traitées au glutathion). Des expériences de choix avec des disques de papier filtre ont montré que les insectes pouvaient distinguer nettement des différences de concentration en sucres, du même ordre d'importance que celles provoquées par le polluant, et y répondre. Ainsi, la cause primaire par laquelle SO₂ augmente les performances d'*E. varivestis* sur soja semble être l'augmentation de la concentration foliaire en sucres stimulateurs, ce qui serait, au moins en partie, la conséquence de l'interférence du polluant avec la charge du phloème et le transfert du sucre à partir des feuilles atteintes.

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