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**EFFECTS OF SULFUR DIOXIDE AND
NITROGEN DIOXIDE ON VEGETATION**

**Progress Report
for Period 1 June 1977 - 29 February 1978**

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INTRODUCTION

DOE Contract EE-77-S-02-4368 was initiated on 1 June 1977 to study four different, but interdependent, areas of research relating to the emission of SO₂ and NO₂ into the atmosphere and their effects on vegetation. The areas of research selected are related to fossil fuel combustion and are believed or are known to occur in the field: (1) the mode of action of SO₂ in relation to its metabolic fate; (2) the influence of NO₂ on the phytotoxicity of SO₂, and the combined effects of these pollutants on crop yield under field conditions; (3) the effect of SO₂ on the plant with respect to its susceptibility to plant diseases; and (4) the effect of SO₂ on the behavior and development of the Mexican bean beetle on soybean and on the production of volatile terpenoids in conifers with respect to their suitability for colonization by insects.

It is difficult to coordinate all the elements of a diversified program, such as the hiring of new personnel and delivery of specialized equipment without some problems. For example, the selection and subsequent availability of post-doctoral associates were controlled more by the status of their doctoral theses than by our needs, and the promise of delivery of equipment by some companies was more related to commercial than scientific realities. By 1 September 1977, the two post-doctoral associates and a research assistant had begun work. During the intervening period, more emphasis was placed upon some areas of research than upon others; e.g., the interaction of SO₂ with plant diseases was studied more extensively than the mode of action of SO₂. But as of the submission of this Progress Report, all areas of investigation are being actively pursued.

Some changes in personnel have been made since the submission of the original proposal more than two years ago and these are shown in the Budget and Personnel sections. The net result has been an increase in man-years for the first year from the originally proposed 5.6 to 7.1, the increase being at senior scientist level. In addition, another senior scientist has been assigned to the project (supported by BTI) to initiate a program on the impact of SO₂ and NO₂ on roots, root communities, and mycorrhizae. Preliminary work on this subject is reported in this Progress Report and new research is proposed.

A collaborative study was begun with Dr. James Lassole, Department of Natural Resources, Cornell University, on the effects of SO₂ and NO₂ on photosynthesis and water relations of conifers. The only costs to BTI will be for supplies and the use of some facilities.

The Mode of Action and Metabolism of SO₂ in Plants

Three different but interdependent areas of research are currently under way in our laboratory in an effort to understand the mechanisms of uptake, action, and resistance of plants to sulfur dioxide.

Mechanism of Uptake of SO₂

The mechanism of SO₂ uptake by leaves is controversial and poorly understood. Several investigators have proposed that SO₂ penetrates the cell membrane as bisulfite ion (HSO₃⁻). It is their view that bisulfite, formed from the reaction of SO₂ with the aqueous phase of the cell wall, penetrates the plasmalemma by passive diffusion.

This explanation for SO₂ uptake is a very limited one because it does not explain how a charged molecule such as HSO₃⁻ can diffuse against the negative electrochemical potential of the cell membrane. Bisulfite anions appear to penetrate the plasmalemma by an active transport mechanism as do other anions (e.g., Cl⁻, SO₄⁻² etc.) and their uptake isotherm is a hyperbolic function. The most probable mechanism of SO₂ uptake by the cell membrane is by diffusion of SO₂ as a gas and not as bisulfite ions, because SO₂ is an uncharged molecule and can easily penetrate the membrane lipid bilayer. The uptake isotherm of a diffusion process is always linear.

In order to elucidate the mechanism of SO₂ uptake, we will compare the uptake isotherms (rate of uptake at various concentrations) of gaseous SO₂ using intact soybean and corn plants with those of bisulfite using leaf disks and Na₂³⁵SO₃. If SO₂ penetrates the cell membrane by diffusion, its uptake isotherm will appear to be linear. If the isotherm is hyperbolic, then SO₂ is taken up by an

active transport mechanism in the form of bisulfite ions.

The experiments described above will be carried out during the next three months. Fumigation chambers for determining the SO_2 uptake isotherms have been constructed. Each chamber has a volume of 64 liters and is made of Plexiglas covered internally with teflon sheet to prevent SO_2 adsorption by the walls of the chamber.

Mechanism of SO_2 Action in Relation to Its Metabolic Fate.

The mechanism by which SO_2 injures leaves is unknown. It has been shown that leaves of spinach and soybean exposed to $^{35}\text{SO}_2$ rapidly convert $^{35}\text{SO}_2$ to $^{35}\text{SO}_4^{-2}$, with subsequent incorporation into S-containing metabolites (methionine, cysteine, glutathione, etc.). In addition to these metabolites, H_2S is produced from SO_2 as well as from other inorganic S-containing salts (Na_2SO_3 and Na_2SO_4). Light appears to be necessary for this H_2S evolution.

The formation of SO_4^{-2} from SO_2 in the dark or in the light indicates that oxidation of bisulfite takes place inside the cell. It has been shown that bisulfite oxidation can proceed via enzymatic reactions in mammalian systems (sulfite oxidase in liver mitochondria) and non-enzymatic reactions in vitro (free radical-mediated), but neither has been shown to occur in plant tissues in vivo. On the other hand, the formation of sulfur-containing amino acids in the dark or light and the evolution of H_2S from SO_2 only in the light strongly suggest that bisulfite is reduced in vivo. Thus, the conversion of SO_2 involves two main steps: (1) the oxidative step which is O_2 -dependent and (2) the reductive step which is light-dependent.

It is not known which step is injurious to the green tissues. We postulate that the non-enzymatic, free radical-mediated, bisulfite oxidation is responsible for the SO_2 injury.

In an effort to elucidate the metabolic step responsible for SO_2 action, we are currently comparing the rate of transformation of exogenously-supplied $^{35}\text{SO}_2$, $^{35}\text{SO}_3^{-2}$ and $^{35}\text{SO}_4^{-2}$ using corn and soybean plants. During the last six months, methods were developed for the separation and quantitative determination of the water-soluble inorganic and organic sulfur compounds, as well as the lipid-soluble compounds in soybean and corn plants.

The water-soluble sulfur compounds are extracted from the leaves with 10^{-2} M phosphate buffer, pH 7.2, in 70% ethanol containing 10 mM of N-ethylmaleimide (NEM). The use of NEM was found to be necessary for the quantitative recovery of sulfite, cysteine, and glutathione, which are extremely labile to oxidation in their free form. The ethanol extract was further treated with chloroform to remove excess NEM and lipid-soluble compounds. The water phase contained SO_4^{-2} , $\text{SO}_3\text{-NEM}$, CyS-NEM , GSH-NEM , methionine, and its sulfoxides. The sulfur compounds were separated by a combination of two-dimensional paper chromatography and high voltage paper electrophoresis.

The incorporation of ^{35}S (SO_2 , SO_3^{-2} , and SO_4^{-2}) into the lipids was followed by extracting the insoluble residue after ethanol extraction with chloroform-methanol (2:1). This extract was combined with the chloroform extract above and it was washed with 0.1 M NaCl three times to remove H_2O -soluble compounds. The purified lipids were separated by thin layer chromatography. The ^{35}S -containing compounds were detected by means of autoradiography.

Preliminary experiments using $^{35}\text{SO}_4^{-2}$ with detached leaves of soybean and corn showed that after 1 hr of exposure to $^{35}\text{SO}_4^{-2}$, glutathione, cysteine, and methionine were labelled. A considerable amount of radioactivity was recovered in the lipid fraction as sulfolipid. We are presently comparing the rate of transformation of radioactive $^{35}\text{SO}_3^{-2}$ and $^{35}\text{SO}_2$ with $^{35}\text{SO}_4^{-2}$ and this study should continue throughout the current contract year and into the second year.

Mechanism of Resistance

The mechanism of resistance of plants to SO_2 is unknown and is probably associated more with a biochemical mechanism than with the rate of uptake.

We have recently found that water homogenates of leaves of soybean, corn, and pinto bean containing 5 mM bisulfite produce ethylene and ethane. Ethane production is 10 to 15 times higher than that of ethylene. Soybean and pinto beans homogenates produced 20 times more ethane than corn. It may be coincidental that corn plants are relatively more resistant to SO_2 than are the other two species. Ethane production is an extremely sensitive indicator of lipid peroxidation in liver homogenates.

The increased production of ethane and ethylene raises the prospect that bisulfite, and by extension, SO_2 , peroxidizes the polyunsaturated fatty acids of the membrane lipids and destroys the compartmentalization of the plant cells. It is possible that species resistant to SO_2 have a larger pool of natural antioxidants or lower amount of polyunsaturated fatty acids.

The Influence of Atmospheric NO₂ on the Phytotoxicity of SO₂

Many studies have demonstrated the phytotoxicity of SO₂ to crop plants, but only recently has attention been focused on the potential for enhanced damage due to the interactive effects of two or more pollutants. Several researchers have shown that SO₂ and NO₂ mixtures cause more than additive effects on crop species. Recent dose/response studies of SO₂ exposures on cultivars of the same species or on closely related species have indicated that partial resistance to SO₂ injury can be attributed to rapid and effective closure of stomata. Therefore, when assessing susceptibility to any pollutant, rigorous measurement of leaf stomatal resistances should be considered.

The main objective of this research is to determine whether and to what extent NO₂ alters the phytotoxicity of SO₂ with respect to foliar injury, reduced yield, or lowered quality of economically-important crop species. Because the Boyce Thompson Institute will move from Yonkers to Ithaca, NY during the 1978 growing season, six cultivars of soybean that can be grown to maturity in the field in both locations were selected for study: Beeson, Calland, Amsoy, Corsoy, Wells, and Hark.

Pre-exposure measurements of the leaf resistances of these soybean cultivars have shown that after the leaves have fully expanded there is a gradual but steady increase in leaf resistance with age. Furthermore, the upper foliar surfaces have higher stomatal resistances than the lower surfaces. Presumably, more pollutant would enter the leaf through the lower leaf surface due to the lower resistance to gas exchange.

These six cultivars have been exposed to 0, 0.7, 3.0 and 5.0 ppm SO₂ for four hours in our controlled environment chambers (70% RH, 20 C). Leaf resistance measurements were made on the youngest fully-expanded trifoliate leaves before, during, and after these exposures. No injury was found on the control plants or on those exposed to 0.7 ppm while the two higher concentrations induced injury over large areas of the leaves. The plants exposed to 3.0 ppm SO₂ were used to establish tentative rankings of relative susceptibility for all six cultivars. The rankings according to increasing susceptibility were Hark>Calland>Corsoy>Beeson>Wells>Amsoy.

All plants exposed to 3.0 ppm SO₂ were ranked by visual assessment of the percentage of leaf area injured. A subsample of leaves was photocopied, the images excised, and leaf area measured with a Lambda leaf area meter. By repeating the leaf area measurements with the injured areas removed, a percentage of the total leaf area injured could be determined. This was used to verify that the visual grading system being employed was adequate.

Stomatal resistance of Hark leaves increased more than did leaves of other cultivars during the SO₂ exposures, and Hark was also ranked as the most resistant to SO₂. This is consistent with results found with other species by other workers, although there appeared to be no differences in leaf stomatal resistance among the more susceptible cultivars.

The Effect of SO₂ on the Plant-Pathogen Interaction

Interest in plant-pathogen-air pollutant interactions has increased in recent years because of the wider distribution of air pollutants and increased interest in the relationship between the environment and plant disease. As more coal is utilized in existing and in new power generating facilities and as industry expands in agricultural areas, an understanding of this relationship should provide important input to air pollution risk prediction for agricultural systems.

During the first year of this contract, cultures of wheat and corn were tested for susceptibility to sulfur dioxide and two plant-pathogen-SO₂ interactions were examined. In addition, two species of conifers were obtained for study in the third year of the contract. These trees were not tested for SO₂ susceptibility as outlined in the proposal because of the greater time required for establishment in pots than had been anticipated.

Response of Wheat and Maize to SO₂

Seven cultivars of wheat (four hard red spring types and three soft white winter types) were grown in a standard soil mix in a greenhouse at about 22 C with a 16-hr photoperiod. Two weeks after seeding the plants were transferred to controlled environment growth chambers and conditioned at 27 C, 75% RH, and 20 klux illumination for 14 hrs/day and 22 C, and 80% RH during a 10-hr dark period. After two days, they were exposed to 0.0, 0.2, 0.4, or 0.6 ppm SO₂ for 30, 78, or 102 hrs and then transferred to the greenhouse. Exposures were conducted in groups of three, one control chamber and two SO₂ chambers, and were rotated weekly until each concentration

had been used twice. Five days after each exposure, plants were evaluated for foliar injury and the top growth was collected, dried for at least 48 hr at 75 C in a forced-air oven, and dry weights were recorded. The relative susceptibility of the seven cultivars, expressed as an ED₅₀ (the dose at which 50% of the plants were injured) was determined by probit analysis and is shown in Table 1. It is apparent that there is considerable variability in the response of wheat to SO₂ but it appears that the hard red spring wheats are generally more resistant than the soft white winter wheats. Foliar injury appeared as tip necrosis or as necrotic areas near the tip. In either case, the necrotic tissue was bleached to an almost white color. In general, the injury was not severe and rarely extended beyond 25% leaf necrosis.

Analysis of dry weights (Table 2) revealed significant reductions from controls in the cultivar Yorkstar after exposure to 0.6 ppm for 102 hr. In many cases, plants exposed to 0.2 ppm had greater dry weights than controls. It appears that this was an effect of a particular growth chamber, but rotation of chambers should have precluded that possibility.

Similar experiments were conducted using four maize hybrids obtained from Agron Seed Co. Experimental methods and conditions were the same as those used for wheat except SO₂ concentrations of 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 ppm for 6, 30, 54, 78, and 102 hr were used. Initial screening was done during the summer on three hybrids. In December, the fourth hybrid (ATC-75) was added, and one of the original three (RX-57) was included as a standard. Due to different conditions in the greenhouse in December, dry weights were somewhat lower for the second series of exposures.

Table 1. Relative susceptibility of wheat cultivars based on number of plants responding to a given dose

CV	Type ^A	ED ₅₀ ^B	Slope
Era	HRS	50.81 a	0.02767
Prelude	HRS	36.37 b	0.04985
Thatcher	HRS	36.31 bc	0.02913 ^C
Waldron	HRS	32.0 bc	0.04322
Yorkstar	SWW	31.24 bc	0.04897
Ticonderoga	SWW	30.52 bc	0.05904
Arrow	SWW	30.14 c	0.05264

^A HRS = Hard red spring wheat. SWW = Soft white water wheat.

^B Dose = Concentration (ppm) X length of exposure (hr).
ED₅₀'s followed by the same letter are not significantly different (p = 0.05).

^C Probit regression for Thatcher was not significant, indicating deviation from linearity.

Table 2. Effect of four concentrations of SO₂ at three lengths of exposure on dry weight of seven wheat cultivars

CV	SO ₂ conc. (ppm)	Time of exposure				
		30 hr	78 hr	102 hr		
Dry weight (g)						
Era	0.0	0.089	0.112	ab ^A	0.095	ab
HRS ^B	0.2	0.113	0.138	a	0.114	a
	0.4	0.101	0.099	ab	0.098	ab
	0.6	0.105	0.084	b	0.076	b
MSE ^C		0.00083	0.0013		0.00063	
Waldron	0.0	0.168	0.164	b	0.152	ab
HRS	0.2	0.178	0.215	a	0.173	a
	0.4	0.142	0.165	b	0.135	ab
	0.6	0.159	0.127	b	0.108	b
MSE		0.0019	0.0016		0.0022	
Thatcher	0.0	0.127	0.144		0.132	ab
HRS	0.2	0.144	0.182		0.166	a
	0.4	0.125	0.143		0.127	ab
	0.6	0.130	0.140		0.092	b
MSE		0.0015	0.0017		0.0015	
Prelude	0.0	0.165	0.167		0.168	
HRS	0.2	0.179	0.207		0.186	
	0.4	0.138	0.172		0.164	
	0.6	0.171	0.156		0.122	
MSE		0.0023	0.0027		0.0035	
Arrow	0.0	0.146	0.201	b	0.159	ab
SWW	0.2	0.163	0.268	a	0.178	a
	0.4	0.178	0.197	b	0.138	ab
	0.6	0.167	0.148	b	0.117	b
MSE		0.0028	0.0029		0.0021	
Ticonderoga	0.0	0.156	0.154		0.151	ab
SWW	0.2	0.147	0.172		0.174	a
	0.4	0.149	0.158		0.153	ab
	0.6	0.136	0.126		0.103	b
MSE		0.0015	0.0020		0.0024	
Yorkstar	0.0	0.162	0.145		0.157	a
	0.2	0.154	0.158		0.177	a
	0.4	0.161	0.145		0.145	ab
	0.6	0.141	0.120		0.095	b
MSE		0.0025	0.0022		0.0021	

^A Means followed by the same letter are not significantly different ($p = 0.05$) based on Tukey's test for comparison of means. Absence of letters indicates no significant differences. All comparisons are made within cultivar and time period. Mean of 8 plants.

^B Type of wheat. HRS = Hard red spring wheat. SWW = Soft white winter wheat.

^C MSE = Mean square for error or experimental estimate of σ^2 .

In the first exposures, foliar injury was observed on only a few plants and calculation of an ED₅₀ was not possible. The second series of exposures resulted in more visible injury (marginal and tip necrosis), although necrotic areas rarely exceeded 1% of the leaf area. Observed thresholds for visible injury are illustrated in Table 3. Exposure to SO₂ had little effect on dry weight of top growth (Table 4), even after 102 hrs at 0.5 ppm.

Tissue samples were also taken for determination of sulfur content. Analysis of these samples will be completed before June and will provide information on the relationship of sulfur content in tissue to visible injury.

Conclusions

Young plants of both wheat and maize may be considered to be resistant to SO₂ because of the small amount of foliar injury induced by large doses of SO₂ and the minimal effect of the pollutant on dry weight. These results may or may not extend to other growth stages and care should be taken in estimating effects of SO₂ on subsequent growth stages based on these data. It appears that genetic variability for response to SO₂ exists, particularly in wheat. Similar variability may exist in maize, but the doses of SO₂ used in this study did not induce a response great enough to distinguish varietal differences.

Effects of SO₂ in Infection of Wheat by Puccinia graminis f. sp. tritici

Two of the seven cultivars of wheat, Thatcher and Prelude, were selected for use in studies of wheat stem rust because Thatcher is known to have a high degree of generalized, or non-specific, resistance to P. graminis, while Prelude is considered to be devoid

Table 3. Visible response of two maize hybrids to six concentrations of SO₂ at five lengths of exposure

ATC-75						
Length of exposure (hr)	SO ₂ conc. (ppm)					
	0.0	0.1	0.2	0.3	0.4	0.5
6	-	-	-	-	-	+ ^A
30	-	-	-	-	-	+
54	-	-	+	-	+	+
78	-	-	+	+	+	+
102	-	-	+	+	+	+
RX-57						
6	-	-	-	-	-	<u>+</u> ^B
30	-	-	-	-	-	-
54	-	-	-	<u>+</u>	<u>+</u>	+
78	-	-	+	<u>+</u>	<u>+</u>	+
102	-	-	+	+	+	+

^A + Indicates at least two of eight plants injured, however slightly.

- Indicates no injury observed.

^B + Indicates one of eight plants injured.

Table 4. Effect of six concentrations of SO₂ at five lengths of exposure on dry weight of four maize hybrids

Hybrid	SO ₂ conc. (ppm)	Time of exposure				
		6 hr	30 hr	54 hr	78 hr	102 hr
RX-57	0.0	1.69	1.62	2.06	1.79ab	1.57 ab ^A
	0.1	2.46	1.66	1.76	1.47 b	0.90 b
	0.2	1.66	1.48	1.75	1.60 b	1.60 ab
	0.3	1.32	1.72	1.48	1.58 b	1.37 ab
	0.4	1.73	1.75	2.70	2.90 a	2.16 a
	0.5	1.78	1.76	1.97	1.27 b	1.20 ab
MSE ^B		1.390	0.584	0.733	0.406	0.341
RX-94	0.0	1.34	1.49	1.90	1.84	1.41
	0.1	1.86	1.18	1.33	1.52	1.12
	0.2	1.26	1.82	2.07	1.67	1.60
	0.3	1.48	1.53	1.65	1.48	1.12
	0.4	1.57	1.74	1.77	2.05	1.61
	0.5	1.57	1.85	1.58	1.12	1.20
MSE		0.910	0.476	0.396	0.429	0.225
EX-114	0.0	1.44	1.56	1.80 ab	1.79	1.44
	0.1	1.26	1.28	1.15 b	1.31	1.32
	0.2	1.68	1.85	2.37 a	1.36	1.71
	0.3	1.47	1.21	1.51 ab	1.31	1.11
	0.4	1.48	1.25	1.60 ab	2.17	1.78
	0.5	1.53	1.45	1.38 b	1.21	1.36
MSE		0.383	0.309	0.212	0.310	0.421
ATC-75	0.0	0.28	0.31	0.41	0.41	0.60
	0.1	0.43	0.39	0.42	0.42	0.69
	0.2	0.31	0.29	0.39	0.50	0.59
	0.3	0.26	0.35	0.40	0.56	0.60
	0.4	0.33	0.35	0.34	0.47	0.63
	0.5	0.32	0.36	0.45	0.57	0.66
MSE		0.009	0.007	0.012	0.011	0.023
RX-57 ^C	0.0	0.40	0.49	0.54	0.58	0.77
	0.1	0.33	0.35	0.69	0.67	0.86
	0.2	0.42	0.65	0.61	0.65	0.83
	0.3	0.46	0.47	0.47	0.66	0.87
	0.4	0.49	0.51	0.58	0.75	0.89
	0.5	0.45	0.41	0.46	0.67	0.89
MSE		0.021	0.023	0.023	0.034	0.056

^A Means followed by the same letter are not significantly different ($p = 0.05$) based on Tukey's test for comparison of means. Absence of letters indicates no significant differences. All comparisons are made within hybrid and time period.

^B MSE = Mean square error or experimental estimate of σ^2 .

^C ATC-75 was tested approximately 5 months after the other hybrids. RX-57 was included for comparison to first test. Growing conditions were reproduced as closely as possible but greenhouse conditions during the winter were different resulting in much less growth.

of generalized resistance to the pathogen. Generalized resistance is usually considered to be more desirable than specific resistance and current breeding programs are attempting to incorporate this trait.

Plants of the two cultivars, cultured as previously described, were conditioned for two days at 25/20 C, 75%/80% RH and 20 klux for 14 hrs/0 klux for 10 hrs (day/night). After conditioning, they were exposed to 0.10 ppm SO₂ for 102 hrs and then removed to the greenhouse.

Inoculations were made two days before exposure, one hr after exposure and five days after exposure. Two methods of spore application were employed: 1) 8 mg of spores were suspended in 2 ml of Soltrol 170 oil and applied as a spray at the rate of 0.2 ml/plant, 2) spores were applied as uniformly as possible to the undersurface of a leaf with a cotton swab. Control plants were treated in an identical manner, except that spores were excluded from the oil or swab. After inoculation, the plants were placed in a mist chamber for 16 hr at 17 C and then returned to growth chambers or the greenhouse. Ten days after inoculation, the number of pustules per plant was recorded and the aboveground portions of four individual plants per treatment were collected for sulfur analysis and dry weight determination. In "swab" experiments, spores were collected from pustules and incubated on water agar plates for 24 hr to determine per cent germination. The number of pustules produced on SO₂-fumigated plants vs unfumigated plants was less, in all but one case, on Thatcher. The reduction was significant in only two cases, but the trend was consistent and repeatable. In the case of Prelude, the results were much more

variable and, in several cases, exposed plants were more heavily infected than control plants. Statistical significance could not be shown for these differences and trends were not obvious in the data (Table 5). Analysis of data on dry weight, sulfur content, and spore germination did not show significant interactions of the pollutant and pathogen and trends in the data were not apparent (Table 6).

Conclusions

Exposure of Thatcher wheat to 0.1 ppm SO₂ for 102 hrs appears to reduce infection, although the effects were not great. Infection of Prelude appears to be unaffected by SO₂ exposure. The interaction of SO₂ and the pathogen did not manifest itself in either dry weight of top growth or sulfur content of the plant. Germination of spores produced on exposed plants was not different from that of spores from control plants. The differences seen between representatives of two types of resistance and the possible effects on the epidemiology of the disease should be investigated in the future, preferably in field experiments.

Effects of SO₂ on Infection of Bountiful Bean by Southern Bean Mosaic Virus

Although not included in the original proposal, a study of Southern Bean Mosaic Virus (SBMV) was beginning at the time the contract was initiated. These experiments were completed using contract resources and the results are therefore included.

Bountiful bean plants were grown in a synthetic soil mix in a charcoal-filtered greenhouse until unifoliate leaves were fully expanded, and then transferred to controlled environment growth chambers. Plants were exposed to 0 ppm or 0.15 ppm SO₂

Table 5. Effect of SO₂ exposure on infection of wheat of Puccinia graminis f. sp. tritici, Race 15B-2.

CV	Inoculation B	SO ₂ conc. (ppm)	Time of inoculation A		
			-2 days	+1 hr	+5 days
			No. of pustules/plant D		
Thatcher I C	Spray	0.0	4.58*	1.33	1.91
		0.1	1.08	0.25	0.50
Thatcher II	Spray	0.0	33.66*	1.58	4.08
		0.1	5.16	0.16	1.33
Thatcher I	Swab	0.0	5.50	48.08	17.75
		0.1	1.58	39.08	25.91
Thatcher II	Swab	0.0	29.92	46.67	13.67
		0.1	18.83	41.33	13.50
Prelude I	Spray	0.0	5.33	1.83	24.25*
		0.1	0.50	1.91	1.58
Prelude II	Spray	0.0	61.75	64.66	7.33
		0.1	69.75	63.83	14.91
Prelude I	Swab	0.0	78.83	114.83	9.91
		0.1	64.75	79.00	4.41
Prelude II	Swab	0.0	61.75	64.66	7.33
		0.1	69.75	63.83	14.91

A Time of inoculation - - Pre-exposure. + = Post exposure.

B Method of spore application Spray = Oil spray. Swab = Cotton swab.

C I and II indicate repetitions of each experiment.

D Mean of 12 plants.

* Indicates significant difference ($p = 0.05$). Comparisons made between exposed and non-exposed plants at the same time of inoculation.

Table 6. Effect of SO₂ exposure and *P. graminis* infection on dry weight, sulfur content and spore germination on two cultivars of wheat

CV ^A	Inoculation ^B	SO ₂ conc. (ppm)	Dry weight (g) ^C						Sulfur content (%) ^D						Spore germination (%) ^E		
			-2 days		+1 hr		+5 days		-2 days		+1 hr		+5 days		-2	+1	-2
			I	C	I	C	I	C	I	C	I	C	I	C	days	hr	days
Thatcher I	Spray	0.0							0.194	0.239	0.224	0.233	0.253	0.268			
		0.1							0.410	0.451	0.552	0.506	0.437	0.561			
Thatcher II	Spray	0.0							0.289	0.323	0.322	0.290	0.375	0.314			
		0.1							0.522	0.493	0.441	0.412	0.517	0.494			
Thatcher I	Swab	0.0	0.250	0.252	0.417	0.385	0.475	0.625	0.459	0.629*	0.503	0.474	0.464	0.367	89*	90	94
		0.1	0.237	0.280	0.315	0.435	0.440	0.562	0.700	0.692	0.572	0.559	0.512	0.483	71	89	93
Thatcher II	Swab	0.0	0.192	0.235	0.320	0.312	0.452	0.767	0.454	0.449	0.561	0.482	0.388	0.443	93	93	97
		0.1	0.212	0.210	0.247	0.287	0.615	0.722	0.564	0.586	0.528	0.720*	0.522	0.475	94	94	95
Prelude I	Spray	0.0							0.503	0.522	0.553	0.566	0.496	0.512			
		0.1							0.719	0.675	0.657	0.753	0.641	0.726			
Prelude II	Spray	0.0							0.343	0.389	0.374	0.463	0.422	0.375			
		0.1							0.575	0.561	0.674	0.655	0.566	0.574			
Prelude I	Swab	0.0	0.327	0.287	0.600	0.600	0.930	1.015	0.372	0.463	0.406	0.321	0.345	0.422	94	96	98
		0.1	0.372	0.297	0.437	0.510	0.692	0.775	0.468	0.601	0.416	0.396	0.496	0.437	93	94	93
Prelude II	Swab	0.0	0.320	0.315	0.265	0.270	0.422	0.492	0.337	0.328	0.485	0.472	0.394	0.304	96	97	97
		0.1	0.307	0.350	0.295	0.277	0.420	0.570	0.478	0.539	0.523	0.514	0.466	0.381	94	99	97

A I and II indicate repetitions of the same experiment.

B Method of spore application: Spray = Oil spray. Swab = Cotton swab.

C Times of inoculation indicated are relative to SO₂ exposure. I = Inoculated. C = Control. Numbers are means of four observations.

D Mean of two plants for spray inoculation; mean of four plants for swab inoculation.

E Mean of six collections.

* Indicates significant difference ($p = 0.05$). Comparisons made between exposed and non-exposed plants at the same time of inoculation.

continuously at 23.8 C, 70% RH and 20 klux illumination for 14 hr/day. Two groups of plants were placed in each chamber for a period of seven days. Unifoliate leaves were then inoculated with SBMV in 0.1 M phosphate buffer, pH 7, and one group from the control chamber was placed in the SO₂ chamber, one group from the SO₂ chamber was transferred to the control chamber, and the other groups were returned to their original chambers. Non-inoculated controls were maintained in each treatment. After another seven-day period, the plants were removed and harvested. Virus titer in purified leaf extract was determined spectrophotometrically. Sulfur content of leaf tissue was determined by complete oxidation of ground, dried leaf tissue, absorption in H₂O₂, and titration to endpoint with BaCl₂. The experiment was repeated five times.

In all repetitions, virus titer increased in fumigated plants when compared to controls; however, the increase was statistically significant in only one case. Post-inoculation fumigation resulted in the greatest increases in virus titer. In unifoliate leaves, sulfur uptake was increased when the second exposure was preceded by SO₂ treatment rather than control, i.e., the effects of pre- and post-inoculation SO₂ exposure were more than additive. In trifoliolate leaves, there was little or no interaction between pre- and post-inoculation exposure. The data are currently being analyzed to determine if this effect is one of sequential fumigation or virus infection.

Effects of SO₂ on Plant-Insect Interactions

As originally proposed, this work was divided into two parts. In one, the soybean/Mexican bean beetle relationship is being used to investigate the extent to which SO₂ might alter the suitability of the plant as a host. In the other, conifers are being used to study the effects of SO₂ on the production of secondary plant products in conifers and possible relationships with destructive insects.

Soybean/Mexican Bean Beetle

The goals for the first year of this investigation were to determine the susceptibility of different soybean cultivars to the Mexican bean beetle (MBB) and to initiate studies on the feeding habits and development of this insect on SO₂-fumigated and control plants.

The studies on soybean susceptibility to the MBB began with an exhaustive search of published reports and a search by Dr. T. Hymowitz of the Department of Agronomy, University of Illinois, Urbana, through their data bank retrieval system for soybean lines susceptible and resistant to this insect. These searches and personal conversation with scientists conducting research on the subject disclosed that none of the commercial soybean cultivars are resistant to the MBB and only one recently-introduced cultivar, Shore, is tolerant at low insect population levels. Although some P.I. lines have shown considerable resistance, for reasons of availability, practical application, and coordination with other areas of this project, the commercial cultivars are most suited for our investigation. Therefore, studies were designed to be responsive to variations in the level of susceptibility to the

MBB by closely monitoring for differences in antibiotic effects, feeding preferences, development time, and fecundity of the insect on different cultivars. These tests, as well as those to be used in achieving the second goal of the current year's research, require precise control of rearing temperature, which can itself affect profoundly insect growth and development. A growth chamber was ordered to fill this need, but a series of delays prevented its delivery until mid-February. Accordingly, progress on this part of the research has been hampered.

In the meantime, a colony of the MBB was established and the tests proposed for the year's work were conducted in a preliminary manner, permitting difficulties to be corrected and the procedures to be modified where necessary. In addition, the time was utilized to develop needed background information on the MBB on a preferred host, Phaseolus vulgaris. Although we have had a considerable amount of experience in rearing this insect for routine assays, the studies to be conducted as part of this project demand a greater knowledge of reproduction, feeding habits, and growth and development of the MBB.

Reproduction. Under favorable conditions, mature females deposited one egg mass every 2.3 days. Ninety-four per cent of the egg masses on intact plants were deposited on the undersurface of the leaves. Unless the insects were stressed for food, the eggs were deposited on leaves where very little or no feeding had occurred. It is not clear whether the presence of eggs deters feeding, but it is quite evident that a shortage of "undisturbed" leaf tissue will deter oviposition. Accordingly, females held in Petri dishes would not deposit eggs for as long as five days if provided only

enough leaf tissue/day for feeding. Under these conditions, when oviposition occurred, the eggs were frequently scattered rather than in masses. Normal oviposition could be achieved on excised leaves in crystallization dishes by providing each female with a new leaf each day. These females deposited an average of one egg mass every 2.1 days.

Although the number of matings/female has been shown to affect egg viability, we found that availability of males and subsequent mating did not affect the length of the preoviposition period or the number of eggs deposited/day. In fact, virgin females began ovipositing at the same time as multiply-mated females.

Feeding behavior. Feeding habits of adult males and females were studied prior to adopting the procedure for feeding preference assays.

The Mexican bean beetle feeds by scraping parenchymatous tissue into a "windrow" and then compressing the tissue to ingest the plant juices. By feeding in this manner, the beetle leaves a network of leaf veins and windrows of crushed tissue that prevent direct measurement of damaged leaf area by electronic area meters. It was found that a highly significant linear correlation ($r = .985$) existed between area meter readings of damaged discs with and without the damaged area trimmed from the undamaged tissue. Consequently, a regression equation was calculated that permits accurate conversion of untrimmed values to actual area consumed.

The area of leaf tissue consumed by adult males and females was also determined daily for 14 days starting from the time of pupal eclosion. It was found that consumption was higher in immature

adults (prior to the onset of oviposition), especially by the males (4.90 and 2.91 cm²/day for immature and reproductive males, respectively; 6.97 and 5.53 cm²/day for immature and reproductive females, respectively). Although the effects were not quantitated, it was also observed that females did not feed for a period of 30 to 60 minutes prior to oviposition or for 30 to 60 minutes after oviposition (the effects are not reflected in the daily averages of area consumed because the reproductive cycles were not synchronized). On the basis of these results, immature females 3-4 days old will be used in feeding preference assays.

Growth and development. The proposed studies require that the MBB be reared on excised leaves in closed dishes, so growth and development under these conditions were compared to that on intact plants. The total development time on intact Pinto beans (Phaseolus vulgaris) approximated that reported by other workers using similar rearing conditions, provided that the larvae were not overcrowded. Larval development time was very sensitive to food availability, i.e., larval density relative to the food supply affected the development time. Larval development time was greatly prolonged (16 days), growth retarded (average pupal weight of 29.0 mg), and growth rate lowered (0.136 as opposed to 0.151 under favorable conditions) when larvae were reared in 90 X 15 mm Petri dishes with a constant 3 cm² of leaf surface/larva. When the leaf area was adjusted as required through the larval stadia, up to 15 larvae could be reared/90 X 50 mm crystallization dish without retarding development time or growth (12 days and average pupal weight 42.5 mg, respectively).

The growth chamber will be fully operational prior to March 1, so the remaining quarter of the current year will be used to screen soybean cultivars for susceptibility to the MBB. It is anticipated that 2/3 of this screening will be completed by June 1.

Volatile Constituents of Conifer Foliage

Sampling system. Preliminary investigations were concentrated on the establishment of a rapid, reliable method for analysis of needle volatiles and on the selection of tree species for further study. A comparison between headspace samples and pentane extracts of needles indicated that the solvent extracts gave more reproducible results. Approximately 0.5 g of chopped needles were allowed to soak in 2 ml pentane or hexane for 30 min and the resulting extract was suitable for direct injection into the gas chromatograph. Headspace samples of the chopped needles appeared to be adequate for analysis of the monoterpene hydrocarbons, but due to their limited volatility, oxygenated terpenes and sesquiterpenes were not readily detected. The pentane extraction method was therefore used for all further studies.

Conditions for gas chromatography. Several columns were compared for separation of the terpenes present in conifers. The best polar column appeared to be FFAP (free fatty acid polyester), and good non-polar separation was obtained with an OV-1 column. In each case, temperature programming from 80° to 160° at 4°/min provided a clear separation between the monoterpenes, oxygenated terpenes, and sesquiterpenes.

Variation in volatile composition. Observations on the variability of volatile profiles of needles from different trees or

branches of the same tree were made using readily available Douglas-fir, white pine, and Scotch pine. Gas chromatograms obtained from extracts of needles from different branches of the same tree were essentially identical qualitatively. However, some variation was noted from tree to tree. In the case of Douglas-fir, sabinene appeared to be absent in some individuals but present in others. Less variation was found between individuals of the two pine species.

Collection of volatiles. In order to determine whether solvent extracts can indicate the composition of volatiles emanating from the needles of growing trees, a system was devised to collect the total volatiles from whole plants. The plants are placed in a glass or clear plastic chamber through which air is drawn continuously by means of a vacuum. The volatile-laden air is bubbled through a tube containing pentane, and any pentane evaporating is condensed in a Vigreux column so that it returns to bubbler tube. In this way, continuous collections of the volatiles can be made in pentane without the problems of breakthrough which are encountered during collection on solid adsorbents such as Porapak. Preliminary results with this system indicate that considerable differences may exist between the profiles obtained by extraction and by air entrapment.

Selection of insect pest. Investigations into the most suitable insect pest of conifers for these studies have resulted in the selection of the spruce budworm, Choristoneura fumiferana. This insect meets the requirement of representing a major pest of several conifer species, it is relatively easy to rear on a synthetic diet, and is known to occur in New York State. Preparations are now under way to start a colony of the spruce budworm at the Institute.

Effects of SO₂ on Roots

A study of the effect of atmospheric SO₂ on root development, physiology, metabolism, and ecology is being initiated and supported by the Boyce Thompson Institute to complement the contract sponsored by DOE.

Interest in the indirect effect of air pollutants upon roots has been almost nil in spite of the realization that growth and development of above-ground plant parts are dependent upon their supporting root system. An understanding of this relationship with reference to air pollution effects should provide a significant input into air pollution risk predictions and our understanding of long-term and short-term effects.

Over the past year, root studies were done to support another project that required the quantitative measurement of root exudates from lowland rice plants. Root exudates were considered a potential energy source for nitrogen fixation by free-living soil microorganisms in rice paddies. Through these studies, an expertise in handling and studying root systems was developed which will be used in 1978 to augment the DOE-supported study on the effects of SO₂ and NO₂ on plants.

Cooperative work with John Laurence in late 1977, led to the collection, examination, and the measurement of roots from wheat and maize plants used in the study of plant pathogen - SO₂ interactions.

Response of Wheat and Maize Roots to SO₂

Roots from plants in experiments described by John Laurence in "Effects of SO₂ on Plant-Pathogen Interactions" were collected,

washed, separated, oven-dried for 48 hours at 75° C, and weighed.

Roots were from hard red spring wheat cultivars Prelude and Thatcher exposed to SO₂ and Puccinia graminis and from corn hybrids ATC-75 and RX-57 exposed to SO₂. In all cases, the effect of SO₂ and/or disease had little or no effect on the dry weight of roots or the shoot/root ratio, but the data have not been analyzed statistically.

If effects of SO₂ upon root development are to be measured at levels of SO₂ below the threshold for visible foliar symptoms, a much more sensitive measure than dry weight is required. Several approaches will be examined including the distribution and respiration of ¹⁴C-labelled photosynthate throughout the plant, and changes in root respiration, mycorrhizal populations, and root exudates.

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