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Sulfur Dioxide Effects on Petunia Pollen Germination and Seed Set

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Sulfur dioxide (SO_2) is one of the most important, biologically-active air pollutants (Fowler and Cape 1982; Urone 1976). The SO_2 effect on certain areas of plant growth and development has been examined in a limited number of species. Some of the general effects reported include: changes in stomatal aperture, reduction in dry plant weight, inhibition of some photosynthetic enzymes, depression of net photosynthetic rates, and chlorophyll decomposition expressed by necrosis and chlorosis (Bell 1982; Black 1982; Godzik and Krupa 1982; Posthumus 1982; Wellburn 1982). Information pertaining to SO_2 effects on sexual reproduction is extremely limited even though this complex process is critical especially in annual species (Bonte 1982). This study reports the SO_2 effect on both *in vitro* and *in vivo* pollen germination characteristics and *in vivo* seed set in *Petunia hybrida* Vilm.

MATERIALS AND METHODS

For the *in vitro* pollen germination studies, hydrated pollen grains from clone W166H were placed in a germination medium (10% sucrose + 0.01% boric acid) on slides which were immediately placed in closed Petri dishes at 26°C. One group of Petri dishes contained 20 ppm SO_2 generated by mixing appropriate amounts of HCl and $Na_2S_2O_5$ (Adam 1976) while the second control group contained the ambient SO_2 level. After 2 h of incubation, at least 10 pollen germination counts (100 each) from the control and 20 ppm SO_2 groups were taken after fixing and staining with a 1% aniline blue solution (0.1% aniline blue + 10 ml lactic acid + 10 ml glycerin + 10 g phenol + 10 ml distilled water). After 3 h of incubation, at least 9 pollen tube length measurements from the control and 20 ppm SO_2 groups were taken after fixing and staining with 1% aniline blue. This experiment was repeated on each of 3 days. Statistical comparisons were made between the control and 20 ppm SO_2 groups using a F test.

For the *in vivo* pollen germination studies, pollen grains from the clone W166H were placed on styles from clone T2U which were immediately placed in closed plexiglass chambers. Three forms of stylar attachment were tested; detached from the flowers,

attached to flowers which were detached from the plant, and in flowers attached to the plant. Each chamber was targeted to contain a certain SO_2 level by mixing appropriate amounts of HCl and $Na_2S_2O_5$ and was equilbrated for 17 h prior to the pollinated style introduction. For each SO_2 level, a corresponding control chamber containing the ambient SO_2 level was present so that more precise paired comparisons between each SO_2 level and a control were possible. Seventeen hours after pollination and introduction into the chamber, the styles were removed. Using fluorescent microscopy, at least 30 pollen germination counts (100 each) and at least 25 pollen tube length measurements from each SO_2 level and its corresponding control were taken. At the time of stylar removal, the actual SO_2 level in each chamber (excluding the control) was determined using the sulfate concentration method (Adam 1976). Statistical comparisons were made between each SO_2 level and its corresponding control using a F test.

For the *in vivo* seed set studies, the same procedure was used with seed set obtained from pollinated styles attached to the flowers on plants after 30 days in the chambers. The number of seeds produced in at least 20 flowers was determined at each SO_2 level and its corresponding control. Statistical comparisons were made between each SO_2 level and its corresponding control using a F test.

RESULTS AND DISCUSSION

Relatively low SO_2 levels (20 ppm) resulted in highly significant reductions in *in vitro* pollen germination and pollen tube length (Table 1). A greater % reduction was found for pollen germination (45%) than for pollen tube length (32%).

Table 1. *In vitro* pollen germination and pollen tube length after exposure to 20 ppm SO_2.

Characteristics	Comparisons		% reduction
	Control	20 ppm SO_2	
Pollen germination (%)	53	29**	45
Pollen tube length (μ)	88	60**	32

**F value between control and 20 ppm SO_2 significant at the 1% level.

With only one exception among the 16 comparisons, highly significant reductions in *in vivo* pollen germination, pollen tube length, and seed set were found (Table 2). In general, increasing SO_2 levels increased the % reduction but the magnitude of the increases was not consistent. For example, the effect of increasing SO_2 levels was more pronounced for pollen germination than pollen tube length at all stylar attachment forms. At similar SO_2 levels, the % reduction was greater for seed set and less with pollen tube length. For example, at about 200 ppm SO_2, the % reductions in seed set, pollen germination (on plant) and

Table 2. *In vitro* pollen germination, pollen tube length, and seed set after exposure to various SO_2 levels. Pollen germination and pollen tube length measurements were made with the styles in three attachment forms; detached from the flowers (detached styles), in the flower detached from the plant (detached flowers), and in the flowers attached to the plant (plant). Seed set was obtained from pollinated styles attached to the flowers on the plants.

Characteristic	Stylar attachment	SO_2 level (ppm)	Comparison		% reduction
			Control	SO_2 treatment	
Pollen germination (%)	Detached styles	113	72	35**	51
		160	77	38**	51
		495	86	1**	99
	Detached flowers	157	76	43**	43
		403	76	38**	50
	Plants	102	78	70**	10
		213	80	54**	33
Pollen tube length (μ)	Detached styles	113	824	737**	11
		160	848	665**	22
		495	862	30**	97
	Detached flowers	157	1314	1128**	14
		403	1019	728**	29
	Plants	102	1370	1305	5
		213	1220	961**	21
Seed set (number/flower)		81	165	130**	21
		208	156	77**	51

**F value between the control and SO_2 treatment significant at the 1% level.

pollen tube length (on plant) were 51, 33, and 21, respectively. As a rule, pollen germination at all stylar attachment forms was reduced considerably more than pollen tube length at comparable SO_2 levels. For example, at 113 ppm SO_2, the % reduction in pollen germination and pollen tube length in detached styles was 51 and 11, respectively. The form of stylar attachment considerably altered the SO_2 effect on both pollen germination and pollen tube length. Generally, the % reductions were more pronounced with detached styles and least pronounced with styles on plants.

The results of this study clearly indicate that many aspects of the plant reproductive process are influenced by elevated SO_2 levels. The extreme sensitivity of *in vitro* pollen germination to relatively low SO_2 levels found in this study is probably the result of SO_2-induced pH reductions in the germination medium. Exposure of the *in vitro* germination medium to very low SO_2 levels for a brief period markedly lowered the pH and this acidification was considered the primary cause of reduced pollen germination and tube growth in a number of species (Bonte 1982). Species differences in tolerance to acidity in the *in vitro* germination medium were reported and, from this, it can be concluded that Petunia is quite acid-sensitive. In this study, pollen germination was more sensitive to acid conditions in the medium than pollen tube length suggesting that these two related processes differed in their response to SO_2 acidification. The *in vivo* pollen germination and tube length study reported here indicated that pollen germination was more sensitive to elevated SO_2 levels than pollen tube length and this sensitivity was associated with the form of stylar attachment. SO_2 is known to induce many complex changes in plant growth and development and probably many undiscovered modifications as well (Bell 1982; Black 1982; Bonte 1982; Coffin and Stokinger 1977; Godzik and Krupa 1982; Posthumus 1982; Wellburn 1982). Logically, pollen germination should be more sensitive to elevated SO_2 levels compared to pollen tube growth because germination occurs on the surface of the stigma in close contact with the atmosphere and plant. As a result, adverse SO_2 effects from either area can be readily transmitted to the germinating pollen grain. On the other hand, pollen tube growth occurs only in close contact with the plant and should be insulated from atmospheric SO_2. In this study, a considerable reduction in seed set at relatively low SO_2 levels were found. Although a few reports (Bonte 1982) of SO_2 reducing seed weight are available, there is limited information about the extent of seed set reduction and the possible mechanisms involved. Relatively low SO_2 levels are known to disrupt the vegetative and reproductive nuclei in the pollen tube (Bonte 1982), and this disruption may result in abortion which, in turn, is expressed as reduced seed set. More research in this critical area is essential to elucidate the specific effects of SO_2 on plant reproduction and to determine the possible impact of elevated SO_2 levels on crop production (Last 1982).

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