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The effects of long-term open-air fumigation with SO_2 on a field crop of broad bean (*Vicia faba* L.)

II. Effects on growth components, leaf area development and elemental composition

BY M. J. KROPFF[1,3*], J. MOOI[2], J. GOUDRIAAN[1], W. SMEETS[3], A. LEEMANS[3] AND C. KLIFFEN[2]

[1] *Department of Theoretical Production Ecology, Agricultural University, P.O. Box 430, 6700 AK Wageningen, The Netherlands*

[2] *Research Institute for Plant Protection, P.O. Box 9060, 6700 GW Wageningen, The Netherlands*

[3] *Department of Air Pollution, Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands*

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SUMMARY

Faba bean crops (*Vicia faba* L.) were exposed to elevated SO_2 concentrations in three different years in an open-air field exposure system for the controlled release of air pollutants. The treated crops were exposed to an average SO_2 concentration of 165 $\mu g\ m^{-3}$ in 1985; 62 $\mu g\ m^{-3}$ in 1986 and 74 $\mu g\ m^{-3}$ in 1988. The ambient SO_2 concentration was about 10 $\mu g\ m^{-3}$. Plant height, number of internodes and number of pods were not affected by SO_2. The specific leaf area was reduced in the plants exposed to SO_2 at the end of the growing season. Leaf area development was strongly affected during the pod-filling period in 1985 and 1988 as a result of leaf injury and defoliation in the fumigated plots. In 1986 a similar trend in leaf area reduction was observed in the early reproductive phase.

N and Mg content of the different organs was unaffected by SO_2. The S content was strongly elevated in the leaves and pods of the fumigated plants, and the Ca content of the leaves was reduced by SO_2. Chlorophyll content of different leaf numbers was unaffected by SO_2.

Key words: *Vicia faba*, sulphur dioxide, air-pollution, open-air fumigation, elemental composition, leaf injury.

INTRODUCTION

The influence of atmospheric pollutants, especially SO_2, on plant growth has been the subject of numerous studies in the past, most of which have shown that plant growth is depressed as a result of long term exposures to concentrations as low as 40 $\mu g\ SO_2\ m^{-3}$ (Bell, Rutter & Relton, 1979). However, other workers have reported increases in the growth of plants exposed to low levels of SO_2, even when the sulphur nutrition of the plants is adequate (McLeod *et al.*, 1988). One of the main causes of reported differences in responses of plants to air pollutants is the influence of the particular exposure system on the microclimate, since the effect of air pollutants on plants is largely affected by environmental factors such as windspeed, humidity and radiation (Black, 1982). In most experiments in which the effects of air pollution on plant growth are studied, some form of enclosure is used in controlling the pollutant, ranging from indoor growth cabinets to open-top chambers in the field. The extrapolation of the effects observed in these studies to the field situation may lead to over- or under-estimation of the effects (Olszyk *et al.*, 1986).

* Correspondence should be sent to: M. J. Kropff, Department of Theoretical Production Ecology, Agricultural University, P.O. Box 430, 6700 AK Wageningen, The Netherlands.

In a previous paper (Kropff *et al.*, 1989) we reported on the effects of SO_2 on the growth and seed yield of broad bean exposed to long-term elevated SO_2 concentrations. In three years, the crops were exposed to mean SO_2 concentrations of 165 $\mu g\ m^{-3}$ (in 1985), 62 $\mu g\ m^{-3}$ (in 1986) and 74 $\mu g\ m^{-3}$ (in 1988). In all years no effects on plant growth were found during the vegetative phase. A final reduction in total dry matter of 17% was observed in 1985 and 9% in 1988. In 1986 a severe infection of the control crop with the fungal pathogen *Botrytis fabae* occurred in the pod-filling period. Slight *B. fabae* infections were also observed in the control plots in 1985 and 1988. The objective of this part of the study was to determine the effects of SO_2 on dry matter growth of the different plant parts, morphological parameters and mineral content of plant organs.

MATERIALS AND METHODS

Faba bean crops (*Vicia faba* L. cv. Minica) were exposed to elevated SO_2 concentrations in three contrasting growing seasons in 1985 and 1986, using the open-air exposure system developed by Mooi & Van der Zalm (1986). Experimental details were given in a previous paper (Kropff *et al.*, 1989). In the open-air exposure system, plots of 8 × 8 m were exposed to mean SO_2 concentrations of 165 $\mu g\ m^{-3}$ in 1985, 62 $\mu g\ m^{-3}$ in 1986 and 74 $\mu g\ m^{-3}$ in 1988. The control plot, located at 250 m distance from the system, was exposed to SO_2 at background concentrations of SO_2 only which were 16 $\mu g\ m^{-3}$ in 1985, 7 $\mu g\ m^{-3}$ in 1986 and 9 $\mu g\ m^{-3}$ in 1988.

The bean plants were grown at a density of 20 plants per m^2 in plastic containers (55 × 22 × 25 cm) filled with a commercial potting mixture (Trio 17) in order to avoid confounding effects of differences in soil conditions between the plots. Water was supplied by a drip-irrigation system. In 1985 the plants emerged on 16 April, in 1986 on 5 May and in 1988 on 15 May. Plant growth was determined up to 29 July in 1985, 21 July in 1986 and 25 August in 1988 by frequent harvesting. The plots were divided into 4 blocks each containing one subplot for each harvest (6 in 1985 and 5 in 1986). In 1988 five plants were harvested from one container of the three blocks. Between the last 2 harvests in 1986, plants in the control plots became infected with the fungal pathogen *B. fabae*.

After collection of the plants in the field, they were divided into leaves, stems, and pods. Leaf area was measured separately for each plant. As necrosis and yellowing were unevenly distributed over the leaf (mostly starting at the margins), the total area of the green, yellow and necrotic tissue was measured by passing the leaves through a Delta-T Devices moving belt planimeter. In 1988 the fraction of yellow and necrotic tissue was estimated. Plant height, the number of internodes (which is a measure of phenological development) and the number of pods were determined on all harvested plants. In 1986 the number of leaves was also counted. In 1986 leaf area and leaf dry weight were determined for 4

Table 1. *The response of leaf, stem, pod and total dry weight of broad bean* (Vicia faba) *exposed to ambient or elevated SO_2 concentrations in 1985 (165 $\mu g\ m^{-3}$), in 1986 (62 $\mu g\ m^{-3}$) and in 1988 (74 $\mu g\ m^{-3}$), in relation to plant age expressed in days after emergence*

	Leaf dry weight (kg ha^{-1})		Stem dry weight (kg ha^{-1})		Pod dry weight (kg ha^{-1})		Total dry weight (kg ha^{-1})	
Plant age (d)	Control	Fumigated	Control	Fumigated	Control	Fumigated	Control	Fumigated
1985								
24	96	84***	32	26***	—	—	128	110***
38	494	404***	315	241***	—	—	809	645***
45	935	814***	960	722***	—	—	1895	1536***
65	1946	1906	3488	3150*	92	52***	5526	5108*
85	2432	2296	5066	4952	1936	1392**	9434	8640
105	2020	1456**	4626	4442	7888	6108***	14534	12006**
1986								
21	144	168	59	64	—	—	203	232
39	817	752	766	647	65	46	1648	1445
53	1646	1401*	2657	2177**	335	404	4658	3982
67	1799	1646	3399	2844***	3265	3334	8454	7824
1988								
40	968	1065	1147	1119	—	—	2115	2184
61	1759	1691	3618	3730	129	145	5506	5566
81	1743	1589	4848	6055*	2897	1728*	9488	9372
102	1255	662*	4699	4839	7707	6920	13661	12421

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$.

leaf numbers separately (number 5, 10, 15 and 20 counted from the bottom of the plants). From leaf dry weight and leaf area the specific leaf area was calculated (SLA in cm^2 leaf g $leaf^{-1}$), which is a measure of leaf thickness. In 1986, only plants which were not infected with *B. fabae* were sampled from the control plot at final harvest to permit the analysis of the effects of SO_2 on elemental composition.

Subsamples of the harvested plants were analysed for N, S, Ca and Mg content in the Chemical Laboratory of the Research Institute for Plant Protection. The unwashed leaves were dried and ground in a laboratory mill to pass a 2 mm sieve. Both S and N were analysed with a Carbo Erba Elemental Analyser. Ca was analysed spectrophotometrically with a Technicon Autoanalyser GT II. The chlorophyll *a* and *b* content of leaf samples was determined according to Bruinsma (1963).

RESULTS

The response of the dry weight of plant organs during the growing season to SO_2 exposure is given in Table 1. Total dry weight of the plants was significantly reduced in 1985 in the vegetative phase of growth and at final harvest when a total dry matter reduction of 17% was observed. In 1986 and 1988, slight reductions of total dry matter were not significant. Although the dry weight of all organs was reduced in the exposed plots at final harvest in 1985, significant reductions were observed only in leaf dry weight (28%), due to defoliation, and pod dry weight (23%). Pod dry matter was slightly greater in the exposed plants in 1986. In 1986 only a small, but significant, reduction in stem dry weight was observed. In 1988 leaf dry weight was significantly reduced by 47%. Dry matter content of the exposed plants was not influenced by SO_2 (data not shown).

In all years the time course of leaf area development in both the control and the fumigated crop showed the same pattern (Fig. 1): a slight depression of the leaf area index (LAI) at the beginning of the growing season and a strong reduction of the LAI at the end of the growing period. This was due to severe leaf injury and defoliation of the oldest leaves. For 1986 only, the onset of defoliation in the fumigated plots can be detected from Figure 1b. However, in the field we observed severe leaf injury in the second part of the pod-filling period (after harvest 4) in the fumigated plot.

The response of plant height, number of internodes, number of pods, number of leaves and the SLA to SO_2 fumigation are given in Table 2. The effect of SO_2 on plant height was small. The number of internodes was not influenced in 1985 and 1988 and slightly reduced in 1986. The number of leaves was reduced from about 60 days after emergence

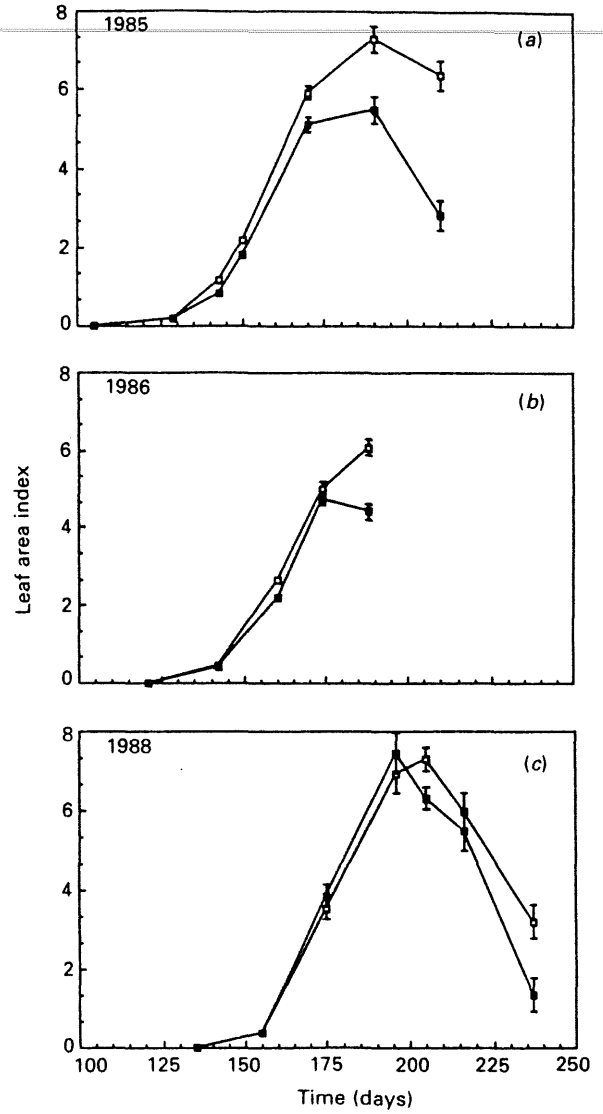

Figure 1. Time course of the Leaf Area Index (m^2 leaf m^{-2} ground) in control (□) and fumigated (■) plots in 1985 (*a*), 1986 (*b*), 1988 (*c*). Standard errors are given as bars. Time is expressed as the day of the year.

onwards, when the leaves in the exposed plots showed the first symptoms of defoliation. Pod numbers were hardly influenced by SO_2 in both years except for an enhanced number of small pods formed in the beginning of the pod-filling period in 1986. The specific leaf area was reduced in the reproductive growth phase of the exposed plants in all three years.

The effects of open-air exposure on elemental content of the plant organs in 1985 and of different leaf numbers in 1986 are presented in Tables 3 and 4. These results show no effects of SO_2 on N and Mg content of the different organs during the whole growing season. Differences in Ca content were observed only in the leaves at the end of the growing period in 1985. Ca content of leaves from fumigated plants increased less than Ca content of leaves from control plants. The S content was strongly affected in the leaves, pods and seeds of the exposed plants.

Table 2. *The response of plant height, number of internodes, number of leaf layers, number of pods and SLA of faba beans exposed to ambient or elevated SO_2 concentrations in 1985 (165 μg m^{-3}), in 1986 (62 μg m^{-3}) and in 1988 (74 μg m^{-3}), in relation to plant age expressed in days after emergence*

	Plant height (m)		Number of internodes		Number of leaf layers		Number of pods		Specific Leaf Area (cm^2 g^{-1})	
Plant age (d)	Control	Fumigated	Control	Fumigated	Control	Fumigated	Control	Fumigated	Control	Fumigated
1985										
24	0·05	0·04	1	1	—	—	—	—	208	204
38	0·16	0·11***	6	4	—	—	—	—	236	205
45	0·38	0·32***	8	6	—	—	—	—	228	221
65	0·91	0·82***	15	14	—	—	—[a]	—[a]	303	267**
85	1·26	1·22	19	19	—	—	22	22	300	239***
105	1·31	1·24***	21	21	—	—	17	15	314	194***
1986										
21	—	—	—	—	—	—	—	—	310	256***
39	—	—	8	7*	8	7	—	—	328	294*
53	—	—	16	17***	10	13***	23	36***	311	342*
67	—	—	22	21	17	15*	25	23	341	273***
81	—	—	22	20***	15	13***	18	18	314	281*
1988										
40	53	51	10	10	10	10	—	—	362	363
61	119	121	17	18	15	14	—	—	377	388
81	129	138	19	21	13	11*	—	—	310	278
102	129	124	23	22	15	6*	—	—	189	134

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

[a] Number of pods was not counted.

Table 3. *The response of mineral content of leaves, stems and pods of faba beans exposed to ambient or elevated SO_2 concentrations in 1985 (165 μg m^{-3}), in relation to plant age expressed in days after emergence*

	N (mg g^{-1})		S (mg g^{-1})		Ca (mg g^{-1})		Mg (mg g^{-1})	
Plant age (d)	Control	Fumigated	Control	Fumigated	Control	Fumigated	Control	Fumigated
Stems								
24	61·8	62·0	4·6	5·0	3·0	2·9	2·2	2·2
38	44·7	50·6***	3·3	3·5	—[a]	—[a]	—[a]	—[a]
45	31·6	34·1	2·5	2·4	3·3	3·6	1·9	1·4
65	17·0	17·8	1·2	1·5	3·3	3·3	1·4	1·4
85	12·7	13·6	1·5	1·8	4·4	3·7	0·9	0·8
105	7·7	9·6*	1·4	2·3**	4·2	5·1*	1·2	1·2
Leaves								
24	71·8	70·9	4·9	6·8***	6·6	6·1*	3·1	3·0
38	64·3	63·7	4·5	6·5***	—[a]	—[a]	—[a]	—[a]
45	58·1	57·7	4·2	6·1**	9·6	9·6	2·1	2·1
65	58·6	61·2	4·2	9·4***	11·7	9·9**	4·0	3·7
85	52·0	50·3	3·7	9·6***	14·5	9·9*	4·4	3·3*
105	44·6	45·7	3·3	12·7***	21·1	15·8***	5·3	4·2*
Pods without seeds								
65	58·8	56·8	3·0	3·9	8·9	9·1	4·0	3·4
85	44·9	45·5	2·0	2·9***	4·1	3·8	1·5	2·4
105	26·0	26·9	1·4	2·8***	2·7	3·0	2·2	2·3
Seeds								
85	63·0	64·0	3·2	3·8***	2·2	1·6	2·0	—
105	49·0	48·0	2·2	3·2***	1·3	1·3	1·1	1·2

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as: * $P < 0·05$, ** $P < 0·01$; *** $P < 0·001$.

[a] No data available.

Table 4. *The response of mineral content of leaves of faba beans exposed to ambient or elevated SO_2 concentrations in 1986 (62 μg m^{-3}), in relation to leaf position (leaf number counted from the bottom upwards) and plant age expressed in days after emergence*

	N (mg g^{-1})		S (mg g^{-1})		Mg (mg g^{-1})	
Plant age (d)	Control	Fumigated	Control	Fumigated	Control	Fumigated
Leaf number 5						
39	51·3	51·2	2·9	5·3***	6·0	4·9
53	32·8	27·0	2·1	4·7***	6·7	7·9
67	21·9	24·3	1·7	5·7***	9·8	8·5
Leaf number 10						
39	59·7	59·8	4·0	4·8**	2·5	2·2
53	49·0	54·0	3·8	6·7***	3·9	3·7
67	40·6	40·2	3·7	7·6***	5·6	5·5
81	36·8	33·6	2·4	7·5***	5·7	5·9
Leaf number 15						
53	51·5	59·2*	3·5	6·0**	2·7	3·2
67	52·1	54·0	5·2	8·7***	4·7	4·8
81	51·1	47·5	3·6	8·5***	5·9	5·8
Leaf number 20						
67	53·6	61·9	3·7	5·9***	4·8	4·7
81	53·5	54·1	3·7	8·4***	6·3	6·3

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$

The S content of the stem was less influenced. Data in Tables 3 and 4 indicate a smaller accumulation of S in the leaves of the exposed plants in 1986 when the SO_2 concentration was much smaller. In 1986 chlorophyll content was determined throughout the growing season for 4 leaf numbers. No effect of SO_2 on chlorophyll content of the leaves was detected (Table 5).

Table 5. *The response of chlorophyll content of leaves of faba beans exposed to ambient or elevated SO_2 concentrations in 1986 (62 $\mu g\ m^{-3}$), in relation to leaf position and plant age expressed in days after emergence*

	Chlorophyll content (mg g^{-1})	
Plant age (d)	Control	Fumigated
Leaf layer 5		
39	13·7	12·2
Leaf layer 10		
39	13·1	12·4
53	17·8	13·9
67	11·2	12·6
81	8·4	6·1
Leaf layer 15		
53	14·6	11·0*
67	12·1	15·9*
81	12·7	10·3
Leaf layer 20		
67	15·5	18·7
81	13·3	11·7

Where analysis of variance showed a significant difference between the treated plot and the control plot values the treatment plot means are indicated as: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

DISCUSSION

Total dry weight of the exposed plants was reduced by exposure to SO_2 during the growing period. However, effects were only significant at final harvest and early in the growing season of 1985 (Table 1). Reduction of total dry matter early in the season of 1985, may be explained by the leaf epidermal damage observed in 1985, 29 days after emergence of the crop. The contact between the abaxial epidermis of the leaves and the mesophyll cells was broken. This effect was observed both in the control and in the fumigated plot, but the effect was more marked in plants exposed to SO_2. Although the dry weight of almost all organs was reduced in the exposed plots throughout the growing season, significant reductions were observed only in leaf and pod dry weight in 1985, in stem dry weight in 1986 and in leaf dry weight in 1988. The reduction in total dry weight at final harvest in 1985 was mainly due to the depression of pod dry weight. The growing season of the 1986 crop was much shorter than in 1985 and 1988.

Reduction of plant growth was accompanied by a large reduction in leaf area at the end of the growing season. This was mainly due to effects on the older leaves, which first showed red brown coloured necrotic spots, starting at the margins of the leaf. The symptoms clearly differed from those associated with known diseases (Gerlach, personal comm.). In many other studies, reductions in crop growth during fumigation were accompanied by leaf injury (Bell & Clough, 1973; Brisley, Davies & Booth, 1959; Davis, 1972), but growth reductions without visible injury, have also been reported (Sprügel *et al.*, 1980; Bleasdale, 1972; Lockyer, Cowling & Jones, 1976; Tingey & Reinert, 1971). Sometimes an enhanced leaf area has been observed during exposure to SO_2 in controlled environments (Whitmore & Mansfield, 1983; Pande & Mansfield, 1985). The timing of reductions of growth and leaf area in this study suggests a causal relationship between the two. The observed reduction in leaf area at the end of the experiment in the fumigated plots caused reductions in the amount of absorbed radiation causing reduced rates of canopy photosynthesis. This may partly explain the depression in dry matter growth and seed yield. However, direct effects on photosynthesis and/or translocation may also have contributed to the yield losses observed in this study. Quantitative assessment of the impact of the reduced leaf area on crop growth and production can be determined with the help of simulation models, which calculate potential rates of crop growth at a given LAI on the basis of the amount of radiation absorbed by the leaves. Such an analysis of the data presented here will be given in a subsequent paper (Kropff, in prep.).

Plant height was slightly reduced in the fumigated plots in 1985 and the number of internodes was only reduced slightly in 1986. Kress, Miller & Smith (1986) reported small reductions in the height of soybeans in fumigation plots with SO_2 concentrations up to 1500 $\mu g\ m^{-3}$ for 4 h a day. Heggestad *et al.* (1986) reported no effects on plant height of tomatoes exposed to 300 $\mu g\ SO_2\ m^{-3}$ for 5 h a day for 5 days a week. The final number of pods was not influenced by SO_2 fumigation (Table 2). A much larger number of pods per plant was observed in 1986 in the beginning of the pod-filling period in the fumigated plots. As a result of abortion of young pods, the final number was not influenced by SO_2. Kress *et al.* (1986) report small effects of SO_2 on seed numbers of soybeans produced, but Heggestad *et al.* (1986) reported reduced fruit numbers in tomato at the high concentrations they applied. The number of leaves was significantly reduced as a result of an enhanced leaf loss in the fumigated plots.

The SLA is determined by environmental factors and changes during plant development. The SLA in both years was measured from pooled leaf material, and thus encompasses the effects of plant development, senescence or SO_2 on leaf size and dry weight. In 1985 the SLA of the leaves in the control plot increased with time, which indicates the formation of thinner leaves at the end of the growing season. The SLA was nearly constant in the control plots in 1986. Its value was comparable with the SLA of the 1985 crop at the end of the growing season. The SLA was reduced at the end of the growing season in the fumigated plots. This effect was more marked in 1985. The reduction of the SLA

in the fumigated plots at the end of the growing season may have been caused by the necrosis which reduced the leaf size, in the lower leaf numbers in the fumigated plants, or it may reflect a reduced translocation of assimilates from the leaves to other organs.

The concentration of N and Mg in the tissue of the different organs was not influenced by fumigation of the plants. Hardly any differences between the fumigated plants and the control plants were observed for any organs and at all harvest dates in 1985 (Table 3). The sulphur content was strongly enhanced in the SO_2-treated plots, especially in the leaves and pods. Uptake of SO_2 by leaves and sulphur accumulation in leaves are well known phenomena (Guderian, 1977). The sulphur level in the plants, however, may be affected by many factors such as translocation, dilution by new growth, losses through leaching, gaseous emission and exudation by roots (Garsed, 1984). Heggestad *et al.* (1986) observed no enhanced S levels in tomato fruits, but Sprügel *et al.* (1980) found higher S concentrations in the seeds. In the present work, the calcium content of leaves at the end of the growing season was significantly lower in the fumigated plots. This may be a result of the increased rate of defoliation instead of gradual senescence. For the different leaf numbers the same conclusions can be drawn with respect to the effect of SO_2 exposure on N, Mg and S content. The highest sulphur levels were found in relatively young leaves (number 15) in 1986, obviously as a result of the delay of 32 days after emergence in the start of fumigation. The chlorophyll content was unaffected in the treated plants in 1986, which suggests that foliar injury was not preceded by a slow process of chlorophyll breakdown.

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