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RESPONSE OF TWO POPULATIONS OF *TRIFOLIUM REPENS* L COLLECTED FROM POLLUTED AND UNPOLLUTED SITES TO LOW LEVELS OF SO_2 AND NO_2 SINGLY AND IN COMBINATIONS

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The polluted and the unpolluted population of *Trifolium repens* L. were exposed to 6.8 pphm SO_2, 6.8 pphm NO_2 and 6.8 pphm SO_2 + 6.8 pphm NO_2 for prolonged period in the winter and summer. The most severe reduction in growth was recorded when both the populations were exposed to SO_2 and the least after the NO_2 exposure during winter. The interaction between SO_2 and NO_2 was significant. NO_2 treatment apparently cancelled or reduced the toxic effects of SO_2. The summer experiment showed different results. NO_2 treatment showed enhanced in growth of both the populations while the combined treatment reduced it. SO_2 treatment on the other hand reduced the growth in the unpolluted population and promoted in the polluted population.

In conclusion there was no firm evidence of the genetic adaptation. However, the polluted population appeared to be slightly more tolerant to SO_2 and NO_2 pollution.

INTRODUCTION

A great deal of work has been reported on the toxic effects of air pollutants on plants other than grasses. This has mostly been with reference to higher concentrations of pollutants. A limited amount of work has been published where species other than grasses were exposed to low levels of NO_2 or SO_2. In almost all cases, injury or reduction in growth was noticed.[1-5]

Trifolium repens L. is an important agricultural species in grassland communities of virtually all types. It is possible that it might be equally, if not more, susceptible to air pollution than many grasses. Effects of air pollutants on this species have been neglected so far. The objective of this study was to determine the effects of low levels of SO_2 and NO_2 singly or in a mixture over an extended period on two populations of *T. repens* which had been collected from the natural environment. Attempts have also been made to ascertain whether any adaption to atmospheric pollutants had occurred.

MATERIALS AND METHODS

Clones of *T. repens* were collected from a polluted and an unpolluted site and were propagated in the unheated greenhouse. Later on small fragments of both the populations with some leaves and root were repotted into 7.5 cm diameter pots containing John Innes No. 1 compost. These plants were propagated and hardened off in th unheated greenhouse for 31 days. Plants of each population were divided visually into four similar groups. Plants in each group were further subdivided into five sets; ten plants in each set were placed in the fumigation chambers in seed trays. The trays were arranged in a circle occupying the same general position in all the chambers. The plants were watered regularly to avoid any water stress. Four successive harvests were made after 35, 63, 91 and 119 days. At each harvest 10 plants of each population from each chamber were chosen randomly. The roots were washed and the plants were separated into shoot, root and dead matter. The dry weights of all plants parts were obtained after oven-drying at 80° for 24 hours. The leaf areas were determined on Paton Electronic Planimeter. The root/shoot ratio, leaf weight ratio, sepcific leaf area and leaf area ratio were also calculated.

The fumigation system was composed of four hemispherical chambers. The ambient air drawn through these chambers was filtered by charcoal to remove any trace of pollutant. There was no supplimentary lighting or heating and the rate of air movement through each chamber was about two complete air changes per minute. In each chamber fans were placed which created a sufficient air movement to prevent the formation of high boundary layer resistances across the leaves. The level of pollutants in the chambers were as follows:

a) Charcoal filtered air, b) 6.8 pphm SO_2, c) 6.8 pphm NO_2, d) 6.8 pphm SO_2 + 6.8 pphm NO_2. The pollutant concentrations in each chamber were monitored periodically using Technicon Air Monitor IV for SO_2 and Meloy Chemilumenizer NA 520-2 for NO_2

In the summer it was not possible to fumigate both the populations simultaneously because of the limited availability of space in th fumigation chambers. Hence, the polluted and the unpolluted populations were exposed to pollutants in two summers for 80 and111 days respectively. Later, the roots were washed and the plants were separated into shoot and root and the dry weight were obtained. The data was analysed using analysis of variance techniques.

RESULTS AND DISCUSSION

Both populations of *T. repens* showed reduction in growth when exposed to (a) 6.8 pphm SO_2, (b) 6.8 pphm NO_2 and (c) 6.8 pphm SO_2 + 6.8 pphm NO_2 during the winter, after only 35 days exposure (Fig.1). The most severe reduction in growth was observed after the SO_2 and the least after the NO_2 treatment. The SO_2 treatment showed progressively greater effects after 91 and 119 days as compared with the first two harvests after 35 and 63 days. In most of the variables measured, the polluted population showed greater growth than the unpolluted population at all harvests. After 35 days exposure to SO_2, dry leaf weigth, root/shoot ratio and the leaf area ratio were significantly reduced in both the populations whereas the dead dry weight increased significantly. The dead dry weight decreased in both the populations after 35 days exposure to NO_2. After 63 days in the SO_2 treatment only the root/shoot ratio, leaf weight ratio and specific leaf area showed significant difference. At the third and the fourth harvests after 91 and 119 days respectively, SO_2 pollution significantly reduced the growth of both the populations. Interaction between SO_2 and NO_2 showed a significant difference in some of the variables at all harvests, particularly

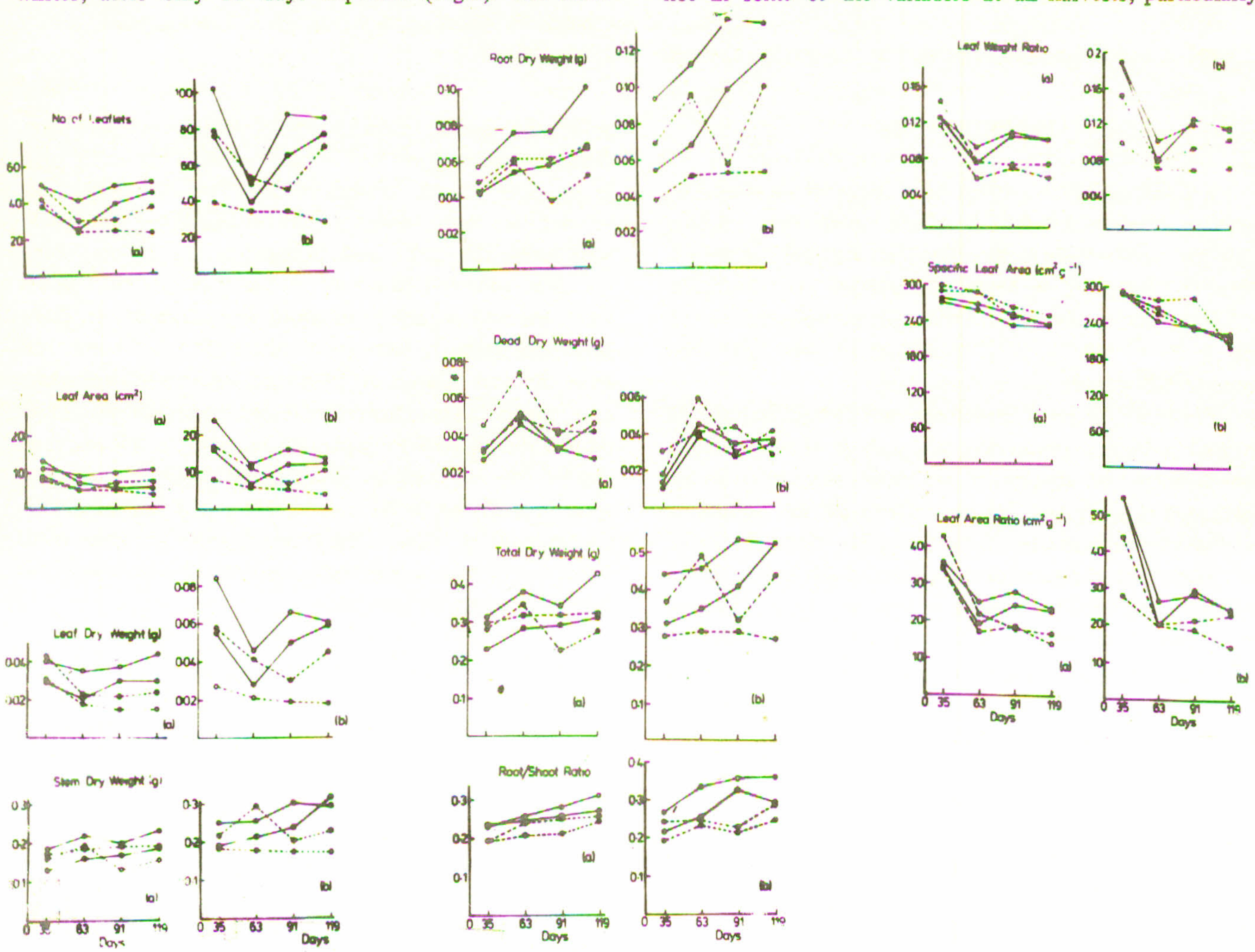

Fig. 1. Growth comparison between two populations of *T. repens* (a) Unpolluted population (b) polluted population, grown at different levels of air pollution (o–o; control; ●–● 6.8 pphm NO_2; o......o 6.8 pphm SO_2; ●......● 6.8 pphm SO_2+6.8 pphm NO_2) for 119 days.

after 91 and 119 days. The interaction indicated that the magnitude of the SO_2 effect on the growth of both populations depended on NO_2 concentration. In the absence of NO_2, SO_2 reduced the growth rate significantly. Similarly NO_2 in the absence of SO_2 suppressed the growth. However, SO_2 in the presence of NO_2 caused a smaller reduction in growth rate than did the SO_2 alone. NO_2 apparently counteracted or reduced the effect of SO_2 on growth of both the populations of *T. repens*. The NO_2 treatment also showed significant difference ($p < 0.05$) in the leaf weight ratio after 119 days which was principally associated with the reduction of leaf weight when exposed to the combined treatment of SO_2 and NO_2. Analysis of variance for all harvests of both the populations is presented in Table 1, which also indicates highly significant differences ($p < 0.001$) in the population, SO_2 and the interaction between SO_2 and NO_2 in almost all the measured variables. Such significant differences have already been explained at each harvest.

Percentage reduction (relative to controls) for all treatments of different variables measured in shown in Table 2, for both populations after 119 days exposure. The SO_2 treatment produced the greatest reductions in all the variables measured for both the populations. The polluted population seemed to be more sensitive to the SO_2 treatment than that of unpolluted population. However, the polluted population showed less damage under the NO_2 and SO_2 + NO_2 treatment. The stem and the total plant dry weights were slightly increased under the NO_2 treatment in the polluted population, whereas no such increase was indicated by the unpolluted population. The percentage of dead dry weight was increased in the unpolluted population when exposed to the SO_2 pollution, whereas the polluted population showed high dead dry weight in the combined treatment.

The summer experiments showed that although the time and duration of pollution exposure were different there were similar patterns of stimulation in growth in the NO_2 treatment and reduction in the combined treatment (Tables 3 and 4). However, the response to SO_2 treatment differed in the two populations, the unpolluted population showed a reduction in all te variables measured, whereas in case of the polluted population, the shoot and total dry weight increased while the root dry weight decreased slightly. The SO_2 treatment produced a significant difference ($p < 0.05$) in the shoot and total dry weights in the unpolluted population. This significant difference can be correlated with reduction in growth in the SO_2 as well as in the combined treatment.

The unpolluted and the polluted population of *T. repens* showed a reduction in growth when exposed to different pollution conditions, particularly to SO_2 pollution. It seems likely that the toxicity of the air pollutants during cold weather might be responsible for this reduction in growth. Materna & Kohout[6], and Keller[7] also found damage to conifers during winter emissions of pollutants. Atmospheric relative humidity increased during the winter and this might results in a plant growing in such conditions being more susceptible to the pollutants possible as a consequence of aerosol deposition. O'Gara (reported by Magill *et al.*[8] found that plants are three times more senstive to SO_2 pollution when the relative humidity is 100% compared with a relative humidity of 30%. This might indicate enhanced stomatal intake of SO_2 at high humidity[9,10]. The dead dry weight was high in winter in the unpolluted and the polluted population when exposed to the SO_2 and the combined treatment of SO_2 and NO_2 respectively. The increase in dead dry weight is probably due to the more rapid death of leaves in the polluted air. Matsushima & Hirada[11] also observed leaf fall in citrus trees with an increase in SO_2 concentration without visible injury. Bleasdale[12] showed that aerial parts of plants, particularly the leaves were adversely affected by smoke pollution, especially in the winter. Hence, the rate of leaf senscence was high relative to the rate of formation of new leaves.

The reduction in growth of both the populations of *T. repens* was not as great under NO_2 exposure as under SO_2 fumigation during the winter period. However, the stem and the total plant dry weight showed little stimulation under NO_2 treatment. Capron & Mansfield[13] found a reduction in growth when tomato plants were exposed to low concentrations of oxides of nitrogen in winter conditions. It is suggested that the low temperature and subsequent slow growth of plants in winter made them more susceptible to the pollutants. Anderson & Mansfield [14] also suggest that oxides of nitrogen are more toxic to plants when growth is slow.

The combined effect of SO_2 and NO_2 was not additive or synergistic in contrast to the evidence of the previous workers. In the present study, a less than additive effect (antagonism) was observed for both populations of *T. repens*. It was found that SO_2 alone reduced the growth rate significantly more than when applied with NO_2. It is suggested that either competition occurs between the two pollutants for an active site or that NO_2 protects or compensates for the damaging effects of SO_2 in both the populations of *T. repens*.

The experiments which were conducted during the summer, produced different results from the winter ex-

Table. 1 Analysis of variance of different variates of two populations of *T. repens* when grown at different levels of air pollution

Source of variation	d.f	No. of leaflets	Leaf area	Leaf wt.	Stem wt.	Root wt.	Dead wt.	Total plant wt.	Root/ shoot ratio	Leaf wt. ratio	Specific leaf area	Leaf area ratio
Havest	3	10.230●	17.518●	10.147●	2.377	10.148●	19.105●	3.119☆	11.323●	72.667●	48.166●	106.299●
Population	1	58.873●	35.646●	47.603●	24.877●	34.601●	12.576●	22.719●	11.135●	24.363●	3.402	13.809●
SO_2	1	33.865●	36.581●	52.896●	7.813★	39.395●	12.595●	12.777●	41.964●	78.858●	9.496★	46.359●
NO_2	1	0.452	0.005	0.070	0.052	0.691	5.923☆	0.115	0.427	7.925★	4.954☆	3.015
Harvest. pop	3	1.274	1.478	0.979	0.177	0.682	1.281	0.326	0.207	1.974	2.348	3.384☆
Harvest.SO_2	3	3.415☆	2.374	3.600☆	2.323	3.908★	0.490	2.827☆	1.760	1.907	0.955	1.644
Pop.SO_2	1	6.4☆	8.686★	9.736★	2.761	9.498★	0.613	4.878☆	3.097	6.222☆	1.158	8.211★
Harvest.NO_2	3	1.011	0.483	0.457	0.576	0.284	0.136	0.419	0.231	2.085	1.738	3.213☆
Pop.NO_2	1	1.435	0.742	1.111	1.491	0.290	2.653	1.475	1.072	1.325	0.059	1.247
SO_2.NO_2	1	28.776●	35.845●	40.911●	16.093●	40.702●	0.012	22.179●	15.870●	16.608●	0.043	15.740●
Harvest.pop.SO_2	3	1.789	2.607☆	2.070	0.434	0.837	0.250	0.459	1.705	6.967●	0.632	8.902●
Harvest.pop.NO_2	3	0.397	0.391	0.672	1.276	1.314	3.174☆	1.574	0.387	0.575	0.927	0.695
Harvest.SO_2.NO_2	3	0.545	1.568	0.944	0.078	0.235	0.781	0.055	0.230	0.786	0.889	1.448
Pop.SO_2.NO_2	1	4.444☆	3.314	4.185☆	0.528	4.155☆	1.445	1.753	2.295	0.694	0.435	0.146
Residual	291											
Total	319											

Levels of pollution were as follows: SO_2 6.8 pphm; NO_2 6.8 pphm; $SO_2 + NO_2$ 6.8 pphm of each.
Significance levels: ☆ = $p < 0.05$; ★ = $p < 0.01$; ● = $p < 0.001$.

Table 2. Percentage reductions (relative to control) of different growth variables measured for two populations of *T. repens* after being exposed to atmospheres containing (a) 6.8 pphm NO_2, (b) 6.8 pphm SO_2 (c) 6.8 pphm SO_2 + 6.8 pphm NO_2 for 119 days.

	Unpolluted population							Polluted population						
	No. of leaflets	Leaf area	Leaf wt.	Stem wt.	Root wt.	Dead wt.	Total plant wt.	No. of leaflets	Leaf area	Leaf wt.	Stem. wt.	Root wt.	Dead wt.	Total plant wt.
NO_2	13.1	30.7	31.3	20.3	34.3	41.4	27.0	10.1	9.2	3.6	7.7*	13.0	5.5	0.2*
SO_2	55.0	65.5	66.9	33.1	49.1	12.5*	35.5	67.4	71.1	69.8	41.7	60.4	22.4	48.3
SO_2+NO_2	28.3	47.2	46.9	17.7	32.6	15.8	24.1	18.7	24.2	25.9	13.8	25.4	13.4*	16.3

* Percentage increase.

Table 3 (a). Shoot, root and total dry weights and root/shoot ratio of *T. repens* (polluted population) when grown for 80 days at different levels of pollution.

Treatments	Shoot dry wt. (g)	Root dry wt. (g)	Total dry wt. (g)	Root/shoot ratio
Control	0.8	0.148	0.95	0.17
NO_2	1.04	0.17	1.21	0.17
SO_2	0.86	0.145	1.00	0.16
$SO_2 + NO_2$	0.63	0.119	0.75	0.19

Table 3 (b). Percentage reductions (relative to control) of shoot, root and total dry weights for *T. repens* (polluted population) after being grown at different levels of pollution for 80 days.

Treatments	Shoot dry wt.	Root dry wt.	Total dry wt.
NO_2	30.0*	14.9*	27.4*
SO_2	7.5*	2.0	5.3*
$SO_2 + NO_2$	21.3	19.6	21.1

*Percentage increase.
Levels of pollution were as follows: NO_2 6.8 pphm; SO_2 6.8 pphm; SO_2+NO_2 6.8 pphm of each.

Table 4 (a). Shoot, root and total dry weights and root/shoot ratio of *T. repens* (unpolluted population) when grown for 111 days at different levels of pollution.

Treatments	Shoot dry wt. (g)	Root dry wt. (g)	Total dry wt. (g)	Root/shoot ratio
Control	0.79	0.204	0.99	0.226
NO_2	1.21	0.25	1.46	0.211
SO_2	0.53	0.118	0.65	0.259
$NO_2 + NO_2$	0.47	0.118	0.59	0.242

Table 4 (b). Percentage reductions (relative to control) of shoot, root and total dry weights for *T. repens* (unpolluted populations) after being grown at different levels of pollution for 111 days.

Treatments	Shoot dry wt.	Root dry wt.	Total dry wt.
NO_2	53.2*	22.6*	47.5*
SO_2	32.9	42.2	34.3
$SO_2 + NO_2$	40.5	42.2	40.4

*Percentage increase.
Levels of pollution were as follows: NO_2 6.8 pphm; SO_2 6.8 pphm; SO_2 + NO_2 6.8 pphm of each

periments. The NO_2 treatment enhanced growth in both the populations. This suggests that NO_2 could be beneficial to plants as a nitrogen source during active growing seasons. The SO_2 treatment on the other hand increased the growth rate slightly in the polluted population, whereas a reduction in growth was observed in the unpolluted population. This response to SO_2 could be due to the fact that the populations of *T. repens* were exposed during two different years, and the duration of exposure was also different. Alternatively, the response could be due to a genetic difference between the populations. The combined treatment of SO_2 and NO_2 reduced growth in both the populations, particularly that from the unpolluted, where it produced a greater reduction than SO_2 exposure alone. This response was different from that found in the winter where the combined effect of SO_2 and NO_2 was less than that of SO_2 exposure alone. This suggests that the two pollutants act differently on *T. respens* at different times of the year.

Variations in sensitivity to pollutants are not surprising in these populations. O'Conner *et al.* [15] also found considereable variations in sensitivity to SO_2 pollution. For example, one seedling of *Eucalyptus gomphocaphala* sustained 60 % leaf necrosis after 1 hour's exposure to 3ppm SO_2; while another seedling sustained only 40 % necrosis after 2 hours exposure. Similarly, paired populations of *Melalenca incana, Acaica pravissima* and *Agonis flexuosa* (all grown from seed), when subjected to SO_2 treatment showed considerable variability. Extensive review by Treshow[16] showed great varietal difference in plant susceptibility to pollutants. Bressan *et al.* [17] concluded that the main reason for these varietal differences in response to pollutants is the relative absorption of gas by the plant tissues.

Although the polluted population was larger in growth form compared with that from the unpolluted, it showed no clear evidence of adaptation to either of the pollutants. The only suggestion of adaptation which the polluted population did show was the slight stimulation in growth when exposed to SO_2 pollution during the summer period. No such enhancement in growth was observed in the unpolluted population.

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