GROWTH OF WHITE CLOVER (TRIFOLIUM REPENS L.) EXPOSED TO LOW CONCENTRATION OF SO_2 AND NO_2 AT DIFFERENT LEVELS OF SULPHATE AND NITRATE NUTRIENTS

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Separate treatment of SO_2 and NO_2 pollution at low concentration (6.8 pphm) had stimulated the growth of *Trifolium repens* L. slightly at low and medium levels of sulphate and nitrate, whereas at high level of nutrients, the plants showed reduction in growth. When plants were treated with a mixture of SO_2 (6.8 pphm) and NO_2 (6.8 pphm), all the measured variables were greatly reduced at all levels of nutrients. On the other hand, dead dry matter was greatly increased.

In conclusion it is suggested that *T. repens* which was collected from a polluted area might have adapted itself to the local environment. In addition, the low level of SO_2 and NO_2 could be used by plants in the form of sulphate and nitrate respectively under favourable growing conditions.

Key words: White clover growth, Sulphate and nitrate, SO_2 and NO_2 pollution.

INTRODUCTION

The situation is rare when only one pollutant is present at a time. In most of the circumstances more than one pollutant is known in the atmosphere, already contaminated by a large number of substances. Agricultural land adjacent to urban areas is increasingly exposed to atmospheric pollution of urban origin. The effects of individual as well as the interaction of two or more gases on crops are poorly understood. Fowler and Cape [3] have tabulated the areas of rural land affected by different levels of pollution, and in Western Europe, the levels of sulphur dioxide and nitrogen dioxide above 0.04 ppm affect over one million hectares of land, which is about one per cent of the total area. The air pollutants in the field situation are normally dispersed and diluted to a large extent by the outside air. Therefore, it is more desirable to study the impact of low level of air pollutants on plants rather than their higher concentrations. Moreover, the application of fertilizers is not evenly distributed throughout the field; some areas get more fertilizers than the others. It becomes more difficult to actually ascertain the degree of the effects of air pollution on crops in such situations.

The aim of this study was to investigate the effects of low level of SO_2 and NO_2 on one of the important pasture plants at different nutrient levels of nitrate and sulphate.

MATERIALS AND METHODS

Clones of *T. repens* were collected from a site which was polluted by the oxides of sulphur and nitrogen. The clones were propagated in the unheated greenhouse. Later on small fragments of this population were repoted into 7.5 cm diameter pots containing John Innes No. 1 compost, and were propagated and hardened off in the unheated greenhouse for some days. These plants were divided into four similar groups. Plants in each group were further subdivided into three sets. Ten plants in each set were placed in the fumigation chambers in seed trays.

The fumigation system was composed of four chambers. The ambient air drawn through these chambers was filtered by charcoal to remove any pollutant. There was no supplementary lighting or heating and the rate of air movement through each chamber was about two complete air changes per min. 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 average concentrations of pollutants within the chambers were as follows:

(1) control, charcoal filtered air; (2) 6.8 pphm No_2;
(3) 6.8 pphm SO_2; and (4) 6.8 pphm SO_2 + 6.8 pphm NO_2.

The pollutant concentrations in each chamber were monitored periodically using a Technic on Air Monitor IV for SO_2 and a Meloy Chemilumenizer NA 520-2 for NO_2.

On set of plants in each chamber was supplied with 500 ppm sulphate and 250 ppm nitrate solutions in the form of sulphuric acid and ammonium nitrate respectively. The second set was treated with the double concentrations of sulphate and nitrate, while the third set was not treated with any of the nutrient at all. Such treatments of sulphate and nitrate were repeated after about 40 days. The plants were watered regularly to avoid any water stress.

After 80 days of fumigation, all the plants were harvested. 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-dried at 80^{0} for 24 hr. The leaf areas were determined on a Paton Electronic Planimeter. The root/shoot ratio, leaft weight ratio, specific leaf area and leaf area ratio were calculated. The data was analysed using analysis of variance techniques.

RESULTS

The results showed (Table 1 and 2) that all the pollutants had different effects on the growth of *T. repens* when grown at low, medium and high levels of nitrate and sulphate nutrients. In most of the growth variables measured, separate treatments of NO_2 and SO_2 enhanced the growth slightly at low and medium levels of nitrate and sulphate. On the other hand, at high level of nutrients, the growth had been reduced when fumigated with NO_2 and SO_2 pollutants individually. Moreover, the combined treatment of SO_2 and NO_2 together greatly reduced all the measured variables at all levels of nutrients except that of dead dry matter. Dead dry matter was significantly increased ($p<0.001$) in the combined treatments of SO_2 and NO_2 at all levels of nutrients. In most of the variables, low level of nutrients promoted the growth more than that of the medium level of nutrients when treated with NO_2 and SO_2 alone. Furthermore, the NO_2 treatment had greater enhanced effects on the growth of *T. repens* as compared to SO_2 pollution.

Table 1 Effects of air pollution on the growth 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 80 days at low (L), Medium (M) and high (H) levels of sulphate of nitrate.

Treatments	Control			NO_2			SO_2			$SO_2 + NO_2$		
	L	M	H	L	M	H	L	M	H	L	M	H
No. of leaflets	173	186	244	152	172	200	136	196	184	90	120	160
Leaf area (cm²)	51.0	59.1	78.7	75.9	63.2	62.9	57.9	64.2	64.4	31.0	42.7	52.3
Leaf Dry wt. (g)	0.19	0.22	0.29	0.27	0.21	0.22	0.21	0.23	0.22	0.09	0.13	0.18
Stem dry wt. (g)	0.58	0.60	0.86	0.75	0.63	0.68	0.63	0.68	0.76	0.42	0.56	0.54
Root dry wt. (g)	0.15	0.17	0.22	0.17	0.15	0.16	0.15	0.20	0.19	0.12	0.17	0.18
Dead dry wt. (g)	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.12	0.12	0.07
Aerial dry wt. (g)	0.80	0.84	1.17	1.04	0.87	0.92	0.86	0.93	1.01	0.63	0.80	0.79
Total dry wt. (g)	0.95	1.01	1.39	1.21	1.02	1.08	1.00	1.13	1.20	0.75	0.96	0.96
Root/shoot ratio	0.17	0.19	0.17	0.17	0.19	0.17	0.16	0.21	0.17	0.19	0.21	0.20
Leaf weight ratio	0.19	0.20	0.19	0.19	0.20	0.19	0.18	0.19	0.16	0.12	0.14	0.15
Specific leaf area	266.9	271.6	272.3	277.8	311.5	283.1	254.3	285.8	287.4	333.8	341.4	307.5
Leaf area ratio	51.3	55.6	50.9	53.9	61.7	54.6	46.6	54.4	47.9	41.7	48.9	46.0

Table 2. Percentage reduction (relative to control) of different growth variables measured for *T. repens* after being exposed to atmosphere containing (a) 6.8 pphm NO_2 (b) 6.8 pphm SO_2 pphm SO_2 + 6.8 pphm NO_2 for 80 days at Low (L), Medium (M) and High (H) levels of Sulphate and Nitrate.

Treatments	No. of leaflets (cm²)			Leaf area (g)			Leaf dry wt. (g)			Stem dry wt. (g)			Root dry wt. (g)			Dead dry wt. (g)			Aerial dry wt. (g)			Total dry wt. (g)		
	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H
NO_2	12.1	7.5	18.0	48.8*	6.9*	20.1	42.1*	4.5	24.1	29.3*	5.0*	20.9	13.3*	11.8	27.3	33.3	0.0	33.3	30.0*	13.6*	21.4	27.4*	1.0*	22.3
SO_2	21.4	5.4*	24.6	13.5*	8.6*	18.2	10.5*	4.5*	24.1	8.6*	13.3*	11.6	0.0	17.6*	13.6	33.3	0.0	0.0	7.5*	10.7*	13.7	4.8*	11.9*	13.7
$SO_2 + NO_2$	48.0	35.5	34.4	39.2	27.7	33.5	52.6	40.9	37.1	27.6	6.7	37.2	20.0	0.0	18.2	300.0*	500.0*	133.3*	21.3	4.8	32.5	21.1	5.0	30.9

* = Percentage Increase

Analysis of variance for all the levels of nutrients showed that leaf weight ratio was significant at all pollutants treatments. SO_2 treatment greatly reduced ($p<0.001$) the leaf weight ratio. The interaction between SO_2 and NO_2 was also significant ($p<0.05$). This showed that when both treatments were present together, the leaf weight ratio was significantly reduced. Specific leaf area was also found significant at all treatments. All the pollutants greatly increased the specific leaf area, particularly when both treatments were present together. This is due to the fact that specific leaf area is the ratio between leaf area and the leaf dry weight. All the pollutant treatments reduced the leaf dry weight as compared to unpolluted treatment. Therefore, naturally, the specific leaf area would be higher in the case of pollutant treatments. Leaf area ratio was significant only under the SO_2 treatment. SO_2 pollutant reduced the leaf area ratio significantly ($p<0.01$). All other treatments showed no significant effects on the leaf area ratio.

Analysis of variance for each level of nutrient was also performed separately. The pattern of significant differences was more or less similar to that of the analysis of variance for all levels except that of the high level of nutrient. At higher level of sulphate and nitrate, dead dry weight and specific leaf area showed significant differences ($p<0.05$) at the SO_2 treatment only. Interaction between SO_2 and NO_2 was also significant ($p<0.05$) only in the case of dead matter production.

DISCUSSION

T. repens showed better growth at low and medium levels of nitrate and sulphate nutrient when fumigated with low concentrations of NO_2 and SO_2 pollutants in fumigation chambers. The soil in which *T. repens* was grown became deficient after some time. The only source from where the plants could take in the nutrient supply was the surrounding atmosphere. The air in the controlled fumigation chamber was clean and there was hardly any source of pollutant in the chamber. It has been estimated [6] that sulphur dioxide is taken in directly by the foliage rather tnan indirectly from the deposited sulphur in the soil. Nutrients, particularly nitrates and sulphates, were necessary for plant growth. The absorption of nutrients directly or indirectly through the soil in significant when plants grow faster as compared to slow growing plants. Therefore, in summer, plants take full advantage of the light and temperature and increase in size significantly. Similar conclusions were drawn by Robert in 1975 when he fumigated birch plants with SO_2 pollutant during the growing period.

Booth et al [2] showed that SO_2 react with water to form the toxic sulphite ions which are then oxidized to sulphate. This sulphate could be toxic to plants if present in higher amount. However, if present in low concentration, this may be used by the plants as a source of sulphur [2]. The increased dead weight in the present study could be correlated with the work of Matsushima and Hirada [4] who fumigated two-year old citrus trees with 0-5 ppm SO_2 for two hours daily for about a month. They found increased leaf fall with the increasing concentrations of SO_2. Bleasdale [1] also found more leaf senescence in the polluted air than in the scrubbed air. Thomas *et al.* [7] have suggested that such accelerated senescence could be caused by SO_2 involving a reduction in the buffering capacity of the leaf sap.

In conclusion it could be suggested that the population of *T. repens* which was collected from an area exposed to sulphur dioxide and oxides of nitrogen since long time might have adapted itself to polluted conditions. Moreover, the enhancement in growth at low sulphate and nitrate soil might be due to the utilization of air pollutants by plants directly through the aerial parts or indirectly through the soil particularly in favourable growing season. However, if the soil sulphates and nitrates are excessively higher, then the plants growth would be reduced even if the plants are growing under low SO_2 and NO_2 pollution.

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