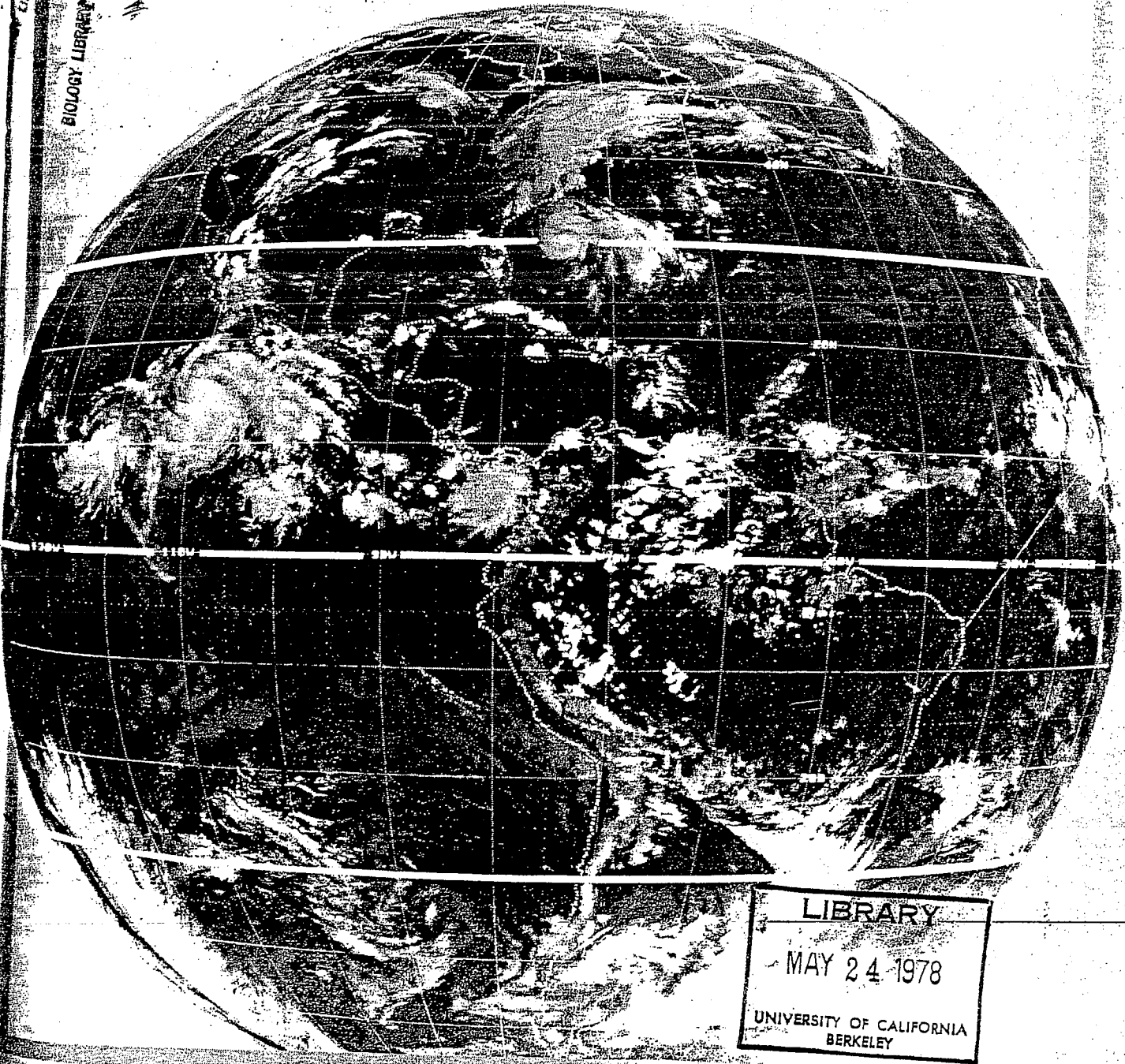


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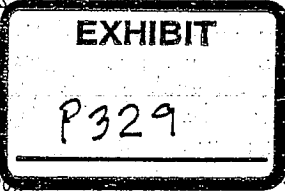
Atmospheric electricity and sun-weather relationships



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Extreme pollution sensitivity of grasses when SO₂ and NO₂ are present in the atmosphere together

PREDICTIONS of the possible effects of atmospheric pollution on vegetation have usually been based on the results of experiments in which plants were fumigated in the laboratory. For many years it was thought that exposures to SO₂ concentrations below 0.30 p.p.m. were not detrimental to higher plants^{1,2}, which led to the belief that this form of pollution is unlikely to cause any appreciable damage to crops in Britain where, even in heavily populated areas, monthly mean SO₂ concentrations are usually below 0.15 p.p.m., and daily means rarely exceed 0.30 p.p.m. (ref. 3). Recently, however, evidence has accumulated that concentrations of SO₂ below 0.15 p.p.m. can be damaging to plants⁴⁻⁷, casting doubt on the comfortable conclusions drawn from earlier studies. We found that laboratory experiments can underestimate the amount of injury resulting in the field, and report here that combinations of SO₂ with NO₂ give more realistic effects.

There are three reasons for underestimation in the laboratory. First, many experimental fumigations were conducted in chambers in which the air was inadequately stirred. This would have resulted in a slow transfer of pollutants into the leaves because of a high diffusion resistance in the boundary layer. The importance of breaking down the boundary layer resistance to a level comparable with that for leaves out of doors has not generally been recognised. We have shown elsewhere⁸ that the growth of S23 ryegrass was unaffected by 0.11 p.p.m. SO₂ in a wind speed of 10 m min⁻¹, whereas the same concentration in a wind speed of 25 m min⁻¹ caused a reduction in yield of about 30% over a period of 4 weeks. Second, most investigators (including ourselves) have fumigated plants with constant levels of pollutants, using 24-h mean values from field monitoring as a guide to concentration. In the field, and especially near industrial sources, the levels of air pollution are in a continual state of fluctuation and levels up to 10 times the daily mean can occur for periods of 1 h or more. We are currently studying the effects of these 'peaks' of high concentration on plant growth. Finally, experiments in which plants are fumigated with only one pollutant at a time could produce incorrect estimates of the contribution to plant damage in the field where exposure to several pollutants often occurs simultaneously. Most of the combustion processes which generate SO₂ also produce a mixture of nitrogen oxides (NO_x), since high

temperatures cause atmospheric O₂ and N₂ to combine to form NO, which is then slowly oxidised to NO₂. When SO₂ and NO₂ are present in the atmosphere together, their short-term effects on plants have been shown to be at least additive⁹, and sometimes synergistic¹⁰. The effects on plant growth of prolonged exposures to low concentrations of these two ubiquitous pollutants in combination have not, as far as we know, been investigated. We have now carried out some preliminary studies, the results of which suggest that the combined effects of these two gases could be the cause of serious losses in crop production in polluted areas, and that when SO₂ interacts with NO₂ there is a large increase in the toxicity to plants.

Four commonly sown pasture grasses were studied: *Dactylis glomerata* L. var. Aberystwyth S37 (Cocksfoot), *Lolium multiflorum* Lam. var. Milamo (Italian ryegrass), *Phleum pratense* L. var. Eskimo (Timothy) and *Poa pratensis* L. var. Monopoly (Smooth-stalked meadowgrass). Plants were raised from seed in a cold greenhouse and used for experiments 6 weeks after sowing. The fumigation system consisted of four specially designed greenhouses, unheated and rapidly ventilated with ambient air which was purified by passage through activated charcoal filters. Fans carefully placed within each greenhouse created air movement sufficient to reduce the boundary layer resistance around the leaves to a value like that in a grass sward out of doors. Controlled levels of pollutants were added for 103.5 h weekly, clean air being given for the remaining 64.5 h. The concentration of each gas used during the 103.5 h of exposure was 0.11 p.p.m. The treatments, with concentrations calculated as weekly means, were as follows: (1) control, charcoal-filtered air; (2) 0.068 p.p.m. SO₂; (3) 0.068 p.p.m. NO₂; (4) 0.068 p.p.m. SO₂+0.068 p.p.m. NO₂. The atmosphere in the control glasshouse was found to contain less than 0.001 p.p.m. SO₂ and NO₂. Fumigations were continued for 20 weeks from October 1976 to March 1977. At the end of the experiment, 10 plants of each species were taken at random from each treatment and leaf areas and total dry weights were determined. Percentage increases or decreases, relative to the controls, were then calculated.

Table 1 shows that when the two pollutants were applied singly, their effects on dry weight varied among the four species. While the yields of *D. glomerata* and *Poa pratensis* were reduced by both SO₂ and NO₂, *L. multiflorum* was not significantly affected by either pollutant alone and *Phleum pratense* was affected by SO₂ only. These reductions in yield were not associated with significant reductions in leaf area, except in the case of *Poa pratensis* exposed to SO₂. Treatment with combined SO₂ and NO₂,

Table 1 Percentage reductions (relative to control) in total dry weights and leaf areas of grasses exposed to atmospheres containing SO₂ and NO₂ alone or in combination for 20 weeks

	SO ₂	NO ₂	SO ₂ + NO ₂	Effect*
	Dry weights			
<i>Dactylis glomerata</i>	40§	22†	78§	S
<i>Lolium multiflorum</i>	5 NS	10† NS	52†	S
<i>Phleum pratense</i>	51§	1† NS	86§	S
<i>Poa pratensis</i>	46§	38§	84§	A
	Leaf areas			
<i>D. glomerata</i>	20 NS	1† NS	76§	S
<i>L. multiflorum</i>	22 NS	1† NS	43†	S
<i>Phleum pratense</i>	11 NS	30† NS	82§	S
<i>Poa pratensis</i>	28†	17 NS	84§	S

Concentrations were as follows: SO₂ 0.068 p.p.m.; NO₂ 0.068 p.p.m. SO₂ + NO₂ 0.068 p.p.m. of each.

* Effect: A, Additive effect of pollutants (that is, there was no evidence of interaction when SO₂ and NO₂ were presented together); S, synergistic effect of pollutants (that is, a statistically significant interaction).

Levels of significance established by analysis of variance of the 2 × 2 factorial experiment: †, P < 0.05; ‡, P < 0.01; §, P < 0.001. †† Increase.

however, caused large statistically significant reductions in total dry weight, associated with reductions in leaf area, in all four species. With the exception of *L. multiflorum*, the reductions were greater than 75%. It is interesting that the effects of the two pollutants in combination were greater than their summed individual effects, except in the case of *Poa pratensis* which was the species most sensitive to the individually applied pollutants.

If the toxicity to plants of this mixture of two pollutants had been estimated from their effects when applied separately, it would have been seriously underestimated for three out of four species. In the case of *L. multiflorum* no effect at all would have been predicted from the response to SO_2 and NO_2 when applied alone.

The relative sensitivities of the four grasses were surprisingly different. Although *Phleum pratense* exhibited the greatest reduction in dry weight when exposed to SO_2 , it was one of the species not significantly affected by NO_2 . With such marked differences in response to the three treatments, we suggest that in a mixed sward in the field, different mixtures of SO_2 and NO_2 could result in quite different changes in species composition. Prediction of responses under field conditions would, however, require knowledge of the degree of variation of response within each species, and the capacity for resistant individuals to be selected. Such information could only come from a very much larger investigation.

Many aspects of the response of plants to air pollution are still unresolved, but it is clear that if laboratory experiments are to provide information applicable to field conditions, much more care must be taken over the choice of fumigation conditions. Although the other two points we have enumerated are also important, we suggest that interactions between individual pollutants might represent the largest source of error.

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Simultaneous occurrence of C_3 and C_4 photosyntheses in relation to leaf position in *Mollugo nudicaulis*

SINCE the discovery of C_4 photosynthesis^{1,2} higher plants have been categorised into C_3 plants, which form 3-phosphoglycerate (PGA) as the first labelled intermediate, and C_4 plants, which form 4-carbon acids as the predominant early photosynthetic products. C_4 plants can also be distinguished by features such as Kranz leaf anatomy, low photorespiration, high photosynthetic efficiency and reduced ^{14}C discrimination³. Although 943 C_4 plants are known to be distributed among 18 families, only 18 genera from 10 families include both C_3 and C_4 plants⁴. Kennedy and Laetsch⁵ described *Mollugo verticillata* as intermediate for C_3 and C_4 photosynthesis. But Brown and Brown⁶ felt that it was a typical C_3 plant. Recently three *Panicum* species

(*P. milioides*, *P. hians* and *P. laxum*) have been found to have photosynthetic characteristics intermediate between C_3 and C_4 plants⁷⁻⁸. We now report that a single plant of *Mollugo nudicaulis* can have some leaves with C_3 characteristics and others with C_4 characteristics according to their position on the stem.

Plants of *M. nudicaulis* L. were collected from their natural habitat on the campus of Sri Venkateswara University (approximately 12-h photoperiod with temperatures of 35 °C by day and 25 °C by night). Plants chosen were about 8 weeks old with unbranched stems each bearing 12-14 leaves, the first being the youngest, fully unfolded leaf. The second to fourth leaves were taken from 4-week-old seedlings of *Oryza sativa* L. variety IR-22, and *Setaria italica* Beauv. variety AKP-2, grown in seed pans in similar conditions.

After preillumination for 30 min in air containing $^{12}\text{CO}_2$, leaves were exposed to $^{14}\text{CO}_2$ for 5 s (at an irradiance of 200 Wm^{-2} under tungsten lamps and at 33 °±1 °C) and their early photosynthetic products were analysed as before⁹. Photosynthetic rates were computed from the total $^{14}\text{CO}_2$ fixed during 5 s. Photorespiratory rates were determined by Kennedy and Laetsch's modification⁵ of Zelitch's method¹⁰. Stomata were opened before the assimilation period by passing CO_2 -free air over the illuminated leaves for 45 min. Air flow was then stopped, and the leaves were allowed completely to assimilate 2 μmol of $^{14}\text{CO}_2$ for 45 min. Then the $^{14}\text{CO}_2$ evolved in the light was compared with the $^{14}\text{CO}_2$ evolved in darkness. The $^{14}\text{CO}_2$ -trapping agent consisted of 50 ml of 1.0 N KOH with 0.6% isoamyl alcohol. Although the pools of photosynthetic intermediates were not estimated during this experiment, the technique of measuring $^{14}\text{CO}_2$ evolution in the light and in the dark was useful for detecting the occurrence of photorespiration in C_3 as well as C_4 plants and has been used as an arbitrary measure of photorespiration^{5,10,11}. Chlorophyll was determined after extracting into 80% acetone¹² and the chlorophyll *a/b* ratio was estimated as before¹³.

Table 1 presents the photosynthetic characteristics of *M. nudicaulis* leaves at different positions on the stem. The features can be compared with those of *O. sativa*, a C_3 plant, and *S. italica*, a C_4 plant. Based on leaf position and photosynthetic characters, we placed them in three categories: young leaves (at positions 1-4 on the stem), old leaves (at positions 11-13 or 14) and middle-aged leaves (positions 5-10).

The young leaves had normal anatomy with palisade and spongy parenchymatous tissue and with no sheath cells around the vascular bundles. On the other hand, old leaves had typical Kranz anatomy with chlorenchymatous bundle sheaths specialised for starch accumulation. Middle-aged leaves had an indistinct chlorenchymatous bundle sheath which did not stain for starch.

There was no regular variation in chlorophyll content (per unit fresh weight) except that the younger leaves tended to have more chlorophyll than did the older leaves. The chlorophyll *a/b* ratio also showed little correlation with leaf age, although the highest ratio was in the 12th leaf.

Young leaves incorporated most of the ^{14}C into PGA and sugar phosphates, and they had lower photosynthetic rates and a higher photorespiratory ratio than did old leaves (Table 1). On the other hand, the old leaves formed mostly C_4 acids (malate and aspartate) as early photosynthetic products, and they had higher photosynthetic rates and a low photorespiratory ratio. The old leaves resembled the leaves of *S. italica* while the young leaves were similar to those of *O. sativa*.

Our data suggested the occurrence of both C_3 and C_4 pathways in leaves of different ages on *M. nudicaulis*. Young leaves were C_3 , old leaves were C_4 and middle