

SHORT COMMUNICATION

Some effects of increased atmospheric carbon dioxide on white clover (*Trifolium repens*) and pea (*Pisum sativum*)*Summary*

The effects of atmospheric carbon dioxide enrichment on nitrogen fixation by white clover and pea, and on stomatal aperture, transpiration loss and nitrate reductase activity in white clover, are described and the implications discussed.

Introduction

Varying the carbon dioxide supply to plants markedly alters their growth and performance. Wilson *et al.*¹⁸ were probably the first to demonstrate a beneficial effect from increased atmospheric CO₂ level on legume nitrogen fixation. More recently, Hardy and Havelka⁴ and Havelka and Hardy⁶ found that raising atmospheric CO₂ to *ca* 1200 ppm gave marked increases in plant weight, N₂ fixation per plant and per unit nodule weight and extended the period of N₂ fixation. Apart from the earlier work¹⁸, herbage legumes have not been studied in this respect and may differ from grain legumes in some relevant aspects. For example, white clover does not show a light-induced diurnal variation in acetylene reduction, a property that appears to vary considerably between legumes^{2 5 8 9 16 17}.

The present investigation was undertaken to obtain information on the effects of increased CO₂ supply on N₂ fixation, and on plant processes which may affect it, in white clover. Peas were included in the investigation to permit comparison of herbage with grain types of legumes.

Materials and methods

White clover (cv Blanca) and pea (cv Dark Skinned Perfection) were sown in 175 mm diameter pots containing N-free coarse quartz sand, covered with a 25 mm layer of polythene pellets to prevent growth of algae on the sand surface and evaporation of water. The pots were placed in a growth chamber having a 16-hour day, light intensity of 16,500 lux and day and night temperatures of 21° and 13° respectively. Both species were inoculated with rhizobial strains, known to be effective with them, at sowing and again seven days later.

After establishment the clovers and peas were thinned to six and three plants per pot respectively. The pots received water only for seven days after

sowing, after which a N-free nutrient solution was given daily. The pots were placed on low supports to facilitate free drainage of excess nutrient. The amount of nutrient solution added depended on the stage of growth but was always sufficient to produce drainage of excess solution from the pots.

A simple device was constructed to provide an increased CO₂ level in the atmosphere. The growth chamber was completely divided into two equal compartments by 500 gauge polythene sheeting. A series of 63 mm diameter polyvinylchloride pipes containing a number of 1 mm holes were placed in each compartment between the rows of pots. The pipes were connected to a common air supply which was adjusted to give a small but equal air flow from each hole. Carbon dioxide was introduced into the air pipes in one of the growth chamber compartments through 5 mm diameter copper pipes ending in 0.1 mm jets, and adjusted to give an atmospheric concentration of 1200 ppm. The gas and air flows were controlled by a relay switching system and coincided with the light period in the growth chamber. The system was tested before installation of the experiment and found satisfactory. The CO₂ concentration in the untreated growth compartment was not increased above ambient. Atmospheric CO₂ concentration was measured by a 'Kitagawa' gas detector (manufactured by Komyo Rikagaka Kogyo KK, Tokyo 152, Japan). This instrument, while lacking the sensitivity of more sophisticated equipment, proved very suitable for detecting the relatively large differences in CO₂ concentration used in the present experiment.

Carbon dioxide and N (175 ppm N as ammonium nitrate) treatments were imposed, separately and together, on the clover fifty days after sowing. The N treatment was not applied to the pea.

Clover was harvested 55, 63, 66 and 70 days after sowing, using six pots at each harvest. Peas were harvested 46, 49 and 62 days after sowing, using four pots for each harvest.

Acetylene reduction was measured on whole plants, after careful removal of sand from the roots. Incubation conditions were one hour at 21° in an atmosphere of 90% air and 10% acetylene. Ethylene was measured as previously described⁹.

The procedures used for dry matter and N estimations have been published³. Nitrate reductase was measured on duplicate samples by the method of Mulder *et al.*¹¹, but using N- (1-naphthyl) ethylene-diamine hydrochloride to develop colour in the test extracts.

Stomatal openings were examined by the method of Sarvella *et al.*¹⁵. Water loss from the pots was measured by weighing.

Results

Clover. Carbon dioxide enrichment increased the yields of dry weight and N per plant (Table 1). Percentage N in the dry material was slightly higher in the CO₂ treatments at the first harvest. In subsequent harvests lower values were found in the CO₂ treatment in the absence of added N but higher levels obtained where mineral N had been supplied. In all treatments, percentage N tended to decrease with time. The weight of nodules per gram of root (nodule density) decreased throughout the first three harvests but

TABLE 1

Effects of CO₂ and N on dry weight (g/pot) and acetylene reduction (μM C₂H₄/pot/h) by white clover

Treatment	Harvest	Total dry weight	Nodules	Nodules/ root (g/g)	Nitrogen	%N	C ₂ H ₄	C ₂ H ₄ /g nodule
Control	1	5.6	0.20	0.20	0.21	3.8	45.5	223
	2	11.0	0.23	0.14	0.45	4.1	43.3	163
	3	13.9	0.29	0.12	0.55	4.0	43.8	151
	4	18.2	0.54	0.18	0.68	3.7	65.4	121
- N + CO ₂	1	6.3	0.22	0.20	0.26	4.1	62.9	286
	2	12.2	0.25	0.14	0.46	3.8	60.4	178
	3	16.6	0.38	0.13	0.61	3.7	62.3	164
	4	21.0	0.55	0.16	0.72	3.4	88.9	162
+ N	1	5.2	0.16	0.16	0.21	4.0	19.9	124
	2	12.0	0.14	0.08	0.49	4.1	9.4	56
	3	15.6	0.15	0.06	0.58	3.8	9.3	62
	4	19.9	0.30	0.12	0.73	3.7	9.2	31
+ N + CO ₂	1	5.7	0.16	0.15	0.24	4.2	23.4	146
	2	11.5	0.13	0.15	0.46	4.0	11.1	81
	3	18.0	0.23	0.09	0.67	3.7	19.1	83
	4	21.8	0.23	0.09	0.84	3.9	34.7	151

showed an increase at the fourth, and was virtually unaffected by the CO₂ treatment.

Acetylene reduction per plant and per gram of nodule (specific nodule activity) was also increased by CO₂. These increases were manifested at the first harvest which was taken five days after the CO₂ treatment was applied and were consistently maintained throughout the four harvests. In both the control and CO₂ treatments, acetylene reduction per plant remained relatively static throughout the first three harvests but showed a large increase at the last harvest. Specific nodule activity in both these treatments decreased consistently with time.

Nitrogen on its own also increased total dry weight, but to a lesser degree

TABLE 2

Water loss by clover plants (ml/pot)

Treatment	Time (hours)		
	3	4.5	5.5
Control	59.2	75.8	87.5
+ CO ₂	38.3	49.2	60.0

TABLE 3

NO₃-reductase levels in leaves, petioles and roots of white clover

Harvest	Treatment	$\mu\text{M NO}_2^- \cdot \text{g fresh wt}^{-1} \cdot \text{h}^{-1}$		
		Leaves	Petioles	Roots
1	+ N	0.80	0.08	0.14
	+ N + CO ₂	0.61	0.10	0.17
2	+ N	0.85	0.15	0.15
	+ N + CO ₂	0.61	0.12	0.14

than CO₂, and reduced nodule weight per plant and nodule density. These trends were reflected in the rates of acetylene reduction per plant and per gram of nodule. Raising the CO₂ level, in the presence of N gave further increases in dry weight and N yields, and in N content. Nodule density was not consistently increased but acetylene reduction per plant and per gram of nodule was.

Raising the level of CO₂ markedly reduced clover stomatal opening size. Examination of stomatal imprints under the microscope showed the shorter axis was reduced by about half in the higher CO₂ atmosphere. Water loss from these plants was also reduced (Table 2). Leaves of plants receiving N showed lower NO₃-reductase levels in the CO₂ treatment (Table 3). Levels in petioles and roots were much lower and showed no trend. The enzyme was not detected in plants not receiving N.

Pea. Carbon dioxide enrichment had, in many respects, similar effects on pea (Table 4). Dry weights, yields of nodules and of N and acetylene reduction per plant were increased. Nodule density was also greater in the CO₂ treatment and increased throughout the course of the experiment in contrast to the clover, which showed no trend. Percentage N was generally decreased by CO₂. Specific nodule activity was markedly lower in this treatment and, as in the case of clover, decreased with time.

TABLE 4

Effects of CO₂ on dry weights (g/pot) and acetylene reduction ($\mu\text{M C}_2\text{H}_4/\text{pot/h}$) by pea

Treatment	Harvest	Total dry weight	Nodules	Nodules/ root (g/g)	Nitrogen	%N	C ₂ H ₄	C ₂ H ₄ /g nodule
Control	1	6.3	0.39	0.22	0.23	3.7	19.8	50.6
	2	11.2	0.69	0.38	0.44	3.9	26.5	38.4
	3	27.6	1.06	0.66	0.97	3.5	37.0	34.9
+ CO ₂	1	9.0	0.73	0.35	0.31	3.4	31.4	43.0
	2	12.3	0.94	0.49	0.53	4.3	33.3	35.4
	3	31.8	1.57	0.87	1.02	3.2	45.1	28.7

Discussion

The results show that raising the CO₂ level can have, in many respects, the same effects on herbage as on grain legumes. Yields of plant dry weight and of N were increased while percentage N in the dry matter was decreased. Some differences were also found between the two species in the present investigation. Thus CO₂ enrichment had no effect on nodule density in clover but markedly increased the specific activity of the nodules. In the case of the pea, nodule density was increased by CO₂, whereas specific nodule activity was decreased. In previous work using red clover, Wilson *et al.*¹⁸ found that CO₂ enrichment increased total nodule number and N₂ fixation but did not determine nodule mass. Mulder and van Veen¹², using red clover, found that CO₂ bubbled through the liquid rooting medium, increased total N₂ fixation but not nodule mass, indicating increased specific nodule activity. When pea was used nodule number was not affected, and total nodule mass slightly increased. In another study with pea¹⁴, but over a shorter period than the present work, CO₂ enrichment increased nodule numbers and mean nodule mass but not specific nodule activity. Hardy and Havelka⁴ obtained increased nodule mass and specific activity with soyabean. The present results are in good agreement with the previous findings, but it is apparent that the effects of CO₂ enrichment depend to some extent on the test species, and on the conditions and duration of the experiment. Further investigation of these aspects is necessary.

The decreased N₂ fixation per plant and per gram of nodule in the presence of mineral N was expected. Carbon dioxide caused an eventual reversal of this trend. This may have been due to a direct CO₂ effect (*i.e.* increased energy supply) or to reduced mineral N uptake also. The lower NO₃-reductase activity in the CO₂ enriched plants receiving mineral N is interpreted as indicating a reduced uptake of mineral N. This conclusion is consistent with the observed reductions in stomatal aperture and transpiration current, both well known effects of CO₂ enrichment^{1 13}. As there is good evidence that, within broad limits, transpiration *per se* does not affect the N₂ fixation process^{7 10}, an expected consequence of the reduced mineral N uptake would be a lower energy requirement for its elaboration in the plant and an eventual increase in carbohydrate available for export to the nodules.

It thus seems possible that changes in acetylene reduction activity can result from altered mineral N uptake which, in turn, depends to some degree at least, on transpiration current. Such inter-related factors as these have special importance when N₂ fixation, and the effects of environmental factors on it, is being measured on plants grown in the presence of mineral N, *e.g.* in soil.

It can be concluded that CO₂ enrichment can enhance N₂ fixation in the legume by one or more of three ways *viz* by increasing numbers and/or mass of nodules on the plant root, by increasing sources of energy, reductant and carbon skeletons for the reduction and elaboration of symbiotic N and by decreasing uptake of mineral N and thus reducing the competition for carbohydrate required for its elaboration in the plant. The first two of these

possibilities may result in increased plant yield and total N₂ fixation while the latter, though possibly not increasing total output, can be expected to increase the symbiotic component of the nitrogen yield of legumes grown in the presence of mineral N.

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C. L. MASTERTSON and MARIE T. SHERWOOD
Agricultural Institute,
Wexford, Ireland

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