SHORT COMMUNICATIONS

THE EFFECT OF CARBON DIOXIDE TREATMENT OF SEEDS ON FLOWERING IN SUBTERRANEAN CLOVER*

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Dormancy breaking in the germination of small-seeded legumes by low concentrations of CO_2 has been reported by Ballard (1958) and Grant Lipp and Ballard (1959). They suggested the usefulness of this treatment in seed testing, and for securing uniform material for experimental purposes. Any possible effect which this treatment may have on flowering would be unimportant in the former application; but in some instances would be of relevance for the latter (cf. Morley and Evans 1959). Since a wide range of substances is known to affect flowering, it would be desirable to establish whether CO_2 has such an effect. The participation of CO_2 in chemical vernalization (Leopold and Guernsey 1954), its dark fixation during photoperiodic induction (Gregory, Spear, and Thimann 1954; Langston and Leopold 1954), and its sex reversal activity (Gusev 1949) give added point to such an enquiry.

Evidence from two experiments with subterranean clover suggests that CO_2 treatment, even when prolonged considerably beyond that required for germination, is without effect on flowering.

Experiment 1

Imbibed non-dormant seeds of the introduction Portugal C.P.I. 19471‡ were supported on irrigated filter paper in jars containing, nominally, air, 3% CO_2 (in air), and 30% CO_2. The gas mixtures were obtained by displacing calculated amounts of CO_2 into the jars, and every second day the gaseous contents were flushed with air and the required CO_2 concentration restored. To reduce etiolation the jars were illuminated for 12 hr daily at 100 f.c. at a temperature of 25°C, the temperature during the dark being 20°C. Another group of imbibed seeds was held at 3–5°C in the dark. After 17 days the seedlings were transplanted and grown in a glasshouse at day and night temperatures of approximately 25 and 20°C respectively, and under day lengths which decreased from 15 to 11 hr.

The experiment was discontinued after 190 days, at which time no plants in any of the air- or CO_2-treatment groups, and only 57% of those in the low-temperature group, had flowered, all the latter having taken place between days 95 and 136. Apparently this strain has a low-temperature requirement which was not fully satisfied by the initial period at 3–5°C and the subsequent growth conditions. This result at least permits the inference that CO_2 treatment does not substitute for low temperature, nor does it in other ways markedly accelerate flowering under the stated conditions.

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Experiment 2

Imbibed non-dormant seeds of the cultivar Clare were placed on moist vermiculite in jars through which gases of the following nominal composition were passed continuously—air, 3% CO_2 (in air), 10% CO_2, 30% CO_2.

The jars were held at 25°C and illuminated at approximately 15 f.c. for 10·8 hr daily. After 10 and 20 days, and after 20 days for another group of imbibed seeds held at 3–5°C in the dark, seedlings were transplanted and grown in a glasshouse at day and night temperatures of approximately 25 and 20°C respectively, and at day lengths which increased from 10·8 to 14 hr. For both experiments the roots in the higher CO_2 treatments were ageotropic and stunted. In this experiment etiolation was more severe, and losses in all treatment groups occurred at transplanting. There were no survivors in the 30% CO_2 group.

TABLE 1

EFFECT OF CO_2 AND OF LOW TEMPERATURE ON TIME AND POSITION OF FIRST OPEN FLOWER IN SUBTERRANEAN CLOVER, CULTIVAR CLARE

Treatment	Mean No. of Days to First Open Flower		Mean Effective Node	
	Duration of Treatment		Duration of Treatment	
	10 Days	20 Days	10 Days	20 Days
Air	116·7	129·2	21·0	22·8
3% CO_2	122·0	128·8	20·5	24·0
10% CO_2	121·5	130·7	22·0	23·3
Low temperature	—	114·7	—	18·0

The number of days elapsing from the commencement of treatment to the appearance of the first open flower on each surviving plant was recorded, together with its position. The first flower to open was never inserted directly on a primary axis and about 70% were inserted on a first-order lateral. For these cases the "effective node" was recorded as the sum of the nodes on the primary axis to the insertion of the lateral concerned, plus the number of nodes along that lateral to the insertion of the flower. The values of less regular positions are excluded from the data of Table 1; but not the associated time data.

The most important result is that neither CO_2 treatment differed significantly from the air treatment, either in time or position of flowering (the variance due to treatment duration having been eliminated).

Thus it may be concluded from this experiment that CO_2 treatment neither hastens nor delays flowering; but a conclusion that CO_2 treatment is incapable of substituting for a low-temperature treatment is less securely founded here than in experiment 1.

Although the vernalization requirement of the cultivar Clare is slight, and flowering under favourable photoperiods can occur without exposure to cold, some acceleration of flowering by the low-temperature treatment was to have been expected (Morley and Davern 1956; Morley and Evans 1959). In fact the plants of the low-temperature group flowered earlier by about 15 days ($P < 0{\cdot}001$), corresponding to about five nodes ($P < 0{\cdot}001$), than plants pooled from all gas treatments of 20 days. But there is a delay in flowering of about 10 days ($P < 0{\cdot}01$), corresponding to about two nodes ($0{\cdot}1 > P < 0{\cdot}2$), associated merely with the duration of gas (including air) treatments. Dissection of samples of seedlings at the times of their removal from the jars showed that they added about 1–2 nodes during the second 10 days of treatment. It is as if after transplanting all plants proceeded towards flowering at the same rate; but that while in the jars the processes leading to flowering became dissociated from growth processes. No simple explanation for this is available.

However, this feature does not detract from the general conclusion that treatment of subterranean clover seeds and seedlings with CO_2 for periods up to 3 weeks does not markedly affect the subsequent flowering.

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