

EFFECTS OF TEMPERATURE ON GROWTH AND NUTRIENT UPTAKE IN SUBTERRANEAN CLOVER DURING RECOVERY FROM PHOSPHORUS STRESS

I. GROWTH CHANGES

By D. BOUMA* and E. J. DOWLING*

[Manuscript received September 23, 1968]

Summary

During a pretreatment period of 18 days, subterranean clover plants (*Trifolium subterraneum* L., cv. Mt. Barker) were grown at a deficient and an optimum phosphorus supply in three temperature regimes (27°C day/22°C night, 21/16°C, 15/10°C). They were then transferred to full solutions (optimum phosphorus) and plants from each pretreatment grown for a further 7 days in each of the three temperature regimes.

Shoot dry weights and leaf areas at the end of pretreatment were greater the higher the temperature. However, the plants grown with a deficient phosphorus supply showed a smaller temperature response than the non-deficient control plants, suggesting an increase in phosphorus requirements at higher temperatures.

Over the subsequent 7 days the relative growth rate of the previously deficient plants was smaller, and differed more from that of the non-deficient plants, the higher the temperature during pretreatment. Relative rates of leaf expansion over the same period were not influenced by differences in the phosphorus status at the beginning.

For both phosphorus pretreatments the relative rates of increase in dry weight and in leaf area were smallest at the lowest temperature. Most of the effects of temperature and phosphorus nutrition on relative growth rates were reflected in similar, though somewhat smaller, effects on net assimilation rates.

The distribution of dry matter to the roots was restricted after transfer of non-deficient plants from the highest to the lowest temperature and this was accompanied by a considerable accumulation of dry matter per unit leaf area. This did not occur in the plants recovering from phosphorus stress and the proportion of dry matter distributed to the roots was greater than in the non-deficient plants.

It was concluded that differences in yield between levels of phosphorus supply, as well as growth responses after removal of a phosphorus stress, may be expected to decrease with a decrease in temperature.

I. INTRODUCTION

In a study of the growth changes in subterranean clover during the early stages of recovery from phosphorus and sulphur stresses, relative rates of increase in plant dry weight were smaller the greater the previous stress. This was related to reductions in both components of the relative growth rate, the ratio of leaf area to plant dry weight, and the net assimilation rate. There was also evidence that the recovery in the assimilatory capacity of the leaves was slower after a sulphur stress

* Division of Plant Industry, CSIRO, P.O. Box 109, Canberra City, A.C.T. 2601.

than after a phosphorus stress (Bouma 1967a). The relative rates of leaf area expansion differed little between previous stress levels, and this was attributed to a preferential distribution of assimilates to new leaves formed after the removal of the stress.

The distribution of nutrients taken up during the recovery was largely directed to older leaves formed before the removal of the stress, presumably due to the demands of renewed metabolism. By contrast, most of the nutrients taken up during the same period by plants that had not been under stress were distributed to new shoots (Bouma 1967b, 1967c). These experiments were carried out under near-optimum temperature conditions.

These results were expected to vary with the temperature. However, little is known of the interacting effects of temperature and phosphorus nutrition on the growth of subterranean clover. From an agronomic point of view, temperature may have a marked effect on the response potential of the plant to the removal of a nutrient deficiency. The experiment of the present series of papers examined the effect of temperature on the growth of subterranean clover at different levels of phosphorus supply and on the growth changes during the early stages of recovery from phosphorus deficiency.

II. METHODS

Subterranean clover seeds (*Trifolium subterraneum* L., cv. Mt. Barker) were germinated in river sand at a temperature of 25°C during the day and 20°C at night (6 p.m.–6 a.m.). Seven days after sowing, seedlings selected for uniformity were transplanted to two phosphorus pretreatment solutions (August 8, 1963). Nutrients were applied at half strength initially and increased to full strength on the seventh day of pretreatment. This was done to prevent possible leaf injury in the very young seedling stage. The final phosphorus levels were 1 and 5 p.p.m. phosphorus. These are referred to as P₁ and P₂ respectively. The pretreatment period lasted 18 days, during which 170 and 850 µg phosphorus was supplied at the low (P₁) and high (P₂) phosphorus levels respectively. The phosphorus pretreatments were intended to provide moderately deficient and non-deficient plants. The levels chosen were based on results of several other experiments under comparable conditions, using the same experimental techniques. The latter, as well as the concentrations of other nutrients, have been previously described (Bouma and Dowling 1966).

Immediately after transplanting from sand to pretreatment solutions, seedlings in both phosphorus solutions were placed in three different temperature regimes: 15/10°C, 21/16°C, and 27/22°C. The first number in each temperature regime was the day temperature and the second number the night temperature (6 p.m.–6 a.m.). The three temperature pretreatments are referred to as t₁, t₂, and t₃ respectively. At the end of the 18 days of pretreatment the plants were transferred to full nutrient solutions (5 p.p.m. phosphorus). Immediately after transfer, the plants from each temperature pretreatment were assigned at random to each of the same three temperature regimes. However, the temperatures during this period are referred to as T₁, T₂, and T₃, in capital letters, to distinguish this period from the pretreatment period. Of the P₁ and P₂ plants pretreated at each temperature, one-third were placed at T₁ after transfer to full solutions, another third at T₂, and the remainder at T₃. The plants were then grown for a further period of 7 days. There were eight replicates, each replicate consisting of two plants per container.

Growth changes during the 7 days after transfer were described by estimates of leaf area and dry weight per plant. Leaf areas were estimated by visual comparison of individual leaves on the intact plant with a set of photographic standards (Williams, Evans, and Ludwig 1964). The first estimate for all plants was made on the day of transfer (day 0). Mean leaf areas for subsequent occasions were corrected by regression analysis (McIntyre and Williams 1949; Bouma and Dowling 1966). Harvests were carried out on the day of transfer (day 0) and at the end of the

experimental period (day 7). The harvested plants were separated into roots, leaves, and petioles. The leaf fraction included leaflets and petiolules. Dry weights were obtained after drying in a forced draught oven at 75–80°C. The mean dry weights were adjusted by covariance analysis on leaf areas in a manner similar to that for the leaf areas.

The concepts and functions of the analysis of plant growth were used to examine the growth changes during the 7 days after transfer. The subject of growth analysis has been reviewed by Williams (1946) and by Watson (1952). The symbols used in this paper are as follows:

A = leaf area (cm^2);

W = dry weight (mg);

R_A = relative leaf area growth rate (leaf area increase per unit leaf area in unit time: day^{-1});

R_W = relative growth rate (increase in dry weight per unit dry weight in unit time: day^{-1});

E_A = net assimilation rate (increase in plant dry weight per unit leaf area in unit time: $\text{mg cm}^{-2} \text{day}^{-1}$);

A/W = leaf area ratio (ratio of leaf area to dry weight per plant: $\text{cm}^2 \text{mg}^{-1}$).

By definition, $R_W = E_A(A/W)$.

Statistical analysis of the data was carried out to enable comparisons between treatment effects within temperature pretreatments. For results based on adjusted leaf areas and dry weights, the variance for each of the six treatment combinations ($2P \times 3T$) was calculated, and from these the least significant differences between pairs of adjusted treatment means within the three pretreatment temperature regimes. The differences in least significant difference (L.S.D.) values between pairs of means were generally small in relation to treatment effects, and for the sake of clarity only the largest L.S.D. value in each temperature pretreatment is shown in the relevant figures.

III. RESULTS

(a) Dry Weight Changes

(i) Pretreatment Effects

Figure 1 shows the effect of phosphorus levels (P_1 and P_2) and temperatures (t_1 , t_2 , and t_3) on the dry weight of shoots and roots at the end of the pretreatment period.

The lowest pretreatment temperature (t_1) resulted in considerably smaller plant dry weights, both at P_1 and at P_2 , than the higher pretreatment temperatures. At both phosphorus levels the temperature response during pretreatment was greater from t_1 to t_2 than from t_2 to t_3 . Most of the temperature response at both phosphorus levels was due to the effects of higher temperatures on shoot growth, particularly in the case of the non-deficient P_2 plants. This is shown in the following comparison of percentage increase in shoot and root dry weight between t_1 and t_2 over the pretreatment period at the two phosphorus levels:

Phosphorus level	P_1	P_2
Increase in shoot dry weight (%)	97	130
Increase in root dry weight (%)	83	40

There is some evidence in Figure 1 that the phosphorus deficiency on day 0, judged by differences in plant weight between phosphorus levels, was greater at t_2 and t_3 than at t_1 . Differences between phosphorus levels were not significant after pretreatment at t_1 but were relatively greater and highly significant at t_2 and t_3 . Most of the response to phosphorus during the three temperature pretreatments was due to its effect on shoot growth. This was particularly noticeable at t_2 and t_3 ,

where the shoots of the P_2 plants weighed nearly 50% more than those of the P_1 plants. The difference in root weight between phosphorus levels at t_2 was only 12%, whilst at t_3 the roots of the P_2 plants weighed less than those of the P_1 plants.

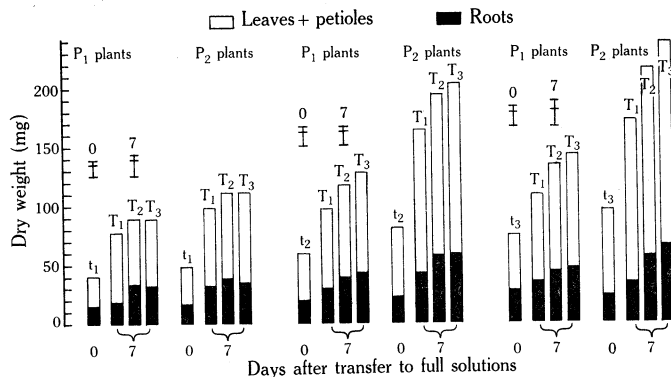


Fig. 1.—Dry weights of plants at the end of pretreatment at two phosphorus levels (P_1 and P_2 —1 and 5 p.p.m. respectively) in three temperature regimes (indicated by t_1 , t_2 , and t_3 —15/10, 21/16, and 27/22°C respectively), and the dry weights at the end of 7 days after transfer from each pretreatment combination to complete solutions placed at each of the three temperatures (indicated by T_1 , T_2 , and T_3 respectively). Significant differences ($P \leq 0.05$ and $P \leq 0.01$) are shown by vertical bars, the numbers referring to days 0 and 7; only the largest value is shown for comparisons between pairs of adjusted treatment means within each temperature pretreatment (see Section II).

(ii) Changes after Transfer to Complete Solutions (Fig. 1)

Increases in dry weight for P_1 and P_2 plants were greater the higher the temperature to which the plants were transferred. However, there was a clear indication that the ability of the P_1 plant to respond to the removal of the phosphorus deficiency

TABLE 1

DISTRIBUTION OF DRY MATTER BETWEEN PLANT PARTS FOR THE 7 DAYS AFTER TRANSFER TO FULL SOLUTIONS

Values are increase in dry weight of plant part, expressed as a percentage of the increase for the whole plant

Plant Part	P ₁ Plants Transferred from t_1 to:			P ₁ Plants Transferred from t_3 to:			P ₂ Plants Transferred from t_1 to:			P ₂ Plants Transferred from t_3 to:		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Shoots	64	61	65	75	72	70	68	66	70	85	74	70
Roots	36	39	35	25	28	30	32	34	30	15	26	30

was restricted by low temperatures. For example, the P_1 plants raised at t_2 had a dry weight of 59 mg on day 0 and this had increased by 37, 57, and 68 mg 7 days after transfer to complete solutions at T_1 , T_2 , and T_3 respectively. The corresponding P_2 plants raised at t_2 increased their dry weight on day 0 from 81 mg by 84, 113,

and 122 mg respectively. Similar trends were apparent for the plants raised at t_3 and to a lesser extent for those raised at t_1 .

The effects of pretreatments and temperatures during the recovery on the distribution of dry matter between shoots and roots during this period are shown in Table 1. In the plants raised at t_1 a somewhat greater proportion of dry matter was distributed to the roots of the P_1 plants than to those of the P_2 plants, but there

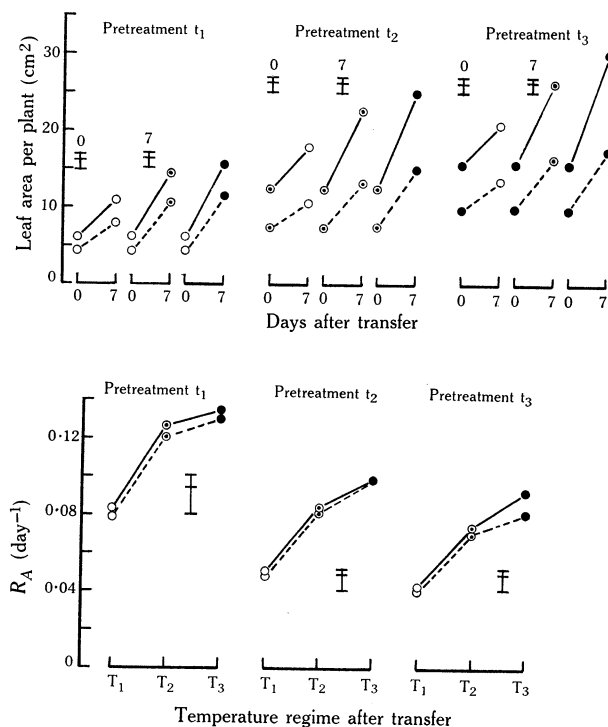


Fig. 2.—Leaf areas and relative leaf area growth rates (R_A) for the 7-day period after transfer from the pretreatments shown to complete solutions at each of the three temperatures T_1 (\circ), T_2 (\circ), and T_3 (\bullet). Phosphorus pretreatments: --- P_1 ; — P_2 . Significant differences are shown as for Figure 1.

was little or no effect of temperature differences during the recovery. In the plants transferred from t_3 , however, temperature differences during the recovery affected the distribution of dry matter quite markedly, particularly in the plants that had not been under stress. The proportion of dry matter distributed to the roots after transfer of the P_2 plants raised at t_3 to T_1 was only half (15%) of that for the corresponding plants transferred to T_3 (30%). This was largely balanced by a greater proportion of assimilates retained in the leaves (53 and 42% after transfer to T_1 and T_3 respectively). The trends for the P_1 plants raised at t_3 were in the same direction, but far less pronounced.

(b) Changes in Leaf Growth

Leaf areas on the day of transfer were smaller the lower the temperature during pretreatment and smaller for P_1 than for P_2 plants (Fig. 2). The ratios of leaf areas

of P_1 plants to that of P_2 plants for day 0 were 0.72, 0.60, and 0.64 at t_1 , t_2 , and t_3 respectively, indicating that the stress was more severe at t_2 and t_3 than at t_1 . This is in line with a similar conclusion based on the comparison in dry weight shown earlier.

Transfer to complete solutions resulted in marked increases in leaf area which, within each pretreatment temperature, were greater the higher the temperature during recovery. This is further illustrated by the comparisons of R_A , the values for

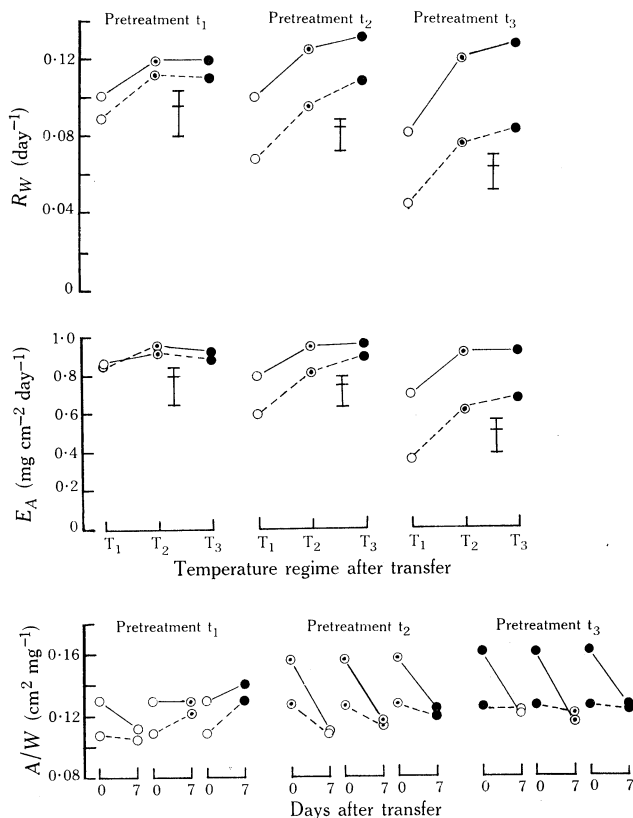


Fig. 3.—Relative growth rates (R_W), net assimilation rates (E_A), and leaf area ratios (A/W) for the 7 days after transfer from the pretreatments shown to complete solutions at each of the three temperatures T_1 (\circ), T_2 (\circ), and T_3 (\bullet). Phosphorus pretreatments: --- P_1 ; — P_2 . Significant differences ($P \leq 0.05$ and $P \leq 0.01$) are shown by vertical bars.

the plants transferred to T_2 or T_3 being considerably greater than for the corresponding plants transferred to T_1 . For the plants raised at t_2 and t_3 the values of R_A after transfer to T_3 were at least twice as high as for the corresponding plants transferred to T_1 .

The differences in R_A between P_1 and P_2 were small, with the possible exception of the plants raised at t_3 , where the value of R_A for the P_1 plants transferred to T_3 was somewhat lower than for the corresponding P_2 plants. This is in line with earlier results (Bouma 1967a) for phosphorus and also for sulphur. There was strong evidence that during the recovery from nutrient deficiencies assimilates were preferentially distributed to new leaf tissue.

(c) *The Relative Growth Rate and Its Components*

Differences in R_W between P_1 and P_2 were greater the higher the temperature at which the plants had been pretreated (Fig. 3). This was largely due to the fact that R_W for the recovering P_1 plants was lower the higher the temperature before the removal of the phosphorus stress. For example, the P_1 plants raised at t_1 increased their dry weight during recovery at T_1 and T_3 by 8.9 and 11.0% per day respectively. The corresponding values for the P_1 plants raised at t_3 were only 4.5 and 8.4% respectively. Although differences in the stage of development may have been involved, it appears more likely that the depressing effect of higher pretreatment temperatures on R_W of the recovering P_1 plants, which did not occur in the P_2 plants, was largely a reflection of the greater severity of the phosphorus stress in the plants raised at the higher temperatures.

R_W of the recovering P_1 plants as well as that of the P_2 plants was lower at T_1 than at T_2 or T_3 . This was particularly noticeable for the plants raised at t_2 and t_3 , and was probably in part a reflection of the unfavourable growing conditions at T_1 compared with T_2 or T_3 .

The effects of phosphorus pretreatments and temperatures during recovery on R_W were clearly reflected in similar, though somewhat smaller effects on E_A . The decline in E_A of P_1 plants with higher pretreatment temperatures, if due to an increase in the phosphorus stress during pretreatment, is in line with earlier results (Bouma 1967a). In experiments with subterranean clover plants raised at several levels of phosphorus supply, E_A during recovery was smaller the greater the previous phosphorus stress.

Differences in mean leaf area ratios between P_1 and P_2 were not greatly affected by temperature treatments during the period after transfer.

IV. DISCUSSION

On the basis of multiple regressions over a period of 52 weeks, Black (1955) found no effect of temperature on the relative growth rate of subterranean clover (cv. Bacchus Marsh). The plants were of comparable morphological age and were grown outdoors within a range of mean weekly temperatures from 8.2 to 25.2°C. He concluded that the rate of growth of this species in the early vegetative stage was mainly determined by the amount of radiant energy received. Morley (1958) studied five different cultivars of subterranean clover and found considerable differences between them in their relative growth rates at different temperatures. The plants were grown in comparable glasshouses kept at three temperatures with means of 16.2, 18.6, and 24.2°C, the day temperature being 5°C higher than the night temperature. From his results it appears that Black's choice of Bacchus Marsh as experimental plant was rather unfortunate, because its relative growth rate was little affected by the three temperature regimes used by Morley (1958). For the other cultivars differences in R_W between temperatures were reflected in differences in E_A as well as in A/W .

The present experiment was carried out under the relatively low levels of radiant energy prevailing at the end of winter, in August. The seedlings (cv. Mt. Barker) placed at the three pretreatment temperatures, were 7 days old from sowing and had been raised under the same conditions (25°C day/20°C night). At the end of

pretreatment (18 days) in the three temperature regimes shoot dry weights and leaf areas of P_1 and P_2 plants were as shown in Table 2.

The Mt. Barker cultivar was not included in the investigation by Morley (1958). However, the present results leave little doubt that even the moderately low temperature of 15/10°C (59/50°F) can cause marked growth reductions of this cultivar. Figure 3 showed clearly that reductions in R_W induced by transfer to the lowest temperature, or by differences in phosphorus status, were reflected in similar, although somewhat smaller, reductions in E_A .

TABLE 2
DRY WEIGHTS OF SHOOTS AND LEAF AREAS OF P_1 AND P_2 PLANTS AFTER
18 DAYS GROWTH AT THE TEMPERATURES SHOWN

	Temperature			Percentage Increase, $t_3 - t_1$
	t_1 (15/10°C)	t_2 (21/16°C)	t_3 (27/22°C)	
Dry weight (mg)				
P_1	26	40	51	96
P_2	32	59	74	131
Leaf area (cm ²)				
P_1	4.5	7.5	9.9	120
P_2	6.2	12.6	15.6	152

It is also clear from the above comparison and from the comparison between Figures 2 and 3 that lower temperatures depressed R_A more than R_W . There were no differences in R_A during the recovery period between P_1 and P_2 for any of the pretreatment temperatures (Fig. 2). R_W of the P_1 plants was always less than that of the P_2 plants, the differences being greater the higher the temperature during pretreatment, probably because the severity of the phosphorus stress increased with pretreatment temperatures (see comparison in Table 2). This suggestion is in line with earlier results, where it was found that R_A during recovery did not depend greatly on the severity of the previous phosphorus stress, while R_W differed more from that of non-deficient plants the greater the previous deficiency in the phosphorus supply (Bouma 1967a).

The effect of temperature on the translocation of carbohydrates has been a matter of some controversy. In reviewing the older literature Esau, Currier, and Cheadle (1957) found many conflicting results, but concluded that most of the more recent evidence indicated a restricted translocation at low temperatures. Humphries (1963), using single rooted leaves of dwarf bean plants, established an increased translocation from the leaf to the roots, and also an increase in E_A , in the treatments that stimulated root growth. These treatments included higher temperatures and pretreatment with indolylacetic acid. His results and also those of Thrower (1965) demonstrated the importance of sink size, for example the demand for assimilates in enhanced root growth, in determining the rate of movement of assimilates.

Evidence presented here strongly suggests a restriction of assimilate transport after transfer from a high to a low temperature, but only in plants that had been raised at an adequate level of nutrition. For example, the P_2 plants raised at t_3

distributed during the 7 days after transfer to T_1 , T_2 , and T_3 15, 26, and 30% respectively of the total dry matter production to the roots. The accompanying percentages of dry matter retained by the leaves were 53, 43, and 42% respectively. There was little effect on the distribution of dry matter to the petioles. In another experiment under the same experimental conditions (Bouma and Dowling, unpublished results), except that it was carried out in spring 2 months later, almost identical results were obtained: plants raised with adequate nutrition at t_3 distributed 20, 25, and 33% of the total dry matter to the roots during the 7 days after transfer to T_1 , T_2 , and T_3 respectively. The percentages of dry matter retained by the leaves were 53, 45, and 40% respectively, there being little effect on the petioles.

The temperature-induced restriction of assimilate distribution to the roots was accompanied by a considerable accumulation of dry matter in the leaves. For example, the P_2 plants raised at t_3 showed a value of 2.7 mg dry matter per square centimetre of leaf area on the day of transfer to T_1 , T_2 , and T_3 . This value had changed to 4.0, 3.8, and 3.4 mg cm⁻² respectively 7 days later. Even greater amounts of dry matter accumulated in the second experiment quoted above (Bouma and Dowling, unpublished results), carried out 2 months later at higher levels of radiation. The value on the day of transfer was 3.5 mg cm⁻² and this had changed to 5.4, 4.5, and 3.9 mg cm⁻² 7 days after transfer from t_3 to T_1 , T_2 , and T_3 respectively.

By contrast, the distribution of dry matter after the transfer of the P_1 plants raised at t_3 to complete solutions was comparatively little affected by temperatures during the 7-day recovery period. The distribution percentages for the roots were 25, 28, and 30%, and those for the leaves 51, 46, and 46% at T_1 , T_2 , and T_3 respectively. The amounts of dry matter per unit leaf area also changed little during the recovery and were not affected by temperature differences during this period. The value on the day of transfer to full solutions was 3.2 mg cm⁻² and this had changed to 3.4, 3.5, and 3.4 mg cm⁻² after 7 days at T_1 , T_2 , and T_3 respectively.

The differences in the temperature response between P_1 and P_2 were most likely due to differences in sink size. Transfer of the non-deficient P_2 plants from the highest to the lowest temperature reduced the demand for assimilates in the roots as well as in other plant parts. However, R_W and E_A were not depressed as much as R_A , so that dry matter could accumulate in the leaves. Removal of the phosphorus stress in the P_1 plants by the transfer to complete solutions appears likely to have induced a considerable demand for assimilates for renewed growth of all plant parts, even at the lower temperature during the recovery. This resulted in a relatively greater flow of assimilates to the roots after transfer to T_1 than in the corresponding non-deficient P_2 plants, thereby also preventing the accumulation of dry matter in the leaves found in the P_2 plants.

Some of the implications of the temperature-phosphorus interactions presented in this paper may be of significance from an agronomic point of view. In most of the areas of southern Australia where subterranean-clover-based pastures are grown, temperatures would be well below the lowest temperature of the present experiment (59/50°F) for most of the growing season. More favourable temperatures may occur early in the season during autumn and particularly towards the end, in spring. Table 2 showed clearly that the temperature response with respect to both leaf area and shoot dry weight was greater for the plants raised with adequate phosphorus

than for the stressed plants. In practice, an adequate phosphorus supply would be particularly important during the more favourable temperature conditions early and late in the season.

The results also provide evidence to conclude that yield differences due to differences in phosphorus supply, as well as yield responses after removal of a phosphorus stress, may be expected to decrease with a decline in temperature.

V. ACKNOWLEDGMENTS

The authors are grateful to Dr. R. F. Williams for his criticism of the manuscript, and to Mr. G. A. McIntyre, Division of Mathematical Statistics, CSIRO, for his advice in the statistics of the experiment.

VI. REFERENCES

- BLACK, J. N. (1955).—The interaction of light and temperature in determining growth rate of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. biol. Sci.* **8**, 330–43.
- BOUMA, D. (1967a).—Growth changes of subterranean clover during recovery from phosphorus and sulphur stresses. *Aust. J. biol. Sci.* **20**, 51–66.
- BOUMA, D. (1967b).—Nutrient uptake and distribution in subterranean clover during recovery from nutritional stresses. I. Experiments with phosphorus. *Aust. J. biol. Sci.* **20**, 601–12.
- BOUMA, D. (1967c).—Nutrient uptake and distribution in subterranean clover during recovery from nutritional stresses. II. Experiments with sulphur. *Aust. J. biol. Sci.* **20**, 613–21.
- BOUMA, D., and DOWLING, E. J. (1966).—The physiological assessment of the nutrient status of plants. II. The effect of the nutrient status of the plant with respect to phosphorus, sulphur, potassium, calcium, or boron on the pattern of leaf area response following the transfer to different nutrient solutions. *Aust. J. agric. Res.* **17**, 633–46.
- ESAU, K., CURRIER, H. B., and CHEADLE, V. I. (1957).—Physiology of phloem. *A. Rev. Pl. Physiol.* **8**, 349–72.
- HUMPHRIES, E. C. (1963).—Dependence of net assimilation rate on root growth of isolated leaves. *Ann. Bot. (N.S.)* **27**, 175–83.
- MCINTYRE, G. A., and WILLIAMS, R. F. (1949).—Improving the accuracy of growth indices by the use of ratings. *Aust. J. scient. Res.* **B 2**, 319–45.
- MORLEY, F. H. W. (1958).—Effects of strain and temperature on the growth of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. agric. Res.* **9**, 745–53.
- THROWER, S. L. (1965).—Translocation of labelled assimilates in the soybean. IV. Some effects of low temperature on translocation. *Aust. J. biol. Sci.* **18**, 449–61.
- WATSON, D. J. (1952).—The physiological basis of variation in yield. *Adv. Agron.* **4**, 101–45.
- WILLIAMS, R. F. (1946).—The physiology of plant growth with special reference to the concept of net assimilation rate. *Ann. Bot. (N.S.)* **10**, 41–72.
- WILLIAMS, R. F., EVANS, L. T., and LUDWIG, J. (1964).—Estimation of leaf area for clover and lucerne. *Aust. J. agric. Res.* **15**, 231–3.