

A STUDY OF THE INTERACTION OF COLD STRESS, AGE, AND PHOSPHORUS NUTRITION ON THE DEVELOPMENT OF *LOTONONIS BAINESII* BAKER

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[Manuscript received 13 June 1972]

Abstract

The effects that specific amounts of cold stress had on the development of the tropical legume *L. bainesii* were assessed for plants of four ages grown in a nitrogen-deficient soil. They were dependent primarily upon symbiotically fixed nitrogen, when receiving phosphorus at five treatment levels. The plants received normal lighting and a 12-hr day length under controlled environment, and the cold stress was gradually increased from nil to frosting. Root temperatures were similar to those in the field.

Both univariate and multivariate approaches were adopted in analysis of data for fresh and dry weight, water content, nitrogen, and phosphorus. Besides large differences due to phosphorus, there were large differences between ages and between temperatures for a majority of the variates. The net response to chilling was thus multiple in character rather than singular; a complex of age, nutrient, and temperature.

For the majority of variates studied, cold impaired development in young plants more than in old, apparently because young plants not receiving phosphorus were only initiating nodulation. Although the development of older, phosphorus-treated plants was less affected by cold, they were more susceptible to injury by frost, apparently because of enhanced tissue nitrogen levels and succulence.

L. bainesii could withstand an extensive amount of cold stress, and productivity and nitrogen fixation were clearly favoured during stress by high levels of phosphorus fertilization. This was most successful with mature plants. The plant did not break down because of cold as do many tropical legumes.

Multivariate analyses provided a qualitative, simply expressed summary of the overall pattern of results; univariate analyses provided a detailed, precise account of the magnitudes of effects, but required much intricate computation.

I. INTRODUCTION

Bryan (1961) has described the distinctive features of the legume *Lotononis bainesii* as a component of subtropical pastures. He showed that high yield of dry matter and nitrogen may be obtained in a nitrogen-deficient soil through effective symbiosis with a highly specific red strain of *Rhizobium* (Norris 1958). Bryan also refers to the cold and frost tolerance of *L. bainesii*, and its capacity to grow over a wide range of day length and temperature. Bryan *et al.* (1971) found yield of *L. bainesii* to be more closely associated with radiation than with other meteorological factors. They also found that mean weekly minimum temperature needed to rise above 9°C for resumption of active growth after winter.

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Cold and frost tolerance are desirable qualities for subtropical pasture plants in the Australian environment, where average night temperatures in some areas may be low enough to seriously restrict growth of introduced tropical species. The incidence of frost in both the subtropics and tropics of eastern Australia is sufficient to limit pasture development seriously (Coleman 1964; Henzell 1967). Bryan and Sharpe (1965) have demonstrated the sensitivity of Pangola grass to temperature: "little growth was made when the average minimum screen temperature was less than 52°F, or the average night temperature less than 58°F". Bryan and Shaw (1964) comment that although *Paspalum plicatulum* is drought tolerant it is damaged by frost. Davies (1960) stated that among legumes on the subtropical coast, with a mean of five frosts per annum, only *L. bainesii* and *Indigofera spicata* withstand mild frosts and "re-grow during the winter after being cut back by the sudden insolation frosts".

Henzell (1962) found that *L. bainesii* and *I. spicata* fixed about 80% as much nitrogen as white clover when growing in nitrogen-free sand in the glasshouse, even though their strain of *Rhizobium* was not fully effective. He commented that a number of factors might limit this result in the field, cold being more likely than drought to do so.

Field conditions are better approximated if the growing plant receives normal lighting and day length whilst being subjected to cold stress. The stress should also be increased gradually, if a sudden cold shock is to be avoided. Special facilities were developed by one of us (C.T.G.) for this purpose.

The age of the plant when chilled may affect its reaction to cold, for young spring growth is often regarded as more susceptible to chilling and frosting than is the maturer growth of autumn. This may also be confounded with the nutrient status of the plant's tissues, for the growth pattern of a legume is dependent on the influence of phosphorus supply on nitrogen fixation, when in a nitrogen-deficient substrate. Shaw *et al.* (1966) have shown the importance of phosphorus fertilization to growth and nitrogen fixation in *Stylosanthes humilis*. Andrew and Robins (1969) found that in *L. bainesii* the higher levels of phosphorus fertilization increased nitrogen in the plant tops more than dry matter yield.

The present paper describes the effects that specific amounts of cold may have on the development of *L. bainesii* in a nitrogen-deficient soil when dependent primarily upon symbiotically fixed nitrogen. The comparisons are for plants of differing physiological ages and a range of phosphorus treatments.

II. MATERIALS AND METHODS

Lotononis bainesii Baker inoculated with *Rhizobium* strain CB 376 was sown in polythene-lined pots containing 2.42 kg oven-dry soil from the A horizon of a light-textured solodic soil from Rodd's Bay, Qld., and was covered with a 1.5-cm layer of acid-washed quartz gravel. Analyses of the type profile for this soil showed total nitrogen 0.06%, total phosphorus 0.006%, and pH 6.4. Nutrients excluding nitrogen were added as a blanket dressing at the rates: 0.199 g K₂SO₄, 0.008 g ZnSO₄, 0.008 g CuSO₄, 0.0006 g Na₂MoO₄.2H₂O. Phosphorus treatments were applied as NaH₂PO₄.2H₂O at sowing at the following rates per pot: nil (P₀), 0.145 g (P₁), 0.289 g (P₂), 0.434 g (P₃), 0.578 g (P₄). Temperature was a constant 25°C and light approximately 3500 f.c. per 13 hr per day. Plants were thinned to four per pot.

The experimental layout was a single replicate of a factorial treatment design, comprising the five levels of phosphorus × five harvests at increasing degrees of cold stress × four ages at the

commencement of cold treatment. The number of pots was thus 100, re-randomized within each age group at 6-day intervals. The ages were taken separately in random order, with treatment commencing at days 31, 38, 45, and 52 from sowing. The treatment of the successive 25 pots of each age group in the one frost room involved a long period of time (some 10 months).

Throughout the treatment period a 12-hr day was imposed as this is more nearly the normal autumn day length in south-east Queensland. Radiation-type frost conditions were also simulated with the increasingly cold nights and warm, bright days of autumn. An initial constant temperature of 25°C was gradually lowered over 24 days, until the plants were at a minimum of between 0 and -1°C for 4½ hr at the close of the dark period, and at a maximum of 18.3°C for 3 hr at the middle of the light period. Figure 1 presents the details of the temperature regimes imposed.

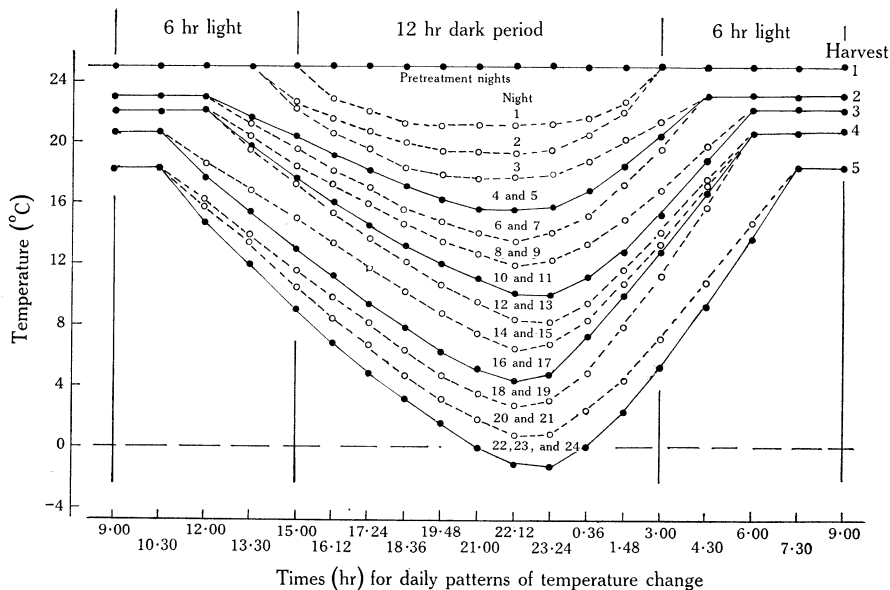


Fig. 1.—Temperature regimes imposed for 24 days of chilling treatment involving gradual departure from a constant 25°C.

The pots were insulated against cold during treatment, because under field conditions soil temperatures are much less affected than air temperatures by radiation-type frosts. They were inserted to their full depth through close-fitting holes in a Coolite lid (2.5 cm thick) into a large tank of water (1080 litre capacity) immersed to a depth of 5 cm into the water. There was thus a zone some 7 cm deep between the lid and the water surface that acted as a buffer against sudden temperature change within the pot. The tank was well insulated, under thermostatic control not to drop below 12°C, and was stirred by pumping. The lid normally fitted closely but could be raised and the pots removed for watering to field capacity by weight.

An increasing amount of cold was imposed at each harvest interval and thus the plants became "hardened" against the cold rather than being suddenly exposed to it. If cold stress be expressed as degree-hours departure from a constant 25°C, the days of harvest and amounts of cold were: day 0, nil deg-hr; day 5, 320 deg-hr; day 11, 1190 deg-hr; day 17, 2580 deg-hr; day 24, 4990 deg-hr (approximating to a quadratic curve of increase). In the age 45 group, the health of plants in pots receiving phosphorus at P₀ and P₄ levels was observed in response to gradually increasing frosting over a further 10-day period, being ultimately subjected to a minimum temperature of -11°C.

At harvest, tops fresh weight was recorded, and dry weight of tops and roots after drying under forced draught at 80°C. Nodulation was good with phosphorus addition. Nodule recovery was good, and as the nodules closely invest the root system in *L. bainesii* (Norris 1958) they were included with the root system.

Nitrogen and phosphorus were estimated on the ground tops and root samples by auto-analysis of Kjeldahl digests with selenium catalyst, after the method of Hegarty *et al.* (unpublished data).

III. STATISTICAL ANALYSIS

There were two sets of variates to be considered. The first (case 1) comprised seven variates: fresh and dry weight of tops, dry weight of roots, percentage nitrogen in tops and roots, and percentage phosphorus in tops and roots. The second (case 2) comprised four derived variates: the total nitrogen and phosphorus contents of the tops and roots were obtained by multiplying dry weights by the appropriate percentage.

The variates were first considered separately by univariate analysis. The need to impose a gradual cold stress rather than a sudden cold shock makes it inevitable that cold stress be confounded either with age at harvest or with age at imposition of the stress. In the design adopted, cold stress is confounded with age at harvest. However, for the 20 initial age \times cold stress combinations the ages at harvest fall into six groups characterized by approximate values of 38, 43, 49, 55, 62, and 69 days from sowing. From a plot of variate against age at harvest for each cold level [e.g. Fig. 2(a)] the effects of increasing cold for the six constant ages (38, 43, 49, 55, 62, and 69 days from sowing) were read directly or by interpolation between two adjacent points [e.g. Fig. 2(b)].

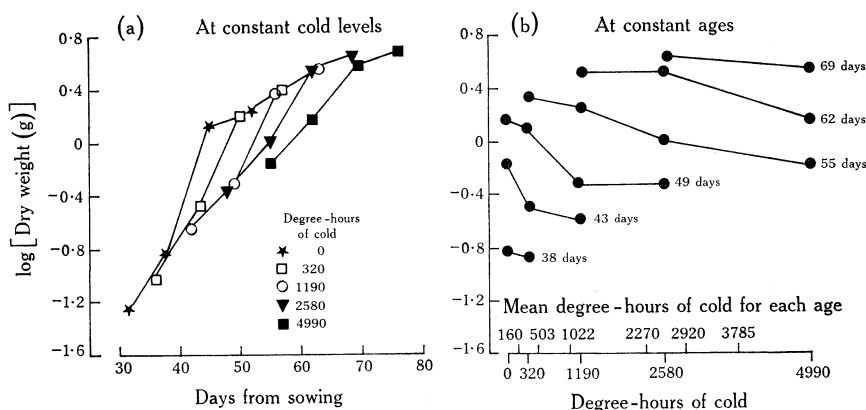


Fig. 2.—Effects of cold on dry weight of tops. This figure illustrates the method of deriving effects of increasing cold at constant age (b) from a plot for each cold level against age at harvest (a). All variates were considered in this way in univariate statistical analysis.

Preliminary analyses of variance showed that there were large differences between initial ages and between temperatures for a majority of the variates, and a study of the cold effects at the six constant ages also revealed marked interactions between the two factors. This, coupled with the large effect of adding phosphorus, indicated that the following comparisons contained most of the information in the trial:

- (1) at P_0 ;
- (2) over the levels P_{1-4} , as mean (P_M) and as linear (P_L) responses;
- (3) the difference ($P_M - P_0$), which we call the P_D effect.

For a majority of variates there was a systematic change over the six constant ages in the slope of the variate's response to increasing cold. This change was assessed for any variate by performing a further regression of these slopes ("linear cold coefficients") on the corresponding ages. By adopting this approach the picture of events was clarified because the regression relationships were appropriately summarized, thus conveniently reducing the amount of graphical presentation otherwise necessary. Sensitive tests of error variance could also be applied to the comparisons of systematic change in linear coefficients with increasing age.

The error variance used was constructed from the second-order interaction, ages \times cold \times phosphorus level, of the original analysis of variance. Tests of the equality of the linear cold

coefficients (parallelism) concerned a component of the age \times cold interaction for the comparisons at P_0 and P_M , and a component of the age \times cold \times phosphorus level interaction for P_L and P_D . Where these interaction components were significant they were invariably associated with a systematic change in the linear coefficients with increasing age. This change was assessed by testing the significance of its linear regression on age using a weighted regression analysis. The linear cold coefficients had different variances due to the different number of data points within each age [e.g. Fig. 2(b)] and hence the weights used were the inverses of these variances.

These linear cold coefficients for P_M , as derived from Figure 2, are shown in Figure 3 together with those for P_0 , P_D , and P_L . However, P_L never showed a significant trend in cold effect with age. Note that the P_D linear cold coefficients were obtained directly as the differences ($P_M - P_0$) for untransformed data, and as $\log(P_M - P_0)$ for log transformed data.

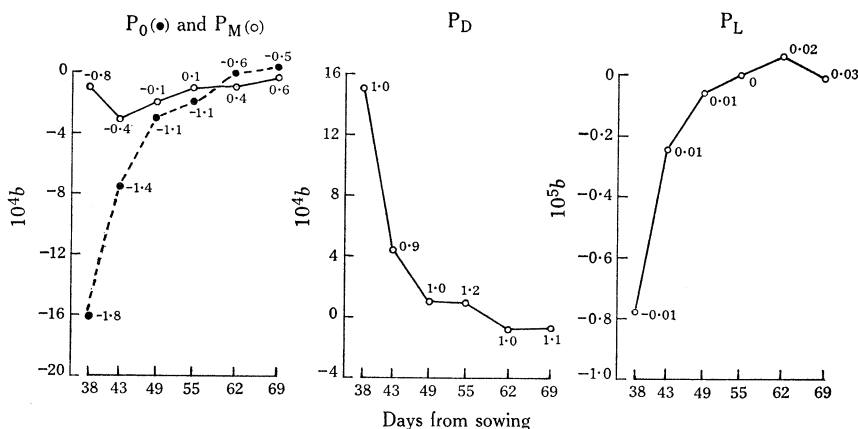


Fig. 3.—Linear cold coefficients for the regression of \log_{10} dry weight of tops per degree-hour on age. This figure illustrates the method for deriving P_0 , P_M , P_D , and P_L from the data for effects of increasing cold at six constant ages of Figure 2(b). All variates were considered in this way in univariate statistical analysis. Note the displacement of the scale for P_D . Mean values are given for each slope.

Beside each point in Figure 3 are the appropriate mean variate values associated with each slope. These enable the displacement due to age to be visualized. The exact location of any line in the two-dimensional space of variate value and cold may also be seen by reference to Figure 2(b) for the mean cold values associated with each age.

The appraisal of the overall plant response was then sought, but multivariate analysis was not undertaken because only one replicate was available. Rather a pattern-forming probabilistic approach was taken in which both the chilling treatments and the ages were considered essentially sequential. In this sense the observations within each sequence were considered to be related, even though they were made on independent pots frequently randomized, i.e. they were statistically independent. The general principles involved in the classification of sequences have been discussed by Dale *et al.* (1970). As the major interest lay in the chilling treatments the system was regarded as 20 elements (ages \times phosphorus levels), each associated with a time vector of five successive sets of observations. Thus the chilling sequence was regarded as a homogeneous Markov chain of the first order.

The two sets of variates were small (seven in case 1 and four in case 2) and showed no skewness, so were standardized according to the suggestions of Burr (1968), and classified by his "incremental sum of squares" fusion strategy (Burr 1970). The program MULTCLAS was used on the Control Data 3600 computer at CSIRO, Canberra. The hierarchy was truncated when there was no longer fragmentation into isolated element occasions, yielding four groups in case 1 and six in case 2.

For each of the 20 elements in the system of time vectors a 4×4 (case 1) or 6×6 (case 2) transition matrix was set up. These were then classified by the procedures of Dale *et al.* (1970) which are available as the program TRANSMAT at Canberra.

IV. RESULTS

(a) Visual Response

Treatment responses were apparent to the eye, as a complex of phosphorus nutrition, age at treatment, and degree of cold stress.

Plants of the P_0 treatment were much smaller than those receiving phosphorus and had a more hardy appearance with a deeper anthocyanin coloration with cold. This was especially so for the oldest plants of the age 52 group, but least for the youngest plants of age 31. P_0 plants were also less susceptible to frost injury than others. With added phosphorus, the degree of injury varied with age, for the leaves in the crown of the plant were burned in age 52 plants, the tip leaves of laterals were burned in age 45 plants, the injury was slight in age 38, and was virtually nil in age 31. P_0 plants, on the other hand, were not injured by frost at the treatment levels imposed and so were behaving as though physiologically younger than the plants receiving phosphorus.

Plants of the age 45 group treated at P_0 and P_4 levels were exposed to severe frosting and compared to see if the differences might be transitory. This proved not to be so. After 7 days, when the daily temperature fell to -7.5°C for 3 hr and cumulative degree-hours of cold totalled 7800, the leaves of P_0 plants were yellowing but not injured, whereas on P_4 plants all laterals were dead. After 10 days, the temperature had been lowered to -11°C for 3 hr, totalling 9250 deg-hr of cold, and all live material was a deep reddish colour. Only 5% of leaves were dead in P_0 , whereas almost all were dead in P_4 . A greater frost tolerance in the absence of phosphorus was thus indicated, even under extreme cold stress.

The older high-phosphorus plants at age 52 were not only more coloured and more injured by frost than others, but their injured leaves had dried more at harvest.

*(b) Univariate Analyses**(i) Dry Weight of the Whole Plant*

The effect of cold on plant growth in response to phosphorus nutrition is shown in Figure 4 in which the logarithm of the dry weight of the whole plant is plotted as an isometric projection against degree-hours of cold and age when treatment commenced. Whole-plant data are presented because effects for tops and roots do not differ greatly from the whole plant when graphed in this way.

There was a normal pattern of development for all age combinations for zero cold stress over a 21-day period in response to phosphorus, so that the slopes of the curves are analogous to a plot of relative growth rates. There was a decided difference in plant size at the earliest age (day 31) between P_0 and P_{1-4} levels and, whilst later final growth rates were higher with added phosphorus, at this early age P_0 plants had the higher growth rates. At other levels of cold, the slopes for age combinations indicate the rates of development as the plant is constrained away from normal by increasing amounts of cold stress. Here again, added phosphorus greatly stimulated growth.

Plants not subjected to cold stress continued to grow at all phosphorus levels, although their rates of growth fell over the period, as might normally be expected.

This is shown to have occurred by the slopes of the curves for zero cold stress, which cover a 21-day period from day 31 on.

This series for normal plants may be compared with plants of the 31-day age group during their subjection to increasing cold over the following 24 days. The cold-treated plants did not attain the normal size even though they grew for 3 days more. The plants of all age groups, however, continued to grow even over the coldest period, their dry weight production being approximately equal to that of normal at day 42.

Figure 4 thus obviously portrays an interesting pattern of events which confirms the visual observations already described. Figure 4 also indicates that there are real but complex interactions of cold, age, and phosphorus which warrant more detailed statistical analysis, for their nature cannot be determined from this figure alone. The characteristics and significances of these events is therefore now examined at both the univariate and the multivariate levels, along the lines described in the statistical portion of the methods.

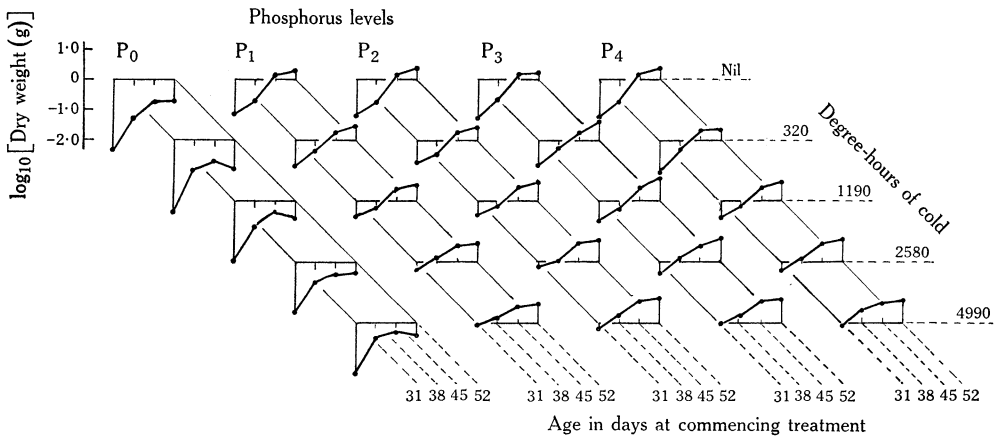


Fig. 4.— \log_{10} dry weight of the whole plant plotted as an isometric projection against degree-hours of cold and the age when treatment commenced.

(ii) Dry Weight of Plant Parts

For P_M and P_0 the change in the linear cold coefficients with increasing age was positive and significant ($P < 0.001$) for the logarithm of dry weight of tops, roots, and whole plant. The nature of this change for tops for P_M may be seen in Figure 2(b). For P_D this change was negative ($P < 0.001$), which resulted from a greater difference due to chilling in younger plants than in older, primarily because of the adverse effect of chilling on young P_0 plants during their early rapid growth phase.

(iii) Changes in Nitrogen Content of the Whole Plant

The changes in nitrogen content of the whole plant paralleled dry weight trends in that linear cold coefficients for P_0 and P_M increased significantly ($P < 0.001$) with age, whilst those for P_D decreased.

In the whole plant, only the P_M linear cold coefficients for relative nitrogen increased ($P < 0.001$), indicating that nitrogen accretion by the plant was more active than was the change in dry weight.

(iv) *Changes in Nitrogen Content of the Plant Parts*

The change in the linear cold coefficients for P_M and P_0 with increasing age are presented in Figure 5 in which the logarithm of the relative and absolute values for nitrogen in tops and roots are plotted against age. The appropriate mean nitrogen value associated with each slope is given on the curves. From these curves P_D values are apparent, and also the range of difference due to phosphorus addition. The logarithmic data tend to minimize the differences present in the untransformed data, but they permit more reliable interpretation of significance tests, because uniformity of comparison is gained with tests of other variates. This applied especially to the relative data.

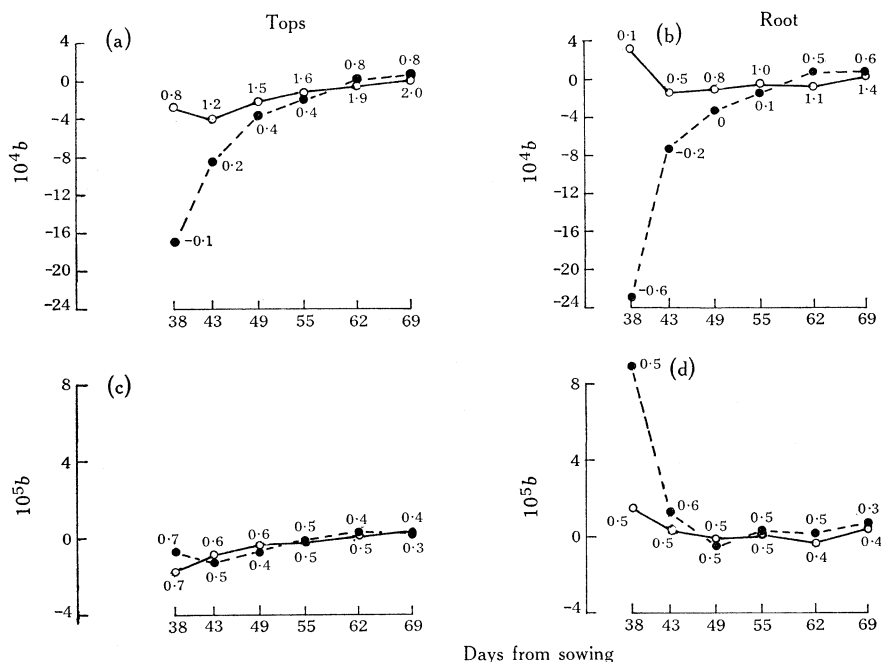


Fig. 5.—Linear cold coefficients for the regressions of \log_{10} nitrogen content (mg) and \log_{10} relative nitrogen content (as a percentage of dry weight) per degree-hour of cold on age in tops (a and c) and in roots (b and d), respectively. Changes in slope of P_M (○) and P_0 (●) are contrasted. Mean values are given for each slope.

The roots showed similar responses in nitrogen content to those of the whole plant. For P_D , the change in slope was still negative ($P < 0.001$), as a result of a greater limitation due to chilling in young P_0 plants, which were commencing to fix nitrogen at that time. The change was never large for P_0 plants, and at day 31 the plants were still very small and poorly nodulated. The similarity of the nitrogen yield to that of dry weight (see above) is apparent, but it should be noted that the

effects on nitrogen were not as severe. This is clear from the relative nitrogen data in Figure 4(d) where P_D increased from negative values to zero differences in older plants ($P < 0.001$). The increases were greater in younger plants ($P < 0.01$).

Thus it can be seen that the roots of the young plants behaved differently from older plants when under cold stress. They were more sensitive to the effects of cold upon dry weight increase when commencing to fix nitrogen, but the greater limitation was on dry weight.

The tops also showed similar responses in nitrogen content to those of the whole plant. There was a greater difference in slope due to cold stress in young plants. However, the tops differed from the roots in that changes in relative nitrogen content were negligible for P_D , i.e. the curves for P_0 and P_M were substantially parallel, their linear cold coefficients changing from negative values for young plants to zero for older plants [Fig. 4(c)]. However, only the change in slopes for P_M was significant ($P < 0.001$).

Nitrogen concentration in tops was thus more impaired by cold in young plants than in old plants. This applied both in the presence and absence of phosphorus. In the roots, however, the limitation of cold was only on young P_0 plants and on their initiation of nitrogen fixation.

The ratio of nitrogen content of tops to nitrogen content of roots confirm this, because the change in linear cold coefficients was significantly positive ($P < 0.001$), ranging from negative values in young plants to zero values at maturity. The passage of nitrogen from roots to tops was thus impaired by cold, mainly in young plants.

(v) *Changes in Phosphorus Content*

The wide range of phosphorus treatments might be expected to influence phosphorus uptake, so that the interest lies primarily in the effect of cold on the general nutritional response.

Mean relative phosphorus contents of the whole plant were: $P_0 = 0.10\%$; $P_1 = 0.22\%$; $P_2 = 0.32\%$; $P_3 = 0.38\%$; and $P_4 = 0.43\%$ dry weight. Values for tops and roots were similar and showed similar rates of increase with added phosphorus, although the range was somewhat greater for the tops than for the roots.

Absolute phosphorus levels were, of course, markedly affected by treatment in this low phosphorus soil. There were significant increases in linear cold coefficients for the whole plant but these need not be enumerated as they were similar to those for dry weight.

The change in the linear cold coefficients with increasing age for P_M and P_0 levels are presented in Figure 6 in which the logarithms of relative and absolute phosphorus levels in tops and roots are plotted against age. With one exception, the responses to cold followed a similar pattern to those for nitrogen and had the same significances. The exception was in P_D for relative phosphorus content of roots which showed no significant trend in cold coefficients. The response of root phosphorus thus followed the dry weight response more closely than the changes in nitrogen content.

Linear cold coefficients for the ratio nitrogen to phosphorus for the whole plant increased for P_M ($P < 0.001$) over age, there being a cold effect on young plants only, which reduced the proportion of nitrogen in them. At the same time,

the mean value appropriate to each slope increased as the plants aged. Thus, the plants changed as they matured, for at all levels of cold they were becoming enriched in nitrogen relative to phosphorus.

For the ratio of phosphorus content of roots to tops, P_M and P_D linear cold coefficients both declined with age ($P < 0.001$). The adverse effect of cold was again on young P_0 plants. The mean values associated with each slope were considerably higher in P_0 than in P_M , indicating that such phosphorus as was available when under short supply was retained in the roots, rather than being passed to the tops.

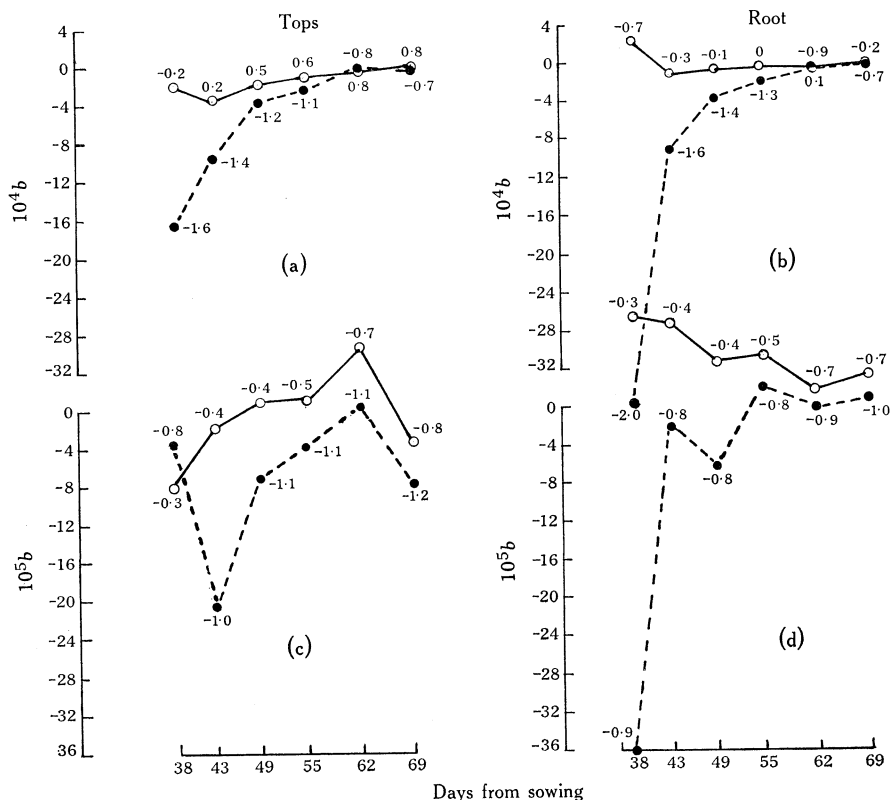


Fig. 6.—Linear cold coefficients for the regressions of \log_{10} phosphorus content (mg) and \log_{10} relative phosphorus content (as a percentage of dry weight) per degree-hour of cold on age in tops (a and c) and in roots (b and d), respectively. Changes in slope of P_M (○) and P_0 (●) are contrasted. Mean values are given for each slope.

(vi) Water Content

Effects of cold and age on relative water content of tops are presented in Figure 7(a) as the linear cold coefficients for P_0 and P_M . Slopes for P_M increased ($P < 0.001$) from negative to zero values, indicating an adverse affect of cold on young plants, and it seems that P_0 responded similarly. However, the water content of P_0 was considerably lower than P_M as is shown by the mean values associated with each

slope. Figure 7(b) presents for the various plant ages the ratio of nitrogen content (m-equiv.) to water content (g). Slopes for P_M increased ($P < 0.01$) as the plant matured and nitrogen levels in the tissues became less affected by cold. From the mean values associated with the slopes it can be seen that despite large changes in water content with both phosphorus and age, the association of water with nitrogen was remarkably constant. This was not the case with phosphorus, which changed with presence or absence of phosphorus.

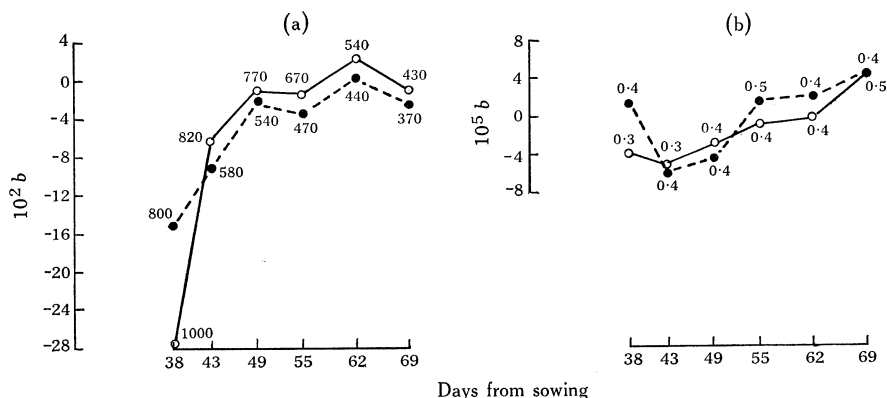


Fig. 7.—Linear cold coefficients for (a) the regressions of relative water content (as a percentage of dry weight) and (b) the ratio of nitrogen content (m-equiv.) to water content (g) per degree-hour of cold on age. Changes in slope of P_M (○) and P_0 (●) are contrasted. Mean values are given for each slope.

(vii) Linear Coefficients

Linear responses to phosphorus always increased with age, although for most variates the age values were not significantly different from zero. However, they were significant for logarithm of phosphorus content (mg) in tops ($P < 0.01$) and roots ($P < 0.01$). The linear coefficient for the logarithm of nitrogen content (mg) in tops reached significance for ages 62 and 69 ($P < 0.05$ and $P < 0.01$ respectively), as did those for whole plant, indicating that the linear coefficient for phosphorus for roots supplemented that for tops. The linear response for the logarithm of dry weight of tops also reached significance for the oldest age ($P < 0.05$).

The linear responses to added phosphorus were not affected by cold at any age.

(c) Sequence Classification

Tables 1 and 2 present group means resulting from the classification of the sets of continuous variates (seven in case 1 and four in case 2) by Burr's (1970) fusion strategy. Table 1 presents means for four groups defined for case 1, and Table 2 means for six groups defined for case 2.

Table 1 shows successive increases in fresh and dry weight for the states A–D, with relatively minor changes in nitrogen and phosphorus percentages. Figure 8 presents the TRANSMAT classification for the basic data in Table 1, displayed at the six-group level, with the successive hierarchical fusions shown above, as the levels T_1 – T_5 .

The level P_1 is anomalous to the classification so will be considered separately to the other levels. P_0 elements of all four ages fell into group I, where the samples passed rapidly into the lowest weight state A and remained there, even if they began in a higher state. Absence of phosphorus thus completely overrode age difference in the growth of the plants.

TABLE 1

MULTICLAS GROUPS FOR A SET OF SEVEN CONTINUOUS WEIGHT AND PERCENTAGE VARIATES

Variate	A	B	C	D
Fresh weight of tops	5.29	13.30	48.72	83.56
Dry weight of tops	0.87	1.50	6.23	15.58
Dry weight of roots	0.37	0.42	1.36	3.25
Nitrogen content of tops (%)	2.78	3.96	3.96	3.32
Nitrogen content of roots (%)	3.09	3.51	2.98	3.14
Phosphorus content of tops (%)	0.11	0.45	0.32	0.20
Phosphorus content of roots (%)	0.17	0.50	0.28	0.23

For the levels of P_2 – P_4 , this situation was completely reversed in groups II, III, IV, and VI, which respectively represent the four successive ages at these levels. At higher phosphorus levels, therefore, age completely overrode phosphorus. Moreover, the successive ages represented successive increase in growth. Group II (age 1) began in the second lowest state B and remained there; group III (age 2) began in B, but achieved the next higher state C at the last treatment; group IV (age 3) began in C but in the last two treatments climbed to the highest state D; and group VI (age 4) passed more rapidly into state D.

TABLE 2

MULTICLAS GROUPS FOR A SET OF FOUR CONTINUOUS NUTRIENT VARIATES

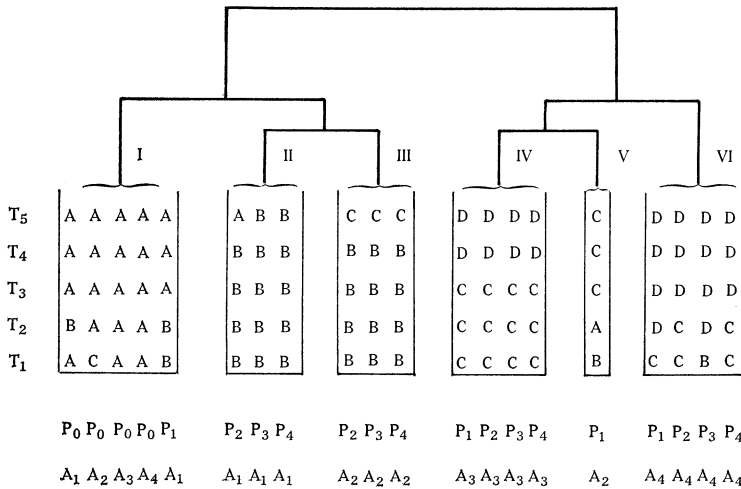
Variate	A	B	C	D	E	F
Total nitrogen in tops	1.72	5.68	18.96	34.87	45.38	79.61
Total nitrogen in roots	0.63	1.69	3.50	6.67	7.20	17.85
Total phosphorus in tops	0.11	0.66	1.70	1.76	3.45	4.99
Total phosphorus in roots	0.04	0.25	0.36	0.43	0.65	1.33

It remains to consider the highly anomalous behaviour of P_1 . This showed an intricate interaction with age. At age 1 it behaved essentially as a P_0 sample, passing rapidly into the lowest weight state A and remaining there; at age 2 it behaved completely anomalously, achieving state C by a route which isolates it in the classification (as group V); at ages 3 and 4 it behaved in exactly the same manner as the high-phosphorus levels. The entire system, in fact, acted basically as a two-level system; the absence of phosphorus overrode age, whereas age overrode phosphorus at high levels. P_1 , the "borderline" phosphorus level, moved from one level of the system to the other as age increased.

Reference to Table 2 shows that states A–F for case 2 represent successive increases in all four mineral contents. The TRANSMAT classification, this time to the five-group level, is also shown in Figure 8 (lower half). Group I was again composed of those elements without phosphorus, i.e. the P_0 treatments at all ages; again, the

plants began and ended in the low-mineral state A, and absence of phosphorus overrode age. Groups II and III once more showed the age-phosphorus reversal of dominance; group II, whose members began in A and rose to B at the third or fourth treatment, was entirely age 1; group III, whose members passed more rapidly

Case 1



Case 2

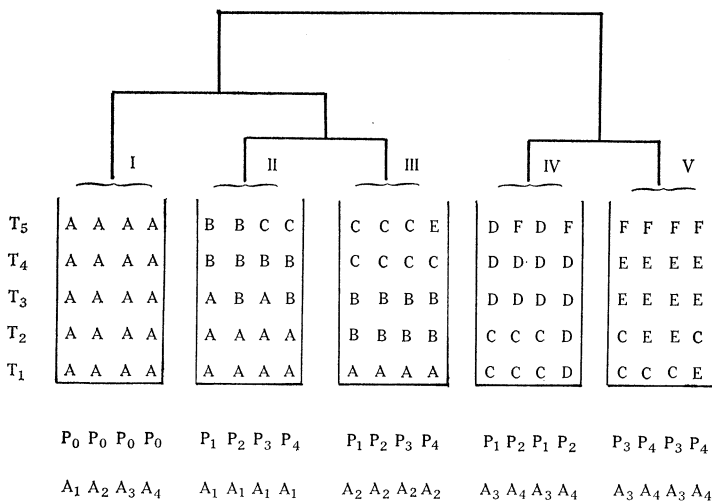


Fig. 8.—TRANSMAT classification for the states A–D in the set of seven continuous weight and percentage variates (case 1) and for the states A–E of the set of four continuous nutrient variates (case 2).

into B and all ultimately attained at least C, was entirely age 2. In groups IV and V, however, the age-phosphorus dominance once more was reversed. Group IV, whose members began in at least C and attained at least D, was P₁+P₂ for ages 3 and 4;

group V, whose members began in C, passed rapidly into E, and all attained F, was $P_3 + P_4$ for ages 3 and 4. The system was now more complex: at P_0 , the phosphorus deficiency overrode age; at ages 1 and 2, given the addition of any phosphorus at all, age overrode phosphorus at all levels; at ages 3 and 4, phosphorus again overrode age.

V. DISCUSSION

(a) *Growth and Nutrient Content*

Several aspects of the response to cold at differing ages appear to be in apposition, but this is not so. On the one hand, linear cold coefficients indicated that it was in young plants that cold impaired development. On the other hand, visual observation indicated that injury due to frosting was more apparent in older plants than in younger. Young P_0 plants were able to resist unusually severe extremes of cold. This would seem to suggest a two-state system with the controlling factors at variance.

In the first case it should be noted that the cold effects on growth and nitrogen fixation were due primarily to P_0 . These operated early in development when the plants were only initiating nodulation and nitrogen fixation, the P_0 plants being small and poorly nodulated. In plants receiving phosphorus nodulation occurred much earlier, probably about days 18–21 when there was no cold stress.

In the second case, the visually observed resistance of young P_0 plants to frost injury was not a feature of active growth but of arrested development. It gave the impression of a physiologically younger condition. P_0 plants appeared to be able to enter more easily a state akin to anabiosis. The impairment to development and nitrogen fixation by cold in young P_0 plants confirms the visually observed arrest to development by cold stress.

The two systems operating, then, were the effects of cold on early development, and the capacity of young, phosphorus-deficient plants to tolerate extremes of cold when not developing.

The interplay between phosphorus and age noted in the sequence classification can be summarized in the following three, essentially qualitative, statements:

- (1) In the absence of phosphorus, plants were cold-sensitive in the earlier stages of growth (shown by the occasional fall from states B or C to state A in case 1, group I); growth was virtually suppressed (no plant again rose out of state A); but the later stages were relatively cold-tolerant (there has been no necessity to define a state still lower than A).
- (2) In the presence of phosphorus, at no age was growth completely suppressed. However, it was still true that young plants were more sensitive to cold than were older plants, in that their growth was reduced (in both cases 1 and 2, the older plants passed more rapidly into the higher states).
- (3) The beneficial effects of added phosphorus were not eliminated by cold treatment (shown in case 2 by the difference between groups IV and V).

In mature plants nitrogen accretion was more active than change in dry weight, and the effects of cold upon it were minimal. Comparisons for phosphorus and nitrogen suggest that phosphorus played a more significant role in maintaining dry weight increase than did the nitrogen fixed, because dry weight paralleled phosphorus

and not nitrogen at all levels of addition. This effect of phosphorus was enhanced with cold and age. The increases of nitrogen noted by Andrew and Robins (1969) at higher levels of phosphorus are thus seen to apply even more markedly under cold stress. They constitute a useful agronomic feature.

The visual observation that frost injury was greater in older, phosphorus-treated plants may thus be explained. Even over the wide range of phosphorus treatment applied, higher succulence accompanied higher tissue nitrogen levels, indicating that this was a phase of active development. The softer tissue that would thus develop would be more susceptible to frosting, and to anthocyanin pigmentation under chilling, as normal pathways of metabolism were then disturbed.

Thus the physiological state best adjusted to tolerance of frosting is that which has become arrested in a juvenile phase of development. But the state most susceptible to frost injury is the actively growing post-juvenile phase. Until frosting occurred this latter phase was relatively tolerant of the type of chilling imposed here. In this regard, cold tolerance has properties analagous to those of water stress as described for wheat by Milthorpe (1950) and for plants generally by Gates (1968).

(b) Pasture Potential under Chilling

Lotononis bainesii withstood a surprising amount of cold stress, and productivity and nitrogen fixation were clearly favoured by high levels of phosphorus fertilization. This was most successful with mature plants. Such plants under field conditions would constitute useful fodder because of their high nitrogen and phosphorus status. Their tissue, moreover, was not destroyed by cold, even in the frosting range, as is often the case with many tropical legumes. No doubt if *L. bainesii* was provided with a reasonable plant cover sensitivity to frost would be further reduced, for it was the exposed laterals that frosted in this experiment, the central leaves being mutually protected.

The pot temperature was here slowly reduced from 25°C to a level approximating that of the field during frosting. Figure 9 presents data kindly supplied by Messrs. G. B. Stirk and R. E. Prebble for grass minima and soil temperature curves at 10- and 20-cm depths at 9 a.m. (when the minimum at 15 cm occurs), and also soil temperature in the pots at 10 cm. Clearly the pot temperatures did not prevent active nitrogen fixation during cold stress, nor should it be expected that field performance would be very different. This feature is highly desirable for a pasture species in subtropical areas.

(c) Nature of the Response

In these studies of interaction both univariate and multivariate approaches yielded complementary, useful information. The response to chilling was multiple rather than discrete: a complex of age, nutrient, and temperature, for the normal pattern of growth response adapted constantly with time to treatment interaction.

Single levels, then, were not as important as higher levels of multivariate interaction in appreciating the effects of chilling. As often occurs with complex biological systems, the interpretative procedures had to be sensitive to numerous interacting changes if the net response was to be appreciated. In appraising this, both univariate and sequence approaches were sensitive to differing levels of integra-

tion, as shown also by Gates *et al.* (1970) for Townsville stylo in response to chilling and drought conditions.

We may describe the differences between the two approaches as follows: first, the sequential analyses provided a qualitative, simply expressed, summary of the overall pattern of results; but they did not provide the detailed account of the magnitude of the effects achieved by the univariate analyses. Secondly, the sequential analyses could be obtained by the use of standard programs very rapidly. Given access to an appropriate computer terminal, the entire set of sequential analyses could be carried out within 1–2 hr; in contrast, the classical statistical analyses required much intricate and extremely time-consuming computation. The choice of approach is thus determined by the immediate practical needs.

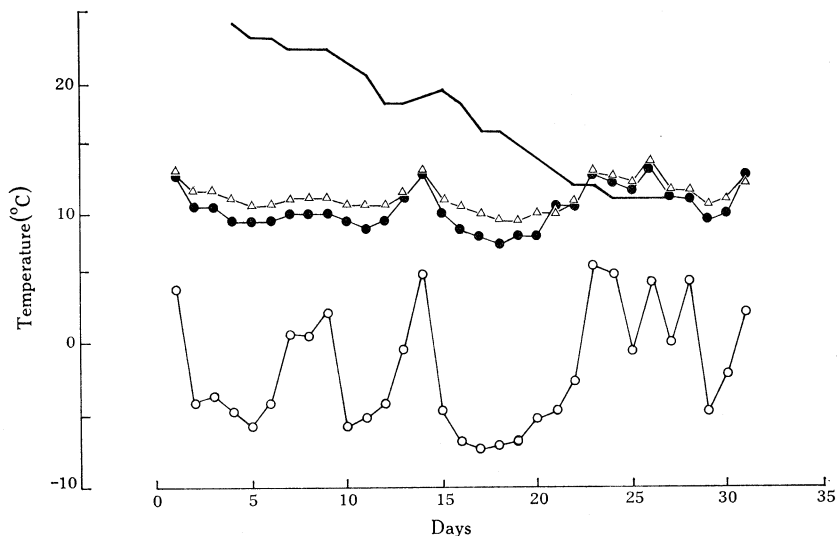


Fig. 9.—Grass minima (○) and soil temperatures at 9 a.m. at 20 cm (△) and 10 cm (●) depth during a period of frosting in the field (at Samford, Brisbane, July 1961) and the minimum soil temperatures at a depth of 10 cm in the pot (—) as cold stress of a similar intensity to the field was imposed. The temperature curve for water in the pot immersion tank coincided with the soil measurement at 15 cm depth.

The results of this experiment suggest the distinct advantage of an experimental approach that observes developmental response over as wide a range of interaction as possible, rather than by varying single factors at a time in an otherwise constant situation. This is especially necessary if data are to be obtained under controlled environment that have relevance to the variable milieu of the field.

VI. ACKNOWLEDGMENTS

We are indebted to Dr. D. O. Norris for provision of inoculum, to Mrs. Marie Smith for primary statistical analysis, and to Dr. W. W. Bryan for helpful discussion. The technical assistance of Mrs. Navarra Gothard and Mr. G. Simpson in the conduct of the experiment and its chemical analysis and the help of Mr. L. Cowper in the maintenance of environmental facilities are gratefully acknowledged.

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