PHYSICAL ENVIRONMENT AND SYMBIOTIC NITROGEN FIXATION

I. THE EFFECT OF ROOT TEMPERATURE ON RECENTLY NODULATED TRIFOLIUM SUBTERRANEUM L. PLANTS

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Summary

The effect of root temperatures (5–30°C) on the growth and symbiotic nitrogen fixation by nodulated plants of four varieties of *Trifolium subterraneum* L., inoculated with each of two strains of *Rhizobium trifolii*, was examined.

Symbiotic nitrogen fixation was reduced at temperatures below 22°C, and at 5°C was only 10–17% of that achieved at 18°C. At 30°C, there was a marked reduction in nitrogen fixation by some host–strain combinations.

Some combinations of host variety and bacterial strain achieved levels of growth similar to that made by plants receiving adequate combined nitrogen, while the others were consistently less effective.

For both dry weight and nitrogen fixation, there was a significant interaction between the varieties and bacterial strains throughout the temperature range and, above 18°C, the degree of this interaction was influenced by the root temperature.

During the 20-day temperature treatment, the increase in dry weight and symbiotically fixed nitrogen was exponential, indicating that any adverse effect of root temperature was directed towards processes controlling the rate of nitrogen fixation and plant growth in any host–strain combination. The principal differences between the treatments was reflected by the slopes of the regression lines.

There was a marked effect of root temperature on further nodule production by these plants. At higher root temperatures nodule production was determined by the combination of host and strain, and appeared to be independent of the amount of symbiotic nitrogen fixation.

I. INTRODUCTION

In studying the effect of the physical environment on the growth of legumes, a relevant question is whether plants dependent upon symbiotic nitrogen fixation and plants receiving adequate nitrogen in a combined form differ in their response to changes in the physical environment. If there are differences, it is important to ascertain the environmental factors responsible, and to determine which aspect of the formation and function of the nodules is affected. Furthermore, the influence of the strain of root nodule bacteria on the response of a particular species, or variety, to the environmental conditions should be known.

Previous investigations have shown that both low and moderately high temperatures, imposed on either the whole plant or the root system only, adversely influence symbiotic nitrogen fixation. Unfortunately, these studies were

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designed so that the differentiation of environmental effects on nodule formation and nodule function was not possible (Mes 1959; Meyer and Anderson 1959; Joffe, Weyer, and Saubert 1961) or adequate controls receiving combined nitrogen were omitted (Jones and Tisdale 1921; Smith and P. B. Gibson 1960). Although these investigations provided interesting information, critical examination of the problems outlined above was not possible. In preliminary reports, A. H. Gibson (1961) and Pate (1961) showed that the strain of root nodule bacteria forming the symbiosis may influence the response of the plants to the temperature stress, in terms of nitrogen fixation and nodule formation respectively.

The experiments reported in this paper are part of a general programme in which the effect of the physical environment on nodule formation and nodule function is being investigated. The present study was designed to examine the effect of root temperatures on growth and nitrogen fixation by nodulated plants of four *Trifolium subterraneum* L. varieties. Plants receiving combined nitrogen were used as a basis for comparison. The interaction between the host varieties, bacterial strains, and the temperature conditions was examined, and observations were made on the effect of root temperature on nodule formation.

II. Materials and Methods

(*a*) *Host Materials*

Certified commercial seed of the *T. subterraneum* varieties Dwalganup, Mount Barker, Tallarook, and Yarloop was obtained from F. H. Brunning Pty. Ltd., Melbourne. Within each experiment the seeds were selected visually for uniformity of size, both within and between varieties.

(*b*) *Bacterial Strains*

The strains used were *Rhizobium trifolii* TA1 (ex. *T. repens* L.) and *Rh. trifolii* NA30 (ex. *T. subterraneum*). Three-day-old cultures of the bacteria grown on yeast extract mannitol agar were used to prepare the inocula for each experiment.

(*c*) *Seed Germination*

The seeds were placed in a desiccator overnight, surface-sterilized by immersion in concentrated sulphuric acid for 15–20 min, thoroughly rinsed in sterile distilled water, and spread on 1% agar in Petri dishes. Following a 48-hr period of cold treatment at 4°C to break dormancy (Loftus Hills 1944), the dishes were transferred to 25°C for seed germination, and inverted to provide seedlings with straight radicles.

(*d*) *Plant Culture*

A modification of Thornton's tube-culture technique (Thornton 1930) was used for growing the plants. 13 ml of seedling nutrient agar (Jensen 1942), supplemented with the addition of 1·0 ml/l of a trace element solution containing boron (0·50 g/l), manganese (0·50 g/l), zinc (0·050 g/l), copper (0·020 g/l), and molybdenum (0·050 g/l), and with the pH adjusted to 6·7, were added to 6 by $\frac{3}{4}$ in.

test tubes. An aluminium foil cap (0·0012 in. thickness) of $1\frac{3}{4}$ in. diameter was held in place on the top of the tube by a rubber ring ($\frac{3}{8}$ in. internal diameter, $\frac{1}{2}$ in. external diameter). A small hole in the foil was plugged with cotton wool. After autoclaving, the tubes were sloped so that the agar extended to the top of the tube.

After 30 hr at 25°C, seedlings with radicles $\frac{3}{8}$–$\frac{5}{8}$ in. long were planted through a freshly made hole in the aluminium cap, so that the radicle grew on top of the agar slope and the cotyledons were outside the tube. This hole was just large enough to accept the radicle, and the foil then maintained a seal around the root as it expanded. The sown tubes were placed beneath a bank of Philips 4-ft 40-W white "Reflectalite" fluorescent lamps (light intensity 900 f.c., photoperiod 16 hr) in a constant-temperature room held at 20°C. A specimen tube (2 by 1 in.), with a moist cotton-wool pad in the bottom, was inverted over each tube in order to maintain a humid atmosphere around the cotyledons. These tubes were removed 3 days after planting, and where necessary, the cotyledons and petioles freed from the seed coat. A uniform population of seedlings was selected. A sterilized Cornwall Luer-Lok syringe with automatic-filling attachment and 4-in. cannula was used to deliver 22 ml of sterile seedling nutrient solution (S.N.S., $\frac{1}{4}$-strength of the nutrient solution used to prepare the agar plus 1·0 ml/l trace element solution, pH 6·7) to each tube through the plugged hole.

As the aim of the experiments was to examine nitrogen fixation and growth of nodulated plants, it was important to have all host–strain treatments at approximately the same level of nodulation when the temperature treatments commenced. To achieve this, it was necessary to inoculate the plants with strain NA30 3 days after planting, or with strain TA1 4 days after planting. The tubes were inoculated by suspending two 3-day-old cultures of bacteria in each 5 l. of S.N.S. used for watering. Where required, uninoculated plants were included for use as combined nitrogen controls; these controls provided a standard with which to compare the performance of nodulated plants.

The seedlings were kept under the above conditions in the 20°C room for 14 days after planting by which time pink nodules had formed on all inoculated plants. A uniform population of plants was again selected and divided into five groups—one group was taken for pretreatment harvest and the other groups allocated to temperature treatments in a controlled-environment cabinet. Ammonium nitrate was added to the uninoculated controls (3 mg nitrogen/tube) and all plants transferred to the root temperature treatments. Within a treatment, the plants were distributed in randomized blocks. The treatments were maintained for 20 days and further additions of sterile S.N.S. were made when necessary. Additional ammonium nitrate was added to the nitrogen controls 7 and 14 days after the treatments commenced.

This modification of the tube-culture technique enabled the application of different shoot and root temperature regimes. Furthermore, growth was up to six times greater than when plants were grown entirely within the tubes in the glasshouse.

(e) *Environmental Conditions*

The controlled-environment cabinet consisted of four water-baths and a bank of lights controlled by a time switch. The temperature in the baths was maintained by circulating brine at 2°C through coils situated adjacent to Braun Thermomix II heater-stirrers, the rate of flow of the brine being regulated according to the root temperature required. The tubes were suspended through holes in a ¼-in. compressed asbestos cover over each bath. The temperature variation within the tubes, both in time and position in the bath, was ±0·3°C. The bank of lights 14–16 in. above the plant culture tubes, contained eight Philips T.L.F. 80-W/33 "Reflectalite" fluorescent tubes and four Mazda 2-ft 75-W incandescent tubes. The light intensity was 950–1150 f.c., measured with an "E.E.L." photometer. The unit was situated in a 10°C constant-temperature room, and air from the room was circulated continuously through the unit. The ambient temperature 1 in. above the bath covers was 22–23°C during the 16 hr light period and 10°C during the 8 hr dark period.

(f) *Nitrogen Determinations*

Groups of three or four plants were pooled for nitrogen determinations. Total plant nitrogen was determined by Kjeldahl digestion, distillation, and titration.

III. Results

(a) *Effect of Low Root Temperatures*

Nodulated and combined nitrogen control plants of the varieties Dwalganup, Mount Barker, Tallarook, and Yarloop were grown at root temperatures of 5, 9·5, 14, and 18°C. The bacterial strains used were NA30 and TA1.

All varieties showed a reduction in growth at root temperatures below 18°C (Fig. 1). Over the 20-day temperature treatment period, the increase in the mean dry weight of plants grown with a root temperature of 5°C was 25–33% of that for the same host–strain treatments grown with a root temperature of 18°C. Within particular temperature levels, there were up to 2½-fold differences between the increments in mean dry weight of the various host–strain treatments (e.g. Yarloop/TA1 compared with Dwalganup/TA1 or Mount Barker/NA30) at all four root temperatures.

In the statistical analysis of the plant dry weights (log transformed) the nitrogen control treatment was included as a strain. The analysis showed significant variety×strain and temperature×strain interactions, while within each variety, strain effects were significant (Table 1).

The principal factor contributing to the variety×strain interaction was the degree to which the dry weight of the nitrogen control treatments exceeded that of the inoculated treatments (Fig. 1), although differences in the behaviour of the two bacterial strains added to the interaction.

The significant temperature×strain interaction was due largely to a smaller difference between the nitrogen control and inoculated treatments at 5°C than at higher root temperatures. However, the interpretation of this interaction is influenced by the more significant interaction between variety and strain.

The amount of nitrogen fixed at 5°C was only 10–17% of that fixed at 18°C, depending on the combination of host and strain (Table 2). Within the temperature treatments, the amount of nitrogen fixed by some host–strain combinations was four times greater than that fixed by others. At higher temperatures, the relative differences were smaller, but even at 18°C, twice the amount of nitrogen was fixed by Yarloop/TA1, Yarloop/NA30, and Tallarook/TA1 than by Dwalganup/TA1 and Mount Barker/NA30.

TABLE 1

ANALYSES OF VARIANCE ON THE TOTAL PLANT DRY WEIGHT (LOG. TRANSFORMED) AND TOTAL PLANT NITROGEN FOR PLANTS GROWN AT ROOT TEMPERATURES OF 5, 9·5, 14, AND 18°C

The nitrogen control treatment was included as a strain in the dry weight analysis. There were 10 replicates per treatment

Source of Variation	Plant Dry Weight		Total Plant Nitrogen	
	Degrees of Freedom	Error Mean Square	Degrees of Freedom	Error Mean Square
Variety (V)	3	1·35319**	3	74·130**
Strain (S)	2	0·88790**	1	13·844**
Temperature (T)	3	3·90033**	3	194·806**
V×S	6	0·09228**	3	7·065**
V×T	9	0·00653	9	6·852**
S×T	6	0·02783**	3	0·570
V×S×T	18	0·00383	9	0·600
Residual	432	0·00610	64	0·512
Strain effects separately for each variety				
Mount Barker	2	0·5286**	1	2·297*
Tallarook	2	0·2646**	1	12·974**
Yarloop	2	0·1643**	1	16·673**
Dwalganup	2	0·2072**	1	3·096*

* $P<0·05$. ** $P<0·01$.

For the analysis of the total plant nitrogen data, the nitrogen control treatments were excluded because percentage nitrogen values of 4·5–5·0 in these treatments indicated that uptake of combined nitrogen was in excess of requirements. Analysis of the data for the two strains showed significant variety×strain and variety×temperature interactions (Table 1). Further analysis of the data indicated that the strain effect within each variety was significant. Strain TA1 was more effective than strain NA30 on all varities except Dwalganup, on which strain NA30 was better (total column, Table 2). In addition, there were marked differences between varieties when each strain was examined separately. The significance of the variety×temperature interaction was due to the increase in differences between the total nitrogen content of Yarloop and Tallarook, and Tallarook and the other varieties, as the root temperature increased from 5 to 18°C

(total rows, Table 2). The failure of this interaction to reach significance in the analysis of the dry weights may be explained by the greater differences between the nitrogen controls and inoculated treatments within the varieties Mount Barker and Dwalganup, than are found between these treatments in Tallarook and Yarloop.

At a root temperature of 5°C, the percentage nitrogen in the plants was low, but increased with an increase in root temperature to 14°C (Table 2). Although nitrogen fixation and plant growth was greater at 18°C than 14°C, there was no further increase in the nitrogen level in the plants. Similarly, differences in the amount of nitrogen fixed by the eight host–strain combinations at any one root temperature above 5°C were not reflected in differences in the percentage nitrogen in the plants.

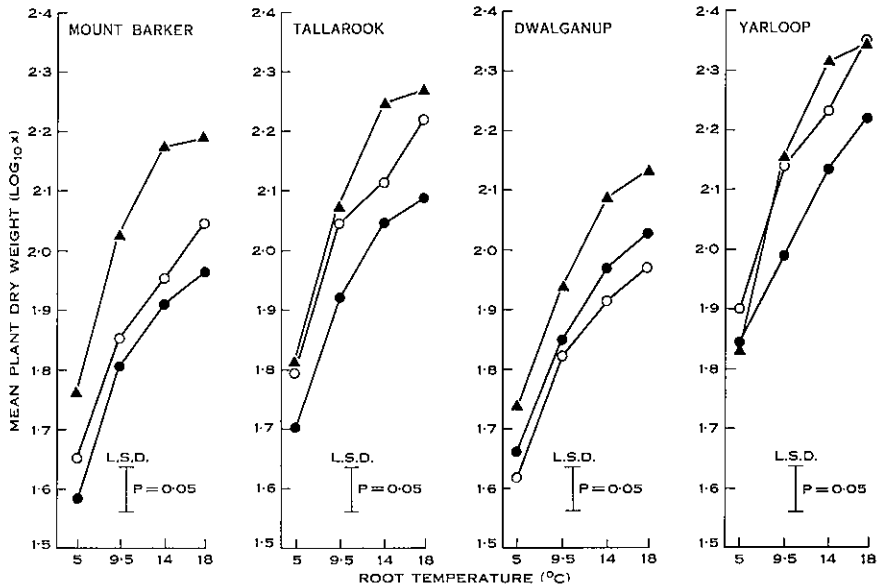

Fig. 1.—Effect of low root temperatures (5–18°C) on plant dry weight for four subterranean clover varieties nodulated by *Rh. trifolii* strains TA1(○) and NA30 (●). ▲Nitrogen control plants.

(*b*) *Effect of High Root Temperatures*

Nodulated and nitrogen control plants of the varieties Mount Barker, Tallarook, and Yarloop were grown at root temperatures of 18, 22, 26, and 30°C. The bacterial strains used were NA30 and TA1.

The effect of higher root temperatures (26 and 30°C) on the growth of plants receiving combined nitrogen varied with the varieties, while the effect on the growth of nodulated plants varied with the combination of host and strain (Fig. 2). Within a particular temperature treatment, there were considerable differences between the host–strain treatments, but these differences were greatest at the 30°C root temperature level. The optimum root temperature under these conditions appeared to lie between 22 and 26°C.

TABLE 2

TOTAL NITROGEN FIXED DURING TREATMENT AND PERCENTAGE NITROGEN LEVELS FOR NODULATED PLANTS GROWN FOR 20 DAYS WITH ROOT TEMPERATURES IN THE RANGE 5–18°C

10 replicates per treatment

Variety	Bacterial Strain	Total Nitrogen at Pretreatment Harvest* (mg/plant)	Total Nitrogen (mg/plant) Fixed at Root Temperatures:					Pretreatment Nitrogen Level (%)	Nitrogen Levels (%) at Root Temperatures			
			5°C	9·5°C	14°C	18°C	Total		5°C	9·5°C	14°C	18°C
Mount Barker	NA30	0·52	0·24	1·27	2·19	2·65	6·35	2·84	1·92	2·72	3·31	3·42
	TA1	0·52	0·48	1·33	2·60	3·31	7·72	2·79	2·13	2·54	3·46	3·39
	Total	—	0·72	2·60	4·79	5·98	14·07					
Tallarook	NA30	0·58	0·52	1·93	3·59	3·93	9·97	2·58	2·09	2·97	3·66	3·63
	TA1	0·68	0·96	2·79	3·99	5·04	12·78	2·83	2·58	3·08	3·56	3·45
	Total	—	1·48	4·72	7·58	8·97	22·75					
Yarloop	NA30	0·73	0·61	2·14	4·20	5·36	12·31	2·58	1·84	2·80	3·52	3·62
	TA1	0·69	1·01	3·05	5·19	6·88	16·13	2·72	2·08	2·68	3·36	3·35
	Total	—	1·62	5·15	9·39	12·24	28·44					
Dwalganup	NA30	0·58	0·47	1·40	2·71	3·14	7·72	3·37	2·18	2·80	3·51	3·47
	TA1	0·55	0·39	1·21	2·32	2·42	6·34	3·26	2·20	2·62	3·44	3·17
	Total	—	0·86	2·61	5·03	5·56	14·06					

* Total nitrogen (mg/plant) in uninoculated seedlings after pretreatment was: Mount Barker, 0·50; Tallarook, 0·55; Yarloop, 0·62; Dwalganup, 0·54.

In the statistical analysis of the plant dry weights (log transformed), the nitrogen control treatment was included as a strain. Analysis of the dry weight data showed a significant second-order interaction between strain, variety, and root temperature (Table 3; Fig. 2).

Analysis of the total plant nitrogen data for the six host–strain combinations (excluding nitrogen controls), also showed a significant second-order interaction between variety, strain, and temperature (Table 3). Within the temperature treatments, the variety×strain interaction was significant at 26 and at 30°C. At

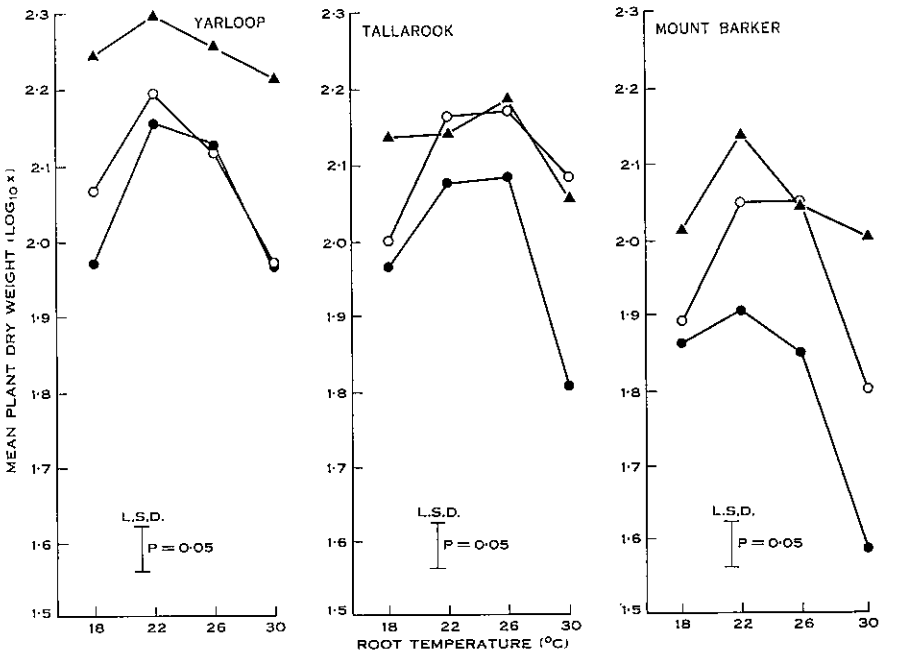

Fig. 2.—Effect of high root temperatures (18–30°C) on plant dry weight for three subterranean clover varieties nodulated by *Rh. trifolii* strains TA 1 (○) and NA30 (●). ▲Nitrogen control plants.

these temperatures, the varieties Tallarook and Mount Barker fixed more atmospheric nitrogen when inoculated with strain TA1 than when inoculated with NA30; both strains achieved a similar level of fixation with the variety Yarloop (Table 4).

Examination of the percentage nitrogen data (Table 4) showed that percentage nitrogen was unaffected up to root temperatures of 26°C. At 30°C, there was a decline in the values for all treatments, the extent of which was influenced by the combination of host and strain.

(c) *Rate of Nitrogen Fixation and Plant Growth*

The variety Mount Barker, nodulated by strains TA1 and NA30, was grown at root temperatures of 5, 14, 22, and 30°C. Twelve replicates of each treatment

were harvested 6, 10, 14, 17, and 20 days after the temperature treatments commenced.

After transformation of the dry weight data to logarithms, the regression between these values and time did not show a significant departure from linearity (Fig. 3). Further analysis indicated that the slopes of the lines differed significantly.

TABLE 3

ANALYSES OF VARIANCE ON THE TOTAL PLANT DRY WEIGHT (LOG TRANSFORMED) AND TOTAL PLANT NITROGEN FOR PLANTS GROWN AT ROOT TEMPERATURES OF 18, 22, 26, AND 30°C

The nitrogen control treatment was included as a strain in the dry weight analysis. There were 11 replicates per treatment

Source of Variation	Plant Dry Weight		Total Plant Nitrogen	
	Degrees of Freedom	Error Mean Square	Degrees of Freedom	Error Mean Square
Variety (V)	2	1·44026**	2	56·163**
Strain (S)	2	1·28140**	1	41·111**
Temperature (T)	3	0·67976**	3	56·558**
V×S	4	0·08218**	2	2·719*
V×T	6	0·02033*	6	1·882
S×T	6	0·05775**	3	1·349
V×S×T	12	0·02939**	6	2·052*
Residual	311	0·00783	48	0·838

* $P<0·05$. ** $P<0·01$.

The total nitrogen data was treated similarly except that the values were transformed to $\log(x+1)$ to account for mean total nitrogen values of less than 1 mg per plant. The b values from the expression $y = a+bx$ were:

Root Temperature (°C)	Strain NA30	Strain TA1
5	0·0087	0·0067
14	0·0287	0·0288
22	0·0276	0·0310
30	0·0049	0·0293

S.E. of difference between b values = 0·0024

A similar experiment with the variety Yarloop confirmed the result that the increase in dry weight and total nitrogen was approximately exponential.

(*d*) *Nodule Formation during Temperature Treatment*

For two experiments, the number of nodule meristems was recorded prior to, and at the completion of, the 20-day temperature treatment.

The effect of root temperature on nodule production by plants which were nodulated at the commencement of the temperature treatment was influenced

TABLE 4

TOTAL NITROGEN FIXED DURING TREATMENT AND PERCENTAGE NITROGEN LEVELS FOR NODULATED PLANTS GROWN FOR 20 DAYS WITH ROOT TEMPERATURES IN THE RANGE 18–30°C

11 replicates per treatment

Variety	Bacterial Strain	Total Nitrogen at Pretreatment Harvest* (mg/plant)	Total Nitrogen (mg/plant) Fixed at Root Temperatures:				Pretreatment Nitrogen Level (%)	Nitrogen Levels (%) at Root Temperatures:			
			18°C	22°C	26°C	30°C		18°C	22°C	26°C	30°C
Mt. Barker	NA30	0·36	2·05	2·28	2·02	0·71	2·81	3·30	3·24	3·29	2·72
	TA1	0·40	2·17	3·31	3·40	1·52	3·17	3·32	3·28	3·35	3·00
Tallarook	NA30	0·46	2·77	3·86	3·64	1·47	3·21	3·43	3·52	3·35	2·87
	TA1	0·57	2·86	4·67	4·73	3·40	3·48	3·39	3·52	3·52	3·25
Yarloop	NA30	0·48	2·81	4·44	4·03	1·94	3·41	3·45	3·38	3·30	2·52
	TA1	0·57	3·51	4·96	3·89	2·08	3·54	3·43	3·43	3·31	2·71

* Total nitrogen (mg/plant) in uninoculated seedlings after pretreatment was: Mount Barker, 0·32; Tallarook, 0·35; Yarloop, 0·40.

by the combination of host variety and bacterial strain (Fig. 4). The combination Yarloop/NA30 showed a definite increase in the number of nodules formed as the root temperature rose from 18 to 30°C, whereas nodule formation by the same variety, inoculated with strain TA1, was relatively unaffected by an increase in root temperature. With the strain NA30 and the variety Dwalganup, further nodule production was inhibited by root temperatures above 22°C, whereas the varieties Tallarook and Mount Barker inoculated with NA30 showed an increase in nodule production above this temperature. With strain TA1, the effect of increasing the root temperature was different for each of the four varieties.

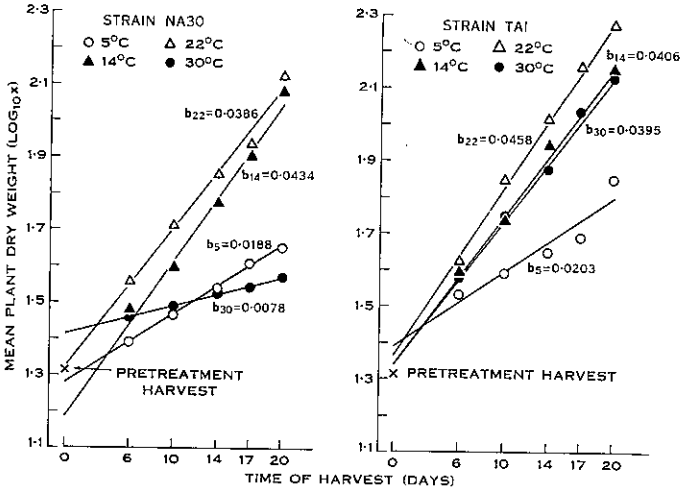

Fig. 3.—Increase in dry weight for Mount Barker plants nodulated by strains NA30 and TA1 at each of four root temperatures (5, 14, 22, and 30°C). Regression lines are fitted according to the equation $y = a + bx$. b_t indicates the slope of the regression line. S.E. of difference between slopes = 0·0031.

In the low temperature range (5–18°C), all host×strain combinations showed an increase in further nodule production as the root temperature rose. Even at 5°C new nodules were formed although the mean increase above the pretreatment level was only 1–2 nodules per plant.

IV. Discussion

(a) *General Effects of Root Temperature on Growth and Nitrogen Fixation*

The experiments reported show that root temperatures exerted a marked effect on the growth of plants receiving adequate combined nitrogen, and also on the growth of those plants dependent on the symbiotic fixation of nitrogen.

While the environmental conditions, such as photoperiod and shoot temperature, in conjunction with some of the root temperatures imposed, were different from those likely to be encountered in nature, it was necessary to adopt this procedure for the effective comparison of results. Furthermore, it is unlikely

that the general principles enunciated will be influenced to a major extent by these other conditions.

Large differences were observed in the growth of the nitrogen control plants of the four varieties. This applied to results at specific root temperatures, and also to effects observed over the range 5–30°C. At all root temperatures, the variety

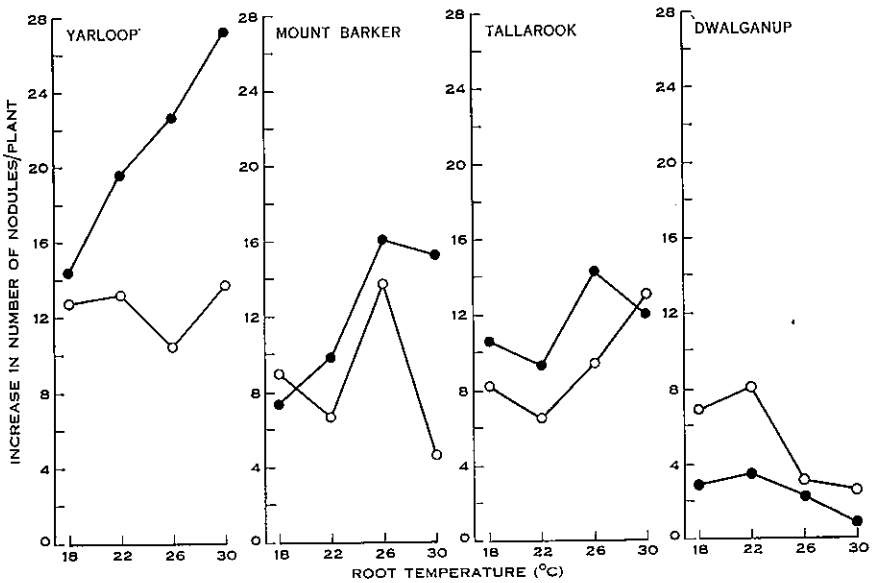

Fig. 4.—Increase in number of nodules during temperature treatment phase. ○ Strain TA1. ● Strain NA30. The number of nodules per plant after the pretreatment phase for the four subterranean clover varieties are as follows:

Variety	Strain TA1	Strain NA30
Yarloop	15	21
Mount Barker	11	11
Tallarook	17	15
Dwalganup	6	5

Yarloop achieved the highest dry weight. This variety also showed the greatest response to an increase in root temperature from 5–18°C, while in the temperature range 18–30°C, it was least sensitive to an increase in root temperature. The growth of the variety Tallarook was only slightly inferior to that of Yarloop.

The growth by nodulated plants at low root temperatures was greatly reduced when compared with that achieved by similar plants at 18°C. In relation to the growth of the nitrogen controls, the magnitude of the reduction of growth varies with the combination of host variety and bacterial strain (Fig. 1). The total plant dry weight of the varieties Tallarook and Yarloop, when inoculated with strain TA1, closely approached that of the nitrogen controls at root temperatures between 5 and 18°C. However, the growth of the remaining host × strain combinations was consistently less than that achieved by their respective nitrogen controls.

Although the amount of nitrogen fixed by all host×strain combinations was low at 5°C, there was a fourfold difference between the poorest and the best of these associations, and up to twofold differences in the amount of nitrogen fixed by the two strains in symbiosis with any selected variety were observed. Under field conditions, many well-nodulated plants die during periods of prolonged low temperature, and this can be attributed to severe nitrogen deficiency (Hely, personal communication). Under such conditions, any advantage gained by a plant through forming a more effective symbiotic association may mean the difference between survival and death of the plants. At higher root temperatures (9·5–18°C), the relative differences in amounts of nitrogen fixed by the host× bacterial strain associations are less, but at this level the absolute differences are more important. These results indicate that the careful selection of the host variety and the bacterial strain could be important in extending the period of plant growth at the beginning, and possibly at the end, of periods of lower soil temperatures.

The interaction between host variety and bacterial strain was statistically significant in both the 5–18°C and 18–30°C root temperature ranges, but in the higher range the interaction included the root temperature effect (Table 3; cf. Gibson 1961). Within the lower range, the relative order of the two strains on any one variety remained constant, whereas within the higher range there was a reversal of order with increasing temperature. For example, strain NA30 was the better strain with Dwalganup up to 18°C, while at 26 and 30°C, strain TA1 formed a more effective symbiotic association with this variety (Gibson 1961). For the current experiments, the interaction with root temperature was due to the greater reduction of symbiotic nitrogen fixation by Tallarook and Mount Barker when inoculated with strain NA30 and grown at 30°C, than when these plants were inoculated with strain TA1. In addition, both TA1 and NA30 had a similar level of effectiveness at 26°C and at 30°C on the variety Yarloop, whereas TA1 was superior at lower root temperatures. Only with the Tallarook/TA1 combination was a reasonable level of symbiotic nitrogen fixation and plant growth maintained at a root temperature of 30°C.

Nutman (1961) examined the symbiotic effectiveness of 15 varieties of subterranean clover in association with several strains of root-nodule bacteria, and concluded that the interaction between host varieties and bacterial strains was of minor importance. However, for the experiments reported herein, bacterial strains different to those examined by Nutman were used, and the finding that host variety×bacterial strain interactions can be of major importance was not necessarily at variance with Nutman's observations. Another aspect on which the present results and those of Nutman differed was the ranking of the varieties. Nutman found that Mount Barker and Yarloop were more effective in their symbiosis with the two main strains examined (SU297 and SU220), than were Dwalganup and Tallarook; in this investigation, Yarloop and Tallarook were consistently better than Mount Barker and Dwalganup. This may be further evidence in favour of strong host×strain interactions within the species. Alternatively, it may reflect differences due to the method of plant culture as Nutman's plants

were grown entirely within the test tubes, and under glasshouse environmental conditions.

There seems little doubt that some combinations of host variety and bacterial strain are able to withstand the stresses imposed by either low or high root temperatures more effectively than other associations. Consideration may profitably be given to the effect of stresses such as these and other environmental factors when testing the suitability of strains of root nodule bacteria for use in inoculants.

(*b*) *Growth Rates*

The exponential increase in dry weight, and total nitrogen, indicated that the effect of adverse root temperatures was continuous throughout the period of temperature treatment. There was no indication that the temperatures examined caused plant growth or nitrogen fixation to be affected to a greater extent with time. Similarly the results did not suggest that the plants, when exposed to the extreme conditions, received an initial set-back from which they were able to recover. Whether subjected to root temperatures of 5°C or 30°C, the adverse effects were directed towards the rates of the processes concerned with symbiotic nitrogen fixation and plant growth.

The linearity of the regression lines between dry weight, or total nitrogen, and time also indicated that the technique of plant culture was satisfactory. The implication of this result was that the differences between treatments after 34 days plant growth were still maximal and not affected by deficiencies in the medium, or by factors other than those of the physical environment.

(*c*) *Nodule Number*

Pate (1961) found an interaction between temperature and bacterial strain, for each of two species, with regard to the number of nodules formed during a whole plant–temperature treatment which commenced when the plants were inoculated. For the experiments reported herein, the temperature treatments were commenced when the plants were well nodulated, at which stage the number of nodules formed by the two strains was similar for any one variety, but considerably different between varieties (Fig. 4). Further nodule formation was influenced markedly by the host×strain combination, and the root temperature.

A comparison of the effect of root temperature on nodule production by the various host×strain combinations, with the effect of root temperature on symbiotic nitrogen fixation by these combinations, indicated that the two characters were not closely related. For example, there were considerable differences between the numbers of nodules formed by the strains NA30 and TA1 on the variety Yarloop at root temperatures 26 and 30°C, but total nitrogen fixed was similar for both host×strain combinations at each temperature (Table 4). Similarly the number of nodules formed by strain NA30 on Tallarook and Mount Barker was equal to, or greater than, the number of nodules produced by strain TA1 on this variety at 30°C root temperature, although with each variety, nitrogen fixation by plants nodulated by strain TA1 exceeded that by plants nodulated by strain NA30.

This result was in accord with the finding of Chen and Thornton (1940) that the important criterion in assessing nodule function was not the number of nodules but the volume, and longevity, of the central tissue containing bacteroids.

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