# PHYSICAL ENVIRONMENT AND SYMBIOTIC NITROGEN FIXATION

## VII.* EFFECT OF FLUCTUATING ROOT TEMPERATURE ON NITROGEN FIXATION

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*Summary*

The effect of exposing nodulated plants to daily periods of high, moderate, or low root temperatures was examined, using *Trifolium subterraneum* and three strains of *Rhizobium trifolii*. With strains whose nitrogen fixation was severely retarded by continuous exposure to high root temperatures, the results from treatments involving exposure of 4, 8, 12, and 20 hr/day to 30°C and continuous illumination were consistent with the effect being on the rate of nitrogen fixation, without any permanent impairment to the symbiotic system. With a 12 hr/day light period, a daily 12-hr exposure to 30°C during the dark period reduced total nitrogen fixation as much as exposure to 30°C during the light period. This indicated that the rate of nitrogen fixation during normal dark periods could be as high as that during periods of illumination. Similar conclusions were drawn from the same type of experiments involving daily exposure to moderate (14 and 16°C) root temperatures.

Daily exposure to 10°C root temperature markedly reduced the overall rate of nitrogen fixation. The magnitude of this effect was influenced by the shoot temperature and by the illumination treatment during exposure. The effect was as great on plants receiving mineral nitrogen as on those dependent on symbiotic nitrogen fixation.

## I. INTRODUCTION

Under natural conditions, root temperatures show considerably less fluctuation than shoot or ambient temperatures. Throughout the studies reported in this series, root temperature conditions examined have involved continuous exposure treatments. However, root temperature fluctuation may be considerable, particularly in prepared seed beds during the period when *Trifolium subterraneum* L. is establishing. Whilst various workers (Mes 1959; Pate 1962) have exposed plants to diurnal fluctuation in temperature, such studies have involved a change in temperature for the whole plant. For the study reported herein, shoot temperatures were held constant, and various measures taken to reduce the effect of the length of the light period.

The aim of the study was to determine to what extent regular exposure to unfavourable root temperatures affected nitrogen fixation, and whether any effect was transient or permanent. Nodulated plants, normally growing with a favourable root temperature, were exposed to high, moderate, or low root temperature for a fixed period each day. The principal variables were the length of daily exposure and the illumination treatment (light or dark period) during exposure.

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## II. Materials and Methods

### (a) *Biological Materials*

The host plant was *T. subterraneum* cv. Mount Barker. Three strains of *Rhizobium trifolii* Dang. were used—strain TA1, a highly effective strain with this cultivar; strain CC17, a highly effective strain up to 22°C root temperature but sensitive to higher root temperature; and strain NA30, a moderately effective strain up to 22°C root temperature but also sensitive to higher root temperatures (Gibson 1965).

### (b) *Plant Culture*

The plants were cultured with the roots growing on agar slopes within, and the shoots exposed outside, test tubes (Gibson 1963), in controlled-environment cabinets in which root and shoot temperatures were controlled independently (Gibson 1965), and with a light intensity of 2000 f.c.

The seedlings were inoculated 3 days after sowing. Uninoculated plants used as nitrogen controls received 1·0 mg nitrogen as $NH_4NO_3$ 8 days after the other plants were inoculated and 8 mg nitrogen at the commencement of the temperature treatments. During the pretreatment phase, conditions were 22°C root temperature, and 22/15°C shoot temperature based on a 16 hr/day light period.

### (c) *Experimental Treatments*

In order to minimize confounding effects of illumination on the diurnally fluctuating root temperature treatments, two complementary experimental approaches were adopted. In one, the plants were provided with continuous illumination and moved twice daily between the different root temperatures. For the second, the plants were illuminated for 12 hr/day and exposed to a non-optimal (low, moderate, or high) root temperature during the light period or the dark period. From the first approach, conclusions could be drawn regarding the effect of length of exposure to the non-optimal root temperature, while the results from the second approach indicated whether such effects were related to illumination during the period of exposure.

#### (i) *With Continuous Illumination*

Plants nodulated by strains TA1 and NA30, or provided with adequate mineral nitrogen, were exposed to either 30 or 16°C root temperature for 4, 8, 12, 16, or 20 hr/day, with the alternate root temperature being 23°C. Control treatments were held at 16, 23, and 30°C continuously. The shoot temperature was 23°C. The treatments commenced 15 days after inoculation, and continued for 14 days. There were eight replicates per treatment.

#### (ii) *With 12 hr Light per Day*

In one experiment, plants nodulated by strains TA1, NA30, or CC17, or provided with adequate mineral nitrogen, were exposed to 30°C root temperature for 12 hr/day during the light period or during the dark period. The alternate root temperature was 22°C, and the shoot temperature for all plants was 25°C. At the same time, an identical set of treatments was established involving 14°C as the suboptimal root temperature; the shoot temperature was 20°C. The treatments commenced 15 days after inoculation and continued for 10 days. There were 10 replicates per treatment.

In an experiment involving exposure to low root temperature (10°C), all plants were nodulated by strain TA1, with half of them receiving adequate mineral nitrogen at the commencement of the temperature-treatment phase. Plants were exposed to 10°C root temperature for 12 hr/day during the light period or during the dark period, with the alternate temperature being 20°C. Controls were grown at 10 and 20°C continuously. The experiment was duplicated at 10 and 20°C shoot temperature. The treatments commenced 11 days after inoculation and continued for 14 days. There were 11 replicates per treatment.

### (d) *Determination of Relative Growth Rates, Nitrogen Assimilation Rates, and Total Nitrogen*

At the commencement of the temperature treatments in all experiments, the plants within a strain treatment were ranked according to leaf size and development. They were then grouped with $n+1$ plants per group, where $n$ = number of treatments. The additional plants were taken

for pretreatment harvest. This procedure facilitated the calculation of relative growth rates ($R_W$, as mg/mg/day) and relative nitrogen assimilation rates ($R_N$, as mg N/mg N/day) (Gibson 1965).

At each harvest the material was dried at 80°C in a forced-draught oven, weighed, and the total nitrogen determined by a colorimetric method in a Technicon Auto-Analyser (Williams and Twine 1967).

## III. Results

### (a) *Effect of Higher Root Temperatures*

(i) *Continuous Illumination Treatment.*—Nitrogen assimilation by TA1 plants and nitrogen controls was not affected by exposure to 30°C root temperature for 4, 8, 12, 16, 20, or 24 hr/day [Fig. 1(*a*)]. However, there was a linear reduction in the rate of nitrogen fixation by the NA30 plants with an increase in the period of exposure to 30°C, such that $R_N$ fell from 0·129 mg N/mg N/day at 23°C to 0·048 mg N/mg N/day at 30°C. The observed total plant nitrogen values showed close agreement with values estimated by using $R_N$ calculated as follows:

$$R_N = (0{\cdot}129x + 0{\cdot}048y)/24,$$

where $x$ = hr/day at 23°C root temperature, and $y$ = hr/day at 30°C root temperature.

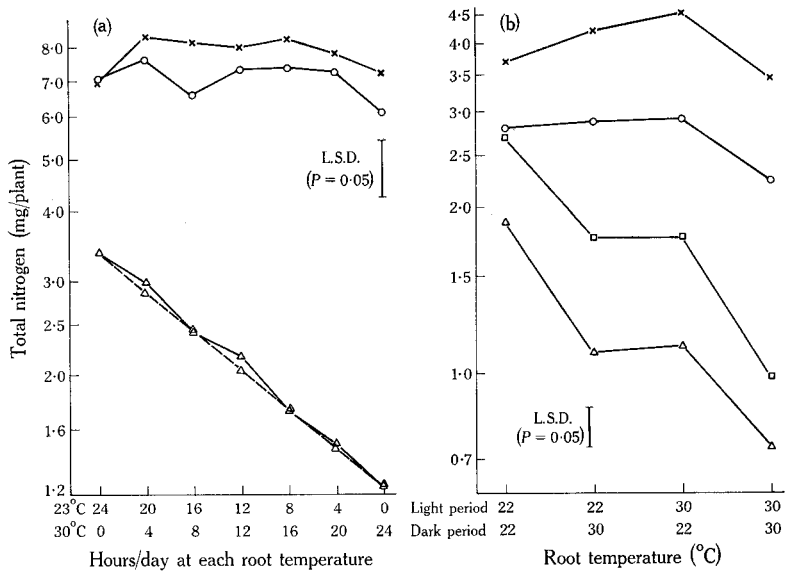


Fig. 1.—(*a*) Plants transferred from 23 to 30°C root temperature for set periods each day. The shoot temperature was 23°C and the illumination continuous. – – – – Theoretical estimate of nitrogen fixation by the NA30 plants (see text). Eight replicates per treatment. (*b*) Plants transferred from 22 to 30°C root temperature during the 12 hr/day light period or dark period. Shoot temperature 25°C. Ten replicates per treatment. × Nitrogen controls. ○ Strain TA1. △ Strain NA30. □ Strain CC17.

(ii) *Light Treatment 12 hr/day.*—Exposure of TA1 plants to 30°C root temperature for 12 hr/day did not affect nitrogen fixation [Fig. 1(*b*)], although continuous exposure to this temperature was detrimental. This latter effect, compared with the

absence of such an effect under continuous illumination, was due to a light period × root temperature interaction (Gibson, unpublished data). A 12 hr/day exposure to 30°C root temperature of NA30 and CC17 plants caused a marked reduction in nitrogen fixation. The $R_N$ of these plants approximated to the mean of the 22 and 30°C control treatments. In all cases with these strains the effect of exposure to 30°C was the same regardless of the lighting treatment during exposure.

For the nitrogen control and TA1 plants exposed to 30°C, there was an increase in the nitrogen distribution index (i.e. in the proportion of the total nitrogen increase that was distributed to the shoots) (Table 1). This effect was greater with the nitrogen controls, and was greatest with continued exposure to 30°C, even though the amount of nitrogen assimilated was reduced to that of the other treatments.

TABLE 1

TOTAL NITROGEN INCREASE IN THE SHOOTS AND ROOTS AND THE NITROGEN DISTRIBUTION INDEX FOR PLANTS EXPOSED TO CONSTANT OR DIURNALLY FLUCTUATING ROOT TEMPERATURES

Nitrogen distribution index values expressed as shoot increase as a percentage of total increase are given in parentheses. Light period was 12 hr/day. There were eight replicates per treatment

| Root Temperature (°C) | | Control Plants: Nitrogen Increase (mg/plant) in | | TA1-nodulated Plants: Nitrogen Increase (mg/plant) in | |
|---|---|---|---|---|---|
| Light Period | Dark Period | Shoots | Roots | Shoots | Roots |
| 22 | 22 | 2·12 (*75*) | 0·71 | 1·60 (*75*) | 0·53 |
| 22 | 30 | 2·73 (*80*) | 0·70 | 1·58 (*73*) | 0·59 |
| 30 | 22 | 2·87 (*78*) | 0·81 | 1·78 (*77*) | 0·52 |
| 30 | 30 | 2·10 (*82*) | 0·46 | 1·23 (*79*) | 0·33 |
| 14 | 14 | 1·41 (*65*) | 0·76 | 1·01 (*65*) | 0·58 |
| 14 | 22 | 1·92 (*72*) | 0·74 | 1·48 (*72*) | 0·59 |
| 22 | 14 | 2·09 (*74*) | 0·75 | 1·36 (*72*) | 0·54 |
| 22 | 22 | 2·10 (*77*) | 0·64 | 1·97 (*76*) | 0·62 |

### (*b*) *Effect of Moderate Root Temperatures*

(i) *Continuous Illumination Treatment.*—For plants grown with continuous illumination and exposed to 16°C root temperature for varying periods, there were two categories of response [Fig. 2(*a*)]. Nitrogen assimilation by the TA1 plants and the nitrogen controls was little affected by exposure to 16°C for 4–20 hr, although it did appear to be reduced by continuous exposure to 16°C (statistically significant for the TA1 plants, $P < 0{\cdot}05$). The NA30 plants exhibited maximum nitrogen assimilation with exposure for 16 hr/day to 16°C; with longer or shorter exposure to this temperature the rate of nitrogen assimilation was less.

(ii) *Light Treatment 12 hr/day.*—For the three strain treatments, there were three different responses to diurnal fluctuation between 14 and 22°C root temperature [Fig. 2(*b*)]. With the NA30 plants, nitrogen fixation at 14°C was similar to that at 22°C, and diurnal fluctuation had no effect. For the TA1 and CC17 plants, nitrogen

fixation by the 14°C controls was considerably less than found with the 22°C controls. However, the TA1 plants showed no effect of the lighting treatment during the exposure for 12 hr/day to 14°C, whereas the amount of nitrogen fixed by the CC17 plants was significantly less when the exposure to 14°C was given during the light period.

Within the nitrogen control and TA1 treatments, the total nitrogen increase in the roots was similar regardless of the root temperature treatment (Table 1). Exposure to 14°C root temperature reduced the total amount of nitrogen fixed, and consequently the proportion translocated to the shoots.

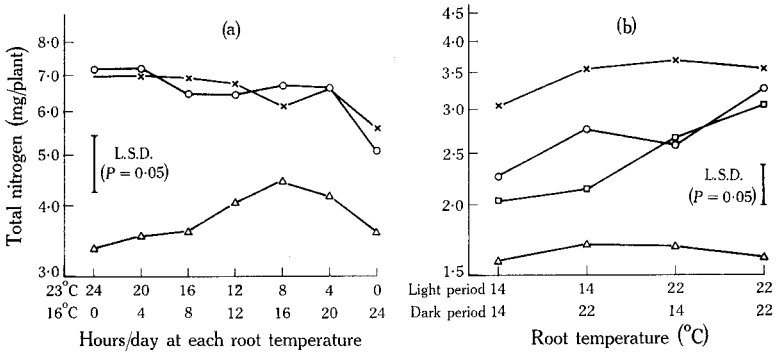


Fig. 2.—(*a*) Plants transferred from 23 to 16°C root temperature for set periods each day. The shoot temperature was 23°C and the illumination continuous. Eight replicates per treatment. (*b*) Plants transferred from 22 to 14°C root temperature during the 12 hr/day light period or dark period. Shoot temperature 20°C. Ten replicates per treatment. × Nitrogen controls. ○ Strain TA1. △ Strain NA30. □ Strain CC17.

### (*c*) *Effect of Lower Root Temperature*

The effect of exposure to 10°C root temperature on nitrogen assimilation varied with the shoot temperature, the length of exposure, the light period, root temperature, and the nitrogen source—symbiotic fixation or mineral nitrogen [Fig. 3(*a*)]. There was an overriding effect of shoot temperature such that the assimilation of both atmospheric and mineral nitrogen was greatly reduced at 10°C relative to that at 20°C. With the latter shoot temperature, exposure to 10°C root temperature for 12 hr/day reduced nitrogen fixation, with the effect being greater when the exposure was given during the light period. A similar trend was evident in plants grown with 10°C shoot temperature, but the magnitude of the effect was small.

At both shoot temperatures, exposure to 10°C root temperature affected dry weight increase [Fig. 3(*b*)] but the effects were less than those observed on nitrogen assimilation. Except for the 10°C controls, the percentage nitrogen values for the TA1 plants exceeded 3·5 [the scales on the ordinates of Figures 3(*a*) and 3(*b*) were drawn to give a direct comparison at 3·5% nitrogen], indicating that nitrogen fixation was probably not the principal factor limiting growth.

At 10°C shoot temperature, the increase in root nitrogen was similar in all root temperature treatments, but there were marked differences in the amount of nitrogen

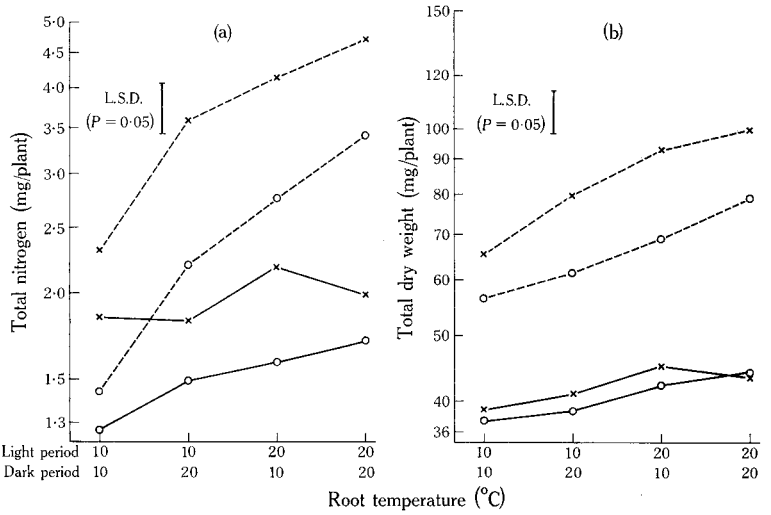


Fig. 3.—Effect of diurnal fluctuation between 10 and 20°C root temperature on (*a*) nitrogen assimilation and (*b*) dry weight increase. All plants nodulated by strain TA1, but half received mineral nitrogen (×), whereas the remainder did not (○). Plants were grown at 10°C (———) and 20°C (– – – –) shoot temperature, with 12 hr light per day.

TABLE 2

TOTAL NITROGEN INCREASE IN THE SHOOTS AND ROOTS DURING EXPOSURE TO CONSTANT OR DIURNALLY FLUCTUATING ROOT TEMPERATURES AT SHOOT TEMPERATURES OF 10 AND 20°C

Nitrogen distribution index values (as percentages) are given in parentheses. There were 11 replicates per treatment

| Root Temperature (°C) | | Control Plants: Nitrogen Increase (mg/plant) in | | TA1-nodulated Plants: Nitrogen Increase (mg/plant) in | |
|---|---|---|---|---|---|
| Light Period | Dark Period | Shoots | Roots | Shoots | Roots |
| | | Shoot temperature 10°C | | | |
| 20 | 20 | 1·15 (*80*) | 0·29 | 0·98 (*82*) | 0·22 |
| 20 | 10 | 1·28 (*77*) | 0·38 | 0·81 (*77*) | 0·24 |
| 10 | 20 | 0·95 (*74*) | 0·34 | 0·77 (*78*) | 0·22 |
| 10 | 10 | 1·03 (*78*) | 0·30 | 0·53 (*72*) | 0·21 |
| | | Shoot temperature 20°C | | | |
| 20 | 20 | 3·32 (*79*) | 0·90 | 2·30 (*79*) | 0·60 |
| 20 | 10 | 2·69 (*74*) | 0·96 | 1·64 (*74*) | 0·58 |
| 10 | 20 | 2·18 (*71*) | 0·88 | 1·30 (*74*) | 0·47 |
| 10 | 10 | 1·30 (*72*) | 0·51 | 0·59 (*65*) | 0·31 |

that was translocated to the shoots (Table 2). Similarly, at 20°C shoot temperature, an increase in the exposure time at 10°C root temperature reduced the nitrogen distribution index, although under these conditions such treatment also reduced the total nitrogen increase in the roots.

## IV. Discussion

While it was known that continuous exposure to high root temperatures reduced the amount of nitrogen fixed by plants nodulated by strains NA30 and CC17 (Gibson 1965), there was no information of the nature of the effect, i.e. whether it was a transient effect on the rate of fixation, or whether it was due to a permanent effect on the integrity of the symbiotic system. The linear decline in the rate of nitrogen fixation with longer daily exposure to 30°C [Fig. 1(*a*)] indicated that the effect was transient, and directed towards some step or steps in the nitrogen fixation reaction. The results were consistent with a change in fixation rate from low to high when the plants are transferred from 30 to 23°C root temperature, and from high to low on return to 30°C. There was no evidence that the integrity of the symbiotic system was affected by the exposure to the high root temperature. The effect was associated with the bacterial component of the symbiosis, as the rate of nitrogen fixation by TA1 plants grown under the same conditions as those retarding nitrogen fixation by NA30 plants was not affected.

Although Virtanen, Moisio, and Burris (1955) showed a reduced ability of excised pea nodules to fix nitrogen after the plants were darkened for 20 hr, little is known of the nitrogen-fixing activity of nodulated plants during normal dark periods. The observation that the NA30 and CC17 plants exposed to a 30/22°C (light/dark) root temperature regime fixed as much nitrogen as those exposed to a 22/30°C regime [Fig. 1(*b*)], suggests that a high rate of nitrogen fixation was achieved by the plants in the former treatment during the dark period. The fact that the 22°C controls fixed nitrogen at a higher rate than the plants in either of these treatments also suggests that nitrogen fixation may proceed at high rates during normal dark periods. A similar conclusion may be drawn from the data for TA1 plants exposed to 14°C root temperature [Fig. 2(*b*)].

With NA30 plants exposed to 16 and 23°C root temperature under continuous illumination, the highest $R_N$ was achieved by those plants at 23°C for 8 hr/day and 16°C for 16 hr/day [Fig. 2(*a*)]. The optimum constant root temperature for nitrogen fixation by NA30 plants is close to 23°C (Gibson 1963, 1969), while there is some evidence that 16°C is close to the optimum temperature for the most efficient fixation of nitrogen by such plants (Gibson 1969). It is feasible that a combination of these conditions could produce the highest rate of nitrogen fixation on a whole-plant basis.

The inhibitory effect of 14°C on nitrogen fixation by the TA1 plants appeared to be associated with the reduction in translocation of fixed nitrogen to the shoots (Table 1). Regardless of the root temperature treatment, the total nitrogen increase in the roots of plants in the four treatments was comparable. This is in accord with a previous finding regarding nitrogen retention in the roots (Gibson 1969), an effect attributed to a seemingly disproportionate production of nodule tissue at the lower temperatures.

The effect of exposure to low root temperatures was markedly influenced by shoot temperature (Fig. 3). Nitrogen fixation was retarded by exposure to 10°C root temperature to a greater extent when the shoot temperature was 20°C than it was with 10°C shoot temperature. However, this was associated with the marked effects of shoot temperature on nitrogen fixation (Gibson, unpublished data). Unlike the effects of exposure to high, and moderate, root temperatures, in this experiment the rate of nitrogen fixation was strongly influenced by the root temperature during the light period. Under the low root temperature conditions, the plants appeared less able to compensate for the reduced fixation rates during the light period than were plants exposed to the moderate root temperatures of 14 and 16°C.

## V. Acknowledgments

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