TEMPERATURE EFFECTS ON THE NITROGEN NUTRITION OF LEGUMES

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ABSTRACT

Nitrogenase extracts have a biphasic N-fixation response to temperature, with a steeper rate of response below 20°C. The reaction is not adversely affected at least up to 40°C. Intact nodules, however, show temperature optima for nitrogenase activity in the 20-30°C range with a tendency to adapt to the cultural conditions. Nodulation, nodule development and nodule degradation are all temperature sensitive. Low unit nitrogenase activities at lower temperatures are compensated for, given time, by increased levels of active tissue. In the short term, legume growth dependent on N fixation is severely restricted at temperatures removed from the optimum for nitrogenase, but in the longer term, growth rates for plants dependent on N-fixation and mineral-N uptake become comparable over a wide range of temperatures. At least in some species, the effect of level of nitrogen supply on apical dominance can also contribute to a broadening of the temperature response curve.

The objective of this review is to put temperature responses of symbiotic N-fixation into the whole plant context, and in particular to cover compensating changes which tend to broaden the temperature response of the whole plant, relative to responses by individual components. Detailed reviews of specific aspects of N-fixation temperature responses are given by Gibson (1971, 1977), and no attempt will be made in this paper to cover the detail included in these earlier papers. At the whole plant level, emphasis will be given to material where adequate mineral-N controls have been included as a contrast to symbiotic associations dependent on N-fixation, thus enabling separation of specific temperature effects on N-fixation from general effects of temperature on the host.

Response of individual components

A brief summary of temperature responses of some components of N-fixation compiled from the reviews of Gibson is given in Table 1.

TABLE 1. Temperature ranges for components of N-Fixation (from Gibson, 1971, 1977)

N-fixation component	**Temperature ranges (°C)**		
	Min.	Optimum	Max.
Growth of free living rhizobium		25-	40
Root infection by rhizobium	7-	30-	33-
Nodulation/Nodule development	7-15	20-30	30-38
Nitrogenase activity (intact nodules)	<5	20-30	30-40

For most components, critical temperatures for tropical species are about 10°C higher than for temperate species, although considerable genetic variation exists both at the rhizobium strain and host level (e.g. Gibson, 1963; Ek-Jander and Fahraeus, 1971). In general, minimum temperature requirements for infection and nodule initiation are higher than those for nodule development and N-fixation.

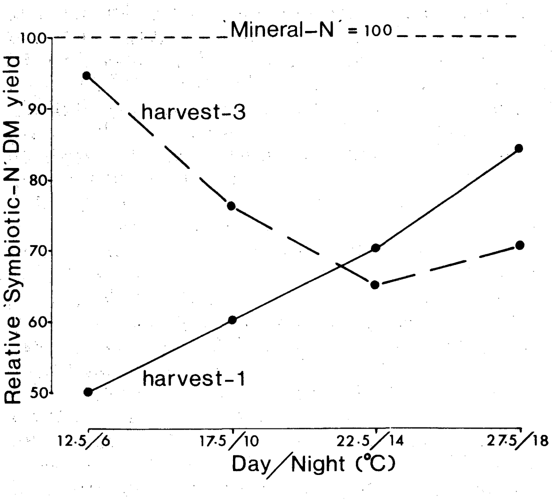
The defined temperature optima for these components contrasts with the response of purified nitrogenase extracts which respond positively to temperature up to 40°C. There is however a reduction in rate of response above 20°C. Nitrogenase responses are similar to those for intact nodules below 20°C (Burns and Hardy, 1975). This led to the conclusion (Hardy and Havelka, 1976) that nitrogenase concentration is not the rate limiting factor above 20°C. Studies with CO_2 supplementation and additional light on soybean *(Glycine max)* suggest that energy supply for nitrogen fixation may be rate limiting above 20°C. Caution should perhaps be used when extrapolating this conclusion to temperatures below 20°C, and particularly to a cool temperate climate where higher temperatures are generally associated with long days, thus reducing energy stress on plants generally.

At higher temperatures nodule development can be disrupted and nodule degradation accelerated (Roughley, 1970; Pankhurst and Gibson, 1973) particularly with some rhizobium strains. In the latter study, this degradation occurred rapidly at 30°C with strain NA30 on subclover *(Trifolium subterraneum)*, despite the apparent presence of large quantities of polysaccharide in the host nodule cells, which suggests a specific effect of temperature rather than an effect of induced carbohydrate deficiency. Nodules formed by TA1 were less severely affected. Developed nodules are resistant to exposure to low temperatures, and even subtropical species can recover full N-fixation activity within one hour after exposure to temperatures as low as 4°C (Ranga Rao, 1977). Nodules on white clover *(T. repens)* may overwinter under snow and recommence activity in spring although this involves synthesis of new bacteroid tissue (Bergersen *et al.,* 1963).

Response of the whole plant

At the whole plant level, these temperature responses (Table 1) carry through in many cases to host yield, in other cases however, yield has been shown to be much less temperature sensitive than would be expected from the nitrogenase response. Subclover under simulated sward conditions (Davidson *et al.,* 1970) was found to be much less temperature sensitive than single spaced plants (Morley, 1958) and this was partly attributed to large leaf canopies in the sward causing complete light interception. In another experiment with subclover (Fukai and Silsbury, 1976), the optimum temperature for growth was 20-25°C when plants were young, but decreased during growth so that by 100 days, yield was inversely related to temperature over the range 15-30°C. A similar result was obtained when comparing first and third harvests when taking repeated harvests of white clover (Figure 1). In this figure the performance with non-limiting mineral-N is used as a control with which to compare symbiotic performance. The first harvest response conforms to that expected from nitrogenase temperature response curves, but by the third harvest the response was opposite. Factors other than the limitations of light interception need to be invoked to explain these results, where complete reversal of response was involved.

Figure 1: The effect of time on the temperature response of white clover plants when dependent on N-fixation relative to those receiving non-limiting mineral-N (Hoglund and Williams, unpub).



Factors modifying the whole plant response

(a) Nodule nitrogenase temperature response curves show an adaptation to cultural temperature (Figure 2). The one cultivar of soybean inoculated with the one strain of rhizobium was used at all temperatures and the adaptation therefore represents plasticity of the symbiotic temperature response. The shift in optimum temperature was equal to about half the difference in growth temperatures. In any situation where competition could cause a genetic shift in the host population, or where a number of rhizobium strains are present, even greater adaptive shifts might be possible. Weber *et al.* (1971) has shown that soil temperature can affect the competitive ability of strains of rhizobium when nodulating soybean.

(b) Initial growth of lucerne dependent on symbiotic-N is slower at lower temperatures (Figure 3), and the divergence between performance on symbiotic-N and that on mineral-N is greater, indicating a specific N-fixation limitation. This is

Figure 2: The effect of growth temperature on the nitrogenase activity of soybean nodules when incubated at a range of temperatures (Gibson, 1976).

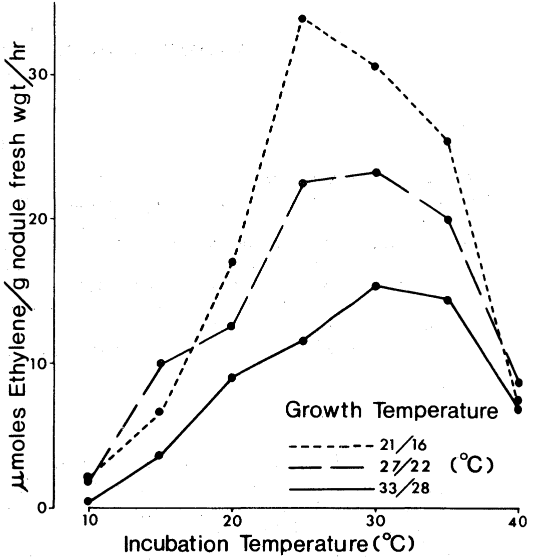
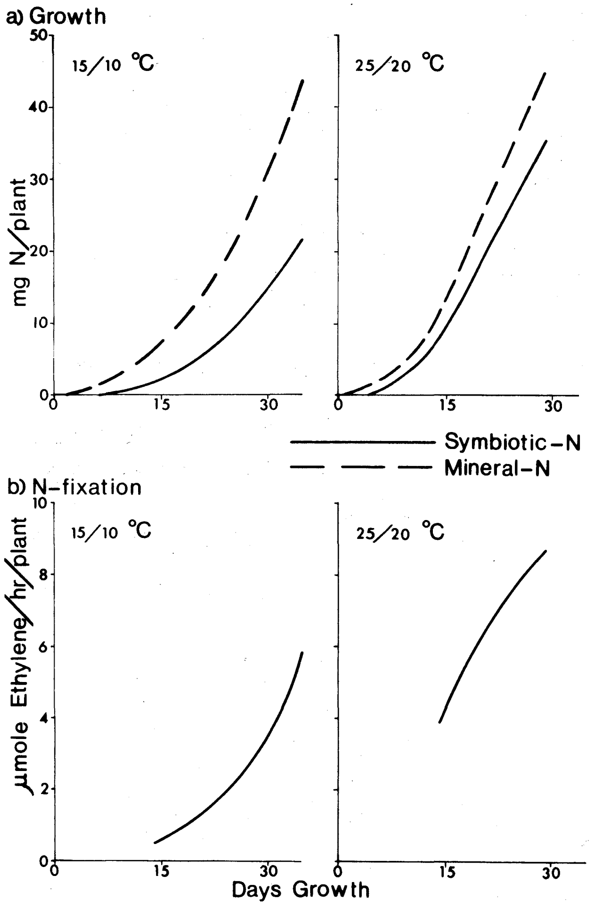


Figure 3: The effect of temperature on growth rates and N-fixation in lucerne (Hoglund, 1973).

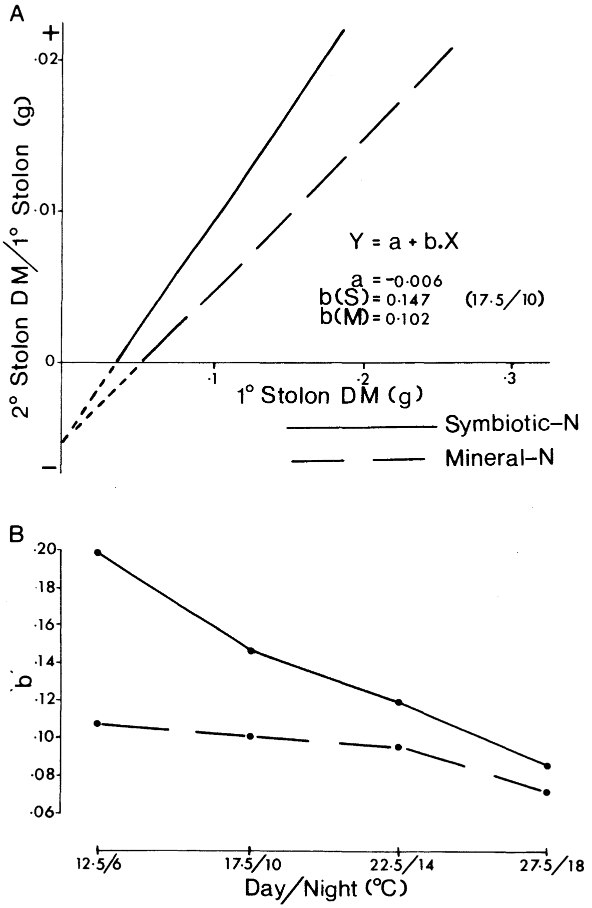


consistent with the lower fixation rates at 15 days. Although N-fixation per unit nodule is less under lower temperatures (11 cf. 19°C), greater volumes of nodule and bacteroid tissue can develop as an apparent compensatory mechanism (Roughley, 1970), thereby reducing the limitation of temperatures below optimum on N-fixation per plant. Therefore the major limitation imposed by temperature down to about 10°C below the optimum probably relates to the time taken to achieve comparable rates of N-fixation per plant. It is doubtful if temperatures in this range have any effect on maximum N-fixation rates per plant *per se*, except insofar as temperature limits maximum growth rates of the host. However, the reduction in rate of development of N-fixation at lower temperatures can in the short term severely restrict growth relative to plants supplied with adequate mineral-N.

(c) Where growth in any period may be partly determined by the development of meristems in a previous period, an improvement in host performance could be expected with time in treatments favouring branching. White clover produced less secondary stolon for any given size of primary stolon when grown with non-limiting mineral-N (Figure 4a). The difference in proportion of secondary stolon between nitrogen treatments decreased with increasing temperature (Figure 4b). As the higher temperature would favour increased N-fixation, both the temperature and nitrogen responses could be interpreted as increased nitrogen increasing apical dominance. The effect of temperature *per se* was not great as shown by plants receiving non-limiting mineral-N.

Any of these factors, alone or in combination, could explain the type of result shown in Figure 1, and will tend to reduce the field sensitivity of N-fixation to temperature for at least at 10°C range on the lower side of optimum. A common feature of these adaptive changes however, is the variation in the degree of adaptation with time.

Figure 4: Branching in white clover. A) Effect of symbiotic-N vs. non-limiting mineral-N at 17.5/10°C; B) Effect of temperature on the regression coefficient (b = slope) for the above relationship (Hoglund and Williams, unpub.) Standard errors of 'b' values are about 20% in all cases.



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