

in translocation between the two compounds probably cannot be the basis for the ineffectiveness of kinetin in counteracting the inhibitory effect of indoleacetic acid on basal regeneration. Hence the mechanisms governing correlative inhibition of lateral bud reactivation and basal protonematal regeneration by the apical region of the gametophore appear to be different from each other.

It should be mentioned here that Gorton and Eakin³ have investigated gametophore initiation in the protonemata (induction of "dauermodifikation"⁴) of *Tortella caespitosa*. Bud initiation was found here to be inhibited by high concentrations of auxin, and was strongly stimulated by kinetin. Kinetin reversed the inhibition of bud formation brought about by naphthalene acetic acid. It seems, therefore, that certain stages in the induction of "dauermodifikation", that is, bud initiation in the protonemata, and lateral bud reactivation in the gametophore may be mediated by the same mechanism.

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² Wickson, Margaret, and Thimann, K. V., *Physiol. Plant.*, **11**, 62 (1958).

³ Gorton, B. S., and Eakin, R. E., *Bot. Gaz.*, **119**, 81 (1957).

⁴ Bünnig, E., "Entwicklungs- und Bewegungsphysiologie der Pflanze", 271 (Springer-Verlag, Berlin-Göttingen-Heidelberg, 1953).

Temperature and Symbiotic Nitrogen Fixation

MODERATELY high temperatures have been found to exert a specific inhibitory effect on symbiotic nitrogen fixation. The results of an experiment illustrating this effect are given in Table 1.

Table 1. EFFECT OF TEMPERATURE, INOCULATION WITH *Rhizobium*, AND POTASSIUM NITRATE ON THE YIELD AND PERCENTAGE PROTEIN NITROGEN OF SUBTERRANEAN CLOVER (MEANS OF FOUR REPLICATES)

Treatment	Yield of dry matter per tube (mgm.)		Protein nitrogen in plants (per cent)	
	20° C.	30° C.	20° C.	30° C.
Without nitrogen or <i>Rhizobium</i>	38.0	35.2	1.85	1.54
Inoculated with <i>Rhizobium</i>	54.5	35.3*	2.66	1.69*
With potassium nitrate	56.8	62.0	2.59	2.55
Standard error expressed as percentage of mean	4.9 per cent		4.3 per cent	

* Interaction between form of nitrogen (*Rhizobium* v. potassium nitrate) and temperature is significant at $P < 0.001$ for both yield and percentage protein nitrogen.

For this experiment, subterranean clover (*Trifolium subterraneum* L. var. *Bacchus* Marsh) plants were grown on nitrogen-free nutrient agar in test-tubes which were stood in temperature-controlled water-baths in a glasshouse. The results show that the uninoculated plants responded normally to nitrogen treatment at both temperatures. By contrast, plants inoculated with *Rhizobium* fixed nitrogen and grew normally only at the lower temperature. The inoculated plants were well nodulated at both temperatures.

Thus the symbiotic nitrogen fixation process was specifically inhibited at the higher temperature.

We have investigated the hypothesis that this inhibitory effect of high temperature is due to a decrease in the supply of soluble carbohydrate to the nodule, resulting from an increase in the rate of respiration. The addition of sucrose to the culture medium in aseptic cultures did not increase the amount of nitrogen fixed at either the high or low temperature. The sucrose increased the yield of the plants but decreased the percentage nitrogen, indicating that the added sucrose was taken up by the plants but was not required directly for nitrogen fixation. We have therefore begun to examine the possibility that the high temperature has induced the deficiency of some other metabolite essential for symbiotic nitrogen fixation.

These findings also raise a number of other interesting problems. One of these concerns the reported harmful effects of high light intensity on symbiotic nitrogen fixation. This was first reported as long ago as 1935^{1,2}, when shading was shown to have a remarkable effect in breaking the nitrogen-hunger period in legumes. This response to shading was interpreted in terms of an effect on light intensity and photosynthesis. In our experiments we have been able to obtain marked responses and apparently normal growth of clover under the conditions of high light intensity of our summer by cooling the plants. It would appear that there is a need to determine in greater detail the relative effects of high temperature and high light intensity on symbiotic nitrogen fixation.

The significance of these findings in relation to the culture of legumes under experimental and field conditions is also an important matter for investigation. We have evidence that these effects are of importance in the culture of legumes in glasshouses in warmer weather, not only where the plants are grown in test-tubes, but also for larger pots. In fact the present work arose from observations made during the course of a pot culture experiment conducted during 1957, when it was found that subterranean clover grown in soil in pots made of clear polystyrene plastic were stunted and pale green in comparison with the larger dark green plants in corresponding enamelled pots. The reason for this difference was traced to the considerably higher soil temperature in the clear plastic pots. Since becoming aware of this effect we have obtained unmistakable evidence of suboptimal symbiotic nitrogen fixation in clover grown in various kinds of pots where soil temperatures have exceeded about 25° C.

We have no direct evidence on the possible effects of high temperatures on symbiotic nitrogen fixation under field conditions. In view of the temperatures involved it would seem that such effects may be of significance, particularly under irrigation in the warmer months, or in tropical and sub-tropical environments.

Full details of the experiments that have been done on this problem will be published elsewhere.

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