The effects of temperature on growth and nitrogen fixation in *Trifolium subterraneum* L. communities.

by

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SUMMARY

Survey of the literature showed there to be much information on the effects of temperature on nitrogen fixation. However, most of this was derived from short-term exposure of single plants, or nodulated roots, to different temperatures. Also, much of this research involved the acetylene reduction assay without consideration of associated evolution of hydrogen. It appears to have been assumed that the relationships between acetylene reduction, hydrogen evolution, and nitrogen fixation are not influenced by the environment nor do they change during ontogeny. Little information was available concerning the response of nitrogen fixation by legumes to temperature when plants are grown as swards at densities comparable with those experienced under commercial conditions. The aim of this investigation was to assess the responses to temperature of swards of Trifolium subterraneum between seedling establishment and dry matter yields regarded as economic in the field.

Swards of T.subterraneum (2,300 plants m^{-2}) were grown at temperatures of $10^{o}C$, $15^{o}C$, $20^{o}C$ and $25^{o}C$ at photosynthetic photon flux densities (PPFD) of 500 or 1000 µmol quanta $m^{-2}s^{-1}$. Growth rates were measured by infra-red analysis of CO_2 exchange, and by increment in biomass with time. Nitrogen fixation was estimated by hydrogen evolution and acetylene reduction assays, and also by increment in organic nitrogen with time.

The growth rates of nitrogen-fixing swards, of 190-475 g dry matter m^{-2}, were inversely related to temperature, and in this respect their response was very similar to that of swards assimilating combined nitrogen. However, swards fixing nitrogen grew more slowly than the latter. This was reckoned to result from the high energy costs of nitrogenase activity, and a lower growth efficiency (g carbon respired

g dry matter retained 24 h^{-1}) for nitrogen-fixing plants. The effect of temperature on the nitrogen fixation rate was similar to that on the growth rate.

Acetylene reduction accurately reflected the response of nitrogen fixation to temperature between 10°C and 20°C, but above 20°C there was a temperature-induced disruption of this relationship. The difference between acetylene reduction and hydrogen evolution is theoretically equivalent to nitrogen fixation, but in long-term experiments the development of hydrogen uptake made this difference an unreliable index of nitrogen fixation.

Hydrogen uptake occurred at high amounts of biomass (greater than 380 g dry matter m^{-2}) at 15°C and 20°C, and also at lower amounts of biomass (greater than 190 g dry matter m^{-2}) at 25°C. Appearance of hydrogen uptake was not quickened when plants were treated with hydrogen. It is suggested that hydrogen uptake developed as a normal event in the physiological ageing of nodules, which was accelerated at high temperature and served in protecting nitrogenase from damage by oxygen.

There was substantial diurnal variation in nitrogen fixation in all environments except 25°C. The data supported a model in which a diurnal change in the transpiration rate resulted in a diurnal rhythm of accumulation of nitrogen in nodules at night and release of nitrogen during the day, and this rhythm affected the nitrogen fixation rate. Nitrogen fixation was finely regulated in the short term through proton reduction.

The hydrogen evolution and acetylene reduction techniques were useful in measuring nitrogen fixation when consistently employed.