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Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature

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Summary Experiments were done to test whether N fixation is more sensitive to high soil temperatures in common bean than in cowpea or soybean. Greenhouse experiments compared nodulation, nitrogenase activity, growth and nitrogen accumulation of several host/strain combinations of common bean with the other grain legumes and with N-fertilization, at various root temperatures. Field experiments compared relative N-accumulation (in symbiotic relative to N-fertilized plants) of common bean with cowpea under different soil thermal regimes. N-fertilized beans were unaffected by the higher temperatures, but nitrogen accumulation by symbiotic beans was always more sensitive to high root temperatures (33°C, 33/28°C, 34/28°C compared with 28°C) than were cowpea and soybean symbiosis. Healthy bean nodules that had developed at low temperatures functioned normally in acetylene reduction tests done at 35°C. High temperatures caused little or no suppression of nodule number. However, bean nodules produced at high temperatures were small and had low specific activity. For *P. vulgaris* some tolerance to high temperature was observed among rhizobium strains (*e.g.*, CIAT 899 was tolerant) but not among host cultivars. Heat tolerance of *P. acutifolius* and *P. lunatus* symbioses was similar to that of cowpea and soybean. In the field, high surface soil temperatures did not reduce N accumulation in symbiotic beans more than in cowpea, probably because of compensatory nodulation in the deeper and cooler parts of the soil.

Introduction

Symbiotic legumes are often more sensitive to unfavorable soil conditions than their nitrogen fertilized counterparts[7]. The deleterious effects of high root temperatures on nitrogen fixation have been shown for numerous legumes[3,15], and genetic variation in tolerance to high rooting temperature has been observed among plant varieties and bacterial strains[1,3,9].

Biological nitrogen fixation by common bean (*Phaseolus vulgaris* L.) is generally weak; this may be due to the sensitivity of the symbiosis to various environmental stresses, including high soil temperature (4). Data collected from various greenhouse studies suggest that the maximum root temperature for optimum N-fixation by common bean is about 30°C[5,6,12]. For tolerant host/strain combinations of other warm-season grain legumes, such as cowpea and soybean, optimum N-fixation can be maintained up to 33°C[1,2,9,12].

A series of experiments was conducted to determine if N-fixation by common bean was more sensitive to high soil temperature than cowpea and soybean symbioses, and if this could account for the poorer symbiotic performance of beans during the summer months[13]. Additional experiments evaluated host and strain differences in tolerance, and the importance of high soil temperature in the field.

Materials and methods

Greenhouse experiments

Greenhouse experiments were done at Davis, California, from September through November 1984, and from March to May 1985. There was ample sunshine, day length varied from 11 to 13 hrs, and greenhouses air temperature fluctuated diurnally from 22 to 33°C. Pots were partially immersed in water baths and the rooting medium was maintained thermostatically at a constant temperature, or on a fluctuating 12 hour day/night cycle. For the fluctuating cycle, heating commenced at 7 a.m. each day, and cooling began at 7 p.m. each night. The potting medium consisted of 1.5 liters of a 4:2:1 fine vermiculite/sand/soil mixture. The soil was a well mixed sample from a field which had been grown to beans, cowpeas, and soybeans the previous summer. All treatments were fertilized with the following low nitrogen nutrient mixture: 190 mg $Ca(NO_3)_2$, 90 mg KH_2PO_4, 82 mg $MgSO_4 . 7H_2O$, 232 mg K_2SO_4, 2.3 mg Fe-EDTA, 1.2 mg KCl, 500 μg H_3BO_3, 110 μg $MnSO_4 . H_2O$, 200 μg $ZnSO_4 . 2H_2O$, 40 μg $CuSO_4 . 5H_2O$, and 30 μg H_2MoO_4. Longer experiments received an additional application of the same mixture at 4 weeks, except that 200 mg $CaSO_4 . 2H_2O$ replaced the $Ca(NO_3)_2$. In addition, +N controls received weekly applications of ammonium nitrate (100 mg N/pot/week). Unless otherwise stated, all treatments were triplicated and pots were randomized within each water bath. All treatments were thinned to 2 plants per pot after emergence and watered to field capacity with distilled water as needed until harvest. Seeds were obtained from University of California Bean Program, Davis.

Experiment 1. Effect of root temperature and rhizobium strain on nodulation and nitrogen fixation. One variety each of common bean (*Phaseolus vulgaris* L.), cowpea [*Vigna unguiculata* (L.) Walp.], and soybean [*Glycine max* (L.) Merr.] was tested at 3 root temperatures with various rhizobium strains and with non-inoculated and + N controls. Root temperature was maintained continuously at 28 or 33°C, or on a 33/28°C (day/night) cycle. The following host/strain combinations were tested: (i) *P. vulgaris* (cv. PI 173076) with strains CIAT 255, CIAT 899 (Centro Internacional de Agricultura Tropical), 128K44 (Nitragin Co., Milwaukee, Wis.), UCD H1, UCD H2, UCD L1. (UCD H1 and H2 were local selections isolated from effective nodules formed at a high temperature, whilst UCD L1 was selected at a lower temperature.); (ii) *V. unguiculata* (cv. C 8043) with strains TAL 309, TAL 169, TAL 201 (University of Hawaii NIfTAL Project, Maui, Hawaii); (iii) *G. max* (cv. Evans) with strains USDA 110, USDA 136 (U.S. Department of Agriculture, Beltsville, Md.), TAL 102. The growth medium was sterilized prior to the planting of 3 surface sterilized seeds per pot. Inoculum was added with a pipette before covering the seeds, at an approximate rate of 10^8 cells per pot. After 28 days plants were harvested, nodulation was observed, and dry weight and Kjeldahl N were determined.

Experiment 2. Effect of root temperature and host variety on nodulation and nitrogen fixation. Six varieties of common bean (PI 173076, PI 205209, Aurora, Puebla 152, Sutter Pink, California Red Kidney) were inoculated with a mixture of heat tolerant rhizobial strains and compared with cowpea (C 8043) and soybean (Evans) symbioses at two root temperatures. Rooting medium temperature was maintained either continuously at 28 C or on a 34/28 C day/night cycle. The following rhizobial strain mixtures that performed well at high temperatures were applied as in Experiment 1: (i) CIAT 899 and UCD H1 for beans; (ii) TAL 169 and TAL 309 for cowpea; (iii) USDA 110 and USDA 136 for soybean. The unsterilized potting medium provided a background mixture of locally adapted strains. After 21 days acetylene reduction capacity (10% acetylene, 15 minutes incubation at 30°C), and nodule number, nodule weight, shoot dry weight and Kjeldahl N were determined.

Experiment 3. Effect of root temperature on nitrogen fixation by established nodules. The purpose was to evaluate effects of high root temperature on N-fixation unconfounded by effects on nodule formation. Plants of common bean (PI 173076, Puebla 152, Red Kidney), cowpea (C 8043) and soybean (Evans) were inoculated with rhizobial strains used in Experiment 2 and grown for 30 days at 28 C (root temperature) in unsterilized potting mix. At this stage 12 uniform plants of each variety were selected. Four of these were harvested for dry weight and Kjeldahl N determination. Uniform, nodulated root segments of these plants were placed in 20 ml airtight incubation jars. Acetylene (10%) was introduced and the containers were incubated at three different air temperatures (27°C, 31°C, 35°C). Gas samples were collected after 5 minutes and 10 minutes, and acetylene reduction rate was determined by the difference in ethylene production recorded at these times. Of the remaining eight plants, four remained in the 28°C water bath and four were transferred to a higher temperature bath, maintained on a 34/28°C day/night cycle. After 10 days plants were harvested and acetylene reduction, nodule weight, shoot dry weight and Kjeldahl N were measured.

Experiment 4. Genetic variation in nitrogen fixation at high root temperature. Eleven *Phaseolus vulgaris* genotypes and twelve genotypes of other species within the Phaseolus genus (*P. lunatus, P. acutifolius, P. filiformis*) were compared with cowpea and soybean at two root temperatures. All treatments were inoculated with a mixture of 4 or more rhizobium strains appropriate for each species. The presence of numerous rhizobium strains in the unsterilized potting mix reduced the influence of strain effects on the expression of the hosts' tolerance to high root temperature. Water baths were maintained continuously at 28 and 33°C. Varieties and species evaluated are indicated in Figure 7. The following cultivars of *P. vulgaris*, supplied by Native Seed Search, Arizona, were considered to be adapted to hot, low desert conditions: I — Tarahumara asufrado; II — Papago vayos; III — Tarahumara mantequilla; IV — Yaqui ojo de cabra; V — Mayo asufrado; VI — Mayocoba bean; VII — Tarahumara amarillo bean; VIII — Tarahumara ojo de cabra. After 30 days plants were harvested and shoot dry weight determined.

Field experiment

Experiment 5. Effect of high soil temperatures on N-fixation in the field. Seeds of common bean (PI 173076), cowpea (C 8043) and non-nodulating soybean (Clark rji) were inoculated with appropriate mixtures of effective and heat tolerant rhizobial strains (CIAT 899 and UCD H1 for bean; TAL 169 and TAL 309 for cowpea) applied to the seed, using peat together with a gum arabic sticker to give approximately 10^8 rhizobia per seed. The seed was planted on July 2, 1985, to a nitrogen deficient field known to contain abundant native strains able to form effective nodules with both bean and cowpea. A split-plot design with four replicates was used with host variety as the main treatment, and nitrogen level (0 or 250 kg N/ha) and soil temperature (unmulched or mulched with 10 t/ha straw) as sub-treatments. Four rows, 30 inches apart, were used for all treatments, and irrigation water was applied at approximately weekly intervals so that the modifying effects of canopy closure and moisture supply were typical of local bean production. Air temperature and within-row soil temperature at 10 cm depth were monitored. After eight weeks, nodulation measurements were made and dry weight and Kjeldahl N determined.

Results

Experiment 1

Inoculated plants of cowpea and soybean appeared green, healthy and well nodulated at all root temperatures. For common bean this was only the case at the low temperature (28°C), and higher temperatures resulted in yellow, stunted plants with numerous small white nodules. Total

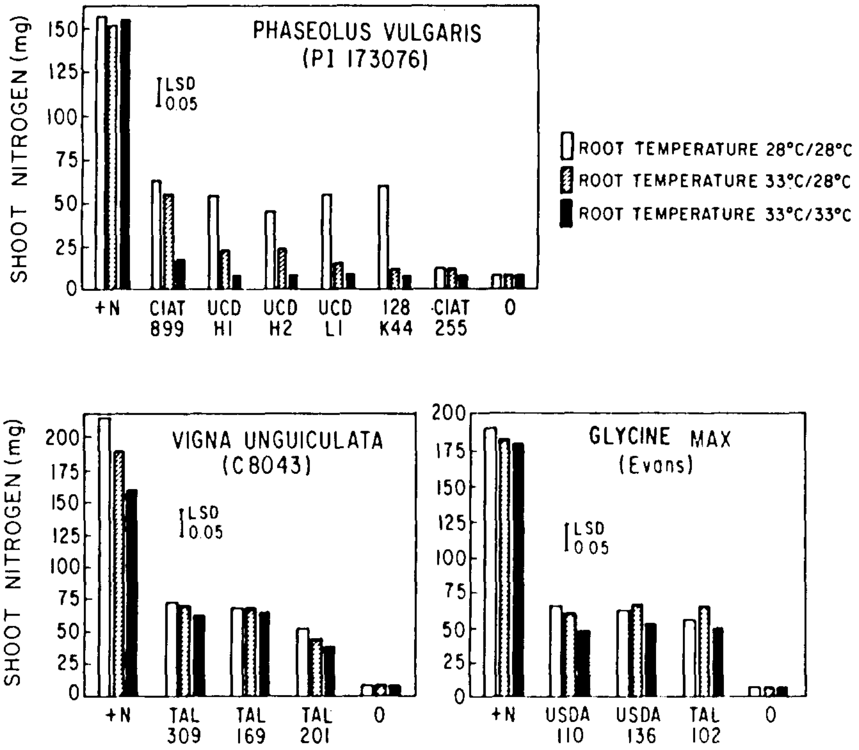

Fig. 1. Effect of rooting medium temperature on N-fixation by various rhizobial strains in association with common bean (*P. vulgaris*), cowpea (*V. unguiculata*) and soybean (*G. max*).

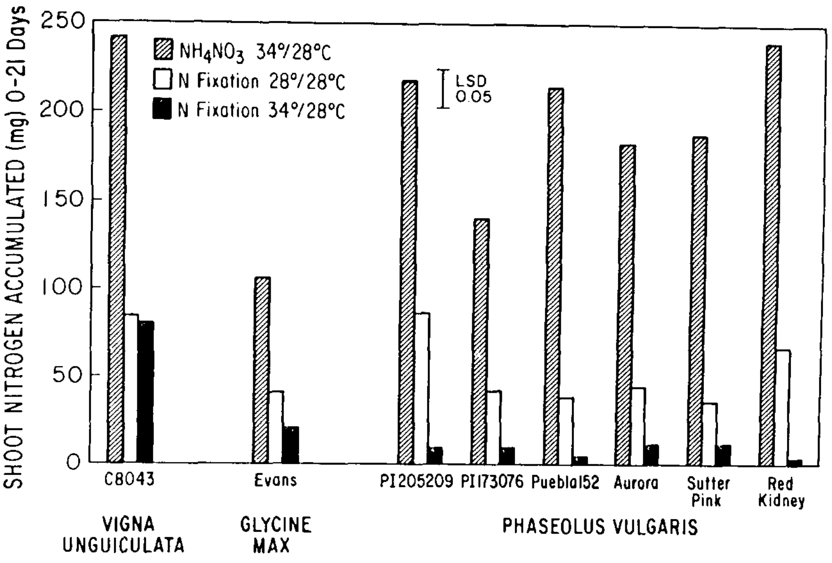

Fig. 2. Effect of rooting medium temperature on early N accumulation by symbiotic plants (0–21 days after emergence).

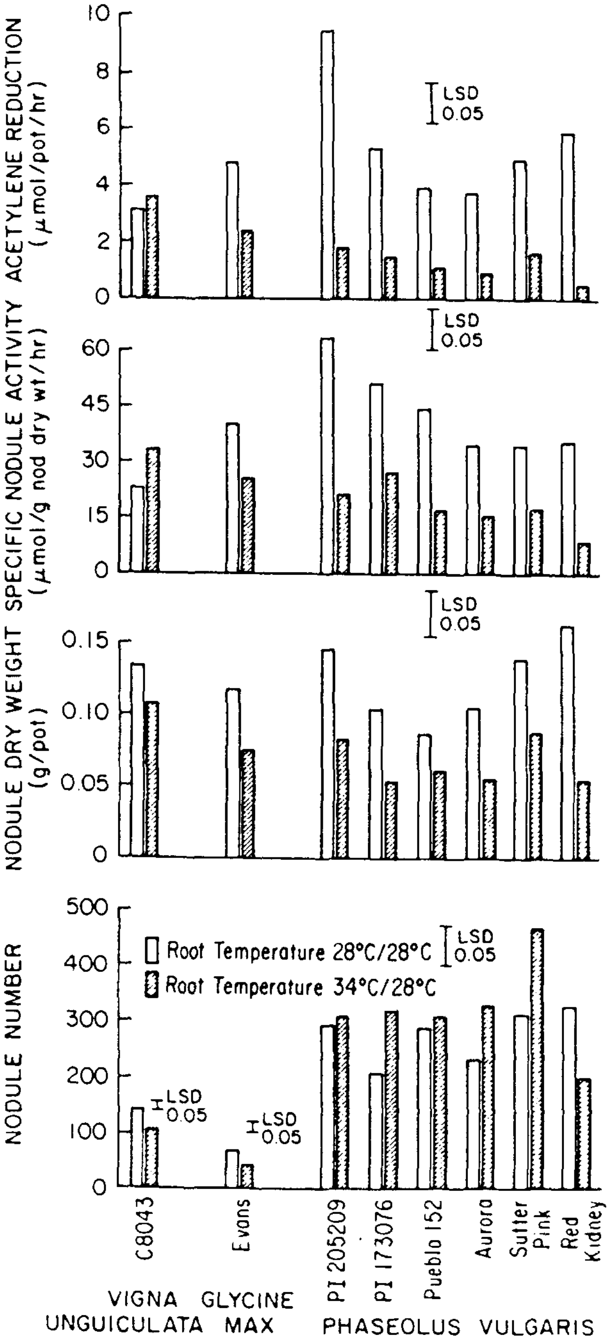

Fig. 3. Effect of rooting medium temperature on nodulation and nitrogenase activity (21 days after emergence).

nitrogen accumulation decreased sharply with increasing root temperature for all the strains of common bean rhizobia, except CIAT 899 which performed well at 33/28°C (Figure 1). There were no significant temperature effects on nitrogen accumulation by cowpea, soybean (except strain USDA 110), or N-fertilized bean.

Experiment 2

Phaseolus vulgaris fixed less nitrogen, and soybean slightly less, at root temperatures of 34 (day)/28 (night)°C than at 28°C continuous (Figure 2). Root temperature had no effect on N accumulation by cowpea. Nodule weight decreased in all *P. vulgaris* cultivars and soybean at the higher root temperature (Figure 3). Only common bean cultivar Red Kidney showed a significant decrease in nodule number; most cultivars produced numerous small nodules at the high temperature (Figure 3). Specific nodule activity (SNA) and acetylene reduction rate (AR), determined after equilibration of root systems at 30°C for 2 hours, was much lower for those common bean varieties grown at the higher root temperature. No negative effects on any aspect of nodulation or nodular activity were observed for cowpea grown at the higher temperatures.

Experiment 3

When plants with nodules formed at 28°C were exposed to higher temperatures (34/28°C) for 10 days, N-fixation by *P. vulgaris* was strongly reduced, but that of cowpea and soybean was only slightly affected

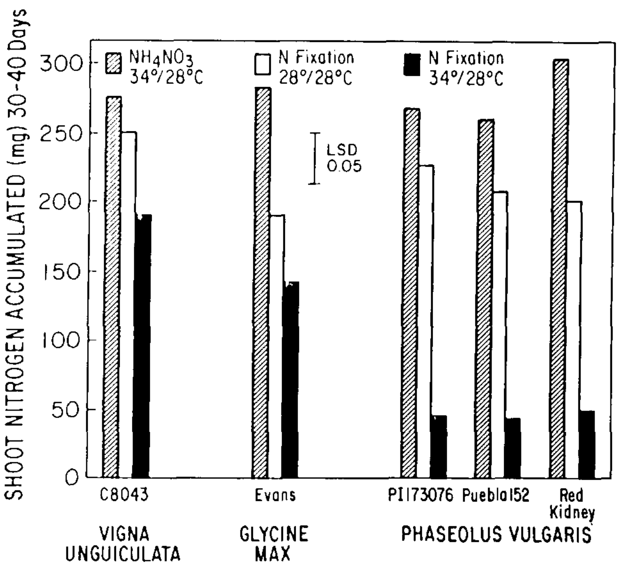

Fig. 4. Effect of rooting medium temperature on N accumulation by symbiotic plants from 30 to 40 days after emergence. (All plants grown at 28/28°C from 0 to 30 days).

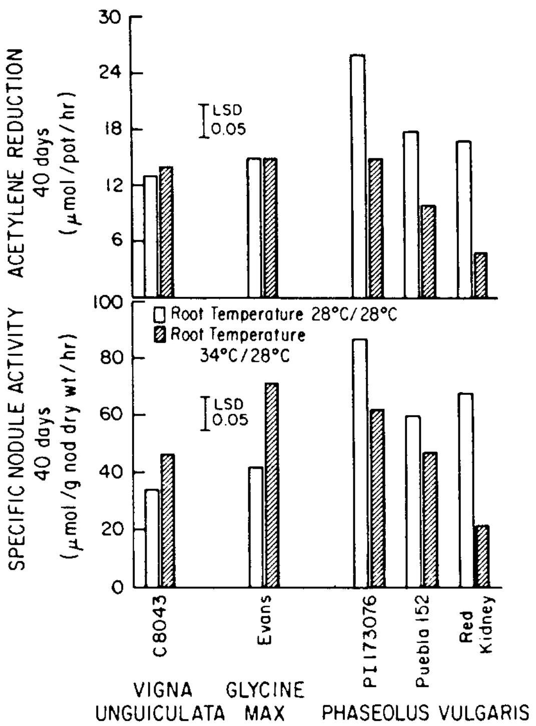

Fig. 5. Effect of rooting medium temperature on nitrogenase activity (30–40 days after emergence).

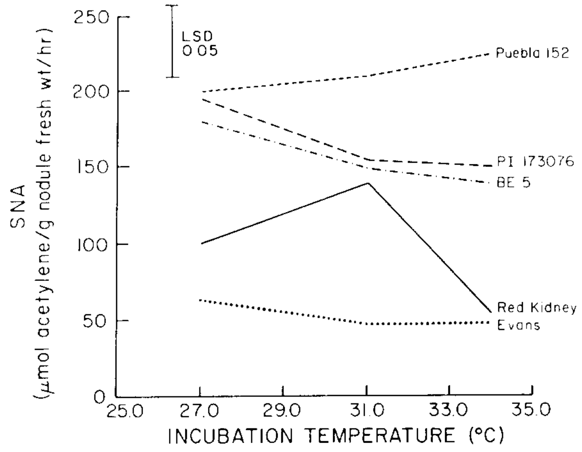

Fig. 6. Effect of incubation temperature (10 minutes) on specific nodule activity of nodules formed at 28°C.

(Figure 4). Bean plants exposed to the higher temperature had significantly lowered specific nodule activity and acetylene reduction although these measurements were made after allowing the root temperature to equilibrate to 30°C (Figure 5). Acetylene reduction by cowpea and soybean was not adversely affected after 10 days at the higher temperature.

Except for one common bean cultivar (Red Kidney), nodulated root segments of plants grown at 28°C had high acetylene reduction rates when incubated for 10 minutes at 35°C (Figure 6).

Experiment 4

Dry weight of all *Phaseolus vulgaris* genotypes was considerably reduced when symbiotic plants were grown at root temperatures of 33°C as compared with 27°C (Figure 7). A similar reduction was observed for two *Phaseolus vulgaris* × *Phaseolus acutifolius* crosses. Other Phaseolus species, like cowpea and soybean, were less sensitive to high root temperature than *Phaseolus vulgaris*.

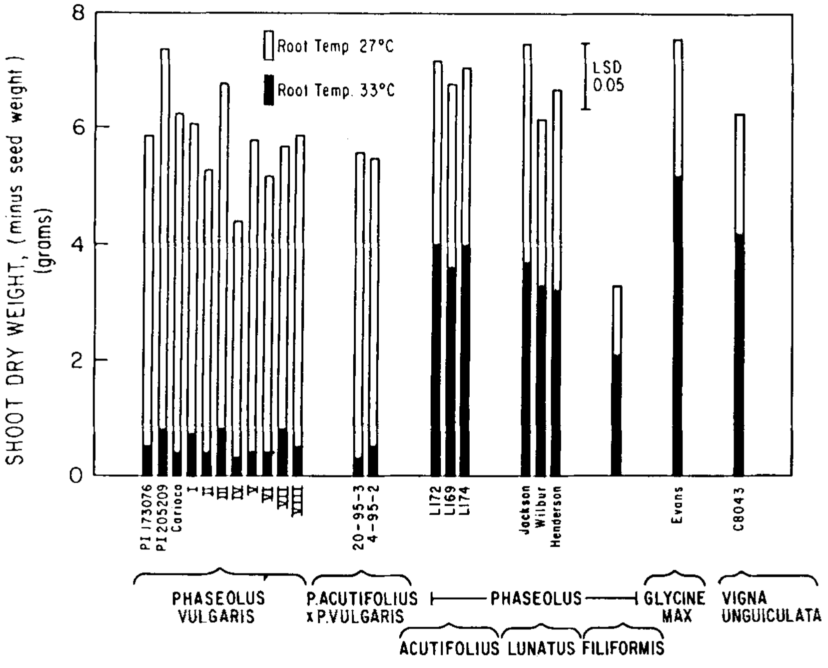

Fig. 7. Effect of rooting medium temperature on the growth of some symbiotic legumes.

Experiment 5

Cooling the soil by mulching did not improve the N-fixation capacity of common bean compared with that of cowpea (Table 1). Compared with a field experiment at the same location the previous year (13), there was no relative improvement in symbiotic beans, although air temperatures were much higher in 1984 than in 1985 (respectively 37.0 and 31.7°C mean daily maximum from time of nodulation to harvest at 56 days). Each irrigation reduced maximum soil temperatures significantly for 3 to 4 days (Figure 8). And canopy closure (day 30) lowered the

Table 1. Field comparison of nitrogen accumulation by inoculated and nitrogen fertilized legumes at three soil temperatures

Species (variety)	Shoot nitrogen accumulation 56 days after emergence (kg/ha)		
	1985 (moderate temperature)		1984 (high temperature)
	Mulched Inoculated/+N	Non-mulched Inoculated/+N	Non-mulched Inoculated/+N
Soybean	36/113	43/111	46/134
(Clark rji)	(32%)[a]	(39%)	(34%)
Common bean	77/133	78/129	86/111
(PI 173076)	(58%)	(60%)	(77%)
Cowpea	123/161	129/162	141/149
(C 8043)	(75%)	(80%)	(95%)

[a] Relative nitrogen accumulation (Inoculated/+N) × 100%.
L.S.D. (0.05) = 11 kg N/Ha.

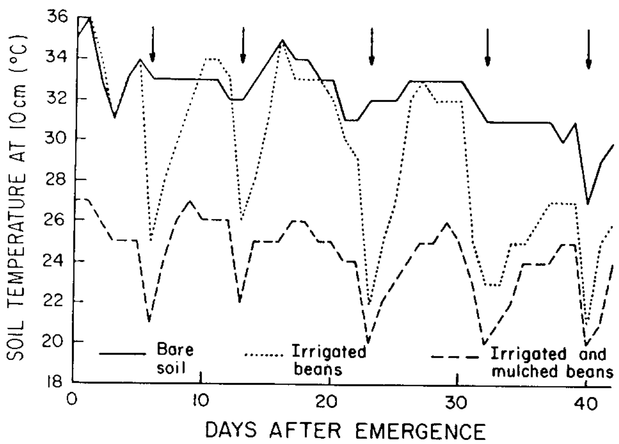

Fig. 8. Daily maximum soil temperature at 10 cm depth. (*Vertical arrows* indicate irrigations).

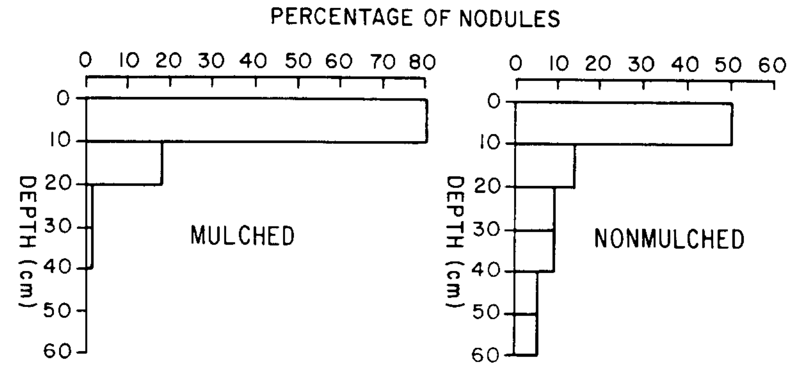

Fig. 9. Effect of mulch on nodule distribution of field grown *Phaseolus vulgaris*.

maximum soil temperature relative to bare soil. Mulching kept soil temperatures below 27°C at 10 cm. When the soil was mulched, nodules concentrated near the surface; in unmulched treatments nodules were present at greater depths (Figure 9).

Discussion

For the *P. vulgaris* symbiosis, high root temperature increased nodule number, but reduced nodule size and plant growth. Bean plants grown at the higher temperatures probably produced more nodules in response to low internal plant nitrogen[3]. Short term incubation at high temperature did not impair nodule function, but long term exposure to high root temperatures did not allow the nodules to develop or maintain normal function[11,15]. These same high root temperatures had no noticeable effect on nodule formation or function of symbiotic cowpeas; for soybeans they slightly impaired the performance of seedlings but not mature plants.

Despite the overall sensitivity of the common bean symbioses, there was significant strain variability in heat tolerance. CIAT 899 was clearly the most tolerant, and local isolates selected at high temperature were marginally more tolerant than a local strain selected at lower temperature.

Continual high temperatures were more detrimental to the symbioses than high day temperatures alternated with cooler night temperatures. Such a fluctuating temperature regime is likely to exist in the field, and should result in less heat-sensitivity than would be expected from greenhouse studies with continuous high temperatures. Beans fertilized with ammonium nitrate were not sensitive to the higher root temperatures, indicating that it is the symbiosis which is sensitive. Evaluation of N-fixation in common bean by comparison with fertilized plants, or with other grain legumes, may therefore be unfairly prejudiced if the temperature of the soil or greenhouse pots is high.

The use of rhizobia selected for tolerance to high soil temperatures may fail because of competition and poor survival of introduced strains in soils; but host differences in tolerance might be successfully exploited by plant breeding. The lack of significant tolerance to high soil temperatures in those *P. vulgaris* genotypes tested is therefore discouraging. However, all tested genotypes within the *P. acutifolius*, *P. filimormis* and *P. lunatus* species formed symbioses tolerant of high root temperatures. Interspecific crosses within the Phaseolus genus are possible, and *P. vulgaris* × *P. acutifolius* crosses are currently being used in a program to improve drought tolerance in common bean (B. Webster, personal communication). Although the two *P. vulgaris* × *P. acutifolius* crosses we evaluated performed poorly at high temperature, they had not been specifically selected for this characteristic, and other evidence suggests that such traits are inheritable[8].

In the field, the cool subsurface soil temperature and the plasticity of nodule distribution with depth should moderate the effect of high temperature on N-fixation. Despite surface temperatures severely detrimental to N-fixation, symbiotic perennial legumes may perform well in the field because of operating nodules in the cooler, deeper soil[10]. Nodule distribution in *P. vulgaris* may respond to variation in soil temperature soil moisture, as indicated by the effect of surface mulching (Figure 9). Thus, even though temperatures in the upper 10 cm of the soil exceeded harmful levels, cooling by mulching did not improve N-fixation. Also, the very hot weather during 1984 did not lead to performance inferior to that of the mild summer during 1985; nitrogen gain by symbiotic beans remained relatively inferior to cowpeas by a constant proportion. These facts suggest that high soil temperature did not limit nitrogen fixation by common bean in the field.

In the greenhouse, *P. vulgaris* usually maintained or increased the number of nodules at higher soil temperatures; the harmful effect was on nodule development. High surface soil temperatures in the field may result in bean plants with numerous small ineffective nodules in the upper surface, and healthy effective nodules in the cooler part. If the healthy nodules supply ample fixed nitrogen and the ineffective nodules consume little photosynthate, plant growth would decline only slightly at high temperature. Likewise when soybean was nodulated with a mixture of effective and ineffective strains[14], the ineffective rhizobia had little influence on N-fixation because effective nodules compensated by increasing their size and activity.

High soil temperatures could affect nitrogen fixation by common bean seriously if they stayed high for a long period. This might result from wide plant spacing, high air temperatures, or soil dryness. Effects of high temperature are likely to be more harmful when the survival and distribution of an introduced inoculum is critical. The nitrogen status of the soil may also be important due to its effect on early plant growth, nodule initiation and canopy closure.

References

1 Dart P J, Day J, Islam R, and Dobereiner J 1976 Symbiosis in tropical grain legumes: Some effects of temperature and the composition of the rooting medium. pp 361–383. *In* Symbiotic Nitrogen Fixation in Plants. Ed. P S Nutman. Cambridge University Press.

2 Dart P J and Mercer F V 1965 The effect of growth temperature, level of ammonium nitrate, and light intensity on the growth and nodulation of cowpea. Aust. J. Agric. Res. 16, 321–345.

3 Gibson A H 1977 The influence of the environment and managerial practices on the legume-rhizobium symbiosis. pp 393–450. *In* Treatise on Dinitrogen Fixation. IV. Agronomy and Ecology. Eds. R W F Hardy and A H Gibson. Wiley, N.Y.

4 Graham, P H 1981 Some problems on nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L.: A review. Field Crops Res. 4, 93–223.

5 Graham P H and Halliday J 1977 Inoculation and nitrogen fixation in the genus Phaseolus. pp 313–334. *In* Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. Eds. J M Vincent, A S Whitney and J Bose. Univ. Hawaii, Coll. Trop. Agric., Misc. Pub. 145.

6 Guss A and Dobereiner J 1972 effects of mineral nitrogen and soil temperature on nitrogen fixation of field beans (*Phaseolus vulgaris* L.). Pesq. Agropec. Bras. 7, 87–92.

7 Lie T A 1971 Symbiotic nitrogen fixation under stress conditions. Plant and Soil. Spec. Vol. 117–126.

8 Lie T A, Hille Ris Lambers D and Houwers A 1976 Symbiotic specialization in pea plants: Some environmental effects on nodulation and nitrogen fixation. pp 319–333. *In* Symbiotic Nitrogen Fixation in Plants. Ed. P S Nutman. IBP. Vol. 7. Camb. Univ. Press.

9 Munevar F and Wollum A G 1982 Response of soybean plants to high root temperature as affected by plant cultivar and rhizobium strain. Agron. J. 74, 138–142.

10 Munns D N, Fogle V W and Hallock B G 1977 Alfalfa root nodule distribution and inhibition of nitrogen fixation by heat. Agron. J. 69, 377–380.

11 Pankhurst C E 1973 Rhizobium strain influence on disruption of clover nodule development at high root temperature. J. Gen. Microb. 74, 219–231.

12 Pankhurst C E and Sprent J I 1976 Effects of temperature and oxygen tension on nitrogenase and respiratory activities of turgid and water stressed soybean and French bean root nodules. J. Expl. Bot. 27, 1–9.

13 Piha M I 1985 Ph.D. Thesis, University of California, Davis.

14 Singleton P W and Stockinger K R 1983 Compensation against ineffective nodulation in soybean. Crop Sci. 23, 69–72.

15 Sprent J I and Minchin F R 1984 Environmental effects on the physiology of nodulation and nitrogen fixation. pp 269–317. *In* Temperate Legumes: Physiology, Genetics and Nodulation. Eds. D G Jones and D R Davies. Pitman.