Effective *Bradyrhizobium* Nodulation of Peanut—Environmental Factors

Report for National Peanut Board Project

Michael San Francisco, Dept. of Biology, Texas Tech Univ., Lubbock, (806) 742-2706
Michelle Radtke, Dept. of Biology, Texas Tech Univ., Lubbock, (806) 742-2226
Calvin Trostle, Texas Cooperative Extension, Texas A&M—Lubbock, (806) 746-6101

Laboratory work with peanut soils across Texas of factors that affect the ability of *Bradyrhizobium* to nodulate and fix N for peanut production have been completed. These factors include soil pH; soil chemical constituents and the particular strain (commercial or experimental) of inoculum; and soil temperature. As a result of this work in addition to TPPB-funded work on Bradyrhizobium and nitrogen (Trostle), two commercial companies began testing of experimental inoculants and formulations for their suitability to West Texas soils.

Objectives:

1) Evaluate the soil, chemical, and environmental parameters unique to high pH West Texas soils for their impact on survivability and effective nodulation of *Bradyrhizobium* strains on different commercial inoculants for peanut;

2) Suggest research-based improvements in field management and application of *Bradyrhizobium* inoculants to enhance development of active nodules.

Project and Research Highlights, 2003:

Bradyrhizobium Research in General—Reports From Other Researchers

A research summary of other *Bradyrhizobium* work was completed. In general, it has been reported that native *Bradyrhizobium* strains capable of nodulating peanut are few and in such low numbers that little effective nodulation is expected without inoculation of peanut-specific *Bradyrhizobium* bacteria at planting. As a rule of thumb, it is believed that in order to achieve at least 50% of the nodules formed that inoculant bacterial counts should be at least 1000X of that native to the soil.

Soil temperatures of 86-95°F are likely to interfere with the development and function of root nodules, rhizobial activity, and may constitute a significant constraint to early growth of legumes in the southern U.S. The critical temperatures for N_2 fixation range between 95-104°F for peanut. The maximum survival temperature reported for *Bradyrhizobia japonicum* ranged from 93 to 120°F.

Most leguminous plants, including peanut, require a neutral or slightly acidic soil for growth. The pH sensitive stage in nodulation occurs early in the infection process and attachment to root hairs is one of the stages affected by acidic conditions. The most favorable soil pH for the growth of *Bradyrhizobia* appears to be much like that of the peanut plant, neutral to slightly acidic. As for acidic soils, soils alkaline in nature may also have critical effects on the survival of introduced *Bradyrhizobia*.

Three liquid Bradyrhizobium inoculant products were tested in this project including **(A)** Urbana Labs 'FrozenPrep,' **(B)** Nitragin 'Lift,' and **(C)** BeckerUnderwood 'HiStick.' In addition, three peanut production soils were tested in this project representing different soil properties. They included Gaines Co., TX (pH 7.7); Hockley Co., TX (pH 7.3); and Dawson, GA (pH 6.1).

In vitro Studies

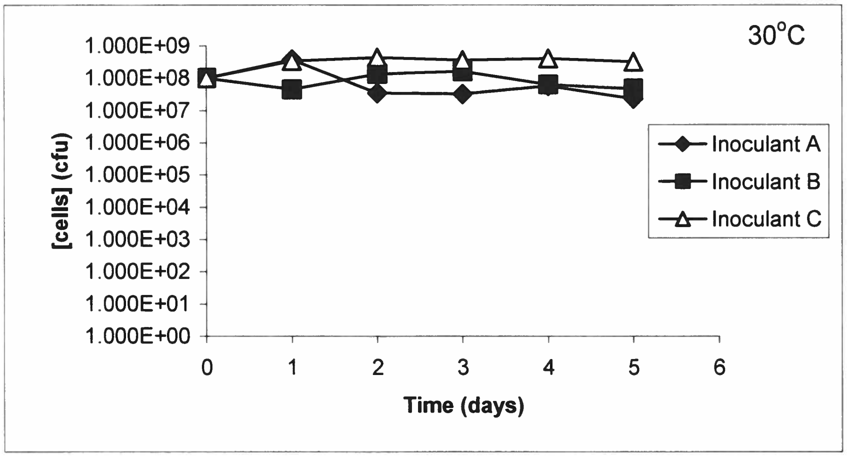
Temperature

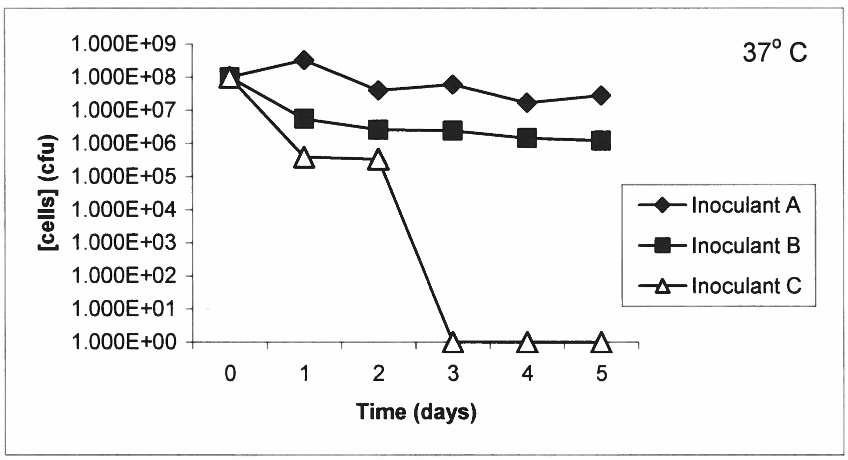
The differences in the ability of the inoculant strains to grow or survive in culture depended on whether the substrate was liquid or solid. Temperature 30° C (86° F) was used as a control temperature while temperatures 37°C (99° F) and 40° C (104° F) were chosen because W. Texas soil temperatures have been shown to be this high at the time of peanut planting. None of the strains tested tolerated or survived high temperatures when grown on solid media. This highlights the need to consider planting conditions and soil temperature at planting. When grown in liquid culture FrozenPrep survived at all temperatures, and showed no change in cell number (Figures 2, 3 and 4). Lift decreased in cell number at temperatures of 37°C and 40°C (Figures 3 and 4) except at 40°C where after 24 hrs there was no detectable survival for the remainder of the experiment (Figure 2). HiStick was temperature sensitive at 37°C and 40°C within 24 hours (Figures 2, 3 and 4).

pH

Effects of acidity on growth and survival of *Bradyrhizobia* have been reported using defined media, however the effects of alkaline conditions have been examined very little. All inoculants tolerated pH's 6, 7, and 8 at 30°C (Figure 4). However, when the temperature was increased there was a large difference between HiStick (C) and the other two inoculants as shown in Figures 2, 3 and 4. HiStick was most sensitive at all pH's when the temperature was 37°C and 40°C, except at pH 6 at 37°C where cell populations were reduced to non-detectable levels within 3 days (Figure 2). Lift (B) was markedly more sensitive that FrozenPrep (A) at all pH's when the temperature was 37°C or 40°C. Lift showed extreme sensitivity to 40°C at pH 6 with the culture not showing detectable levels within 1 day of the treatment (Figure 2). At the "control" temperature of 30°C, there was no difference in all three inoculants at pH 6 and 7 (Figures 2 and 3). However, at pH 8, inoculants B and C appeared to be slightly less tolerant (Figure 4).

Liquid inoculants; A-FrozenPrep; B-Lift; C-HiStick; pH 6





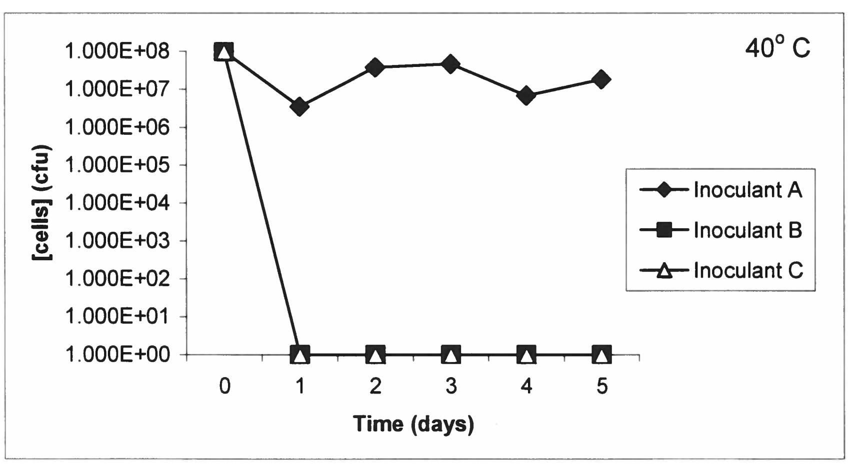
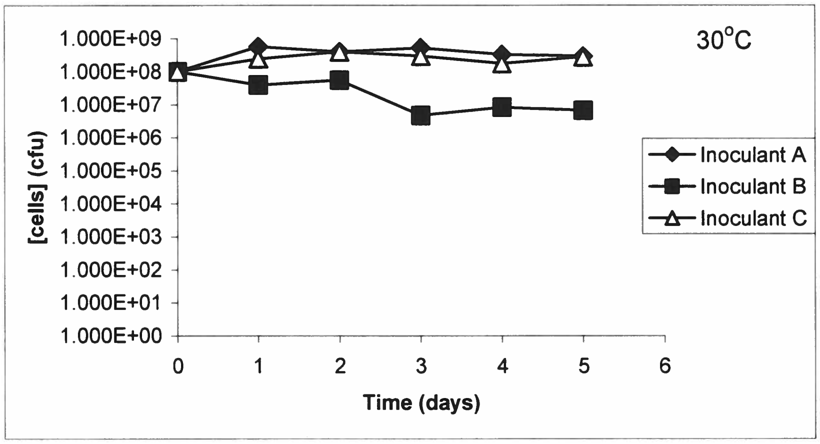
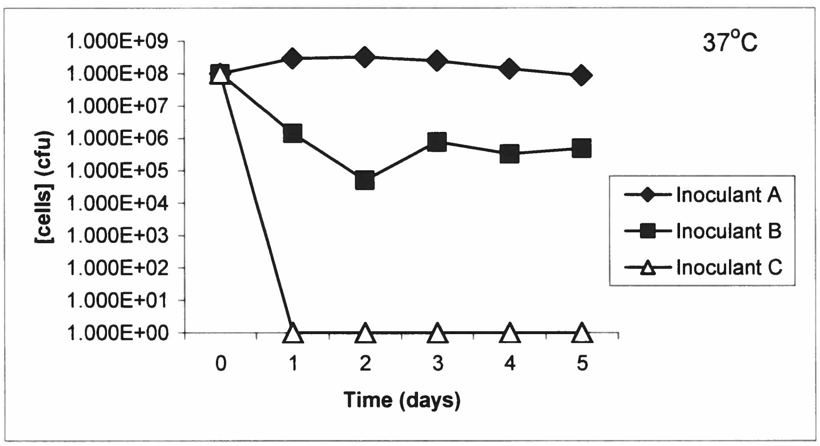


Figure 2. The effect of temperature at pH 6 on survival of bradyrhizobia. Bacterial survival was determined at indicated times in triplicate and the results are the means of two independent experiments.

Liquid inoculants; A-FrozenPrep; B-Lift; C-HiStick; pH 7





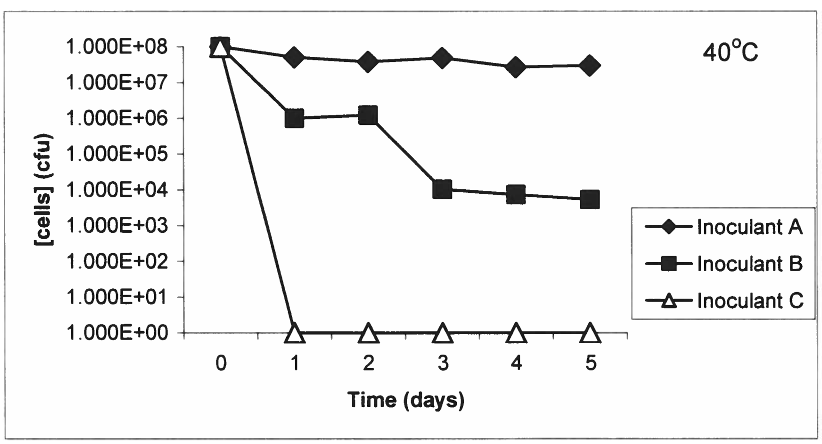
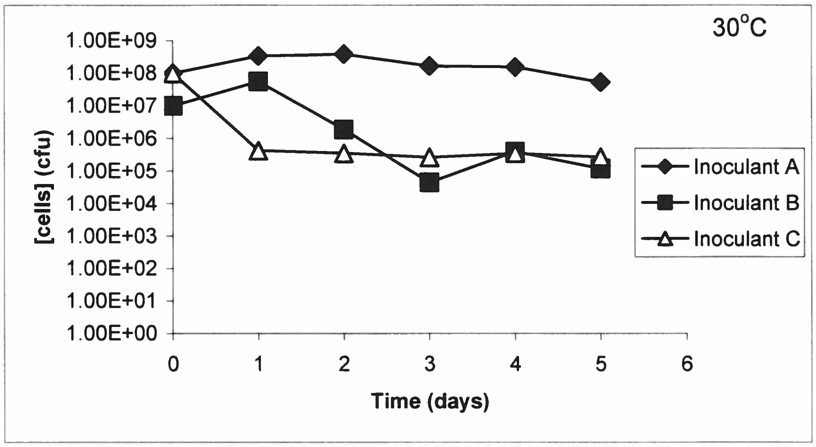
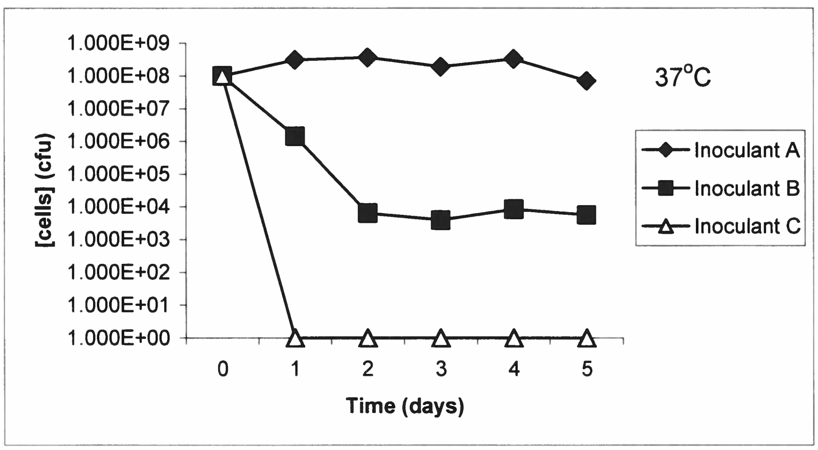


Figure 3. The effect of temperature at pH 7 on survival of bradyrhizobia. Bacterial survival was determined at indicated times in triplicate and the results are the means of two independent experiments.

Liquid inoculants; A-FrozenPrep; B-Lift; C-HiStick; pH 8





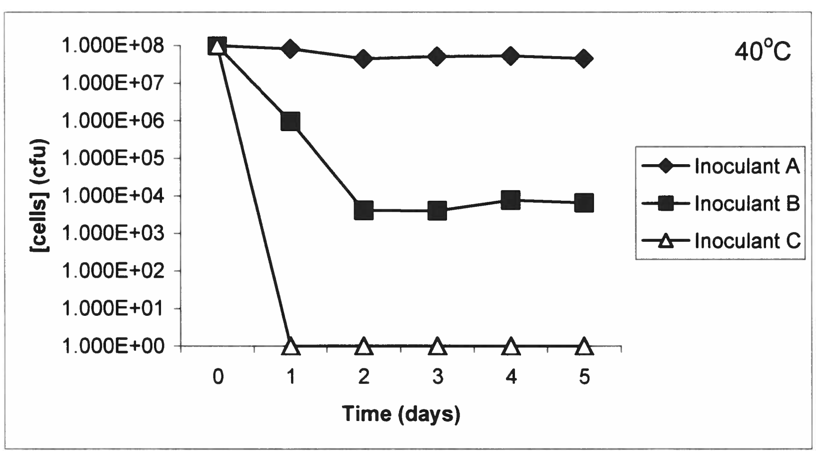


Figure 4. The effect of temperature at pH 8 on survival of bradyrhizobia. Bacterial survival was determined at indicated times in triplicate and the results are the means of two independent experiments.

pH

Survival in Soil

Most literature studies looked at low pH effects on *Bradyrhizobia* growth and to our knowledge this is the first high pH study on peanut *Bradyrhizobia*. Two diurnal temperatures of 25(10) C and 40(25) C were chosen to represent soil temperatures at the time of Texas peanut planting in April and May respectively. Although the inoculants differed in degree of tolerance to pH and temperature when in liquid media, all three inoculants are sensitive to high temperatures when placed in soil. When incubated at 25(10) all three inoculants survived up to 20 days (Figure 5). At 40(25), FrozenPrep survived 4 days, but was not able to tolerate the high temperature after a 10 day incubation period (Figure 6). Lift and HiStick did not survive after only a 24 hour exposure to the 40(25) temperature stress. Controls without inoculation in all cases did not have growth ruling out contamination issues.

Again these trials point toward producers needing to ensure that high temperatures must be minimized after planting enhance Bradyrhizobium survival from the inoculant in the soil.

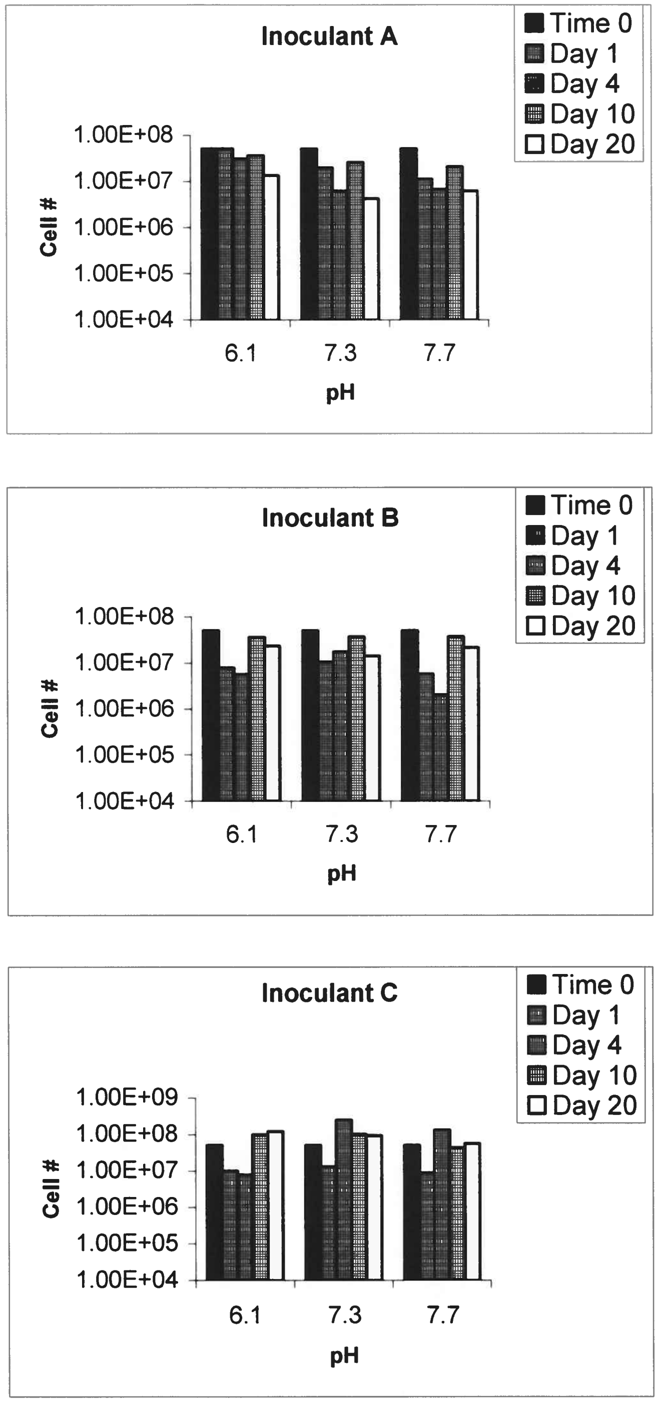


Figure 5. Survival of bradyrhizobia in soil at temperature 25(10).
The results are the means of two independent experiments.

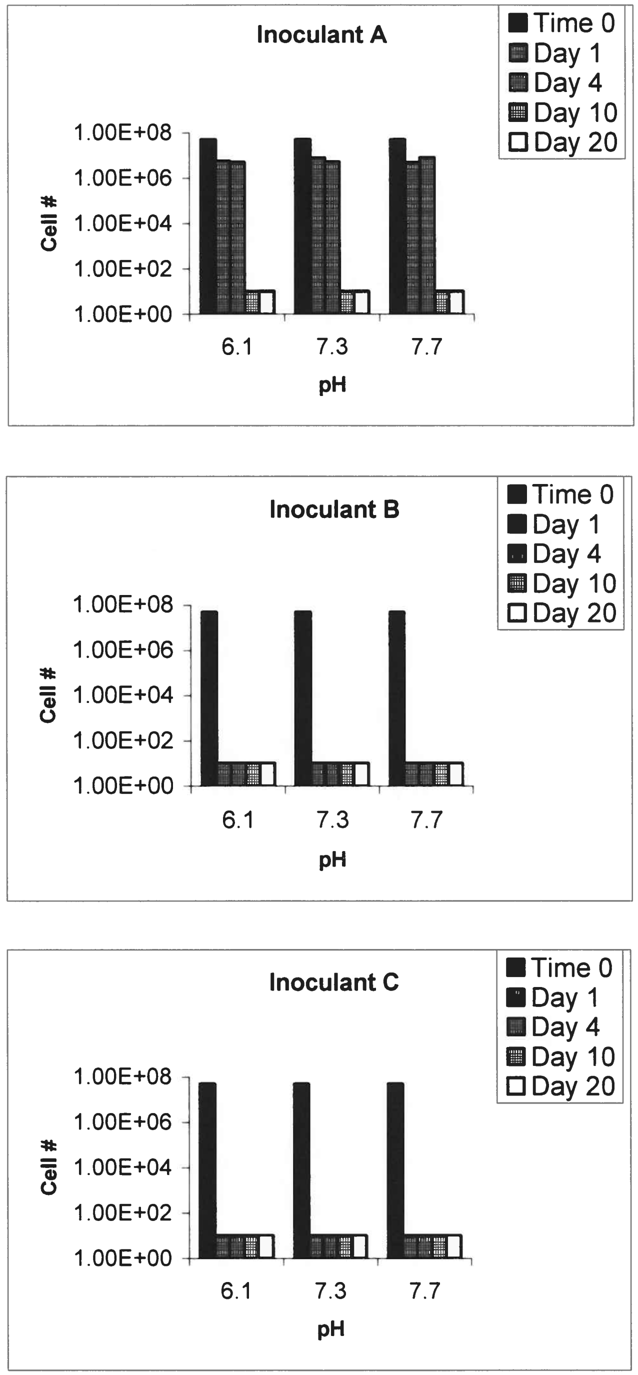


Figure 6. Survival of bradyrhizobia in soil at temperature 40(25).
The results are the means of two independent experiments.

Plant Studies

The effects on peanut nodulation of two temperature regimes were examined using the three commercial *Bradyrhizobia* peanut inoculants. Significant differences in inoculant, pH, temperature, pH X temperature and inoculant X temperature on nodulation were found (Table 4). Insignificant differences were seen in inoculant X pH suggesting that the pH's tested did not influence inoculant effectiveness.

At the 25(10) temperature cycling regime, pH 7.3 and 7.7 were more effective at supporting nodulation compared to pH 6.1. This may be attributed to the higher nitrate content of the pH 6.1 soil from Georgia.. Even moderate amounts of nitrate in the soil have been shown to inhibit root nodulation. At the 40(25) temperature cycling regime nodulation capability was dramatically reduced with all inoculants compared to the 25(10) regime. In spite of this, Lift (B) showed consistently higher nodulation. Effectiveness at all pH's tested showed HiStick to be the least effective. Overall elevated temperatures had a depressive effect on nodulation. Both studies found reduced nodulation and reduced plant growth. Observations that nodule number but not nodule mass were reduced indicates that the process of infection is possibly more adversely affected by high temperatures than the development of nodule mass. Even though there was a difference observed in nodulation between the two temperature regimes the plants were well nodulated compared to the controls. Inoculant A on average was more effective at temperature 25(10) and C was ineffective in nodule number observed. Cell numbers indicated inoculants B and C decreased over time while inoculant A numbers remained constant (data not shown). This may have reduced the infectiveness of the cells.

Plants under the temperature stress regime showed a decrease in shoot weight for all pH's tested, including those plants inoculated with inoculant B. There was also no clear link between ability to survive high temperature incubation and ability to nodulate under temperature stress. Inoculant C was not able to survive incubation temperatures of up to 40°C in in-vitro experiments, but was still able to nodulate under high temperatures. The fact that plant studies were performed under diurnal cycles may account for differences seen.

Overall Conclusions and a Thought for Farmers

It may be concluded that peanut-Bradyrhizobia symbiosis is affected by high root temperatures. The effect is due to failure of adequate nodulation and inability of the nodules to function at this temperature as seen by reduced shoot weight.

The sensitivity does not seem to be effected by the soil pH. At all pH's tested there was no significant difference found between pH and inoculant.

Constraints to nodulation in West Texas of peanut can be attributed to high soil temperatures at the time of planting. If conditions are ideal the peanut plant will show initial root growth in about 60 hours. The soil experiments demonstrated that after only 1 day the inoculants fell to undetectable levels with the exception of inoculant A, which took 4 days to reach undetectable levels. Our plant experiment showed adequate nodulation at all soil pH's tested at 25(10), indicating pH was not a factor. However, significant differences could be seen between the two temperature regimes on nodulation. At temperature 40(25) nodulation was decreased along with shoot weight. Our studies show high temperature rather than pH has a significant impact on *Bradyrhizobia* peanut inoculant survival and the ability to nodulate adequately.

Table 4. The effects of temperature on nodulation of peanut.

Temperature	Inoculant	Soil pH	No.of Nodules	Dry Shoot wt. (mg)
25 (10)	A	6.1	21.56	860
	B		12.89	551.1
	C		12.1	716
	Control		9.5	510
	A	7.3	47.5	620
	B		28.11	584.4
	C		34.37	502.5
	Control		5.6	548
	A	7.7	36	624.5
	B		41.29	693.3
	C		24.22	660
	Control		9.67	773.3
40 (25)	A	6.1	3.85	355
	B		8.78	301.7
	C		3.25	400.4
	Control		2	234
	A	7.3	7.45	361.8
	B		12.91	324.1
	C		1.71	386.4
	Control		4.14	545.7
	A	7.7	5.67	582.5
	B		10.83	465.8
	C		3.92	470.4
	Control		0.83	449.2

F-test significance	DF	F value	p value
Experiment	1	0.05	0.8171
Inoculant	6	4.33	0.0004
pH	4	2.93	0.0224
Temperature	2	23.07	<.0001
Inoculant x pH	12	1.73	0.0645
Inoculant x temp.	6	4.03	0.0008
pH x temp.	4	6.15	0.0001
Shoot wt.	2	10.11	<.0001