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Temperature Effects on *Bradyrhizobium* spp. Growth and Symbiotic Effectiveness with Pigeonpea and Cowpea

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ABSTRACT

Temperature is a limiting factor on legume-*Bradyrhizobium* symbiosis of subtropical plants in the temperate region. Twelve strains of *Bradyrhizobium* spp. that nodulate pigeonpea [*Cajanus cajan* (L.) Millsp], and cowpea [*Vigna unguiculata* (L.) Walp], were evaluated for tolerance to three temperature regimes (20°C/10°C, 30°C/20°C, and 38°C/25°C day/night temperature) by determining their growth following exposure to the regimes. The five most temperature-tolerant strains were further evaluated for symbiotic effectiveness with pigeonpea and cowpea under controlled temperatures. These strains were USDA 3278, USDA 3362, USDA 3364, USDA 3458, and USDA 3472. Plant heights of both crops were generally independent of *Bradyrhizobium* strains and were dependent mainly on temperature regimes. Plant heights were the shortest at the lowest temperature. At the lowest temperature regime, biological nitrogen (N) fixation by pigeonpea was almost completely inhibited. Cowpea genotype IT82E-16 inoculated with USDA 3458 formed the most effective symbiosis. The 30°C/20°C temperature regime was optimum for effective symbiotic association in both crops, and also for *Bradyrhizobium* survival.

Keywords: *Cajanus cajan*, nitrogenase activity, nitrogen fixation, temperature, *Vigna unguiculata*

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INTRODUCTION

Pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculatea*) are important food legume crops generally grown in tropical and subtropical areas of the world, and possess the capacity to fix large amount of nitrogen (N) in a process called biological nitrogen fixation (Kuykendall et al., 2000). In addition, growing pigeonpea and cowpea requires low input and minimal maintenance once crops are established. Like other legumes, these crops require suitable environmental conditions to allow their N-fixing potential to be fully realized. Of the environmental factors limiting legume-*Bradyrhizobium* associations, temperature is the most influential, especially in systems where tropical legumes are introduced into temperate regions (Munevar and Wollum, 1981). Temperature affects the legume-*Bradyrhizobium* symbiosis either directly, by limiting the growth of the microsymbiont and/or indirectly, by regulating the growth of the macrosymbiont (Hashem et al., 1998; Kuykendall et al., 2000). The findings of several other researchers support the regulatory effects of temperature on growth and survival of *Bradyrhizobium* strains in culture (Ahmad et al., 1981; Boonkerd and Weaver, 1982; Osa-Afiana and Alexander, 1982a, 1982b; Singh and Khurana, 1988; Pinto et al., 1998). However, all reported temperature tolerance variability among *Bradyrhizobium* and *Rhizobium* strains.

By regulating the growth of rhizobia in broth culture, temperature ultimately influences their symbiotic associations with host legumes. High temperatures above 35°C decrease nodule weight and number, nitrogenase activity, and shoot-dry matter production in soybean (Diatloff, 1970; Dart et al., 1975; Lindermann et al., 1974), pigeonpea (*Cajanus cajan*) (Dahiya et al., 1981), and leucena (Hashem et al., 1998). Like supraoptimal temperatures, suboptimum temperatures also have negative effects on the legume-*Bradyrhizobium* symbiosis (Dart and Mercer, 1964). Severe decline in cowpea nodulation at temperatures below 21°C have been reported (Dart and Mercer, 1964).

Low and high temperatures can greatly influence pigeonpea and cowpea-*Bradyrhizobium* symbioses. Since variability exists among *Bradyrhizobium* strains and crop genotypes in response to different temperatures (Pinto et al., 1998), it may be possible to identify genotype-strain combinations that can withstand unfavorable temperate limits and are associated with a fair degree of symbiotic efficiency. Such findings will be beneficial to tropical legumes, which experience a wide range of temperatures extending from the low chilling ones of early spring to the higher extremes in summer. Therefore, this study was conducted to examine the variability that exists among cowpea and pigeonpea *Bradyrhizobia* in response to various temperature regimes, and to identify *Bradyrhizobium* strains that are most effective in establishing efficient symbiosis with pigeonpea and cowpea in heat-stressed environments.

MATERIALS AND METHODS

Growth Conditions

All experiments were conducted at each of three temperature regimes: low, 20°C/10°C (day/night), which is typical of temperatures experienced during the early part of the growing season of cowpea and pigeonpea in mid-Missouri, USA (lat 38° 33′ N); optimum, 30°C/20°C; and high, 38°C/25°C, which simulates peak summer temperatures in this region. Controlled environments were provided by Conviron 3000 series growth chambers (Controlled Environment, Incorporation, Pembina, ND). Plants were exposed to 16 h photoperiods with light intensity of 220 μmol. m^{-2} s^{-1}. *Bradyrhizobium* cultures were exposed to the same day/night temperature regimes as plants, but were grown in the dark to simulate darkened conditions to which they would normally be exposed in soil.

Identification of Heat-Tolerant *Bradyrhizobium* spp. Strains

Twelve strains of *Bradyrhizobium* spp. known to nodulate both pigeonpea and cowpea were obtained from the *Rhizobium* Culture Collection of the U.S. Department of Agriculture Soybean Genetics and Improvement Laboratory, Beltsville, MD. These were the pigeonpea *Bradyrhizobium* strains USDA 3362, USDA 3364, USDA 3472, USDA 3559, and USDA 3562 and the cowpea *Bradyrhizobium* strains USDA 3275, USDA 3278, USDA 3280, USDA 3282, USDA 3285, USDA 3449, and USDA 3458. *Bradyrhizobium* strains were grown in extract yeast mannitol broth (YEMB) and on yeast extract mannitol agar (YEMA) (Vincent, 1970). The YEMB medium contained the following ingredients (g L^{-1}): 10.0 mannitol, 0.5 K_2HPO_4, 0.2 $MgSO_4$ .7 H_2O, 0.1 NaCL, and 0.5 yeast extract. Final pH was adjusted to 6.8 and the broth was autoclaved at 121°C for 20 min. *Bradyrhizobium* spp. strains were grown on orbital shakers in duplicate 125 mL Erlenmeyer flasks containing YEMB for 7 d at each test temperature regime. The direct total cell count (mL^{-1}) of the broth cultures was determined by making a 1:10 dilution ratio (1 mL culture to 10 mL sterilized DI H_2O) followed by a further 10% dilution. One drop of the 10% diluent was placed on the etched grid surface of a Petroff Hausser counting chamber (Hausser Scientific, Horsham, PA), evenly spread, then covered with a glass cover slip. The cells in nine randomly selected squares were enumerated with a 40× objective lens of a Nikon Fx-35 phase contrast microscope (Nikon, Inc., Garden City, NY). The formula described in Somasegaran and Hoben (1985) was used to determine the total cell counts per milliliter of suspension.

Screening of *Bradyrhizobium* strains on YEMA media was conducted by making serial dilutions of all 12 strains, and screening at each temperature regime using the method described in Somasegaran and Hoben (1985). A 0.1 mL aliquot from the sixth dilution in the series was spread on the surface of YEMA

plates containing Congo Red. The plates containing each *Bradyrhizobium* strain were divided into three groups, and each group was incubated at each test temperature regime for 7 d. Bacterial colonies were counted with a Fisher Accu-Lite colony counter (Fisher Scientific, St. Louis, MO). Based on total direct cell count and cell viability, the five strains most tolerant to the low and high temperature regimes were further examined for their symbiotic effectiveness on pigeonpea and cowpea.

Symbiotic Effectiveness

The symbiotic effectiveness of *Bradyrhizobium* strains USDA 3278, USDA 3362, USDA 3364, USDA 3458, and USDA 3472 selected from the temperature-screening study was evaluated on pigeonpea genotypes ICPL87 and ICPL8304, and cowpea genotypes Pinkeye Purple and IT82E-16. Seeds from these two early-maturing pigeonpea genotypes were selected from temperature-adaptation studies conducted by the senior author at Lincoln University, Jefferson City, MO (Marsh, 1994). Genotype ICPL87 was initially obtained from Caribbean Agriculture Research and Development Institute, Antigua, and genotype ICPL8304 from the University of Minnesota, MN. The two cowpea genotypes, the standard cultivar 'Pinkeye Purple Hull,' (PEPH) and genotype IT82E-16, were obtained from the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria.

Pigeonpea and cowpea seeds were surface-sterilized as described by Somasegaran and Hoben (1985). They were inoculated by soaking for 15 min in seven-day-old broth culture containing approximately 10^9 cells ml^{-1} of a *Bradyrhizobium* strain. Uninoculated control seeds were soaked in sterilized seven-day-old YEMB for 15 min. Seeds were planted in $51 \times 35.5 \times 9$ cm galvanized trays (Hummerts International, St. Louis, MO) containing a 1:1 sterile mixture of perlite and vermiculite. Seeds of each of the four plant genotypes inoculated with the same *Bradyrhizobium* strain were planted in rows in the same tray. Each row contained 10 seeds per genotype and was replicated three times in the same tray. Seedlings were later thinned to eight plants to reduce overcrowding. The plants were supplied with sterile de-ionized water until the emergence of the first trifoliate; thereafter, they were supplied with a half strength N-free Summerfield nutrient medium (Summerfield et al., 1977). Nutrients and water were supplied through narrow plastic tubing fitted onto a 20 L polystyrene bottle. Cardboard barriers were placed between the galvanized trays to reduce the incidence of cross-*Bradyrhizobium* contamination.

Forty-five days after planting, six plants per replication from each genotype-strain-temperature combination were excised at the base of the shoot, measured for shoot length (cm), and dried at 60°C for 2 d and weighed. Immediately following removal of the shoots, the roots were evaluated for acetylene (C_2H_2) reduction by placing those from individual plants in 125 ml Erlenmeyer

flasks sealed with leak-proof rubber sleeve stoppers. A 10 cc volume of air was removed from the sealed flask, which was immediately replaced with an equal volume of acetylene gas (pure grade) and incubated for 1 h at room temperature. Gas chromatography was performed on 0.5 mL gas samples with a Varian 3600 gas chromatograph (Instrument Group, Sugarland, TX), equipped with a hydrogen flame detector. Separation of the gas was obtained in a 30 m $\times$ 0.5 mm (length $\times$ internal diameter) megabore column (J&W Scientific, Folsom, CA). The column temperature was 60°C and the helium carrier gas-flow rate was 28.7cm sec^{-1}. The standard ethylene (C_2H_4) gas concentration was 1019 ppm. Chromatograph readings were taken as peak height, and nitrogenase activity was measured in μmol C_2H_4 $plant^{-1}$ $hour^{-1}$ and used as an indicator for the amount of N fixed during incubation. After the incubation of roots for acetylene-reduction activity, nodules were removed and counted. All experiments were replicated twice, and the data presented in each table represent the means of the two trials.

Statistical Analyses

Data from all experiments were analyzed using SAS (SAS Institute, Cary, NC) to run the Analyses of Variance (ANOVA). The least significant difference (LSD) tests were used for mean separation where appropriate.

RESULTS

Tolerance of *Bradyrhizobium* to Temperatures Ranges

The low and high temperature regimes significantly decreased cell numbers in the direct microscope counts of *Bradyrhizobium* strains examined, except USDA 3278, USDA 3275, USDA 3285, USDA 3458, and USDA 3362 (Table 1). Strains USDA 3458 and USDA 3362 had significantly higher counts at the low temperature range. The strain USDA 3458 was consistently one of the best growing strains in YEMB under all three temperature regimes. Under the 20°C/10°C temperature regime, strain USDA 3458 had significantly higher cell counts (9.3×10^9 mL^{-1}) than the other strain $\times$ temperature combinations. Strain USDA 3458 also provided the highest cell counts (along with strain USDA 3472) when they were grown at 30°C/20°C, while strain USDA 3275 gave the lowest count. Under the highest temperature regime (38°C/25°*C*), strains USDA 3278, USDA 3364, and USDA 3458 had similar cell counts, which were statistically superior to those of the other strains.

The high temperature regime was detrimental to the viability of *Bradyrhizobium* strains. No viable cells were found for the following five strains: USDA 3275, USDA 3280, USDA 3285, USDA 3449, and USDA 3559. Among the

Table 1
Effect of temperature on growth of *Bradyrhizobium* spp. strains based on direct microscope and plate viable cells counts ($\times 10^9$ mL)

Bradyrhizobium Strains	Direct microscopic counts Number of cells			Plate counts of viable cells Number of viable cells		
	20°C /10°C	30°C /20°C	38°C /25°C	20°C /10°C	30°C /20°C	38°C/25°C
USDA 3275	2.11	1.83	1.46	1.00	4.90	0
USDA 3278	2.17	3.10	3.06	8.30	22.70	0.90
USDA 3280	1.91	3.27	1.61	1.00	2.00	0
USDA 3282	2.13	2.69	1.33	0.80	8.60	0.60
USDA 3285	2.86	2.58	1.29	1.50	11.40	0
USDA 3362	4.07	2.71	2.01	3.60	12.80	0.70
USDA 3364	1.50	3.29	3.45	4.10	15.60	1.10
USDA 3449	2.57	3.18	0.70	1.30	2.70	0
USDA 3458	9.36	4.52	3.04	10.20	15.10	1.10
USDA 3472	2.64	4.46	2.30	17.80	20.50	2.70
USDA 3559	2.35	3.64	1.51	0.80	4.40	0
USDA 3562	1.50	3.98	1.85	1.70	13.10	0.60
LSD 0.05	0.50^Z			0.7^Y		

^{Z}Tests any two means in the columns for the direct microscopic counts of bacterial cells.

^{Y}Tests any two means in the columns for the viable cell counts of bacteria strains.

remaining seven strains that were grown at 38°C/25°C, USDA 3472 had a significantly higher number of viable cells. Across strains the 30°C/20°C temperature regime was optimal for growth, followed by the low temperature and finally by the high temperature regimes.

Symbiotic Effectiveness

Temperature, genotype, and *Bradyrhizobium* strains together significantly affected ($p \leq 0.05$) plant heights of pigeonpea (Table 2). Plants of pigeonpea genotype ICPL87 inoculated with strain USDA 3278 and grown at 38°C/25°C and genotype ICPL8304 inoculated with USDA 3458 and 3472 and grown at 30°C/20°C were significantly taller than the uninoculated control plants. Inoculation, however, did not improve plant height of pigeonpea plants grown at 20°C/10°C. Under the highest temperature regime, ICPL87 plants inoculated with strains USDA 3278 and USDA 3472 were taller than those inoculated with the other strains. Plants grown under the lowest temperature regime were approximately 0.46–0.68 as tall as those grown under the other two temperature regimes, respectively.

Table 2
Effect of temperature and *Bradyrhizobium* strains on height of 45-day-old pigeonpea and cowpea plants

Bradyrhizobium Strains	Plant height (cm)											
	Pigeonpea						Cowpea					
	ICPL 87			ICPL 8304			IT82E-16			'Pinkeye Purple Hull'		
	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C
USDA 3278	14.8	31.8	28.8	14.7	27.6	30.3	7.9	104.5	29.8	8.6	25.9	23.9
USDA 3362	13.2	25.9	25.8	16.5	24.1	29.6	8.8	102.2	23.5	7.8	30.5	20.8
USDA 3364	13.1	25.2	26.4	16.2	26.3	28.3	7.6	103.6	25.7	7.2	34.7	17.4
USDA 3458	16.3	31.8	26.2	16.6	33.2	28.5	8.3	111.8	21.1	7.6	26.9	19.1
USDA 3472	15.6	29.5	29.3	17.4	32.5	27.4	7.7	109.4	24.6	8.9	28.1	19.7
None	16.7	31.7	24.3	15.8	27.7	27.9	9.2	113.5	23.3	10.5	31.5	20.4
				LSD (0.05) = 3.7[Z]						LSD (0.05) = 11.7[Y]		

[Z]Tests any two means in rows and columns for pigeonpea.
[Y]Tests any two means in rows and columns for cowpea.

Within a temperature regime, *Bradyrhizobium* inoculation was ineffective in promoting cowpea plant height (Table 2). However, this trait differed significantly in its response to temperature for each of the *Bradyrhizobium* strains. Cowpea IT82E-16 plants were between 11 and 14 times taller when they were grown at 30°C/20°C than at 20°C/10°C.

Bradyrhizobium strains did not affect shoot dry weight of pigeonpea and cowpea plants grown under the 20°C/10°C or 38°C/25°C temperature regimes (Table 3). However, under the 30°C/20°C regime the cowpeas IT82E-16 inoculated with strains USDA 3362 and USDA 3364 had lower dry weights than the control plants and those plants inoculated with the other strains. For pigeonpea, the lowest plant height occurred with ICPL87 inoculated with strains USDA 3362 and USDA 3364 at 30°C/20°C, which coincided with the lowest shoot dry weight. Shoot dry weights for each cowpea genotype were low at 20°C/10°C and 38°C/25°C and were unaffected by *Bradyrhizobium* strains applied.

Nodulation of pigeonpea was the highest when plants were grown at 30°C/20°C and the lowest at 20°C/10°C (Table 4). Genotype ICPL87 at 30°C/20°C produced more nodules with strain USDA 3278 than with USDA 3472. Under the high temperature regime of 38°C/25°C, the numbers of nodules produced by ICPL 87 in association with USDA 3458 and USDA 3472 were comparable to those grown at 30°C/20°C. There was no difference among *Bradyrhizobium* strains in their symbiotic competence in nodulating the other pigeonpea genotype, ICPL 8304, at 38°C/25°C. When the plants of the two pigeonpea genotypes were grown at 30°C/20°C and individually inoculated with the five selected *Bradyrhizobium* strains, it was found that strains USDA 3362 and USDA 3364 were better microsymbionts with genotype ICPL 87 than with ICPL 8304. However, when plants were grown under the 20°C/10°C temperature regime, N fixation by both pigeonpea genotypes was almost completely inhibited (Table 5). At 38°C/25°C there were no significant differences among the *Bradyrhizobium* strains in the amount of N fixed with ICPL 87, although ICPL 8304 plants fixed higher amounts of N with strain USDA 3472 than with the other strains under this temperature regime.

Cowpea genotypes generally produced more nodules when they were grown at 30°C/20°C than under the other two temperature regimes (Table 4). At 20°C/10°C, cowpea genotype IT82E-16 in association with strain USDA 3458 produced similar or, in some cases, a higher number of nodules and fixed higher levels of N than the other temperature × genotype × strain combinations (Tables 4 and 5). Both cowpea genotypes symbiotically associated poorly with strains USDA 3362 and USDA 3364 at 20°C/10°C. However, their symbiotic responses were similar to the other *Bradyrhizobium* strains at 30°C/20°C.

Of all strains and cowpea genotype combinations, only USDA 3472× genotype PEPH was independent of temperature, producing the same number of nodules and fixing similar amounts of N under all three temperature regimes (Tables 4 and 5). *Bradyrhizobium* strain did not influence the number of nodules produced on IT82E-16 at 30°C/20°C or by both cowpea genotypes at

Table 3
Effect of temperature and *Bradyrhizobium* strain on shoot weight of 45-day-old pigeonpea and cowpea plants

Bradyrhizobium Strains	Shoot dry weight ($g \times 10^{-1}.pl^{-1}$)											
	Pigeonpea						Cowpea					
	ICPL 87			ICPL 8304			IT82E-16			'Pinkeye Purple Hull'		
	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C
USDA 3278	2.3	5.2	3.3	2.2	2.6	3.9	5.1	18.3	6.9	4.1	12.2	7.2
USDA 3362	1.9	2.7	2.6	2.5	1.8	3.9	5.2	12	5.7	3.6	15.9	3.7
USDA 3364	1.9	2.9	2.7	2.2	3.8	3.3	3.4	14.1	5.1	3.2	15.0	3.8
USDA 3458	2.5	4.5	3.3	2.5	2.0	3.4	5.8	19.8	4.8	3.1	16.9	3.8
USDA 3472	2.7	3.5	3.9	3.0	4.1	3.5	4.8	19.8	4.8	3.1	15.0	4.5
None	3.3	4.0	2.9	3.1	2.2	3.8	6.6	20.1	6.1	6.4	14.1	3.7
	LSD (0.05) = 1.5[Z]						LSD (0.05) = 3.5[Y]					

[Z]Tests any two means in rows and columns for pigeonpea.
[Y]Tests any two means in rows and columns for cowpea.

Table 4
Influence of temperature and *Bradyrhizobium* strains on the number of nodules produced by 45-day-old pigeonpea and cowpea plants

Bradyrhizobium Strains	Number of nodules plant^{-1}											
	Pigeonpea						*Cowpea*					
	ICPL 87			ICPL 8304			IT82E-16			'Pinkeye Purple Hull'		
	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C
USDA 3278	10	29	20	8	24	16	28	51	22	12	43	24
USDA 3364	13	24	13	6	14	16	6	42	22	6	31	21
USDA 3458	11	27	26	11	18	20	43	47	20	14	23	24
USDA 3362	11	26	12	6	16	15	12	49	21	8	54	18
USDA 3472	16	21	23	13	20	18	25	48	17	30	33	26
None	0[Z]	0	0	0	0	0	0	0	0	0	0	0
			LSD (0.05) = 7[Y]						LSD (0.05) = 9[X]			

[Z] 0 = No nodule.
[Y] Tests any two means in rows and columns for pigeonpea.
[X] Tests any two means in rows and columns for cowpeas.

Table 5

Nitrogenase activity by 45-day-old pigeonpea and cowpea plants inoculated with different *Bradyrhizobium* strains and grown at three temperature regimes

Bradyrhizobium Strains	μmoleC_2H_2 plant^{-1} hr^{-1}											
	Pigeonpea						Cowpea					
	ICPL 87			ICPL 8304			IT82E-16			'Pinkeye Purple Hull'		
	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C	20°C/10°C	30°C/20°C	38°C/25°C
USDA 3278	0.02	0.73	0.20	0.01	0.78	0.18	0.20	1.48	1.40	0.48	0.90	0.43
USDA 3364	0.01	0.15	0.15	0.01	0.10	0.23	0.14	1.19	0.68	0.19	0.98	0.19
USDA 3458	0.02	1.51	0.38	0.02	0.42	0.41	1.64	0.89	0.67	0.43	0.36	0.98
USDA 3362	0.02	0.13	0.11	0.02	0.08	0.39	0.17	1.02	0.49	0.21	1.17	0.89
USDA 3472	0.02	0.56	0.32	0.02	0.64	0.79	0.30	1.36	1.54	1.12	1.29	1.14
None	0[Z]	0	0	0	0	0	0	0	0	0	0	0
	LSD (0.05) = 0.31[Y]						LSD (0.05) = 0.62[X]					

[Z] No nitrogenase activity.

[Y] Tests any two means in rows and columns for pigeonpea.

[X] Tests any two means in rows and columns for cowpea.

38°C/25°C (Table 4). However, at 38°C/25°C, IT82E-16 fixed more N with USDA 3278 and USDA 3472 than with the other *Bradyrhizobium* strains (Table 5).

At 30°C/20°C, strains USDA 3364, USDA 3458, and USDA 3472 were more effective in nodulating cowpea genotype IT82E-16 than was genotype PEPH. At 38°C/25°C, only strain USDA 3278 was symbiotically associated better with IT82E-16 than with PEPH (Table 5).

DISCUSSION

This study identified and characterized some agronomically important *Bradyrhizobium* strains that effectively nodulate and fix N with pigeonpea and cowpea under heat stress conditions. These strains varied significantly in their ability to grow at elevated temperatures and their effectiveness with pigeonpea and cowpea. The present study indicates that the ability of *Bradyrhizobium* strains to grow profusely in liquid media at certain temperatures is an indication of tolerance to elevated temperatures; this finding was previously documented by Hashem et al. (1998). It is, however, not always the best criterion to use in selecting strains for temperature tolerance (Munevar and Wollum, 1982). Judging *Bradyrhizobium* strains based on their cell viability from suspension plated onto a media surface is generally more accurate and ultimately more reliable than microscope counts; this principle was also documented by Somasegaran and Hoben (1994). It is possible that the two measures may have some association in the present study, thus leading to the selection and use of the easier, more accurate, and faster procedure.

The very low viable cell counts for most of the strains grown on solid media under the highest temperature regime were likely due to their inability to withstand such high temperature levels, resulting in total cell mortality. However, fair growth of these strains under the 30°C/20°C temperature regime indicates that their maximum permissible temperature is above 30°C/20°C but below 38°C/25°C. This result supports the findings of Munevar and Wollum (1982), who demonstrated that soybean *Bradyrhizobium* strains were very sensitive to temperatures above 33°C. Furthermore, Hashem et al. (1998) reported that the symbiotic performance of leucena *Rhizobium* strains correlates clearly with the ability of these strains to grow in culture media at such temperatures. However, LaFavre and Eaglesham (1986) indicated that there was no correlation between the ability of a *Rhizobium* strain to grow on an agar growth medium at high temperature and its symbiotic effectiveness at the same temperature.

The 12 *Bradyrhizobium* strains tested showed a great degree of variability in their growth responses to elevated temperature. The strain having the highest cell count in broth culture at a particular temperature did not necessarily produce the highest number of viable cells using the plate counts. For instance, at 20°C/10°C, strain USDA 3458 had the highest total cell counts,

while USDA 3472 had the highest viable cell counts. This variability may be due to the strains' inherent temperature adaptation qualities, as indicated by Somasegaran and Hoben (1994). Based on cell viability, the order of tolerance among the five best strains were USDA 3278 > USDA 3472 > USDA 3364 > USDA 3458 > USDA 3362 at 30°C/20°C, and USDA 3472 > USDA 3458 > USDA 3278 > USDA 3364 > USDA 3362 at 20°C/10°C. This result shows that the strains producing the most viable cells were common to the optimum and lower temperature regimes. Although individual strains had differing numbers of viable cells under the three temperature regimes, the results indicate that the 30°C/20°C temperature regime was optimum for all of the strains.

The fact that no single strain was dominant under all of the three temperatures regimes provides further evidence for the variability that exists among *Bradyrhizobium* strains in response to different temperatures, a phenomenon that had been observed earlier by several researchers (Ishizawa, 1953; Bowen and Kennedy, 1959; Marshall, 1964; Parker et al., 1977; Day et al., 1978). In terms of both total cell count and cell viability, strains USDA 3278, USDA 3362, USDA 3364, USDA 3472, and USDA 3458 displayed the highest level of tolerance to temperature, particularly to the low and high temperatures, which were of major interest in this study. As stated by Munevar and Wollum (1981), differences in symbiotic responses due to temperature and strain interactions seem to be related to the different temperature characteristics of the strains as measured in pure culture studies. Therefore, the criteria used in this study to characterize the behavior of the strains at different temperatures will have some ecological significance. The ability of a *Bradyrhizobium* to withstand elevated temperatures is largely affected by temperature. At higher cell numbers, mutations may occur at elevated temperatures and nodulate the legume more effectively (Somasegaran and Hoben, 1994). Based on pure culture screening, it was possible to identify strains that were tolerant of high or low temperatures.

In the second phase of the study, the ability of the selected strains to perform as microsymbionts with pigeonpea and cowpea at temperatures to which they showed superior tolerance in pure culture was evaluated. The low temperature had severe inhibitory effects on the growth of cowpea and pigeonpea plants. This inhibition may be due in part to later germination and delayed emergence of seedlings, which were observed during the course of this study (data not shown). Such adverse effects of suboptimal temperatures on growth have been well documented in soybean (*Glycine max*) (Munevar and Wollum, 1981) and in pea (*Pisum sativum*) (Lie, 1971).

The inhibition of N fixation by both pigeonpea genotypes at the suboptimum temperature may be due to the ineffectiveness of the strains, the suppression of nitrogenase activity in the nodules, or a combination of the two. Similar observations have been made in *Phaseolus vulgaris* L. (Thomas and Sprent, 1984) and in soybean (Schweitzer and Harper, 1980). However, the effective symbiosis between *Bradyrhizobium* strain USDA 3458 and cowpea IT82E-16 at 20°C/10°C is proof of the existence of crop genotype and bacterial

strain combinations that are capable of withstanding suboptimum temperatures and functioning with a fair degree of effectiveness.

Contrary to the findings of Dudeja and Khurana (1989), the pigeonpea genotypes used in this study were nodulated at temperatures below 28°C and above 37°C. However, differences in the environmental conditions, crop varieties, and the type of strain used in this study may explain the differences between these two studies. The reduction in nodule numbers at 38°C/25°C compared with 30°C/20°C for some of the strains in this study is in agreement with the findings of Dahiya et al. (1981) for pigeonpea grown at elevated temperatures.

Overall, the 30°C/20°C regime appeared to be optimum for most of the strain × genotype interactions. Based on the nodulation and N-fixation results for both crops, USDA strains 3278, 3458, and 3472 were most effective overall of the five strains as microsymbionts of pigeonpea and cowpea at the three temperatures.

This study also shows that the performance of most of *Bradyrhizobium* strains as microsymbionts of pigeonpea and cowpea at the different temperature regimes is related to their response to elevated temperature in pure culture, and this finding is in agreement with the findings of Hashem et al. (1998). Therefore, pure culture evaluation can be a good preliminary testing procedure to identify strains that are tolerant of sub- or supraoptimal temperatures as potential microsymbionts of pigeonpea and cowpea. This study suggests that it may be possible to select heat-tolerant *Bradyrhizobium* strains that effectively nodulate pigeonpea and cowpea grown in adverse environments where temperature constraints prevail.

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