PN-AAQ-439

Reprinted from

Canadian Journal of Microbiology

Réimpression du

Journal canadien de microbiologie

The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage, and distribution

P. SOMASEGARAN, V. G. REYES, AND H. J. HOBEN

Volume 30 • Number 1 • 1984

Pages 23–30

National Research Council Canada
Conseil national de recherches Canada

Printed in Canada by K.G. Campbell Corporation

The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage, and distribution

P. SOMASEGARAN,[1] V. G. REYES, AND H. J. HOBEN

NifTAL Project, Department of Agronomy and Soil Science, University of Hawaii, Honolulu, HI, U.S.A. 96822

Accepted September 23, 1983

SOMASEGARAN, P., V. G. REYES, and H. J. HOBEN. 1984. The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage, and distribution. Can. J. Microbiol. **29**: 23–30.

Gamma-irradiated peat was used to prepare inoculants for 10 different species of tropical legumes. These inoculants were sent to cooperators in 14 cities in 13 tropical countries. Each cooperator received a package containing a maximum recording thermometer, plating-media components, special instruction sheets, and inoculants. Control experiments were pursued in the laboratory by exposing the various inoculants to 28, 37, and 46°C. Temperatures reached in the inoculants during their transportation varied from 26 (Mexico) to 45°C (Kenya). Arrival time of the inoculants ranged from 6 days (Hissar, India) to 54 days (St. Augustine, West Indies). Although a total loss of viability was reported for the chick-pea inoculant (Saudia Arabia) and a severe decrease in two others (bean and lentil inoculants in Kenya and Saudi Arabia, respectively), over 90% of the inoculants received had viable counts in excess of 1×10^8 cells per gram of moist peat. Laboratory data indicated that 28°C was optimal for multiplication to maximal numbers in excess of 1×10^{10} cells per gram of moist peat. Six inoculants studied for long-term storage showed excellent quality for 24 weeks at 28°C. At 37°C the cell multiplication was comparable with that at 28°C with 8 of the 10 strains only during the 1st week. For all strains, 46°C was lethal. Large reductions in viable counts were observed during inoculant preparation when broth cultures were added to peat.

SOMASEGARAN, P., V. G. REYES et H. J. HOBEN. 1984. The influence of high temperatures on the growth and survival of *Rhizobium* spp. in peat inoculants during preparation, storage, and distribution. Can. J. Microbiol. **29**: 23–30.

De la tourbe traitée par rayons gamma a été utilisée pour la préparation d'inoculants devant être appliqués à 10 espèces différentes de légumineuses tropicales. Ces inoculants furent expédiés à des collaborateurs de 14 cités réparties dans 13 pays tropicaux. Chaque collaborateur a reçu un colis contenant en thermometre enrégistreur en continu, les composants nécessaires aux milieux de culture, des feuillet d'instructions spéciales et les inoculants. Les expériences-témoin ont été conduites en laboratoire en exposant les divers inoculants à 28, 37 et 46°C. Au cours du transport, la température atteinte par les inoculants a varié de 26 (Mexico) à 45°C (Kenya). La durée du transport des inoculants a varié de 6 jours (Hissar, Indes) à 54 jours (St-Augustin, Antilles). Bien qu'une perte totale de viabilité ait été rapportée pour les inoculants de pois chiche (Arabie Saoudite) et une sévère diminution chez deux autres (inoculants des fèves et des lentilles au Kenia et en Arabie Saoudite, respectivement), plus de 90% des inoculants reçus avaient des comptes de viabilité excédant 1×10^8 cellules par gramme de tourbe humide. Les données en laboratoire indiquent qu'une température de 28°C a été optimale pour une multiplication maximale en nombres, excédant 1×10^{10} cellules par gramme de tourbe humide. Six inoculants étudiés sur la base d'un entreposage à long terme ont maintenu une excellente qualité durant 24 semaines à 28°C. A 37°C, la multiplication cellulaire était comparable à celle obtenus à 28°C chez 8 des 10 souches, mais seulement au cours de la 1re semaine. Pour toutes les souches, la température de 46°C a été létale. D'importantes réductions de viabilité des comptes ont été notées au cours de la préparation des inoculants, lorsque les bouillons de culture étaient ajoutés à la tourbe.

[Traduit par le journal]

Introduction

Legume seed inoculation with high quality peat inoculants containing effective and compatible strains of *Rhizobium* can increase yield of legumes. One of the major constraints limiting maximal benefits from biological nitrogen fixation for farmers in the tropics is the availability of high quality inoculants. Usable inoculants must contain high numbers of rhizobia effective in fixing nitrogen with the intended host. Inoculant quality standards vary in different countries, but where such a standard exists, e.g., Australia, inoculants produced from presterilized peat must contain 1.0×10^9 rhizobia $\cdot g^{-1}$ at manufacture and $1.0 \times 10^8 \cdot g^{-1}$ at expiry (Roughley and Pulsford 1982). Under tropical conditions, high temperatures may adversely affect the survival of rhizobia in the packaged inoculant and also on the inoculated seed in the field. Much of the detrimental effects of high temperature may be experienced by the inoculant during storage and distribution since adequate low-temperature facilities are not easily available in the tropics. Inoculants may be exposed to ambient temperatures ranging from 20 to 45°C for varying lengths of time (Ayanaba 1977).

Evaluating *Rhizobium* survival in peat inoculants exposed to high temperatures may be considered as the first stage in identifying potential inoculant strains that would withstand temperature stress on the inoculated seed in the field soil. In tropical and subtropical areas, surface soil temperatures between 40 and 60°C have

[1]Author to whom all correspondence should be addressed.

TABLE 1. Source of *Rhizobium* cultures

Species	Designation	Host legume
Rhizobium sp.	TAL 1000	*Arachis hypogaea* (peanut)*
	TAL 437	*Vigna radiata* (mung bean)
	TAL 999	*Vigna unguiculata* (cowpea)
	TAL 309 (CB 756)†	*Macrotyloma africanum*
	TAL 169 (Nit. 176A22)	*Vigna unguiculata* (cowpea)
	TAL 255	*Cajanus cajan* (pigeon pea)
	TAL 82	*Leucaena leucocephala*
	TAL 22	*Phaseolus lunatus* (lima bean)
	TAL 620 (ICIRSAT 3889)	*Cicer arietinum* (chick-pea)
R. japonicum	TAL 379 (USDA 136b; CB 1809)	*Glycine max* (soybean)
R. phaseoli	TAL 182	*Phaseolus vulgaris* (bean)
R. leguminosarum	TAL 634 (Nit. 92A3)	*Lens culinaris* (lentil)

*Names in parentheses are common names of host legume.

†Parentheses indicates other designation(s) for the same strain: TAL, NifTAL Project, University of Hawaii, Honolulu; Nit., Nitragin Co., Milwaukee, WI; CB, Commonwealth Scientific and Industrial Research Organization, Brisbane, Australia; ICRISAT, International Crop Research Institute for the Semi-Arid Tropics, Hyderabad, India; USDA, U.S. Department of Agriculature, Beltsville, MD.

been reported (Chatel and Parker 1973; Day *et al.* 1978; Diatloff 1970; Philpotts 1967). Selecting tolerant strains of rhizobia to withstand temperature stress during inoculant preparation should be considered, since the heat of wetting killed many of the added rhizobia during the mixing of the peat with the broth culture (Roughley and Vincent 1967).

The influence of prolonged high storage temperatures has been documented in inoculants of *R. japonicum* (Burton 1975; Iswaran et. al. 1969), *R. leguminosarum* (Bajpai *et al.* 1978), a cowpea *Rhizobium* (Roughley 1968), and for a mixture of cowpea rhizobia in commercial inoculants (Wilson and Trang 1980). There is a lack of information on the survival of tropical rhizobia from diverse legume species. Besides, published information on storage studies did not indicate attempts to measure temperature reached in the packaged inoculant during actual distribution (transportation) and also evaluate inoculant quality upon arrival at the destination to make laboratory studies more meaningful.

The objectives of this investigation were to evaluate, amongst tropical rhizobia, the effect of the initial contact of the broth culture with the peat during inoculant preparation, to determine the viability of the rhizobia in the inoculants after distribution to various tropical countries, and to study the influence of short- and long-term exposures of high temperatures on the survival of rhizobia in the peat inoculants.

Materials and methods

Source of rhizobia

The rhizobia (Table 1) used in this study were obtained from the *Rhizobium* germplasm resource at the University of Hawaii, NifTAL Project. Pure cultures of the rhizobia were maintained on ceramic beads (Norris 1963) and broth cultures were initiated by inoculating two ceramic beads into liquid yeast mannitol medium (Vincent 1970) followed by agitation on a rotary shaker.

Peat

Gamma-irradiated (5 Mrad (1 rad = 10 mGy)) peat, prepackaged in 40-g quantities in polyethylene bags (16.5 cm × 11.5 cm × 0.003 cm), was purchased from Agricultural Labs., Regents Park, Australia. The moisture content of the peat was 8% and pH 6.5–6.8. Part of the quantity of the peat was repackaged in smaller polyethylene bags containing 10 g peat per bag. The bags were heat-sealed and reirradiated at 5 Mrad.

Preparation of the inoculant

Peat inoculants of all the strains shown in Table 1 were prepared as follows. Log-phase broth cultures with viable counts 1.0×10^9 cells/mL were obtained in yeast mannitol medium. Broth cultures were introduced aseptically into each bag of peat with a sterile 50-mL plastic syringe (Bekton–Dickinson, Rutherford, NJ) fitted with a 18-gauge, 1.5-inch (1 in = 25.4 mm) needle. Each bag was swabbed with 70% (v/v) ethanol before injecting the broth culture. The puncture was immediately sealed with a R-1212, 0.75 inch diameter self-adhesive label (Avery Label Company, Azusa, CA). Each bag was kneaded thoroughly to ensure even distribution and proper mixing of the broth culture with the peat.

For the short-term growth and survival experiments at 28, 37, and 46°C, the smaller bags (10 g dry peat/bag) were injected with 9 mL of the broth culture to give approximately 50% moisture content (wet weight basis). Following this experiment, long-term storage was conducted in the large bags (40 g dry peat/bag) which were injected with 36 mL of the broth culture to give a similar moisture content as the smaller bags. An incubation temperature of 28°C was used in the long-term storage experiment since this temperature proved the best of three in the short-term experiment.

TABLE 2. Viable counts of rhizobia in the broth culture and after its initial contact with the peat

Species	Designation	$\log_{10}$ no. of rhizobia In broth (mL)	In peat (g moist peat)
Rhizobium sp.	TAL 22	9.14	8.81(8.84)*
	TAL 82	9.15	8.46(8.85)
	TAL 169	9.37	8.62(9.07)
R. phaseoli	TAL 182	8.56	7.74(8.26)
Rhizobium sp.	TAL 309	9.15	8.66(8.85)
R. japonicum	TAL 379	9.43	8.78(9.13)
Rhizobium sp.	TAL 437	9.20	8.62(8.89)
	TAL 620	9.25	8.63(8.95)
	TAL 999	9.39	8.51(9.08)
	TAL 1000	9.31	8.78(9.00)

*Figures in parentheses (expected viable counts) represent 50% of the orginal broth population. Correlation between the observed and expected viable count was significant ($r = 0.85$, $p = 0.01$). Observed and expected viable counts were significantly different (t-test; $p < 0.001$).

Temperature measurements and quality evaluation during inoculant distribution

Microbiologists from 13 countries (Table 4) were invited to cooperate in evaluating the quality of the inoculants upon arrival at their respective destinations. Each cooperator was sent materials in a polystyrene box (Freez-Safe-Thermo-Safe, manufactured by Polyfoam Packer Corp., Chicago, IL) which contained (*i*) duplicate bags (19.0 g moist peat/bag) of the inoculants. Choice of the inoculants sent were based on the legume of economic importance cultivated in the cooperator's country. Cooperators received inoculants for more than one legume in some cases. Inoculant bags were airmailed within 24 h after preparation without prior incubation in the laboratory. Also contained in the box were (*ii*) a maximum registering thermometer (−15 to 105°C, 5.5 inch long, VWR Scientific Inc., San Francisco) and (*iii*) yeast mannitol agar ingredients good for 500 mL of the medium. The ingredients were packaged in three bags, appropriately labeled, and heat-sealed. Bag No. 1 contained a mixture which consisted of mannitol, 5 g; K_2HPO_4, 0.25 g; NaCl, 0.05 g; and $MgSO_4 \cdot 7H_2O$, 0.1 g. Bag No. 2 contained Difco agar (Difco Laboratories, Detroit, MI), 7.5 g. Bag No. 3 which contained 0.25 g Bacto yeast extract was placed inside bag No. 2. One bag of silica gel (8−10 g) was then placed in each of bags No. 1 and No. 2 to prevent moisture damage to the ingredients during distribution. A set of complete instructions which specified various procedures and precautions were included. Some of the important instructions related to the proper method for reading the maximum recording thermometer; storage of the inoculants at refrigerated temperature upon arrival until enumeration; preparation and sterilization of the yeast mannitol agar medium from the ingredients sent; and preparation of the materials (dilution vessels and pipettes) for performing the serial dilutions of the inoculant samples for plate counts. A blank data sheet was sent for recording the data.

Sampling and enumeration of rhizobia

At the author's laboratory, triplicate bags were sampled for each temperature treatment. A 10-g sample from each small bag was aseptically transferred into 90 mL of quarter-strength yeast mannitol broth, shaken for 15 min with a wrist-action shaker, and subjected to the standard 10-fold dilution series. The drop-plate method (Miles and Misra 1938; Vincent 1970; Hoben and Somasegaran 1982) was used for enumerating the viability of the rhizobia on yeast mannitol medium containing Congo Red (25 mg/L). In the long-term storage experiments, 1-g samples of inculants from each of the triplicate bags were removed aseptically, shaken, and serially diluted for plating as described above.

Cooperators made similar viable counts using the drop-plate method, but only on duplicate bags for each strain.

Results

Marked differences in the viable counts between the broth culture and the freshly prepared inoculant were expected when plating was done on the same day (Table 2). This was because the additions of the broth cultures to the peat were 9 mL to 10 g (small bags) and 36 mL to 40 g (large bags), respectively, a dilution of 50% was computed for the added broth culture. This dilution was based on the total volume of moisture added (broth culture plus the moisture from the peat), the number of cells per millilitre of culture, and the given weight of peat. Based on this computation, viable counts equivalent to half of that obtained in the broth culture were expected. This was confirmed by the good correlation ($r = 0.85$, $p = 0.01$) obtained between observed viable counts in the peat and the expected. However, analysis (t-test) of the data of the observed and expected viable counts was highly significant ($p < 0.001$).

Growth and survival of the rhizobia under prolonged exposure to high temperatures is shown in Fig. 1. All strains (except TAL 620) could multiply at two high temperatures (28 and 37°C) but not at 46°C. The most favorable temperature for growth and survival for all strains was 28°C. At 37°C, all strains (except TAL 169 and 620) attained ceiling populations in excess of 1×10^9 cells at the 1st week. However, there was great variation in the abilities of the rhizobia to sustain the high populations at this high temperature to periods beyond the 1st week. The peanut (TAL 1000), bean (TAL 182), and the leucaena rhizobia (TAL 82) showed the best tolerance to 37°C over the other rhizobia as their viability did not decline after 4 weeks. The chickpea strain (TAL 620) was especially sensitive to 37°C, losing its viability immediately following incubation. At the termination of the short-term experiment at 8 weeks, only five strains (TAL No. 1000, 437, 182, 82, and 22) had viable counts in excess of 1×10^8 cells when incubated at 37°C. In addition to adversely affecting the viability, storage at 37°C caused the inocu-

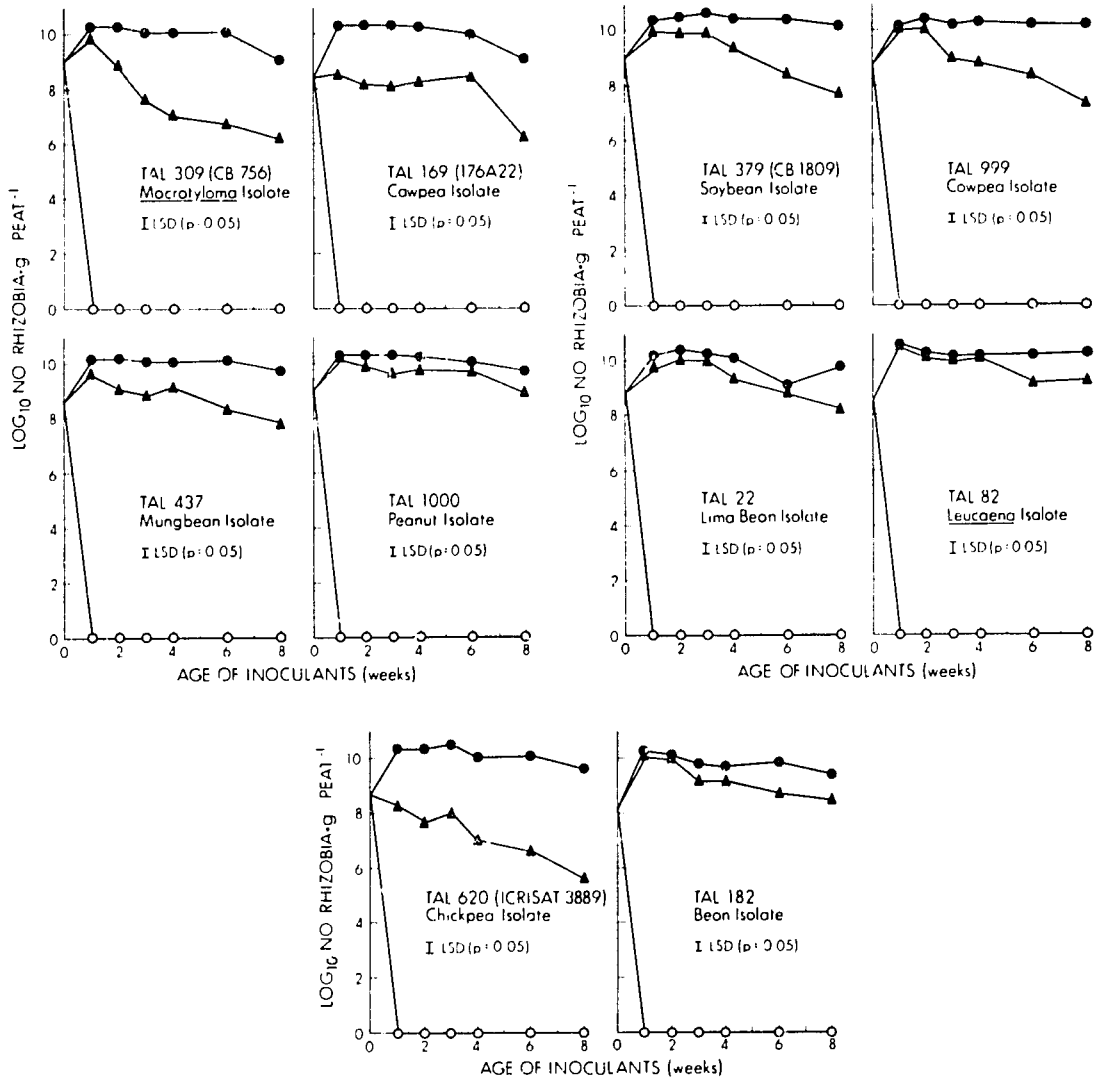

FIG. 1. Effect of high temperatures on the growth and survival of the various rhizobia in the peat inoculants during short-term storage. ●, 28°C; ▲, 37°C, ○, 46°C.

lants to harden and form lumps. Loss of moisture in the inoculants was evident.

Storage for 24 weeks at 28°C did not significantly ($p = 0.01$) affect the survival of all the six strains used in the experiment (Table 3).

Actual temperatures reached in the inoculants during their transportation to the various countries (Table 4) varied from 26 (Mexico) to 45°C (Kenya). Time taken for the inoculants sent from Hawaii to arrive at the various destinations were from 6 days (Hissar, India) to 54 days (St. Augustine, West Indies). There was complete loss of viability in the chick-pea inoculant sent to Saudi Arabia where the maximum temperature reached in the inoculant was 44°C. Viability in the bean and lentil inoculants sent to the same destination was adversely affected by the temperature. A similar decrease in the viability was observed for the bean inoculant sent to Kenya where the temperature in the inoculant was 45°C. Over 89% of the inoculants had viable counts in excess of 1×10^8 rhizobia when evaluated at their destination.

Generally, viability of the rhizobia in the peat inoculants was significantly affected by temperature (Fig. 2). It was evident that the majority of the inoculants were exposed to temperatures between 30 and 40°C. Within this range, there was great variation in the viable counts.

Discussion

Large reductions in numbers of rhizobia resulting from the addition of broth cultures to presterilized peat have been attibuted to toxic degradation and rises in

TABLE 3. Survival of rhizobia in peat inoculants incubated at 28°C during long-term storage

Rhizobia	No. of viable rhizobia ($\log_{10}$·g peat^{-1}) after: 1 week	2 weeks	3 weeks	24 weeks
TAL 22	10.08*	10.21	10.15	10.05
TAL 169	10.26	10.15	10.13	9.51
TAL 309	10.27	10.14	10.19	9.23
TAL 379	10.29	10.28	10.20	9.68
TAL 437	10.23	10.26	10.18	9.25
TAL 1000	10.23	10.29	10.19	9.96

*Figures are the mean of three bags.

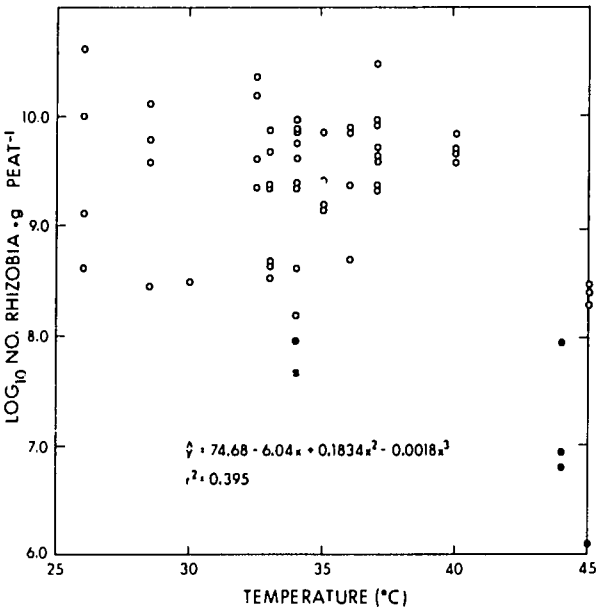

FIG. 2. Relationship of the viability of rhizobia in peat inoculants as affected by temperature during transportation (distribution). Circles (○) indicate viable counts meeting inoculant quality requirements; dots (●) indicate viable counts below inoculant quality requirements.

temperature up to 28°C in the carrier (Roughley and Vincent 1967). Our data indicated that all strains multiplied and reached maximal numbers and also had high viable counts during long-term storage at 28°C. Also, our data suggested that the reduction in the viable counts may not be entirely due to the result of the dilution undergone by the broth culture in the peat. Differences in the viable counts could not be related to the irreversible adsorption of the strains to the peat particles as the peat suspension was shaken for 15 min with a wrist-action shaker. Weaver (1979) demonstrated reversible adsorption by recovering similar numbers prior to and after addition to the peat, using two dispersion methods, with *R. meliloti* and *R. japonicum*. Enumeration by the drop-plate method (Miles and Misra 1938; Vincent 1970) did not influence the viable counts as this method gave comparable results with the pour- and spread-plate methods (Hoben and Somasegaran 1982). Therefore, large reductions in numbers of rhizobia at the time of addition of broth culture to the peat could not be atttributed to a rise in temperature (28°C), dilution undergone by the culture, irreversible adsorption of the cells to the peat, or plating methods. Large reduction in numbers may be caused by toxic degradation as reported by Roughley and Vincent (1967) or by other factors which have not been investigated. However, this initial reduction in numbers of rhizobia following addition to peat should be taken into consideration in inoculant production. Freshly prepared inoculants will have 5 to 10 times less viable rhizobia than are added as broth culture. Further incubation at an appropriate temperature is essential for producing high-count inoculants.

Continuous exposure of inoculants to high temperatures is not common; however, it is one approach of identifying temperature-tolerant strains of rhizobia for use in inoculant production. Except for the chick-pea strain and a cowpea strain, all tested rhizobia were able to multiply to maximal numbers at 37°C during the 1st week of storage and some strains could maintain high viable numbers up to 4 weeks.

The choice of temperatures for laboratory studies was well within the range experienced by the inoculants during their distribution; thus, it was possible to use the laboratory data as useful controls in relation to the data from the transportation experiment.

Laboratory data on strain growth and survival suggested that most of the rhizobia investigated in this work would reach their destinations without any serious deterioration in the viable counts, if the distribution was within a week and the prolonged high-temperature exposure was not beyond 37°C. Though cell multiplication takes place at this high temperature, freshly prepared inoculants may not be distributed to save storage space and economize on transportation time.

A very important parameter that could not be recorded during inoculant distribution (transportation) was the duration of the exposure of the inoculant to high temperature. From the short-term storage experiment with 28, 37, and 46°C, it was clear that the duration of the exposure, temperature, and the *Rhizobium* strain were important factors determining the viability of the rhizobia in the inoculants. Many inoculants were variously exposed to temperatures up to 45°C and in many of these cases, the viable counts were 10^8 rhizobia per gram moist peat or greater. It could be inferred from such data that these inoculants were exposed to the high temperatures for only short periods. These inoculants would meet the lower limits of inoculant quality re-

TABLE 4. Viability of rhizobia in peat inoculants after exposure to high temperatures during transportation

Destination	No. of days to arrive	Maximum temperature reached (°C)	Inoculant viability*
	Soybean inoculant (*R. japonicum*, TAL 379)		
Saudi Arabia (Riyad)†	23	44.0	8.00
India (Hissar)	6	35.0	9.79
Turkey (Ankara)	12	28.5	10.11
Kenya (Nairobi)	26	45	8.52
West Indies (St. Augustine)	54	32.5	10.30
Mexico (Chapingo)	NA‡	26	10.00
Tanzania (Morogoro)	33	34	9.78
Bangladesh (Mymensingh)	20	37	9.58
Philippines (Laguna)	47	34	8.64
India (Banglore)	7	40	9.79
Nigeria (Ibadan)	42	30	8.51
Thailand (Bangkok)	12	37	9.98
Malaysia (Kuala Lumpur)	12	33	9.87
Indonesia (Bogor)	17	34	9.63
India (Banglore)	7	36	9.83
	Cowpea inoculant (*Rhizobium* sp., TAL 169)		
Kenya	26	45	8.26
West Indies	54	32.5	9.30
Mexico	NA	26.0	10.56
Tanzania	33	34	9.30
Bangladesh	20	37	9.26
India (Banglore)	7	40	9.58
Nigeria	42	33	8.54
Thailand	12	37	9.91
Malaysia	12	33	9.40
Indonesia	17	34	9.32
India (Banglore)	7	36	9.40
Philippines	47	34	10.00
	Cowpea inoculant (*Rhizobium* sp., TAL 999)		
Philippines	20	37	9.36
	Peanut inoculant (*Rhizobium* sp., TAL 1000)		
West Indies	54	32.5	9.65
India (Banglore)	7	40	9.69
Nigeria	42	33	8.69
Thailand	12	37	10.51
Malaysia	12	33	9.30
Indonesia	17	34	7.96
	Mung bean inoculant (*Rhizobium* sp., TAL 437		
Bangladesh	20	37	9.65
Philippines	47	34	TNTC§
India (Banglore)	7	40	9.75
Nigeria	42	33	8.70
Thailand	12	37	9.75
Malaysia	12	33	9.73
Indonesia	17	34	9.76
	Bean inoculant (*R. phaseoli*, TAL 182)		
Saudi Arabia	23	44	6.86
India (Hissar)	6	35	9.40
Turkey	12	28.5	9.42

TABLE 4. (*concluded*)

Destination	No. of days to arrive	Maximum temperature reached (°C)	Inoculant viability*
Kenya	26	45	6.28
Mexico	NA	26	8.60
Tanazania	33	34	7.68
India (Banglore)	7	36	8.73
	Leucaena inoculant (*Rhizobium* sp., TAL 82)		
Mexico	NA	26	9.11
Tanzania	33	34	9.88
Philippines	47	34	8.20
	Pigeon-pea inoculant (*Rhizobium* sp., TAL 225)		
Kenya	26	45.0	8.40
West Indies	54	32.5	10.23
India (Banglore)	7	36.0	9.81
	Chick-pea inoculant (*Rhizobium* sp., TAL 620)		
Saudi Arabia	23	44	No growth
India (Hissar)	6	35	9.23
Turkey	12	28.5	9.79
	Lentil inoculant (*R. leguminosarum*, TAL 634)		
Saudi Arabia	23	44	6.92
India (Hissar)	6	35	9.20
Turkey	12	28.5	9.57

*Log_{10} number of rhizobia per gram of moist peat.
†Parentheses indicates name of city in cooperator's country.
‡NA, not available.
§TNTC, too numerous to count.

quirements (Roughley and Pulsford 1982). Soybean, cowpea, peanut, and mung bean inoculants sent to India (Banglore) registered 40°C in the inoculants, but viable counts were high and these inoculants would easily meet the upper limits of inoculant quality requirements. Since it was not possible to determine the duration of the exposure of the inoculants to high tempertures in the transportation experiment, the relationship between the viable counts and temperature was qualitative rather than quantitative though there was a significant correlation. Besides, the variation amongst the different strains to withstand high temperature also affected a quantitative relationship.

This report demonstrates that tropical rhizobia in peat inoculants can multiply and survive at high temperatures. Satisfactory viable counts of *R. leguminosarum* up to 6 weeks at 40 and 50°C, respectively, were reported in two carriers (Bajpai *et al.* 1978). A mixture of *Rhizobium* spp. in commercial granular and powdered peat inoculants remained viable even at 55°C storage temperature (Wilson and Trang 1980) for 15 weeks. Certain strains of *R. japonicum* could survive in liquid cultures incubated at 48.7°C (Munevar and Wollum 1981). Though high-temperature tolerance may be desirable from the viewpoint of inoculant production, tolerant strains need to be examined for retention of infectiveness and effectiveness. It was shown that there was loss of infectiveness in rhizobia in peat inoculants exposed to high temperatures (Wilson and Trang 1980), but effectiveness of the inoculants was not demonstrated. Similarly, loss in infectiveness varied form 1 to 75% in broth cultures of *R. trifolii* incubated at 37°C for 7 days (Zurkowski and Lorkiewicz 1978) and this was attributed to the loss of a large plasmid in *R. trifolii* (Zurkowski 1982). However, cowpea rhizobia grew well at 44°C on yeast extract mannitol agar plates and these strains retained effectiveness better than temperature-sensitive strains when cowpea roots were exposed to 38°C (Eaglesham *et al.* 1981).

Our results clearly demonstrate that the quality of peat inoculants is affected by temperature during transportation but to varying degrees depending on the duration of exposure, destination, and the *Rhizobium* strain. Certain countries may need to take special precautions to preserve inoculant quality, especially during in-country distribution.

Acknowledgements

This work was supported by the United States Agency for International Development, contract No.

ta-C-1207 (NifTAL Project, University of Hawaii). We acknowledge with thanks the cooperation of Ms. Irene and K. P. Tan (University of Malaya, Kuala Lumpur, West Malaysia); Mr. S. V. Hegde (University of Agricultural Sciences, Banglore, India); Ms. Neeru Narula (Haryana Agricultural University, Hissar, India); Mr. C. Mugane (University of Nairobi, Nairobi, Kenya); Mr. Somsak Kotepong (Department of Agriculture, Bangkhen, Thailand); Mr. W. A. Osinaike (Moor Plantation, Ibadan, Nigeria); Mr. E. Semu (University of Dar-es-Salaam, Morogoro, Tanzania); Ms. G. A. Gunawan (Bogor Agriculture University, Bogor, Indonesia); Dr. S. Hoque (Bangladesh Agricultural University, Mymensingh, Bangladesh); Dr. Velittin Gurgun (Ankara University, Ankara, Turkey); Dr. S. J. Quashi-Sam (University of Ghana, Legon, Ghana); Ms. Gayaga Ocampo (Philippines Bureau of Soils, Manila, Philippines); Dr. Ronald Ferrera-Cerrato (Colegio de Postgraduados, Chapingo, Mexico); Mr. M. Essam Abd-elsamee (Riyad University, Riyad, Saudi Arabia); Dr. Richard Graham (University of West Indies, St. Augustine, Trinidad). Dr. A. S. Whitney's assistance and advice on the statistical analysis is gratefully acknowledged.

AYANABA, A. 1977. Toward better use of inoculants in the humid tropics. *In* Biological nitrogen fixation in farming systems of the Tropics. *Edited by* A. Ayanaba and P. J. Dart. John Wiley & Sons Ltd., Chichester. pp. 181–187.

BAJPAI, P. D., B. R. GUPTA, and RAM BALI. 1978. Studies on survival of *Rhizobium leguminosarum* as affected by moisture and temperature conditions. Indian J. Agric. Res. **12**: 39–43.

BURTON, J. C. 1975. Methods of inoculating seeds and their effect on survival of rhizobia. *In* Symbiotic nitrogen fixation in plants. *Edited by* P. S. Nutman. Cambridge University Press, Cambridge. pp. 175–189.

CHATEL, D. L., and C. A. PARKER. 1973. Survival of field-grown rhizobia over the dry summer periods in Western Australia. Soil Biol. Biochem. **5**: 415–423.

DAY, J. M., R. J. ROUGHLEY, A. R. J. EAGLESHAM, M. DYE, and S. P. WHITE. 1978. Effect of high soil temperature on nodulation of cowpea, *Vigna unguiculata*. Ann. Appl. Biol. **88**: 476–481.

DIATLOFF, A. 1970. Relationship of soil moisture, temperature and alkalinity to a soybean nodulation failure. Queensl. J. Agric. Anim. Sci. **27**: 279–293.

EAGLESHAM, A., B. SEAMAN, H. AHMAD, S. HASSOUNA, A. AYANABA, and K. MULONGOY. 1981. High temperature tolerant "cowpea" rhizobia. Proc. Int. Symp. Nitrogen Fixation, 4th, 1980.

HOBEN, H. J., and P. SOMASEGARAN. 1982. Comparison of the pour, spread, and drop-plate methods for enumeration of *Rhizobium* spp. in inoculants made from presterilized peat. Appl. Environ. Microbiol. **44**: 1246–1247.

ISWARAN, V., W. V. B. RAO SUNDARA, S. P. MAGU, and K. S. JAURI. 1969. Indian peat as a carrier. Curr. Sci. **38**: 468–469.

MILES, A. A., and S. S. MISRA. 1938. The estimation of the bactericidal power of blood. J. Hyg. **38**: 732–749.

MUNEVAR, F., and A. G. WOLLUM II. 1981. Growth of *Rhizobium japonicum* strains at temperatures above 27°C. Appl. Environ. Microbiol. **42**: 272–276.

NORRIS, D. O. 1963. A procelain bead method for storing *Rhizobium*. Emp. J. Exp. Agric. **31**: 255–258.

PHILPOTTS, H. R. 1967. The effect of soil temperature on nodulation of cowpea (*Vigna sinensis*). Aust. J. Exp. Agric. Anim. Husb. **7**: 372–376.

ROUGHLEY, R. J. 1968. Some factors influencing the growth and survival of root nodulae bacterial in peat culture. J. Appl. Bacteriol. **31**: 259–265

ROUGHLEY, R. J., and D. J. PULSFORD. 1982. Production and control of legume inoculants. *In* Nitrogen fixation in legumes. *Edited by* J. M. Vincent. Academic Press, Sydney. pp. 193–209.

ROUGHLEY, R. J., and J. M. VINCENT. 1967. Growth and survival of *Rhizobium* spp. in peat culture. J. Appl. Bacteriol. **30**: 362–376.

VINCENT, J. M. 1970. A manual for the practical study of root nodule bacteria. IBP Handbook No. 15. Blackwell Scientific Publications, Oxford, England.

WEAVER, R. W. 1979. Adsorption of rhizobia to peat. Soil Biol. Biochem. **11**: 545–546.

WILSON, D. O., and K. M. TRANG. 1980. Effects of storage temperature and enumeration method on *Rhizobium* spp. numbers in peat inoculants. Trop. Agric. (Trinidad), **57**: 233–238.

ZURKOWSKI, W. 1982. Molecular mechanism for loss of nodulation properties of *Rhizobium trifolii*. J. Bacteriol. **150**: 999–1007.

ZURKOWSKI, W., and Z. LORKIEWICZ. 1978. Effective method for the isolation of non-nodulating mutants of *Rhizobium trifolii*. Genet. Res. **32**: 311–314.