EFFECT OF HIGH SOIL TEMPERATURES ON *RHIZOBIUM* SPP.

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SUMMARY.

Following field observations of poor nodulation of legumes sown on hot days, the possible effects of high soil temperatures on legume bacteria were studied.

Eighty-seven strains of legume bacteria on nutrient agar showed maximum temperatures for growth of 31-38.4 deg. C., 32-32.7 deg. C., 36.5-42.5 deg. C. and 30-42.0 deg C. for clover, pea, medic, and tropical legume strains of Rhizobium **respectively.**

Studies of the decline of viable population in sterile wallum-heath sand at 40 deg. C. indicated the rapid death of pea and clover strains; the death of tropical legume strains within 10 hours; and an initial drop of numbers of lucerne organisms followed by a lesser death rate.

Sowing inoculated seed into moist soil followed by holding at 40 deg. C. before planting showed that strain of legume bacterium, initial concentration of inoculum and time of subjection to high temperatures are significant factors operating in the survival of legume bacteria on the seed.

Methods of overcoming deleterious effects of high soil temperatures are discussed.

I. INTRODUCTION.

In field plots at Coolum in south-eastern Queensland in 1957 it was observed that only a small percentage of seedlings of various tropical legumes, red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) formed nodules when planted following midsummer to late-summer rains. Neither poor moisture nor nutrient deficiency could be invoked to explain the defective nodulation. Watering of a suspension of the same bacterial strain onto rows some weeks later resulted in good nodulation. This suggested that the environmental conditions at planting were deleterious to the legume bacteria.

High soil temperature appeared to be a factor to be considered and consequently a systematic study of the effect of high temperatures on the growth and survival of legume bacteria was commenced. Many of the early studies on the temperature requirements of legume bacteria were directed to finding the optimum temperature for development. The present study was designed to investigate the maximum temperature for growth and the harmful effect of high soil temperature on survival in the soil and on the seed.

II. MAXIMUM TEMPERATURE FOR GROWTH.

References to the maximum temperature tolerance of legume bacteria are somewhat variable. De Rossi (1907) found no growth of organisms from *Vicia faba* L. incubated at 37 deg. C. and frequently none at 35 deg. C. Müller and Stapp (1925) considered 32–33 deg. C. to be harmful to most rhizobia. Gangulee (1926), working with a lucerne organism, obtained a decreased population in soil within seven days with temperatures of 35 deg. C. and 50 deg. C. Studies by Allison and Minor (1940) indicated a maximum temperature for growth of five rhizobium strains, from the clover, pea and bean groups and the strain-specific *Dalea,* to be 31–37 deg. C., and for four lucerne rhizobia 39 deg. C. The experiment, however, was conducted over 48 hours only and a slow growth at the high temperatures could have been overlooked.

More recently Ishizawa (1953) made a survey of 94 rhizobium strains from various legumes. His data show maximum temperatures of growth for the various cross-inoculation groupings as follows: pea, clover and bean 35–37 deg. C., medic 40·5–42·5 deg. C., lupin 32–33 deg. C. and the tropical legume miscellany (including soybean) 32–42·5 deg. C. The work reported here has in some instances confirmed and in others modified these findings.

The primary investigation was the assessment of the maximum temperature for growth on agar slopes. Eighty-seven strains of legume bacteria drawn from four cross-inoculation groupings were used, with most strains being selected from the tropical legume miscellany.

Duplicate tubes of asparagus extract-mannitol-mineral salts agar (Allen 1951) were inoculated, and incubated for at least 14 days in a series of chambers of a multi-temperature incubator. Ten chambers of the incubator were employed, giving a range of temperatures between 22 deg. C. and 45 deg. C. Daily readings indicated a fluctuation of temperature of ±0·7 deg. C. Tubes showing no growth at the completion of the trial period were returned to a temperature suitable for growth. In no case was subsequent growth observed.

The results are summarised in Table 1, and detailed strain data are given in Appendix 1.

Table 1.

MAXIMUM TEMPERATURES FOR GROWTH ON AGAR SLOPES.

Cross-inoculation Group.	No. of Strains Tested.	Maximum Temperature for Growth. (°C.).		
		Lowest.	Highest.	Average.
Medic	8	36·5	42·5	41·0
Trifolium	9	31·0	38·4	33·2
Pea	2	32·0	32·7	32·3
Tropical Legume Miscellany	68	30·0	42·0	35·4

Marked differences between some groups and also within groups are obvious. While being in general agreement with the results of early workers, the maximum temperatures for growth of the pea and the clover groups (average 32·3 deg. C. and 33·2 deg. C., respectively) are lower than those found by Ishizawa (1953). The high maximum temperature for growth of medic organisms is marked. The tropical legume miscellany shows wide variation.

An examination of the data for the tropical legume miscellany with respect to botanical affinities and latitude of occurrence did not reveal any correlation.

III. EFFECT OF HIGH TEMPERATURE ON LONGEVITY AND NODULATION IN SOIL.

(1) Laboratory Trial 1.

Nine strains of legume bacteria of various types were inoculated into sterile wallum-heath sand and incubated at 28 deg. C. and 40 deg. C. This was done by adding 5 ml. of a suspension from a 10-days-old culture of the organism to a series of bottles each containing 20 g. of sterile sand. Counts of viable cells were made at 2-hourly intervals for 12 hours and one at 24 hours, using the Miles-Misra drop plate counting technique. The viable organisms in duplicate bottles for each temperature were counted at each sampling time.

The results presented in Table 2 are the average of the two determinations, counting from 8 drops per dilution in each determination, and are expressed as number of viable organisms per gram of sand.

Figs. 1, 2 and 3 show the death curves for organisms from medic, from clover and pea, and from tropical legume groupings respectively.

The results show the marked killing effect of incubation at 40 deg. C., particularly on pea and clover organisms. The large initial loss of viability of medic organisms followed by a levelling of the curve need not necessarily be interpreted as a selection within a population inherently heterogeneous with respect to high temperature resistance.

The normally recommended level of inoculum for legume seed is 1,000 organisms per seed. The survival time for these would be low in such soils as the test one at Coolum, where temperatures of up to 47·5 deg. C. are recorded at planting depth and where 40 deg. C. is exceeded for up to six hours per day.

Table 2.

EFFECT OF HIGH TEMPERATURE ON LONGEVITY OF LEGUME BACTERIA IN WALLUM-HEATH SAND.

Host of Isolation and Strain.	Maximum Temperature for Growth (°C.).	Temp. of Incubation (°C.).	Incubation Time (Hours).							
			0.	2.	4.	6.	8.	10.	12.	24.
Medicago sativa L. (QA383)	40	28	$2{\cdot}91 \times 10^{6}$†	$2{\cdot}93 \times 10^{6}$	$2{\cdot}79 \times 10^{6}$	$2{\cdot}35 \times 10^{6}$	$2{\cdot}65 \times 10^{6}$	$1{\cdot}95 \times 10^{6}$	—	$4{\cdot}74 \times 10^{5}$
		40	$2{\cdot}91 \times 10^{6}$*	$2{\cdot}70 \times 10^{6}$	$2{\cdot}14 \times 10^{6}$	$9{\cdot}16 \times 10^{5}$	$1{\cdot}79 \times 10^{6}$	$1{\cdot}30 \times 10^{5}$	$1{\cdot}24 \times 10^{5}$	$6{\cdot}06 \times 10^{3}$
M. sativa. (QA679).. ..	36·5	28	$2{\cdot}86 \times 10^{6}$	$2{\cdot}26 \times 10^{6}$	$2{\cdot}25 \times 10^{6}$	$1{\cdot}98 \times 10^{6}$	$2{\cdot}40 \times 10^{6}$	$2{\cdot}13 \times 10^{6}$	$2{\cdot}21 \times 10^{6}$	$6{\cdot}74 \times 10^{5}$
		40	$2{\cdot}86 \times 10^{6}$	$2{\cdot}81 \times 10^{6}$	$1{\cdot}19 \times 10^{6}$	$2{\cdot}16 \times 10^{5}$	$1{\cdot}15 \times 10^{4}$	$3{\cdot}34 \times 10^{4}$	$7{\cdot}33 \times 10^{2}$	$7{\cdot}74 \times 10^{2}$
Trifolium pratense L.(QA382)	32·7	28	$1{\cdot}45 \times 10^{6}$	$9{\cdot}11 \times 10^{5}$	$1{\cdot}16 \times 10^{6}$	$1{\cdot}34 \times 10^{6}$	$7{\cdot}72 \times 10^{5}$	$1{\cdot}10 \times 10^{6}$	$5{\cdot}06 \times 10^{5}$	$5{\cdot}90 \times 10^{5}$
		40	$1{\cdot}45 \times 10^{6}$	$1{\cdot}85 \times 10^{5}$	$7{\cdot}03 \times 10^{4}$	$1{\cdot}87 \times 10^{3}$	0	0	0	0
Trifolium repens L. (QA859)	32	28	$2{\cdot}17 \times 10^{5}$	$2{\cdot}08 \times 10^{5}$	$2{\cdot}12 \times 10^{5}$	$2{\cdot}45 \times 10^{5}$	$3{\cdot}06 \times 10^{5}$	$1{\cdot}61 \times 10^{5}$	$2{\cdot}00 \times 10^{5}$	$1{\cdot}78 \times 10^{5}$
		40	$2{\cdot}17 \times 10^{5}$	$7{\cdot}99 \times 10^{4}$	$1{\cdot}09 \times 10^{4}$	$5{\cdot}63 \times 10^{2}$	$1{\cdot}78 \times 10^{2}$	0	0	0
Pisum sativum L. (QA794) ..	32	28	$1{\cdot}77 \times 10^{5}$	$1{\cdot}47 \times 10^{5}$	$1{\cdot}65 \times 10^{5}$	$2{\cdot}11 \times 10^{5}$	$1{\cdot}14 \times 10^{5}$	$1{\cdot}20 \times 10^{5}$*	$1{\cdot}41 \times 10^{5}$	$1{\cdot}05 \times 10^{5}$
		40	$1{\cdot}77 \times 10^{5}$	$1{\cdot}59 \times 10^{5}$	$1{\cdot}07 \times 10^{5}$	$1{\cdot}12 \times 10^{3}$	0	0	0	0
Pultenaea villosa Willd. (QA323)	35·6	28	$1{\cdot}30 \times 10^{6}$	$9{\cdot}90 \times 10^{5}$	$6{\cdot}37 \times 10^{5}$	$5{\cdot}36 \times 10^{5}$	—	$2{\cdot}03 \times 10^{6}$	$8{\cdot}91 \times 10^{5}$	$4{\cdot}25 \times 10^{5}$
		40	$1{\cdot}30 \times 10^{6}$	$1{\cdot}03 \times 10^{6}$	$6{\cdot}04 \times 10^{5}$	—	$1{\cdot}34 \times 10^{5}$	0	0	0
Centrosema pubescens Benth. (QA549)	38·5	28	$4{\cdot}03 \times 10^{6}$	$3{\cdot}41 \times 10^{6}$	$3{\cdot}43 \times 10^{6}$	$3{\cdot}66 \times 10^{6}$	$3{\cdot}34 \times 10^{6}$	$3{\cdot}16 \times 10^{6}$	$2{\cdot}69 \times 10^{6}$	$1{\cdot}44 \times 10^{6}$
		40	$4{\cdot}03 \times 10^{6}$	$3{\cdot}88 \times 10^{6}$	$2{\cdot}58 \times 10^{6}$	$2{\cdot}44 \times 10^{6}$	$7{\cdot}80 \times 10^{5}$	$8{\cdot}32 \times 10^{4}$	$3{\cdot}84 \times 10^{5}$	$2{\cdot}65 \times 10^{3}$
C. pubescens. (QA837) ..	35·2	28	$4{\cdot}24 \times 10^{6}$	$4{\cdot}72 \times 10^{6}$	$4{\cdot}69 \times 10^{6}$	$4{\cdot}15 \times 10^{6}$	$4{\cdot}08 \times 10^{6}$	$4{\cdot}18 \times 10^{6}$	$2{\cdot}90 \times 10^{6}$	$3{\cdot}45 \times 10^{6}$
		40	$4{\cdot}24 \times 10^{6}$	$4{\cdot}34 \times 10^{6}$	$2{\cdot}70 \times 10^{6}$	$1{\cdot}84 \times 10^{4}$	$4{\cdot}25 \times 10^{3}$*	$1{\cdot}39 \times 10^{4}$*	0	0
C. pubescens. (QA851) ..	33	28	$3{\cdot}54 \times 10^{6}$	$3{\cdot}41 \times 10^{6}$	$3{\cdot}44 \times 10^{6}$	$3{\cdot}66 \times 10^{6}$	$3{\cdot}34 \times 10^{6}$	$3{\cdot}16 \times 10^{6}$	$2{\cdot}70 \times 10^{6}$	$1{\cdot}44 \times 10^{6}$
		40	$2{\cdot}54 \times 10^{6}$	$3{\cdot}23 \times 10^{6}$	$1{\cdot}80 \times 10^{6}$	$5{\cdot}08 \times 10^{5}$*	$5{\cdot}67 \times 10^{3}$	0	0	0

† Viable organisms per gram of sand.
* Results of 1 bottle only.

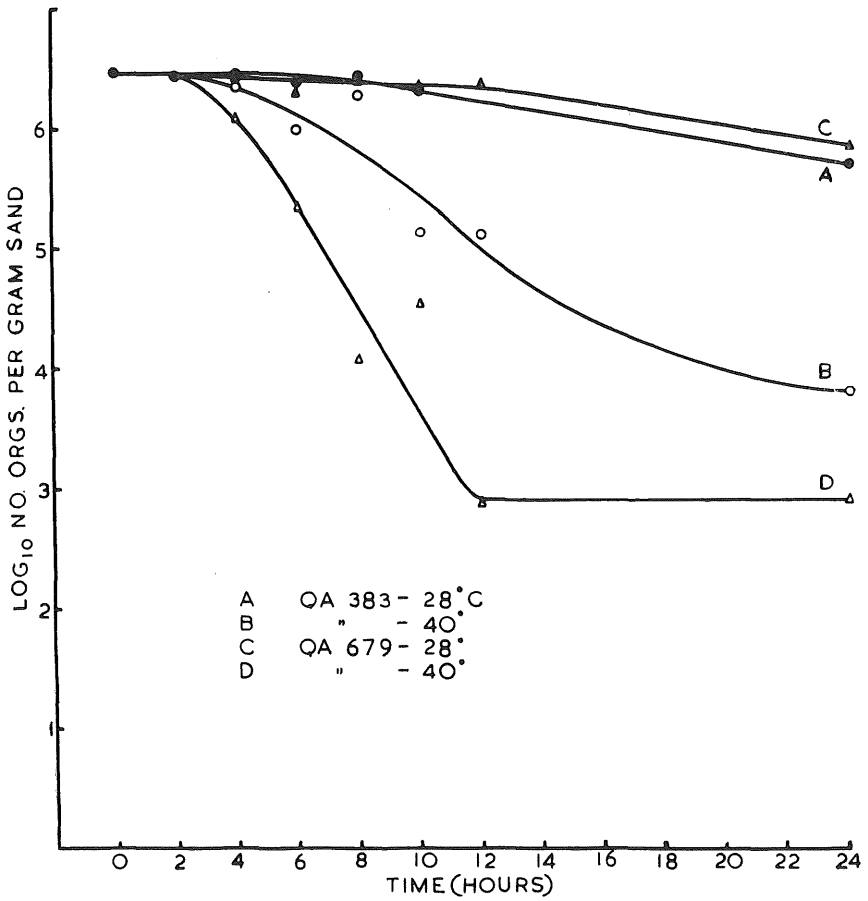


Fig. 1.

Death Curves for Medic Isolates.

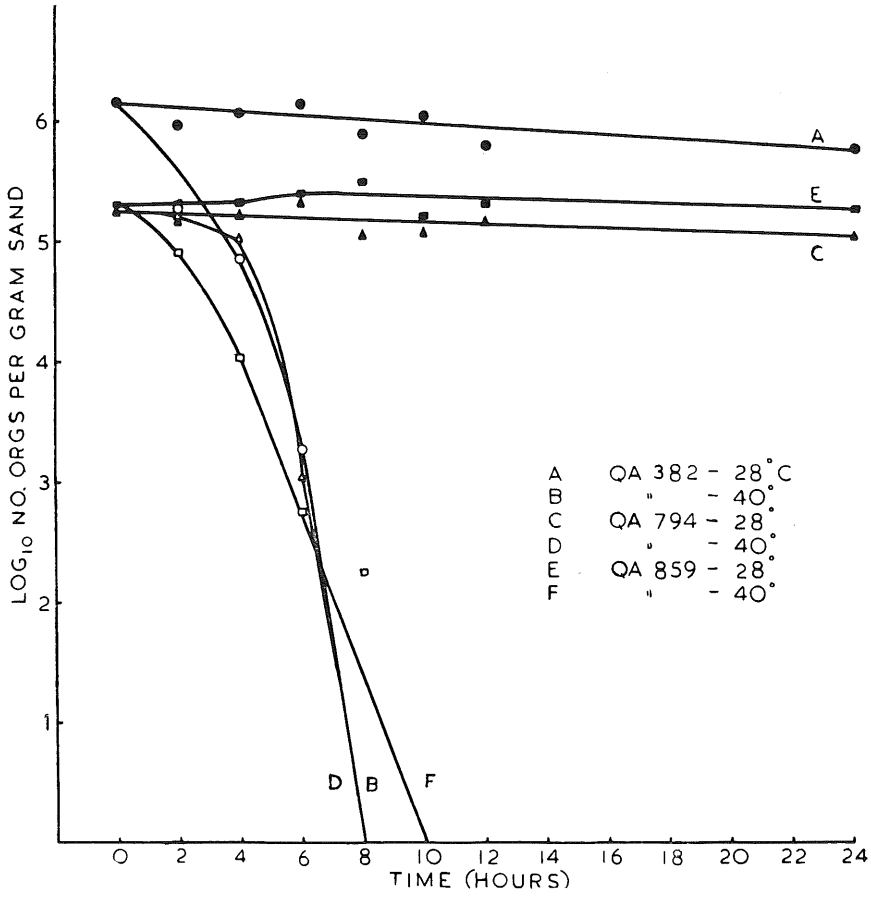


Fig. 2.

Death Curves for Clover and Pea Isolates.

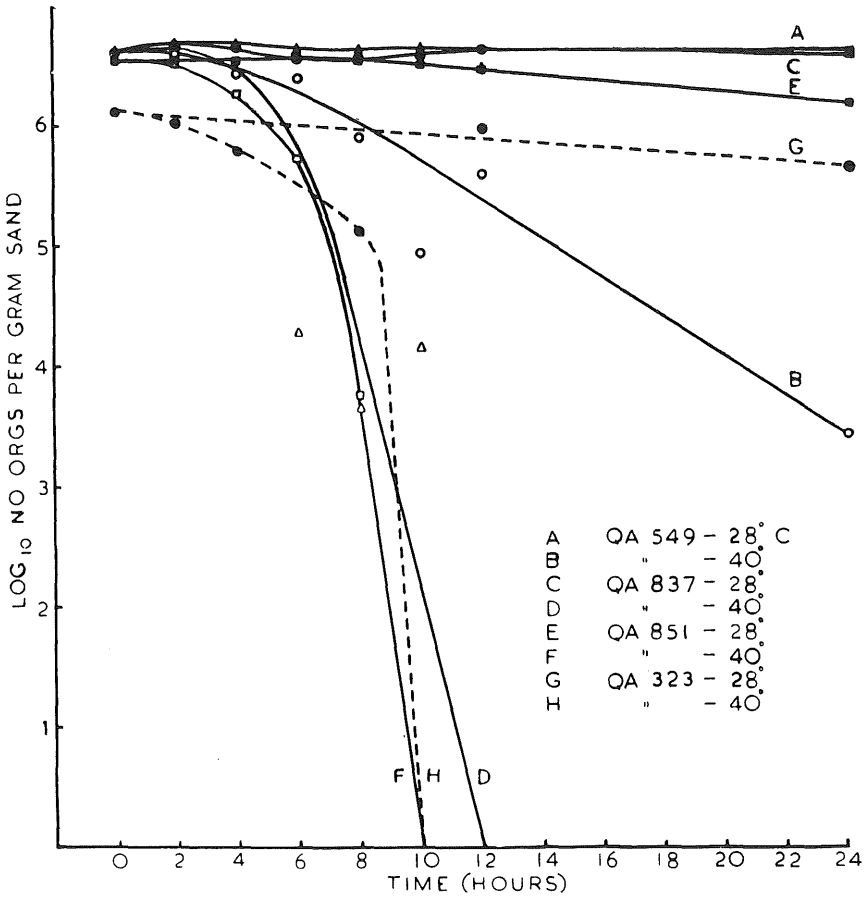


Fig 3.

Death Curves for Tropical Legume Isolates.

(2) Laboratory Trial 2.

Wallum-heath sand was inoculated as in Trial 1 with four strains only, but at two concentrations of bacteria, one being 100 times the other. The sand cultures were incubated at 40 deg. C., and counts made every two hours up to 12 hours. The results are shown in Table 3 and Figs. 4 and 5.

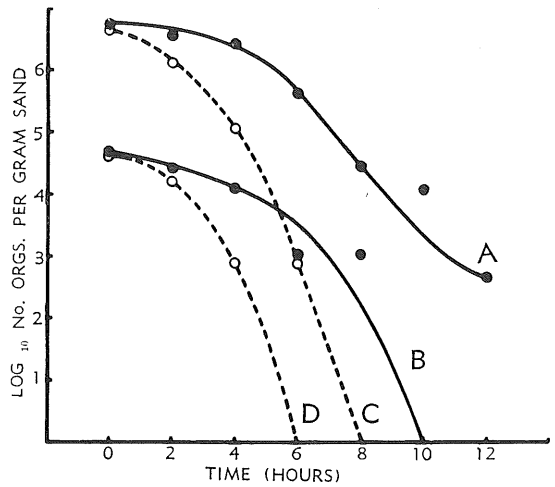


Fig. 4.

Death Curves for Two Concentrations of *Centrosema* Isolates.

A—QA549 at higher concentration.
B—QA549 at lower concentration.
C—QA851 at higher concentration.
D—QA851 at lower concentration.

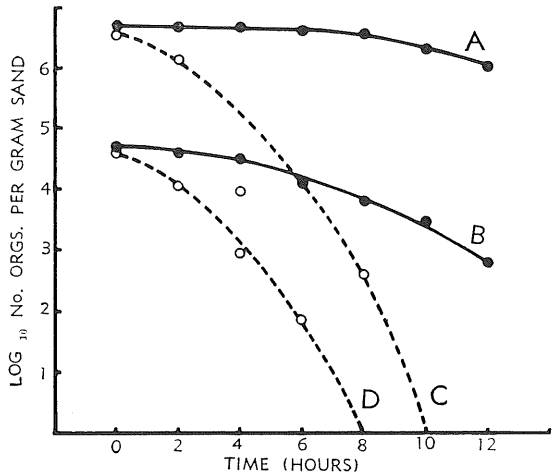


Fig. 5.

Death Curves for Two Concentrations of Medic and Clover Isolates.

A—QA383 at higher concentration.
B—QA383 at lower concentration.
C—QA859 at higher concentration.
D—QA859 at lower concentration.

Table 3.

EFFECT OF HIGH TEMPERATURE ON LEGUME BACTERIA APPLIED TO WALLUM-HEATH SAND AT TWO CONCENTRATIONS.

Host of Isolation and Strain.	Concentration.	Hours Incubated at 40°C.						
		0.	2.	4.	6.	8.	10.	12.
Medicago sativa (QA383)	100x	$5{\cdot}04 \times 10^6$*	$4{\cdot}80 \times 10^6$	$5{\cdot}01 \times 10^6$	$4{\cdot}11 \times 10^6$	$3{\cdot}92 \times 10^6$	$2{\cdot}05 \times 10^6$	$1{\cdot}12 \times 10^6$
	x	$4{\cdot}91 \times 10^4$	$4{\cdot}08 \times 10^4$	$3{\cdot}34 \times 10^4$	$1{\cdot}26 \times 10^4$	$6{\cdot}61 \times 10^3$	$3{\cdot}20 \times 10^3$	$6{\cdot}83 \times 10^2$
Trifolium repens (QA859)	100x	$3{\cdot}41 \times 10^6$	$1{\cdot}32 \times 10^6$	$9{\cdot}72 \times 10^6$	$1{\cdot}19 \times 10^4$	$4{\cdot}16 \times 10^2$	0	0
	x	$3{\cdot}99 \times 10^4$	$1{\cdot}24 \times 10^4$	$9{\cdot}50 \times 10^2$	$7{\cdot}92 \times 10$	0	0	0
Centrosema pubescens (QA549)	100x	$5{\cdot}77 \times 10^6$	$3{\cdot}75 \times 10^6$	$2{\cdot}75 \times 10^6$	$4{\cdot}32 \times 10^5$	$3{\cdot}06 \times 10^4$	$1{\cdot}29 \times 10^4$	$5{\cdot}02 \times 10^2$
	x	$5{\cdot}03 \times 10^4$	$2{\cdot}64 \times 10^4$	$1{\cdot}31 \times 10^4$	$1{\cdot}09 \times 10^3$	$1{\cdot}11 \times 10^3$	0	0
C. pubescens (QA851)	100x	$4{\cdot}47 \times 10^6$	$1{\cdot}38 \times 10^6$	$1{\cdot}21 \times 10^5$	$7{\cdot}20 \times 10^2$	0	0	0
	x	$4{\cdot}18 \times 10^4$	$1{\cdot}68 \times 10^4$	$7{\cdot}99 \times 10^2$	0	0	0	0

* Viable organisms per gram of sand.

These results show that when the initial concentration of the legume bacteria is higher, a longer exposure to high temperature is required for their serious depletion or complete kill. The strain differences found in Trial 1 also occurred in this trial.

(3) Glasshouse Trial.

Following the field observations and preliminary laboratory data on decline of the legume bacteria population in soil, a glasshouse trial was planted to test the effect on nodulation of strain of bacterium, level of inoculation and time of subjection of inoculated seed to the high temperature.

The test plant used was the tropical legume *Centrosema pubescens* Benth. Surface-sterilized seed was inoculated at each of two levels of inoculum (1×10^3 organisms per seed and 1×10^5 organisms per seed) with each of two strains previously found effective for this legume. These strains were QA549 and QA837, with maximum temperatures for growth of 38·5 deg. C. and 35·2 deg. C. respectively. The inoculated seed was sown in sterilized sand plus nutrients and incubated at 40 deg. C. for 0, 3, 6 and 9 hours before planting into aseptic testing units of a modified Leonard jar type. Thirty seeds per jar were sown to yield approximately 10–15 seedlings. Six randomised replications were employed. Records of the presence of nodules and counts of nodules were made after six weeks. These results are shown in Tables 4 and 5 respectively.

Table 4.

PERCENTAGE OF *Centrosema pubescens* PLANTS NODULATED IN GLASSHOUSE TRIAL.

Strain.	Concentration of Inoculum.	Hours Incubated at 40°C.			
		0.	3.	6.	9.
QA837 ..	Low ..	93·1	34·7	8·5	3·0
	High ..	90·9	45·0	14·5	36·6
QA549 ..	Low ..	91·3	82·5	24·2	28·6
	High ..	83·1	83·1	78·4	82·2

Within Low concentration 0>>3>>6, 9
Within High concentration 0>>3>>6 ; 0>>9, 6
Within Strain QA837 0>>3>>9, 6
Within Strain QA549 0>>9,6 ; 3>9 ; 3>>6

Before statistical analyses of the percentage of plants with nodules was proceeded with, the inverse sine transformation was used to equalise variances. Before analyses of numbers of nodules per plant, the log $(1 + x)$ transformation was used. Individual plot results and the summaries of statistical analyses are shown in Appendices 2 and 3 respectively.

Table 5.

AVERAGE NODULE NUMBERS PER PLANT IN GLASSHOUSE TRIAL.

Strain.	Concentration of Inoculum.	Hours Incubated at 40°C.			
		0.	3.	6.	9.
QA837 ..	Low ..	4·0	0·9	0·35	0·0
	High ..	3·0	1·3	0·8	1·5
QA549 ..	Low ..	5·5	3·0	0·5	1·3
	High ..	3·8	3·3	4·5	3·6

Within Low concentration 0>>3>>9, 6
Within High concentration 0>6 ; 0>>9
Within Strain QA837 0>>3>6, 9 ; 3>>9
Within Strain QA549 0, 3>>6 ; 0>>9

Despite variable individual plot values, interactions of strains and times and concentration and times are highly significant for the percentage of plants with nodules. For numbers of nodules per plant, interaction of concentrations and times is highly significant. Here variation between plots of the one treatment arose from some individual plants being heavily nodulated. This may be inherent in the plant (Bowen, unpublished data) or due to a high survival of organisms on particular seeds. The individual plot variations were not unexpected. It is thought likely that some spreading from plant to plant may have occurred. Ideally such experiments would be better conducted with single-plant pots.

Strain QA549, while not as susceptible to high temperature damage as QA837, is affected by a longer exposure. The difference between the strains is one of degree, not of type. It is considered that even with QA549 at the high inoculum rate considerable mortality occurred, but that populations were not reduced below those necessary for nodulation to take place. A longer high temperature exposure on the same day (or perhaps on succeeding days) may have killed these. Under ideal pot testing conditions, it is likely that almost complete kill of the organisms would have been necessary to prevent nodulation.

IV. FIELD TEMPERATURE STUDIES.

For the purposes of the present study information on soil temperatures attained in moist soil at a depth approximating planting (1–2 in.) was required. In the tropics, Ramdas and Dravid (1936) recorded temperatures of up to 64·6 deg. C. at the surface and 45·3 deg. C. at 5 cm. depth in a black soil.

Breakwell and Jenkins (1951) recorded soil temperatures of 112 deg. F. (44·4 deg. C.) 1 in. below ground level in clean-cultivated soil in the Richmond River area of New South Wales (latitude 29 deg. S.). Crofts (1957) recorded 1 in. temperatures of 100–124 deg. F. (37·8–51·1 deg. C.) in fallow in the same soils in the period November 1951 to February 1952. Temperatures recorded by the authors at Coolum, South Queensland (latitude 26·5 deg. S.) in the grey sandy coastal wallum-heath soils, on a typical summer day (February 1958), in moist bare soil showed that temperatures can reach 47·5 deg. C. at 1 in. and 42·5 deg. C. at 2 in. depths. At 1 in., temperatures of over 40 deg. C. were maintained for up to six hours. Results over two years (unpublished records of Chemical Laboratory, Queensland Department of Agriculture and Stock) indicate that from April to September inclusive, temperatures in moist soil in the same area at 1 in. do not rise over 90 deg. F. (32·2 deg. C.), while from November to February, temperatures of over 100 deg. F. (37·8 deg. C.) in moist soil at 1 in. are not unusual.

In order to test ways of overcoming high soil temperatures in the field, temperatures were recorded at Coolum at 1 in. and 2 in. depths with the following paired sites:

(1) Bare soil and an established pasture mixture of para grass (*Brachiaria mutica* Stapf).

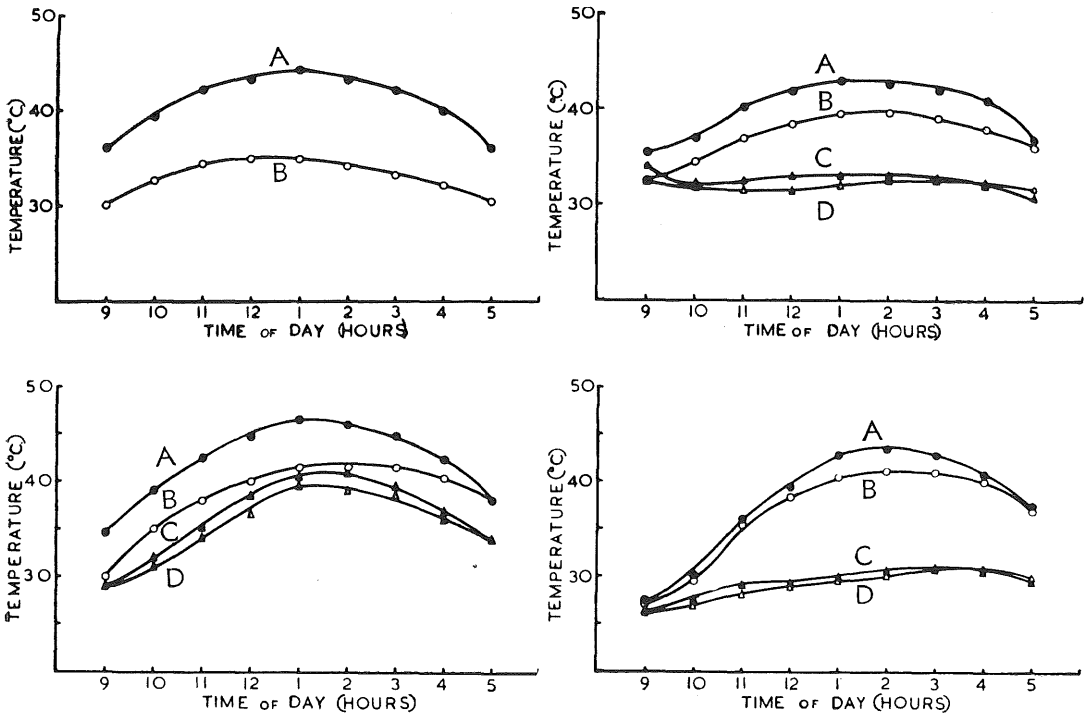
(2) Bare soil and native heath vegetation.

(3) Bare soil and a covering 3 in. plant trash layer.

(4) Bare soil on the western side of an established grass plot and bare soil in grass shade on the southern side of the plot.

The soil was moist and the day (Feb. 5, 1958) a typical cloudless summer day. In each pair the sites were no more than 3 ft. apart to reduce variation due to soil factors.

Figs. 6–9 show the type of results obtained on the respective sites. In all cases lower temperatures were recorded where shade was provided. Under trash, temperatures remained stationary during the day. The peaks in the 2 in. depth curves correspond in time to those at 1 in. While maximum temperatures were recorded at 1 p.m., temperatures under bare ground remain above the maximum for growth of many rhizobium strains for considerable periods.



Figs. 6–9.

Soil Temperatures Measured in the Field at 1 in. and 2 in. Depths at Paired Sites of Bare and Covered Ground.

Fig. 6.—A—Bare ground.
B—Para grass cover.

Fig. 7.—A—Bare soil at 1 in. depth.
B—As A at 2 in. depth.
C—Native heath cover at 1 in. depth.
D—As C at 2 in. depth.

Fig. 8.—A—Bare soil at 1 in. depth.
B—As A at 2 in. depth.
C—Plant trash cover at 1 in. depth.
D—As C at 2 in. depth.

Fig. 9.—A—Bare soil on western side of an established grass plot at 1 in. depth.
B—As A at 2 in. depth.
C—Bare soil in grass shade on southern side of the plot at 1 in. depth.
D—As C at 2 in. depth.

V. DISCUSSION.

The results support the thesis that high soil temperatures attained in summer in subtropical and tropical environments can be a factor hampering the establishment of sown pastures. The aspect studied here has been entirely one of the survival of the legume bacteria. Because of the sparsity of experimental equipment for controlling glasshouse environment, there is practically no information available on the effects of temperature on nodulation of legumes and resultant nitrogen fixation. The most pertinent work was

performed by Jones and Tisdale (1921). The absence of nodulation of soybean at 40 deg. C. in their experiments could well have been because of death of the legume bacteria as well as the effect of high soil temperatures on the plant. Control of abnormally high temperatures must be taken into consideration in glasshouse experiments with legumes.

Within each inoculation grouping, variation in temperature tolerance occurs, and the glasshouse trial on *Centrosema pubescens* indicates that selection of strains tolerant to high temperatures is of possible practical use. The result of further selecting mutants within a strain is now being studied.

Bisset (1952) described endospore formation in the genus *Rhizobium*. Here rests a mechanism for resistance to high-temperature exposure. Great variation between strains in their ability to produce endospores apparently exists—for example, pea and clover rhizobia are claimed to form them only rarely, while rhizobia from other legumes form them more commonly. Furthermore, Bisset stated that as a rule infection occurs only through the agency of variants of typical morphology (heat-susceptible), these being derived from atypical phases (i.e., sporogenous and heat-resistant) either spontaneously or by contact with seedling plants. Selection of strains with sporogenous phases therefore may be a practical aid to overcoming the harmful effects of high soil temperatures. The success of the method would depend on the rate of reversion to the infective type with respect to the duration of high soil temperatures in the course of seed germination.

The concentration of inoculum applied also has an effect. However, the questionable economic practicability of farmers applying inoculum at approximately 100 times the normal concentration limits the value of this approach.

Greater control of the temperature effect may be achieved by agronomic practice. Decreasing temperature with increasing depth of soil suggests that increase of planting depth would overcome high temperature effects. This is limited by a number of factors, including seed size and soil physical properties. The shallow planting necessary with small-seeded legumes makes such legumes more susceptible not only to high soil temperatures near the surface but also to fluctuating surface soil moisture conditions.

Planting into the shade of vegetation, either native pasture or planted pasture, and covering with trash are effective ways to control high temperatures in the surface 2 in.

The season of planting and the time of day at which planting is done are important considerations. The practice in many parts of Queensland is to plant winter pasture species such as red clover and white clover on late summer rains. Under such conditions, trouble could be encountered.

The time required to eliminate or to seriously impede nodulation by the legume bacteria on the seed of *C. pubescens* inoculated at normal concentrations and held at 40 deg. C. varied in the glasshouse trial from three to six hours depending on the bacterial strain. With a soil rising to a temperature of 45-47 deg. C. at planting depths, the time would be expected to be much less. On days when trouble is likely to occur, planting should be carried out as late in the day as practicable. Planting late in the day, however, provides no real guarantee that the trouble will be overcome.

With *C. pubescens* in sterile soil at 28 deg. C. some multiplication occurs outside the seed before germination. Counts indicated a population of viable organisms per seed, under these conditions, of $1{\cdot}4 \times 10^3$, 4×10^3, $3{\cdot}1 \times 10^4$ and $3{\cdot}5 \times 10^6$ organisms at 0, 24, 48 and 120 hours after inoculation, respectively. Up to two days after planting this species, seed populations around the seed are therefore low enough to be killed quickly by possible soil temperatures. With fluctuating soil temperature and less ideal field conditions, it may be expected that this danger period would be increased considerably.

Indications are (Fred, Baldwin and McCoy 1932) that the rate of spread of rhizobium through the soil is very slow indeed. The quickest line of migration from the rigorous soil surface conditions is along the root after root emergence. Therefore, up to this time the rhizobia will remain more or less around the seed. The time for root emergence varies from species to species. For *C. pubescens* it is 4–7 days for normal seeds, hard seeds taking longer, With some species of *Trifolium* and *Medicago,* emergence of the root will occur within two days.

To sum up, selection of bacterial strains which require a longer exposure to high temperatures for complete kill may be of some use, but this may have to be supplemented by agronomic practices such as planting into shade of existing grasses or natural vegetation and other methods which aim at lowering soil temperatures, rather than overcoming the effect of high soil temperature itself. The practice of sod-seeding may have an added value for this reason.

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APPENDIX 1.

MAXIMUM TEMPERATURES FOR GROWTH OF *Rhizobium* SPP.

Host.	Strain Number.	Source.	Maximum Temperature for Growth (°C.).	Latitude of Isolation (°S.).
A. Medic Cross-inoculation Group				
Medicago hispida Gaertn. ..	QA685 ..	Gatton	38·5	27·5
M. sativa L.	QA383 ..	Rothamsted (England) AH	40	
	QA679 ..	Archerfield	36·5	27·5
	QA681 ..	Gin Gin	42·5	25
	QA682 ..	Rockhampton	42·5	23
	QA683 ..	Theodore	42·5	24·5
M. tribuloides Desr.	QA684 ..	Cecil Plains	42·5	27·5
B. Trifolium Cross-inoculation Group				
Trifolium incarnatum L. ..	QA795 ..	Sydney University SU297	32	
	QA790 ..	Sydney University SU298	31	
T. pratense L.	QA382 ..	Rothamsted Clover F ..	32·7	
	QA516 ..	Kingaroy	32·7	26·5
	QA859 ..	Tasmania TAI	32	
T. repens L.	QA444 ..	Lismore	32·7	
	QA483 ..	Kingaroy	32·7	26·5
	QA474 ..	Kingaroy	38·5	26·5
T. subterranean L.	QA442 ..	New South Wales NA34 ..	34·6	
C. Pea Cross-inoculation Group				
Pisum sativum L.	QA384 ..	Rothamsted HX	32·7	
	QA794 ..	Sydney University SU302	32	
D. Tropical Legume Miscellany				
Arachis hypogea L.	QA354 ..	Brisbane	35	27
	QA376 ..	University of Wisconsin ..	33·8	
	QA607 ..	U.S.D.A.	37	
	QA769 ..	Sydney University ..	37	
	QA787 ..	Sydney University SU319	35·5	
Cajanus cajan (L.) Millsp. ..	QA375 ..	University of Wisconsin ..	35·6	
Cassia chamaecrista L. ..	QA634 ..	U.S.A.	33	
Centrosema plumieri (Turp.) Benth.	QA811a	Malaya	35·2	
	QA813 ..	Brisbane	36·5	27
C. pubescens Benth.	QA522 ..	Atherton Tableland ..	42	17
	QA523*a*	Atherton Tableland ..	35	17
	QA853 ..	Atherton Tableland ..	35·2	17
	QA859 ..	Atherton Tableland ..	36·5	17
	QA835 ..	Atherton	35·2	17
	QA616, 1a	Cairns	35·2	17
	QA616, 2a	Cairns	34	17
	QA837 ..	Ayr	32·1	19
	QA838 ..	Ayr	35·2	19
	QA856 ..	Ayr	36·6	19
	QA549 ..	Innisfail	38·5	20
	QA851 ..	Innisfail	33	20
	QA843 ..	Mackay	36·5	21
	QA844 ..	Mackay	36·5	21
	QA848 ..	Rockhampton	38·5	23

APPENDIX 1.—continued.

Host.	Strain Number.	Source.	Maximum Temperature for Growth (°C.).	Latitude of Isolation (°S.).
C. pubescens Benth.—*cont.* ..	QA849 ..	Rockhampton	36·5	23
	QA852 ..	Rockhampton	35·2	23
	QA858 ..	Rockhampton	33·9	23
	QA841 ..	Moolboolaman	36·5	25
	QA842 ..	Moolboolaman	35·2	25
	QA733 ..	Coolum	36·6	26
	QA855 ..	Currumbin	35·2	28
	QA602 ..	U.S.D.A.	35·6	
	QA723 ..	Japan	36·6	
	QA687 ..	Rubber Research Institute, Malaya 272	38	
C. virginianum (L.) Benth.	QA860 ..	U.S.D.A.	35·5	
Clitoria ternatea L.	QA560 ..	Atherton Tableland ..	33	17
	QA606 ..	U.S.D.A.	38	
Crotalaria intermedia Klotschy	QA863 ..	Redland Bay (CB188) ..	41·4	27
C. sagitallis L.	QA642 ..	U.S.A.	35·6	
Cytisus proliferus L.f. ..	QA715 ..	Toowoomba	33	27
Desmodium uncinatum (Jacq.) DC.	QA623 ..	Coolum	32	26·5
D. sessilifolium (Torr.) Torr. & Gray	QA638 ..	U.S.A.	39·5	
Desmodium sp.	QA780 ..	Brisbane	31	27
Glycine javanica L.	QA619, 2a	Coolum	35·2	26·5
Glycine max (L.) Merrill ..	QA597 ..	U.S.D.A.	33·8	
	QA598 ..	U.S.D.A.	38	
	QA647 ..	U.S.A.	33	
	QA648 ..	U.S.A.	35·6	
Indigofera endecaphylla Jacq.	QA861 ..	Beerwah (CB33)	32	27
I. australis Willd.	QA468 ..	Kingaroy	30·5	27
Lespedeza striata Hook. & Arn.	QA604 ..	U.S.D.A.	36	
	QA375 ..	University of Wisconsin ..	32	
	QA765 ..	Sydney University ..	38	
	QA766 ..	Sydney University ..	32	
	QA768 ..	Sydney University ..	30	
Lotus australis Andr. ..	QA865 ..	Rodds Bay (CB440) ..	35·5	24
Mucuna utilis Wall.	QA603 ..	U.S.D.A.	33	
Phaseolus lathyroides L. ..	QA629 ..	Coolum	32	26·5
	QA862 ..	Calliope	35·5	24
P. radiatus L.	QA601 ..	U.S.A.	34	
Psoralea eriantha Benth. ..	QA864 ..	Charleville (CB362) ..	38	27
Pueraria phaseoloides (Roxb.) Benth.	QA548b	Innisfail	34	20
	QA600 ..	U.S.D.A.	32	
Pultenaea villosa Willd. ..	QA323 ..	Brisbane	35·6	27
Sesbania aculeata Auct. Austral.	QA866 ..	Richmond (CB294) ..	38	20·6
Vigna sinensis (L.) Endl. ex. Hassk.	QA314 ..	Brisbane	37	27
	QA608 ..	Grantham	35·6	27
	QA113 ..	Queensland	37	

APPENDIX 2.

NUMBERS OF PLANTS WITH NODULES IN GLASSHOUSE TRIAL.

Treatment.			Replication.						Percentage of Plants Nodulated.
Strain.	Concentration.	Hours.	I.	II.	III.	IV.	V.	VI.	
QA837	Low	0	8 (9)	9 (10)	10 (10)	10 (10)	9 (11)	8 (8)	93·1
		3	3 (10)	5 (7)	4 (15)	3 (7)	0 (4)	2 (6)	34·7
		6	0 (16)	5 (12)	0 (9)	1 (10)	0 (14)	0 (10)	8·5
		9	0 (15)	0 (11)	0 (7)	0 (12)	0 (13)	2 (9)	3·0
QA837	High	0	8 (9)	10 (10)	6 (7)	10 (13)	8 (8)	8 (8)	90·9
		3	6 (7)	4 (10)	7 (11)	4 (8)	0 (13)	6 (11)	45·0
		6	4 (10)	3 (14)	0 (14)	2 (11)	1 (11)	0 (9)	14·5
		9	0 (5)	5 (8)	0 (11)	1 (11)	7 (9)	6 (8)	36·6
QA549	Low	0	8 (8)	13 (16)	11 (12)	13 (14)	10 (10)	8 (9)	91·3
		3	4 (5)	12 (14)	13 (15)	6 (8)	7 (10)	10 (11)	82·5
		6	0 (11)	3 (11)	0 (12)	0 (9)	5 (10)	8 (13)	24·2
		9	2 (15)	2 (9)	2 (8)	5 (10)	4 (6)	1 (8)	28·6
QA549	High	0	7 (7)	8 (9)	10 (11)	8 (10)	9 (10)	7 (12)	83·1
		3	10 (10)	9 (12)	8 (14)	10 (10)	8 (9)	9 (10)	83·1
		6	5 (7)	10 (15)	10 (12)	6 (10)	12 (15)	15 (15)	78·4
		9	9 (11)	8 (9)	11 (11)	11 (14)	12 (15)	9 (13)	82·2

Figures in parenthesis refer to the number of plants per plot.
Low concentration = 1 x 10^3 organisms per seed.
High concentration = 1 x 10^5 organisms per seed.

SUMMARY—MEAN VALUES AND SIGNIFICANT DIFFERENCES IN PERCENTAGE OF PLANTS WITH NODULES.

Transformed Means.

		Concentration.		Strain.		Means.
		L	H	QA837	QA549	
Times—	0	76·8	74·0	77·4	73·4	75·4
	3	48·9	57·0	37·6	68·4	53·0
	6	15·6	41·0	14·0	42·6	28·0
	9	19·0	49·9	18·3	50·6	34·4
Strain—	QA837	31·0	42·6			36·8
	QA549	49·2	68·3			58·7
Means		40·1	55·5	36·8	58·7	47·8

Equivalent Mean Percentages.

		Concentration.		Strain.		Means.
		L	H	QA837	QA549	
Times—	0	94·8	92·4	95·2	91·8	93·6
	3	56·8	70·3	37·2	86·4	63·8
	6	7·2	43·0	5·8	45·8	22·5
	9	10·6	58·5	9·9	59·7	31·9
Strain—	QA837	26·5	45·0			35·9
	QA549	57·3	86·3			73·0
Means		41·5	67·9	35·9	73·0	–

Means.	Strain Equivalent.	Necessary Difference.	
		5%.	1%.
Concentrations v. Times	4·82	13·6	18·0
Strains v. Times	4·82	13·6	18·0
Concentrations v. Strains	3·41	9·6	12·8

Interactions of Strains x Times and Concentrations x Times are highly significant.

APPENDIX 3.

AVERAGE NODULE NUMBERS PER PLANT IN GLASSHOUSE TRIAL.

Treatment.			Replication.			
Strain.	Concentration.	Hours at 40°C.	I.	II.	III.	IV.
QA837	Low ..	0	4·2	3·4	4·9	3·5
		3	0·9	1·6	0·5	0·7
		6	0	1·2	0	0·2
		9	0	0	0	0
	High ..	0	3·8	3·7	2·6	1·9
		3	1·4	1·2	1·9	0·9
		6	1·6	1·2	0	0·3
		9	0	5·9	0	0·1
QA549	Low ..	0	6·5	3·5	7·6	4·4
		3	4·4	2·5	2·5	3·1
		6	0	0·9	0	0·9
		9	0·6	0·7	1·6	2·2
	High ..	0	4·7	4·6	3·5	2·8
		3	3·5	2·5	2·8	4·5
		6	6·6	2·9	4·2	4·5
		9	2·3	5·4	3·4	3·4

Low concentration = 1 x 10^3 organisms per seed.
High concentration = 1 x 10^5 organisms per seed.

SUMMARY—MEAN VALUES AND SIGNIFICANT DIFFERENCES AVERAGE NUMBER OF NODULES/PLANT.

Transformed Means.

		Concentration.		Strain.		Means.
		L	H	QA837	QA549	
Times—	0	·75	·64	·64	·74	·69
	3	·44	·50	·32	·62	·47
	6	·09	·48	·16	·40	·28
	9	·17	·44	·11	·50	·30
Strain—	QA837	·27	·35			·31
	QA549	·45	·67			·56
Means		·36	·51	·31	·56	·44

Equivalent Mean Numbers.

		Concs.		Strain.		Means.
		L	H	QA837	QA549	
Times—	0	4·62	3·37	3·37	1·50	3·90
	3	1·75	2·16	1·09	3·17	1·95
	6	·23	2·02	·44	1·51	·90
	9	·48	1·75	·29	2·16	1·00
Strain—	QA837	·86	1·24			1·04
	QA549	1·82	3·68			2·63
Means		1·29	2·24	1·04	2·63	–

Means.	Strain Equivalent.	Necessary Difference.	
		5%.	1%.
Concentrations v. Times	·053	·15	·20
Strains v. Times	·053	·15	·20
Concentrations v. Strains	·038	·11	·14

Interaction of Concentrations x Times is highly significant.

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