SPECIFICITY AND NITROGEN FIXATION IN THE RHIZOBIUM SYMBIOSIS OF *CENTROSEMA PUBESCENS* BENTH.

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SUMMARY.

The plant and bacterial interactions in legume symbiosis have been studied for the tropical legume *Centrosema pubescens* Benth. and incidental data on nodulation and symbiosis of other tropical legumes also obtained.

C. pubescens and *C. plumieri* (Turp.) Benth. were shown to be highly specific in their bacterial requirements and were more so than *Vigna sinensis* (L.) Endl. ex Hassk., *Phaseolus lathyroides* L., *Desmodium uncinatum* (Jacq.) DC., *Glycine javanica* L. and *Stylosanthes gracilis* H.B.K. The infectivity results are discussed in relation to the classical concept of "cross-inoculation" groups. The cowpea and some other cross-inoculation groups would be better placed in a general tropical legume miscellany within which smaller groups show some relationships between their members.

Twenty-one Rhizobium isolates from *C. pubescens* from different parts of Queensland were tested in sterile sand culture in the glasshouse. A range of effectiveness was recorded and a highly significant correlation was obtained between weight of nodules per plant and aboveground plant weight. Differences between strains in mean nodule weight and numbers of nodules were found. Nodule production by some strains varies markedly with small changes of the environmental conditions. Further studies showed no interference with the nodulation of a highly infective strain, inoculated to the seed, from a non-infective strain added to the soil in heavy concentration.

The agricultural significance of promiscuity and specificity in nodulation is discussed. The legume with highly specific requirements, while being difficult to establish without inoculation by selected strains, may be more free of competition effects when inoculated seed is sown.

I. INTRODUCTION.

Centrosema pubescens Benth., a legume native to South America, has been used in many tropical and subtropical environments as a cover crop. In Queensland, however, it is becoming increasingly important as a legume component of tropical and subtropical pastures, particularly on well-drained coastal and sub-coastal soils with an annual rainfall greater than 45 in. a year. A study has been made of a number of aspects of the Rhizobium symbiosis of this legume, this paper dealing with infection and plant response to inoculation.

II. INFECTION.

In the classical concept of cross-inoculation groups, *C. pubescens* would be placed in the cowpea group on the ability of isolates from this host to nodulate cowpea and other members of this group (Beeley 1938; Ishizawa 1954) and the occasional nodulation of *C. pubescens* by isolates from members of the cowpea group (Wilson 1939a; Ishizawa 1954).

In some areas of Queensland, *C. pubescens* has failed to nodulate even when planted among well-nodulated native legumes regarded as belonging to the cowpea "cross-inoculation" group. Such observations as this led to studies to define the degree of specificity of this legume for nodulation. Two studies were conducted on *C. pubescens* and other tropical legumes, using isolates from a range of tropical legume genera. A third was conducted at the intrageneric level.

(1) Experiment 1. Field Studies on Cross-inoculation with Isolates from Four Tropical Legumes.

This investigation was carried out at Coolum (latitude 26·5°S), in the wallum-heath coastal area of southern Queensland. The vegetation of the site in the virgin state included native legumes belonging to the cowpea "cross-inoculation" group (Bowen 1956).

Surface-sterilized seeds of *C. pubescens, Desmodium uncinatum* (Jacq.) DC., *Glycine javanica* L. and *Stylosanthes gracilis* H.B.K. were inoculated with an isolate from each of these species. These were planted in 11 ft. rows, using six randomised replications.

Fourteen weeks later inspection of the roots provided the data summarised in Table 1.

Table 1.

Nodulation of Introduced Tropical Legumes in the Wallum Area.

Inoculum Treatment.	Host of Isolation.	Test Plant.			
		Desmodium uncinatum.	*Centrosema pubescens.*	*Glycine javanica.*	*Stylosanthes gracilis.*
No inoculum		–*	–	±	+
QA573 ..	*Desmodium uncinatum*	+	–	±	+
QA616, 1a ..	*Centrosema pubescens* ..	+	+	+	+
QA619, 2a ..	*Glycine javanica* ..	+	–	+	+
CB103 ..	*Stylosanthes gracilis* ..	+	–	+	+

* + = Good nodulation.
– = No nodulation.
± = Poor nodulation of a minority of plants.

The need for inoculation of introduced tropical legumes is indicated, as well as the more specific demands of *C. pubescens*. Inclement growth conditions precluded obtaining conclusive data on relative effectiveness of the various strains. Nodules of *G. javanica* produced by naturally occurring organisms were frequently of an ineffective type. Nodulation occurring on all other species was of an effective type, judged by the presence of haemoglobin. Naturally occuring legume bacteria were able to nodulate *Stylosanthes gracilis.* Other observations have indicated that this legume is not highly specific in its nodulation requirements.

(2) Experiment 2. Glasshouse Tests with 27 Isolates from Tropical Legumes.

The second investigation was conducted in the glasshouse, using a modification of the method devised by Leonard (1943) for sterile sand culture testing of Rhizobium strains. Isolates from 27 tropical legumes were inoculated to *Vigna sinensis* (L.) Endl. ex. Hassk., *Phaseolus lathyroides* L., *Centrosema pubescens* and *C. plumieri* (Turp.) Benth. Twenty-five of these isolates were from legumes not of the genus *Centrosema*. The presence of unusual hypertrophies on the roots of *C. pubescens* necessitated repetition of this portion of the trial. The formation of these hypertrophies was confirmed. The results of Trial 2 are summarised in Table 2.

Table 2.

RESPONSE OF *Vigna sinensis, Phaseolus lathyroides, Centrosema pubescens* AND *C. plumieri* TO INOCULATION WITH ISOLATES FROM TROPICAL LEGUMES.

Strain.	Host of Isolation.	Test Plant.			
		Vigna sinensis.	*Phaseolus lathyroides.*	*Centrosema pubescens.*	*Centrosema plumieri.*
QA374 ..	*Cajanus cajan* (L.) Millsp. ..	+*	+	h*	—
QA549 ..	*Centrosema pubescens* Benth. ..	+	+	+	+
QA616, 1a	,, ,,	+	+	+	+
QA560 ..	*Clitoria ternatea* L.	+	+	h	—
QA705, 1a	*Cyamopsis tetragonoloba* (L.) Taub.	+	+	+h	+
QA638 ..	*Desmodium sessilifolium* (Torr.) Torr. & Gray	+	+	h	—
QA623, 1a	*Desmodium uncinatum* (Jacq.) DC.	+	+	h	—
QA744 ..	,, ,,	+	+	h	—
QA780 ..	,, ,,	+	+	h	+
QA720 ..	*Desmodium* sp.	+	+	h	+
QA618, 2a	*Glycine javanica* L.	+	+	h	—
QA619, 2a	,, ,,	+	+	h	—
QA721 ..	*Glycine max* (L.) Merr.	+	+	h	—
QA626, 3b	*Glycine tabacina* (Labill.) Benth. ..	+	+	h	—
QA636 ..	*Indigofera hirsuta* L.	+	+	h	—
QA641 ..	*Lespedeza hirta* (L.) Hornem. ..	+	+	—	—
QA766 ..	*Lespedeza striata* Hook. & Arn. ..	+	+	h	+
QA629, 1a	*Phaseolus lathyroides* L.	+	+	h	—
QA706c ..	*Phaseolus mungo* L.	+	+	h	—
QA548b ..	*Pueraria phaseoloides* Benth. ..	+	+	h	+
QA759 ..	,, ,,	+	+	h	+
QA760 ..	*Pueraria thunbergiana* (S. & Z.) Benth.	+	+	h	—
QA323 ..	*Pultenaea villosa* Willd.	+	+	h	+
QA751 ..	*Stizolobium deeringianum* Bort. ..	+	+	h	—
QA546b ..	*Stylosanthes gracilis* H.B.K. ..	+	+	h	+
QA752 ..	,, ,,	+	+	h	—
QA753 ..	,, ,,	+	+	+	+
	Uninoculated (quadruplicate) ..	—	—	—	—

* + = Presence of nodules.
h = Presence of root hypertrophies.
— = Absence of nodules and root hypertrophies.

The results show the quite specific requirements of *C. pubescens* and *C. plumieri* and the ability of *V. sinensis* and *P. lathyroides* to nodulate with a wide range of isolates. All observed nodulation was of an effective type except for QA705,1a on *C. pubescens* and *C. plumieri,* which was ineffective. Plants bearing root hypertrophies were identical in growth with uninoculated plants.

The histological nature of the hypertrophies shows them to be modified lateral roots. Despite the absence of organised bacterial tissue in them, their absence from uninoculated pots suggests that they are probably stimulated by the presence of the bacteria. Variation between trials in percentage of plants carrying these hypertrophies and the absence of the phenomenon in the field may point to an aborted infection under the ideal glasshouse testing conditions. They will be discussed more fully by the author in a later paper.

(3) Experiment 3. Glasshouse Study with Isolates from Three Species of Centrosema.

This experiment was at the intrageneric level. The method was the same as that used in Experiment 2. Two isolates from *Centrosema pubescens* (QA549 and QA616,1a), three from *C. plumieri* (QA811a, QA813, and QA821) and one from *C. virginianum* (L.) Benth. (QA860) were inoculated to *C. pubescens* and to *C. plumieri,* using four plants per pot and five replications. All strains from *C. pubescens* and *C. plumieri* nodulated both test species well. However, nodulation by the isolate from *C. virginianum* was very poor and did not occur in all pots. All uninoculated pots were free of nodules. *C. virginianum* is a North American species whereas the other two are South American.

(4) Discussion.

The specific requirements of both *C. pubescens* and *C. plumieri* have been shown. This serves to corroborate the data of Beeley (1938), Wilson (1939) and Ishizawa (1954). E. M. Hutton (personal communication) has found *C. pubescens* to be closely self-pollinated, with possibly some ability for cross-pollination in tropical environments. *C. pubescens* thus fits into the hypothesis proposed by Wilson (1939b) of correlation between cross-pollination and a promiscuous nodulating habit, and between self-fertility and specificity in nodulation. On the other hand, Norris (1956) points out that the promiscuously nodulating *Phaseolus lathyroides* has been found by Hutton to be closely self-fertile.

Although reasonably completely reciprocal nodulation is usual with some of the classical cross-inoculation groups, a close examination shows that these groups appear to be based on a minority of species within genera which are closely related botanically (and often geographically). The results reported here support the evidence of other workers that the strict concept of mutual cross-inoculation cannot be applied to the cowpea ''cross-inoculation'' group. This is so much so that its members, together with the soybean cross-inoculation group and possibly the lupin group, the bean group and the strain-specific Sesbania series, would be better regarded as a tropical legume miscellany within which some individual species may nodulate with a wide range of legume bacteria of the cowpea type (promiscuous legumes) and others with very few (highly specific legumes). Within this miscellany, studies within the one genus, e.g. *Phaseolus, Lupinus, Sesbania* and now *Centrosema,* do indicate some degree of order based on botanical and possibly ecological and geographical relationships of small groups. The agricultural significance of promiscuity and specificity is discussed later in this paper.

III. EFFECTIVENESS.

McKnight (1949) gave data showing the low effectiveness of isolates from a number of Queensland native legumes tested on *Vigna sinensis.* Examples of poor effectiveness of many naturally occurring organisms were quoted by Harris (1954).

In some parts of Queensland, *C. pubescens* has become established without inoculation. Such stands are usually well nodulated, the source apparently being from chance infection from naturally occurring organisms. The effectiveness of these naturally occurring strains for *C. pubescens* in Queensland was considered an important aspect for study.

(1) Experiment 1. Relative Effectiveness of 21 Isolations from C. pubescens.

Isolations were made from nodules of *C. pubescens* nodulating naturally in 21 sites from 11 localities in Queensland, from the southern border (latitude 28°S) to Cairns (latitude 17°S). Surface-sterilized seed was planted in sterile testing units of the Leonard type. After emergence, these were thinned to four plants per pot and each plant watered at the base with 1 ml. of a heavy suspension of the test organism. There were six randomised replications. Planting was carried out on Nov. 11, 1957, inoculation 14 days later (7 days after emergence) and harvesting 50 days after inoculation. The first signs of nitrogen fixation were observed 18 days after inoculation. The results are summarised in Tables 3 and 4.

(a) Plant Growth.

Variation in plant growth caused by a plant genetic factor which resulted in poor and often delayed nodulation necessitated two statistical analyses—one based on the 4 plants per pot and the other on the early-nodulating plants as indicated by the presence of well-developed nodules on the crown. Strain QA839 was eliminated from the analyses because of very poor and variable nodulation. Strain QA855 was excluded also because of large individual plot variation.

The results of Experiment 1 show a wide range of relative effectiveness not only among strains collected from widely different areas but also among those collected in the same general area, some strains being up to 60 per cent. more effective than others, under the particular experimental conditions. However, excluding the very poorly infecting strain QA839, all strains are highly effective compared with the uninoculated plots. The order of growth differences between inoculated and uninoculated plants is shown in Fig. 1.

(b) Nitrogen Content.

The analyses show the applied nitrogen controls (equivalent of 1 cwt. ammonium sulphate per acre) to have a much lower nitrogen percentage than inoculated plants. The applied nitrogen was quickly used by the plants, which grew vigorously until the nitrogen was depleted and then grew only slowly. Total nitrogen in all plots, except QA839, exceeded that of the nitrogen added at the equivalent of 1 cwt. of ammonium sulphate per acre.

Table 3.

RELATIVE EFFECTIVENESS OF NATURALLY OCCURRING RHIZOBIA STRAINS FROM *C. pubescens*.

(*a*) *Oven-dry Weight of Plant Tops.*

Strain.	Locality of Isolation.	Mean Weight in Grams per Plant.	Mean Weight Early Nodulating Plants.
Applied Nitrogen ..		0·584	0·584
QA845	Mackay	0·516	0·566
QA851	Utchee Creek (Innisfail)	0·502	0·530
QA616, 1a	Cairns	0·501	0·532
QA549	Utchee Creek (Innisfail)	0·496	0·562
QA850	Utchee Creek (Innisfail)	0·496	0·561
QA836	South Johnstone	0·477	0·477
QA835	Atherton Tableland	0·466	0·485
QA842	Gayndah	0·462	0·555
QA854	East Barron	0·448	0·460
QA837	Ayr	0·439	0·419
QA849	Rockhampton	0·432	0·452
QA841	Gin Gin	0·421	0·421
QA847	Gympie	0·401	0·419
QA843	Mackay	0·400	0·400
QA848	Rockhampton	0·390	0·450
QA844	Mackay	0·390	0·390
QA853	East Barron	0·364	0·403
QA838	Ayr	0·358	0·390
QA852	Rockhampton	0·330	0·339
S.E. Strain Mean		0·040	0·044
Necessary Difference for Significance (5% level)		0·114	0·122
(1% level)		0·150	0·162
QA839	Ayr	0·089	0·109
QA855	Currumbin	0·371	0·399
Uninoculated ..		0·071	0·071

(*b*) *Nitrogen Analyses of Plant Tops.*

Treatment.	Percentage N Based on Oven-dry Weight.
Effectively nodulated	2·9
Applied nitrogen (control)	1·4
Uninoculated	1·2

(c) Number and Weight of Nodules.

These data are presented in Table 4. Three distinct groups are indicated with respect to infection under the conditions of this experiment, namely QA854–QA851, QA842–QA849 and QA839 in Table 4. Strain QA839 was very poorly infective, producing nodules on only half of the test plants. Of these, nodulation was late on half, and numbers of nodules on all nodulated plants was few, the average being 7·5.

There are also significant differences in mean nodule weight.

Table 4.

RELATIVE EFFECTIVENESS OF NATURALLY OCCURRING RHIZOBIA STRAINS FROM *C. pubescens*.

(*a*) *Mean Number and Weight of Nodules.*

Strain.	Locality of Isolation.	Nodule Numbers per Plant.		Nodule Weight (g.).
		Transformed Means*.	Equivalent Means.	
QA854	East Barron	1·472	29·7	·00129
QA836	South Johnstone	1·443	27·7	·00163
QA835	Atherton Tableland	1·391	24·6	·00144
QA549	Utchee Creek (Innisfail)	1·377	23·8	·00152
QA847	Gympie	1·362	23·0	·00140
QA855	Currumbin	1·357	22·8	·00121
QA843	Mackay	1·333	21·5	·00168
QA851	Utchee Creek (Innisfail)	1·318	20·8	·00193
QA842	Gayndah	1·221	16·6	·00186
QA844	Mackay	1·218	16·5	·00180
QA850	Utchee Creek (Innisfail)	1·214	16·4	·00221
QA838	Ayr	1·209	16·2	·00180
QA616, 1a	Cairns	1·197	15·7	·00262
QA853	East Barron	1·194	15·6	·00173
QA852	Rockhampton	1·181	15·2	·00270
QA841	Gin Gin	1·169	14·8	·00240
QA837	Ayr	1·146	14·0	·00286
QA848	Rockhampton	1·109	12·8	·00213
QA845	Mackay	1·088	12·2	·00269
QA849	Rockhampton	1·045	11·1	·00275
S.E. Strain Mean		·071		·000231
Necessary difference for significance (5% level)		·199		·00065
(1% level)		·264		·00086
QA839	Ayr		3·7	·00114

* Prior to analyses of the number of nodules per plant the log transformation was used to equalise the variances.

(*b*) *Nitrogen Analyses of Nodules.*

4·82% N based on oven-dry weight.

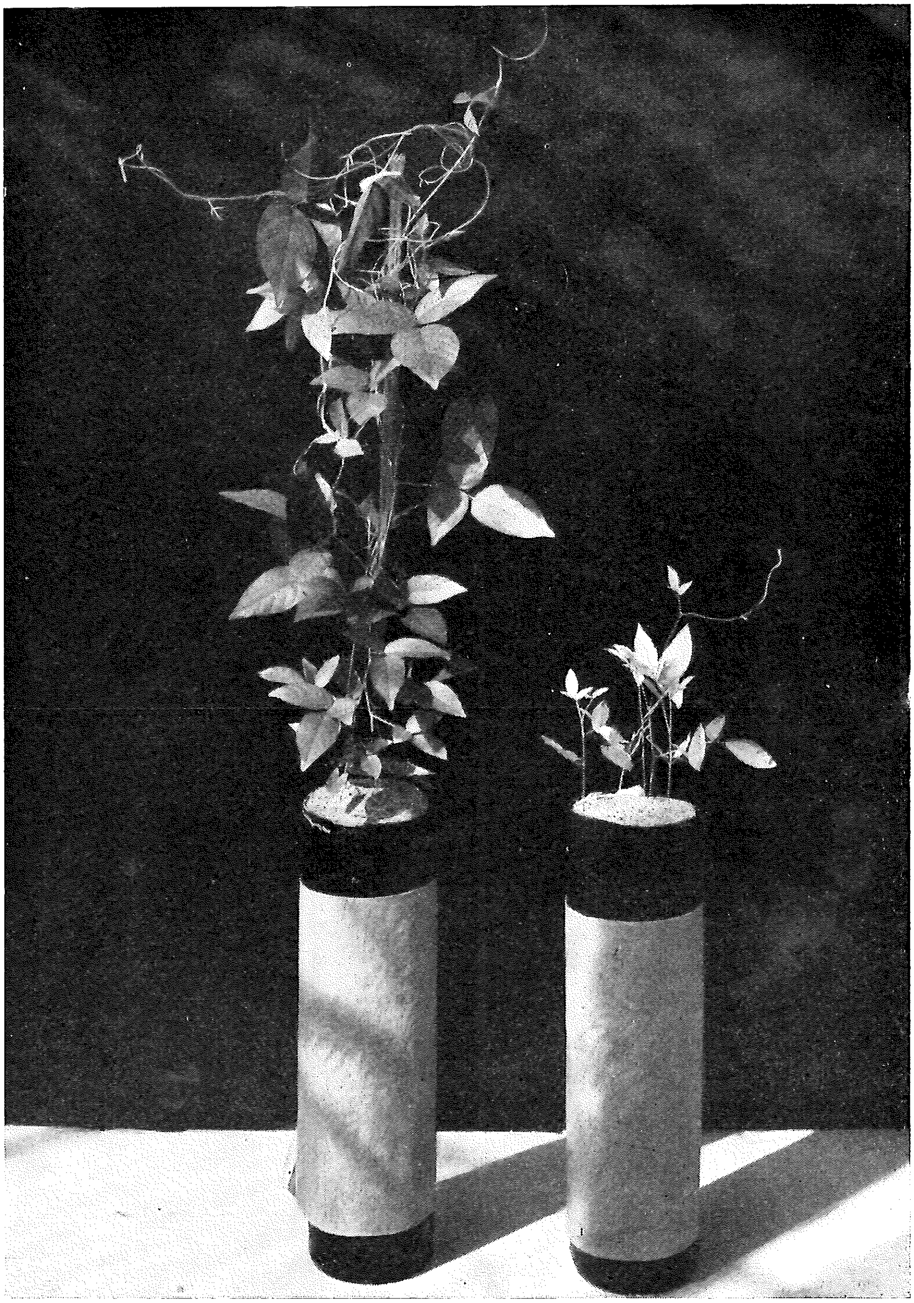

Fig. 1.

Response to Inoculation in Glasshouse Studies. Left: Inoculated. Right: Uninoculated.

(d) Correlation of Plant Growth and Nodule Weight.

Chen and Thornton (1940) showed from considerations of the structure of effective and ineffective nodules of clover, pea and soybean that differences in plant response to these two types of association were due to differences in volume of active bacterial tissue rather than inherent physiological ability of the bacterial strain to fix nitrogen. Four bacterial strains were studied with clover, two with soybean and two with pea. Although this work is often referred to by later workers, similar enquiries into the bases of plant response to different bacterial strains are few.

In the present studies with 17 strains, individual *C. pubescens* plants were harvested and nodule weights recorded for each plant. The average regression of plant weight on nodule weight was found to be highly significant. Differences between regression coefficients for each strain were not significant. The average regression coefficient was 9·09.

This supports the findings of Chen and Thornton (1940) in that the different strains have the same nitrogen-fixing ability weight for weight of active bacterial tissue, the differences in effectiveness resulting from the different amounts of active bacterial tissue formed.

(2) Experiment 2. Isolations from Three Species of Centrosema.

Isolations from *C. pubescens, C. plumieri* and *C. virginianum* were tested on *C. pubescens* and *C. plumieri,* using the modified Leonard technique and five replications. The experiment was planted on Jan. 13, 1958, inoculated 7 days later, and harvested 34 days later still.

The results are presented in Table 5.

All strains except QA860 were effective on *C. plumieri.* While the applied nitrogen treatment (equivalent of 1 cwt. ammonium sulphate per acre) produced the highest plant weight, the nitrogen percentage of plants in this treatment was so low that it was exceeded by the total nitrogen from plants of all strains except QA860. The poor effectivity of QA860 was found to be due to low infection and not lowered nodule size. All strains were effective on *C. pubescens,* QA549 being superior to the others. However, QA860 was poorly infective here also.

The trial did not proceed long enough for total nitrogen in inoculated plots to exceed that of the applied nitrogen treatment. Some tendency in that direction was observed.

(3) Experiment 3. Combined Inoculation of Effective and Non-infective Strains.

Experiments 1 and 2 on effectiveness were carried out using a pure culture method. In field practice this may not be the case. The author (Bowen 1956) has indicated that almost all of the studied indigenous legumes of

Table 5.

EFFECTIVENESS OF RHIZOBIA STRAINS ON *C. plumieri* AND *C. pubescens.*

(*a*) *Oven-dry Weight of Plant Tops.*

1. *C. plumieri.*

Strain.	Host of Isolation.	Mean Oven-dry Weight of 4 Plants (g.).
Nitrogen		3·14
QA821	*C. plumieri*	2·97
QA616, 1a ..	*C. pubescens*	2·92
QA549	*C. pubescens*	2·76
QA813	*C. plumieri*	2·68
QA811, a	*C. plumieri*	2·54
QA860	*C. virginianum*	1·20
Uninoculated ..		0·94
S.E.		0·251
Necessary difference for significance (5% level) ..		0·73
(1% level) ..		0·98

2. *C. pubescens.*

Strain.	Host of Isolation.	Mean Oven-dry Weight of 4 Plants (g.).
Nitrogen		1·82
QA549	*C. pubescens*	0·73
QA811, a	*C. plumieri*	0·58
QA813	*C. plumieri*	0·54
QA821	*C. plumieri*	0·51
QA616, 1a ..	*C. pubescens*	0·48
QA860	*C. virginianum*	0·40
Uninoculated ..		0·28
S.E. (excluding the nitrogen control)		0·065
Necessary difference for significance (5% level) ..		0·19
(1% level) ..		0·26

(*b*) *Nitrogen Analyses of Plant Tops.*

Treatment.	Percentage Nitrogen Based on Oven-dry Weight.	
	C. plumieri.	*C. pubescens.*
Effectively nodulated	3·0	3·0
Applied nitrogen (control)	1·6	2·1
Uninoculated	1·3	1·4

Queensland belong to the tropical legume miscellany with respect to infection. In many areas of Queensland, large numbers of native legumes occur. There is therefore in many places a soil flora carrying high populations of legume bacteria of the tropical legume type. This raises the question of possible interference to nodulation by native Rhizobium strains incapable of forming nodules on the host.

Krasilnikov and Karenyako (1944) and Harris (1954) have reported a stimulative effect on nodulation by other soil organisms. Purchase and Nutman (1957) reported that with avirulent and virulent clover strains the avirulent strain competed with the virulent strain and in mixtures containing a great excess of the avirulent form the formation of nodules was reduced. Kronberger (1936) also found a depression of plant response using a mixed culture.

Experiment 3 was carried out using a sterile sand culture and the modified Leonard technique. QA323, an isolate from the native legume *Pultenaea villosa* Willd. and non-infective on *C. pubescens,* was inoculated to the sand at $3x10^4$ organisms per gram of sand just prior to planting surface-sterilized seed of *C. pubescens* inoculated with the effective strain QA549 at the rate of $1 \cdot 4x10^3$ organisms per seed. Twelve seeds per pot were planted and the seedlings were thinned to 4 plants per pot. Sixteen replications of QA549 alone, QA323 alone, and the mixture were employed.

At harvest 11 weeks later no significant differences were recorded in either plant weight or nodule numbers between QA549 alone and the QA549–QA323 mixture. Mean oven-dried plant weights per pot were 0·674 g. and 0·668 g. respectively and nodule numbers per pot 20·4 and 18·2 respectively. QA323 alone produced no nodulation and was equivalent to uninoculated controls, for which the mean oven-dried plant weight per pot was 0·137 g.

(4) Discussion.

In general, Rhizobium strains isolated from naturally infected plants of *C. pubescens* and *C. plumieri* proved to be effective on *C. pubescens.* Although the small typical ineffective type of nodule has been produced in the glasshouse by the author with a few selected isolates from other tropical legumes, such typical ineffective nodules have not been seen in the field.

In Experiment 1 a highly significant correlation was shown between individual plant growth response to nodulation and the total weight of nodules on the plant. Comparing the 17 bacterial strains examined, differences in the regression coefficients for dependence of plant weight on nodule weight were not significant. This shows that differences in effectiveness between the bacterial strains were due only to differences between strains in the amount of active bacterial tissue formed and not to differences between strains in the nitrogen-fixing ability per unit weight of bacterial tissue. The statistical analysis of data in Table 4 indicates that some of the more effective strains produced significantly more nodules under the experimental conditions while other highly effective strains achieved a high total nodule weight by producing larger nodules.

The very poor effectivity of strain QA839 is explained by a very poor production of nodules (i.e., low infection) and a slight decrease in mean nodule weight. Again, in Experiment 2, poor response of *C. pubescens* and *C. plumieri* to QA860 is mainly a matter of poor infection. Ishizawa (1955) recorded ineffective responses in *C. pubescens,* but a number of strains giving such responses were shown earlier (Ishizawa 1954) to be poorly infective. Examination of the data of other workers (McKnight 1949) on *Vigna sinensis* shows that poor plant response is often a result of poor infective ability of the Rhizobium.

It may be asked, ''How absolute are differences in relative effectiveness?'' The virulence (or ability to produce nodules) of a strain varies under varying conditions. Other workers have shown that a number of soil physical, chemical and biological factors and climatic factors can modify number of nodules. The results of Experiment 2 indicate strain QA549 to be significantly better on *C. pubescens* than QA616,1a at the 5 per cent. significance level. The difference approaches 1 per cent. significance, yet response to the two strains was practically identical in Experiment 1. The difference in Experiment 2 was caused by very poor production of nodules by QA616,1a under the particular set of experimental conditions. Mean nodule weights of QA616,1a and QA549 were of the same order. Other such cases have been recorded in this work.

A need is indicated for a closer study of the property of infection and nodule formation and of plant physiological and environmental factors affecting these. Meanwhile, the selection of a legume bacterium for a particular legume for general agricultural use must depend on the result of trials under several conditions, with an eye to selection of one of high effectiveness and consistently high virulence as suggested by Raju (1936). Such a strain is QA549 for *C. pubescens.*

The mutually effective nodulation of *C. plumieri* and *C. pubescens* by strains effective on either is interesting, considering the very specific requirements of *C. pubescens* for infectiveness and effectiveness. These are both South American species and these results lend further support to the possibility of establishing minor effectiveness sub-groupings within the ''tropical legume miscellany.''

The agricultural significance and importance of promiscuity and specificity may be an interesting field for further study. The ability of *Vigna sinensis* to nodulate effectively with a wide range of legume bacteria has been one factor in its great agronomic popularity. (Conversely, this popularity has led to its wide use as a test plant for Rhizobium isolates from many tropical legumes and their designation to the cowpea ''cross-inoculation'' group.) Similarly, the promiscuous *Phaseolus lathyroides* has been a popular pasture plant in Queensland for some years. On the other hand, some legumes nodulate promiscuously but the association is usually poorly effective. This can lead to quite misleading conclusions regarding the plant's potential. Such legumes appear to be *Samanea saman* (Jacq.) Merrill and *Clitoria ternatea* L. (Allen and Allen 1939).

When considering the mixed culture interaction, the position with establishing *C. pubescens* in the field in Queensland is that it is highly specific in its requirements for nodulation but when nodulating with naturally occurring strains of bacteria such association is likely to be effective, the actual response depending on the environment. Experiment 3 indicates that interference with a highly infective inoculated strain by naturally occurring legume bacteria of the cowpea type not infecting *C. pubescens* is not likely to be of importance. This is in accordance with the findings of Erdman (1946) and Hofer (1945). It is possible that response to strains of variable or low virulence may be reduced by such naturally occurring soil organisms.

Leonard (1930), Dunham and Baldwin (1931), Nicol and Thornton (1941), Burton and Allen (1949) and Harris (1954) have shown that mixtures of poorly effective and highly effective strains may result in an intermediate plant reaction or even an ineffective response. Harris (1954) states—"Where the soil already supports a considerable population of rhizobia, irrespective of whether effective or ineffective types predominate, it is only with difficulty that the desired strain can be superimposed on the natural pattern by seed inoculation."

It would seem that the legacy of the promiscuously nodulating legume is one of possible interference to highly effective inoculated strains by poorly effective naturally occurring legume bacteria. This would be of more economic importance in a perennial legume with a stoloniferous habit. Many pasture legumes fall into this category. The legume with more specific bacterial requirements, while needing inoculation for establishment and display of its potential to plant introduction officers and farmers alike, may be more free of insidious competition effects. More intensive study at the mixed culture level is urgently needed.

IV. PLANT HETEROGENEITY.

The presence of very poorly nodulated plants in the field side by side with heavily nodulated plants has been observed by the author since the commencement of these studies in 1955. This has been observed with seed from several countries and with all strains of legume bacteria for *C. pubescens* studied in the field. In the Rhizobium strain trials in pots, Leonard jars and test tubes, a number of plants failed to nodulate, while others nodulated only sparsely and often only on distal roots despite the presence in the same pot of well-nodulated, vigorously growing plants.

This phenomenon was further investigated, starting with vegetatively propagated material of both well and poorly nodulated plants. Evidence accumulated suggests that a plant factor exists which determines the extent of nodulation in *C. pubescens*. This work will be described in more detail in a subsequent paper.

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REFERENCES.

ALLEN, O. N., and ALLEN, ETHEL K. 1939. Root nodule bacteria of some tropical leguminous plants. II. Cross-inoculation tests within the cowpea group. Soil Sci. 47:63-76.

BEELEY, F. 1938. Nodule bacteria and leguminous cover plants. J. Rubb. Res. Inst. Malaya 8:149-162.

BOWEN, G. D. 1956. Nodulation of legumes indigenous to Queensland. Qd J. Agric. Sci. 13:47-60.

BURTON, J. C., and ALLEN, O. N. 1949. Inoculation of crimson clover (*Trifolium incarnatum* L.) with mixtures of Rhizobium strains. Proc. Soil. Sci. Soc. Amer. 14:191-195.

CHEN, H. K., and THORNTON, H. G. 1940. The structure of ''ineffective'' nodules and its influence on nitrogen fixation. Proc. Roy. Soc. B 129, 208-229.

DUNHAM, D. H., and BALDWIN, I. L. 1931. Double infection of leguminous plants with good and poor strains of rhizobia. Soil. Sci. 32:235-248.

ERDMAN, L. W. 1946. Studies to determine if antibiosis occurs among rhizobia. I. Between *Rhizobium meliloti* and *Rhizobium trifolii*. J. Amer. Soc. Agron. 38:251-258.

HARRIS, J. R. 1953. Influence of rhizosphere micro-organisms on the virulence of *Rhizobium trifolii*. Nature, Lond. 172:507-508.

HARRIS, J. R. 1954. Rhizosphere relationships of subterranean clover. I. Interactions between strains of *Rhizobium trifolii*. Aust. J. Agric. Res. 5:247-270.

HOFER, A. W. 1945. Nitrogen fixation by mixed cultures of Rhizobium. Proc. Soil Sci. Soc. Amer. 10:202-205.

ISHIZAWA, A. S. 1954. Studies on the root nodule bacteria of leguminous plants. II. The relationship between nodule bacteria and leguminous plants. Part 1. From the view of nodule production. a. Cross-inoculation test. J. Sci. Soil, Tokyo, 24:297-302.

ISHIZAWA, A. S. 1955. Studies on the root nodule bacteria of leguminous plants. II. Relationship between nodule bacteria and leguminous plants. Part 2. From the view of nitrogen fixation. b. Experiments on the cowpea group (1). J. Sci. Soil, Tokyo 25:241-244.

KRASILNIKOV, N. A., and KORENYAKO, A. I. 1944. Influence of soil bacteria on the virulence and activity of Rhizobium. Microbiology, Moscow, 13:39-44.

KRONBERGER, M. 1936. Über die gegenseitige Hemmung und Förderung der Knöllchenbakterien. Prakt. Bl. PflBau 14:203-209. (In Allen, Ethel K., and Allen, O. N. 1958. Biological aspects of symbiotic nitrogen fixation. Encyclopedia of Plant Physiology. VIII:48-118.)

LEONARD, L. T. 1930. A failure of Austrian winter peas apparently due to nodule bacteria. J. Amer. Soc. Agron. 22:277-280.

LEONARD, L. T. 1943. A simple assembly for use in the testing of cultures of rhizobia. J. Bact. 45:523-525.

McKNIGHT, T. 1949. Efficiency of isolates of Rhizobium in the cowpea group, with proposed additions to the group. Qd J. Agric. Sci. 6:61-76.

NICOL, H., and THORNTON, H. G. 1941. Competition between related strains of nodule bacteria and its influence on infection of the legume host. Proc. Roy. Soc. B 130:32-59.

NORRIS, D. O. 1956. Legumes and the Rhizobium symbiosis. Emp. J. Exp. Agric. 24:247-270.

PURCHASE, Hilary F., and NUTMAN, P. S. 1957. Studies on the physiology of nodule formation. VI. The influence of bacterial numbers in the rhizosphere on nodule initiation. Ann. Bot., Lond. N.S. 21:439-454.

RAJU, M. S. 1936. Studies on the bacterial-plant groups. II. Variations in the infective power of the nodule bacteria of cowpea group. Zbl. Bakt. 94:337-348.

WILSON, J. K. 1939a. Leguminous plants and their associated organisms. Mem. Cornell Agric. Exp. Sta. No. 221.

WILSON, J. K. 1939b. A relation between pollination and nodulation of the Leguminosae. J. Amer. Soc. Agron. 31:159-70.

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