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Efficiency of Isolates of *Rhizobium* in the Cowpea Group, with Proposed Additions to this Group

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SUMMARY

Forty-one isolates of *Rhizobium* from cultivated, naturalized and indigenous legumes, obtained from various sources, have been tested for their nitrogen-fixing efficiency on *Vigna unguiculata*.

Based on the ability of isolates of *Rhizobium* from indigenous legumes to nodulate and fix nitrogen in association with *Vigna unguiculata*, the following additions to the cowpea cross-inoculation group may be suggested :—

Jacksonia scoparia R.Br., *Platylobium formosum* Sm., *Mirbelia reticulata* Sm., *Pultenaea villosa* Willd., *Pultenaea myrtoides* A.Cunn., *Acacia cunninghamii* Hook., *Acacia podalyriaefolia* A.Cunn., *Acacia granitica* Maid.

The significance of the results obtained in the investigations is discussed.

INTRODUCTION

Isolates of *Rhizobium* vary a great deal in their efficiency, a fact which is of considerable economic importance in agriculture.

As a result of many demonstrations under bacteriologically controlled conditions that strains of any one species of *Rhizobium* may vary in their ability to benefit the host plant with which they are tested, it has become commonplace to refer to " effective " and " ineffective " strains.

Recently, however, the concept of " host plant specificity " has been introduced to explain why a given strain of *Rhizobium* may nodulate unsatisfactorily on, and provide little nitrogen to, one host, and at the same time be capable of nodulating normally and fixing nitrogen satisfactorily on another host plant of a different species within the same cross-inoculation group. A more precise understanding of the problem of strain variation has followed the work of Chen and Thornton (1940), who have shown that the whole of the large differences between the amounts of nitrogen fixed by effective and ineffective strains can be accounted for by the difference in volume and the duration in time of the infected nodule cells. It is therefore suggested that ineffective strains are no less efficient in fixing nitrogen per unit of bacterial mass in unit time than are effective strains.

A soluble substance inimical to the bacteria, formed within the nodules produced by an ineffective strain, has been demonstrated by Chen *et al.* (1940), and this would account for the early arresting of nodule growth and for the short life of the nodule. As individual plants can be found in which a normally effective strain will behave ineffectively, and *vice versa,* it would appear that a maladaptation between the bacteria and the plant results in the condition of " ineffectiveness " (Thornton, 1945).

In Queensland, collecting isolates of *Rhizobium* and testing their efficiency on economically important legumes has been undertaken in connection with the establishment of a legume inoculum service for farmers and orchardists. Previously, Steindl (1940) found a local isolate of *Rhizobium* most effective in association with cowpea (*Vigna unguiculata* Walp.). In the present investigations, isolates of *Rhizobium* have been obtained from cultivated, naturalized and indigenous legumes, and their nitrogen-fixing efficiency, in association with cowpea, determined in greenhouse sand culture tests.

MATERIALS AND METHODS

Collection of Material for Isolation

Root systems were selected in the field, wrapped in moist paper, and brought to the laboratory in tin canisters. Nodules selected for isolation were sound, firm and generally young, mostly flesh coloured on the exterior, and located more or less close to the crown of the plant. Nodules on secondary roots generally were avoided, except in the case of tree species, in which it was frequently found that nodules may not be present near the crown.

Isolation

Selected nodules were washed under running tap water and surface sterilized by agitation for four minutes in 1 : 500 acidified $HgCl_2$ (HCl 2.5 ml., $HgCl_2$ 1.0 gm., tap water 500 ml.) plus " Agral " spreader. The surface sterilized nodules were washed in four changes of sterile water and crushed with a sterile glass rod in 10 ml. of sterile tap water in bacteriological test tubes. Serial loop dilutions were plated from this suspension and incubated at 25°C. until the appearance of colonies, and well isolated colonies were then selected for transfer to agar slants.

The asparagus extract-mannitol medium of Carroll (1934) was used for the isolation of cultures throughout these investigations. Its composition is $C_6H_8(OH)_6$ 10.0 gm., K_2HPO_4 0.5 gm., $MgSO_4 7H_2O$ 0.2 gm., NaCl 0.1 gm., $CaCO_3$ 3.0 gm., agar 15.0 gm., asparagus extract 50 ml., distilled water 950 ml.

Maintenance of Isolates

Cultures were maintained on asparagus extract-mannitol medium at 25° C., and sub-cultures were made at four-weekly intervals.

Sand Culture Experiments

The sand culture tests were carried out in a greenhouse previously sprayed with 5 per cent. formalin.

Containers were 7-inch porous pots painted on the outside with white "Solpah" paving paint. Washed coarse river sand was used in experiments 1 and 2 and washed white silver sand in experiment 3. Containers and sand were sterilized by steaming for 24 hours during a 3-day period.

The seeds were sterilized in 1 : 500 acidified $HgCl_2$, and this was followed by four rinsings in sterile tap water. In experiments 1 and 2, the seeds were inoculated with the appropriate cultures at the time of planting by wetting the seed coat with a suspension of bacteria in sterile water. In experiment 3, inoculation was performed subsequent to germination by the distribution at the base of each seedling of a heavy suspension of the isolate at the rate of 2 ml. per pot.

Three days after germination the seedlings were thinned to three per pot and the surface of the sand in each pot was covered with a layer of sterilized cotton lint, approximately one inch thick, to reduce moisture loss. The plants throughout growth received sterile nitrogen-free nutrient solution ($CaSO_4$ 6.0 gm., $MgSO_47H_2O$ 1.0 gm., KH_2PO_4 1.0 gm., KCl 1.5 gm., Fe, B, Cu, Mn and Zn in trace quantities, tap water 5000 ml.). Every third application was of sterile water.

At the conclusion of the experiment green tops were weighed, the air-dry or oven-dry weights of roots determined, and the results statistically examined. Determinations of nitrogen were made on tops, and in experiments 2 and 3 also on the roots. The distribution of nodules and their types, numbers and average size were recorded.

Table 1.

ORIGIN AND PERIOD IN CULTURE OF ISOLATES IN EXPERIMENT 1.

Isolate No.	Legume from which Isolated	Origin	Months in Culture
R.2	*Crotalaria* sp.	U.S.A.*	61
R.6	*Phaseolus lunatus*	U.S.A.*	61
R.17	*Glycine max*	N.S.W.	17
R.31	*Vigna catjang*	U.S.A.*	61
R.21	*Lespedeza* sp.	N.S.W.	17
R.22	*Lespedeza* sp.	Qld.	18
R.23	*L. stipulacea*	U.S.A.*	61
R.24	*L. stipulacea*	U.S.A.*	61
R.25	*L. sericea*	U.S.A.*	61
R.26	*L. striata*	U.S.A.*	61
R.35	*Vigna* sp.	N.S.W.	17
R.32	*Stizolobium* sp.	U.S.A.*	61
R.42	*Vigna unguiculata*	Qld.	22
R.43	*V. unguiculata*	Qld.	18
R.45	*V. unguiculata*	Qld.	15
R.51	*Aotus lanigera*	Qld.	13
R.70	*Phaseolus lathyroides*	Qld.	7
R.72	*Arachis hypogaea*	Qld.	3
R.100	*Pueraria phaseoloides*	Malaya†	16
R.101	*P. phaseoloides*	Malaya†	16
R.110	*Acacia podalyriaefolia*	Qld.	15
R.113	*Vigna unguiculata*	Qld.	5
R.114	*V. unguiculata*	Qld.	5
C	Not Inoculated	—	—

* United States Department of Agriculture.
† Rubber Research Institute, Malaya.

EXPERIMENTAL

Experiment 1

In this experiment 23 isolates of *Rhizobium* from various Queensland and foreign sources were tested in association with *Vigna unguiculata*. Seven of these isolates were obtained from species of *Vigna*, 13 from other hosts within the cowpea cross-inoculation group, and two from legumes whose status in the cross-inoculation group system was unknown. The remaining strain was an isolate of *Rh. japonicum* (from *Glycine max*). The origin of these strains and their period in culture are shown in Table 1.

R.114 is a re-isolation of strain R.42. Strain R.42 had been cultured for over one year on asparagus extract-mannitol agar ; inoculated into *Vigna unguiculata* ; re-isolated ; and cultured again as R.114 five months prior to this experiment. R.114 was included to determine if there was any difference in nitrogen-fixing efficiency from R.42 as a result of plant passage in the interim.

Seven-day-old slants on asparagus extract-mannitol medium were used for inoculum. Seeds were sterilized, inoculated with a heavy sterile water suspension

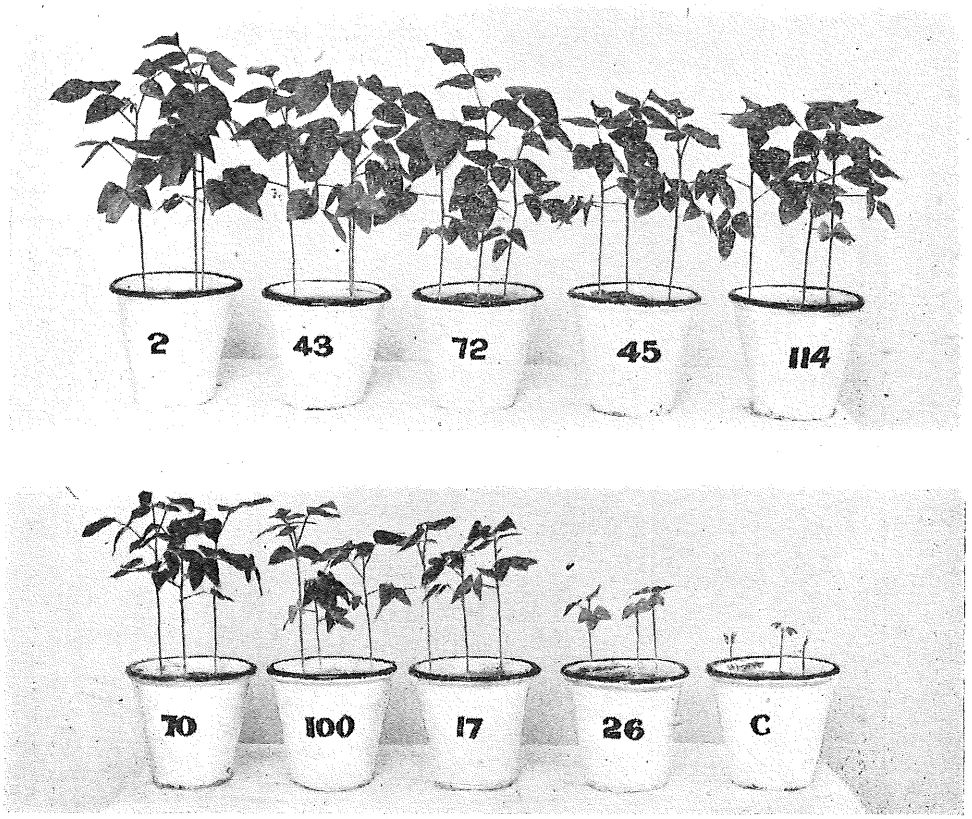

Figure 1.
Top growth of various plants in Experiment 1.

Table 2.

GREEN WEIGHTS AND COLOUR OF FOLIAGE—EXPERIMENT 1.

Isolate No.	Legume from which isolated	Leaf colour	Mean green weight of tops	Green weight differences	
				At 1% level significantly exceeds	At 5% level significantly exceeds
			gm.		
R.2	*Crotalaria* sp.	Green	26.5	R.42 *et seq.*	R.22 *et seq.*
R.45	*V. unguiculata*	"	25.6	R.24 "	R.22 "
R.113	*V. unguiculata*	"	23.5	R.31 "	R.24 "
R.43	*V. unguiculata*	"	23.2	R.110 "	R.70 "
R.114	*V. unguiculata*	"	23.2	R.110 "	R.70 "
R.22	*Lespedeza* sp.	"	20.0	R.17 "	R.32 "
R.42	*Vigna unguiculata*	"	19.6	R.17 "	R.32 "
R.72	*Arachis hypogaea*	"	18.8	R.100 "	R.17 "
R.35	*Vigna* sp.	"	18.7	R.100 "	R.17 "
R.24	*Lespedeza stipulacea*	"	18.1	R.101 "	R.17 "
R.70	*Phaseolus lathyroides*	Light green	17.1	R.101 "	R.17 "
R.31	*Vigna catjang*	"	16.3	R.26 "	R.100 "
R.110	*Acacia podalyriaefolia*	"	15.4	R.26 "	R.101 "
R.25	*Lespedeza sericea*	"	15.2	R.26 "	R.26 "
R.6	*Phaseolus lunatus*	"	15.0	R.26 "	R.26 "
R.32	*Stizolobium* sp.	"	14.1	R.26 "	R.26 "
R.17	*Glycine max*	"	11.6	R.51 "	R.51 "
R.100	*Pueraria phaseoloides*	"	11.6	R.51 "	R.51 "
R.101	*P. phaseoloides*	"	10.6	R.51 "	R.51 "
R.26	*Lespedeza striata*	V. light green	7.0	—	—
R.51	*Aotus lanigera*	Chlorotic	2.7	—	—
R.23	*Lespedeza stipulacea*	"	1.2	—	—
R.21	*Lespedeza* sp.	"	1.1	—	—
C	Not inoculated	"	1.6	—	—

of the appropriate strain, and sown on Oct. 26, 1941, in sterilized coarse river sand in porous pots. There were three replications of each isolate and of the control, and the experiment was laid out as a 24 × 3 randomized block.

The top growth made by several treatments is shown in Figure 1. The tops were cut and weighed when the plants were 89 days old, and the figures statistically examined. The results obtained are set out in Table 2.

The results of the analysis are :—

F for strains 17.79, which is highly significant.

s.e. strain mean = ± 1.821 = 12.22 per cent. G.M.

Necessary difference for significance = 5.2 (5 per cent. level) ; 6.9 (1 per cent. level).

The most efficient strains were isolated from species of *Vigna* and *Crotalaria*. There was, however, considerable variation in efficiency shown by the different isolates from species of *Vigna*. No marked effect on R.42 as the result of plant passage was obtained.

Significant variation occurred in the efficiency of the isolates from *Lespedeza* spp. R.22, R.24 and R.25 benefited the host plant ; the remaining three isolates from this genus were ineffective.

Sears and Carroll (1927) established that *Rh. japonicum* may cause the formation of nodules on cowpea as well as on soybean. The isolate of *Rh. japonicum* used in this experiment caused the formation of scattered clumps of nodules of relatively large size, but was relatively ineffective.

Phaseolus lathyroides was added to the cowpea cross-inoculation group by Allen and Allen (1936). Isolate R.70 was obtained from well nodulated plants, and on *Vigna unguiculata* this isolate was of moderate efficiency. The isolates from *Pueraria phaseoloides* were among the least efficient.

Burrill and Hansen (1917) placed five species of *Acacia* in the cowpea cross-inoculation group. Subsequently 17 additions from this genus were made by Allen and Allen (1936). The strain of *Rhizobium* isolated from *A. podalyriaefolia* was of moderate efficiency in this experiment, and on the basis of this test may be added tentatively, pending the demonstration of the reciprocal cross-inoculation, to the cowpea group.

Experiment 2

The role of indigenous legumes, which comprise a significant portion of the virgin flora of Queensland, in furnishing strains of *Rhizobium* capable of symbiosis with cultivated legumes has not previously been investigated. No information has been available either on the extent to which indigenous legumes symbiose with naturally occurring strains of *Rhizobium* or on the cross-inoculation status of the strains associating with them.

As a preliminary to establishing the cross-inoculation status of a number of isolates from indigenous legumes, in this experiment and experiment 3 the ability of isolates from such sources to symbiose with a member of the cowpea cross-inoculation group was determined.

Isolates from indigenous legumes included in the present experiment were obtained from *Jacksonia scoparia, Acacia granitica, A. cunninghamii, Mirbelia reticulata* and *Platylobium formosum.*

Four isolates tested in experiment 1 (R2., R.43, R.113 and R.114) were re-tested to determine if they had maintained their original nitrogen-fixing efficiency after a further three years' storage on asparagus extract-mannitol medium.

Two isolates from *Centrosema pubescens* from a Malayan source were included, together with three isolates from species of *Crotalaria* and one from *Arachis hypogaea* from Queensland.

In addition to the uninoculated controls, a second series of controls was inoculated with an isolate of *Rh. lupini* (R.201).

The origin of the isolates and their period in culture are shown in Table 3.

Table 3.

ORIGIN AND PERIOD IN CULTURE OF ISOLATES USED IN EXPERIMENT 2.

Isolate No.	Legume from which Isolated	Origin	Months in Culture
R.2	*Crotalaria* sp.	U.S.A.	97
R.43	*Vigna unguiculata*	Qld.	54
R.73	*Arachis hypogaea*	Qld.	39
R.105	*Centrosema* sp.	Malaya	51
R.106	*Centrosema* sp.	Malaya	51
R.113	*Vigna unguiculata*	Qld.	41
R.114	*V. unguiculata*	Qld.	41
R.202	*Jacksonia scoparia*	Qld.	3
R.203	*Acacia granitica*	Qld.	4
R.208	*Crotalaria goreensis*	Qld.	6
R.209	*C. goreensis*	Qld.	6
R.210	*C. usaramoensis*	Qld.	6
R.224	*Mirbelia reticulata*	Qld.	2
R.226	*Acacia cunninghamii*	Qld.	2
R.228	*Platylobium formosum*	Qld.	1
R.201	*Lupinus angustifolius* (Control)	Qld.	6
C	Not Inoculated	—	—

Seed were sown on Oct. 2, 1944, in sterile river sand, each pot receiving the addition of 13 grams of sterile kaolin to provide greater moisture retention. On October 10 the seedlings were reduced to three per pot and inoculated with the appropriate isolate.

Applications of sterile nitrogen-free nutrient solution and of sterile water were made until variations in growth had occurred between the obviously superior and the less effective isolates, when the experiment was terminated. This occurred on November 11, 44 days after inoculation. Heights of plants and the number of nodules on the root systems were recorded, and the green weights of the tops and oven-dry weights of the roots determined. Percentage nitrogen determinations were made on the dry matter.

After the nitrogen supply from the cotyledons was exhausted, all plants showed the yellow or yellow-green colour indicative of nitrogen deficiency. Observations were made at daily intervals during the growing period to establish

Table 4.

SUMMARY OF RESULTS—EXPERIMENT 2.

Isolate No.	Legume from which isolated	Leaf colour	No. of days to green colour	Mean height	Mean No. of nodules per plant	Mean green weight of tops	Dry weight of roots	Nitrogen in dry matter	Green weight differences	
									At 1% level significantly exceeds	At 5% level significantly exceeds
				in.		gm.	gm.	%		
R.113	*Vigna unguiculata* ...	Green	22	9.5	72	17.83	2.77	3.52	R.203 *et seq.*	R.226 *et seq.*
R.114	*V. unguiculata*	"	24	8.5	56	15.67	2.31	3.20	R.210 "	R.203 "
R.226	*Acacia cunninghamii* ...	"	21	9.0	80	14.34	2.12	3.04	R.208 "	R.210 "
R.203	*A. granitica*	"	25	6.0	48	12.41	1.69	2.88	R.209 "	R.208 "
R.2	*Crotalaria* sp.	"	33	7.0	51	11.73	2.10	3.10	R.228 "	R.208 "
R.210	*C. usaramoensis* ...	"	22	7.0	47	10.85	2.08	3.22	R.224 "	R.228 "
R.208	*C. goreensis*	"	25	5.5	37	8.44	1.17	3.01	R.43 "	—
R.209	*C. goreensis*	"	28	5.5	48	8.09	1.50	2.72	R.43 "	—
R.228	*Platylobium formosum* ...	Light green	—	6.0	51	7.17	1.44	2.61	R.105 "	R.43 *et seq.*
R.202	*Jacksonia scoparia* ...	Green	27	5.0	34	7.14	1.34	2.00	R.106 "	R.43 "
R.73	*Arachis hypogaea* ...	Yellow-green	—	5.0	9	6.65	1.65	1.55	—	R.105 "
R.224	*Mirbelia reticulata* ...	Light green	—	6.0	40	5.79	1.35	2.54	—	R.201 "
R.43	*Vigna unguiculata* ...	Yellow-green	—	4.0	0.5	3.68	1.12	*	—	—
R.105	*Centrosema* sp.	Yellow	—	3.5	0	2.85	1.47	*	—	—
R.106	*Centrosema* sp.	"	—	3.3	0	2.73	1.44	*	—	—
R.201	*Lupinus angustifolius* (Control)	"	—	3.0	0	2.58	1.06	*	—	—
C	Not inoculated	"	—	3.0	0	2.57	0.99	*	—	—

* No determination made.

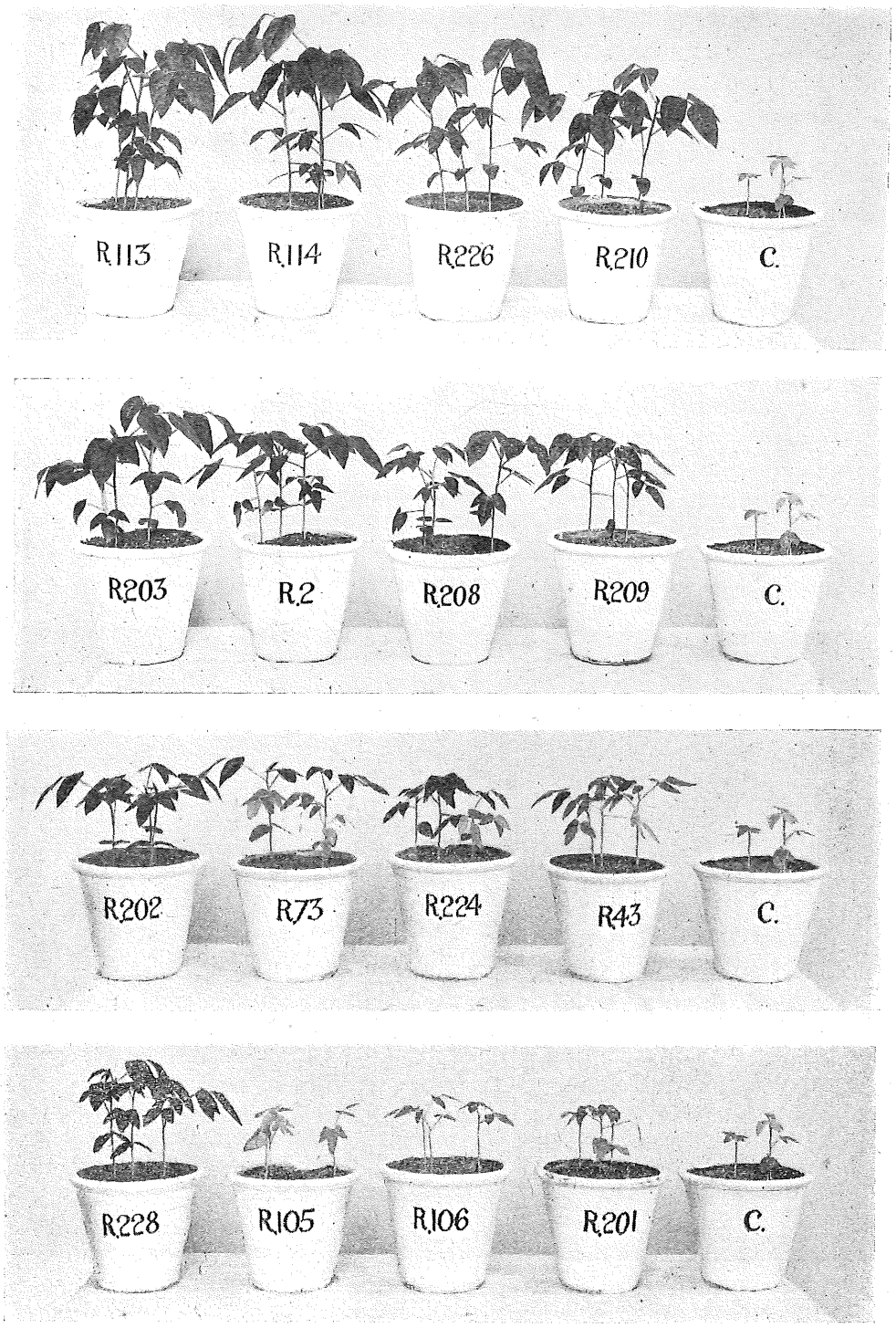

Fig. 2.
Top growth of various plants in Experiment 2.

with some precision when the green colour subsequently appeared in the leaves as a result of the nitrogen provided by the microsymbiote being used in plant metabolism.

The data obtained, together with the statistical examination of the green weights of the tops, are shown in Table 4. The results of the analysis are :—

F for strains = 19.15.

s.e. for strain mean = ± 1.108 = 13.4 per cent. G.M.

Necessary difference for significance = 3.2 (5 per cent. level); 4.3 (1 per cent. level).

Figures 2 and 3 show top growth and nodulation, respectively, of various plants in the experiment.

From this experiment it is concluded that strains of *Rhizobium* within the cowpea cross-inoculation group are associated with certain indigenous legumes. The practical importance of this fact is, of course, that green manure crops of the cowpea group planted on virgin soil may nodulate more or less effectively as the result of the previous growth of certain naturally inoculated indigenous legumes. Based on the ability of these isolates to nodulate and benefit *Vigna unguiculata*, the following tentative additions, pending the demonstration of the reciprocal cross-inoculation, are made to the cowpea cross-inoculation group :—

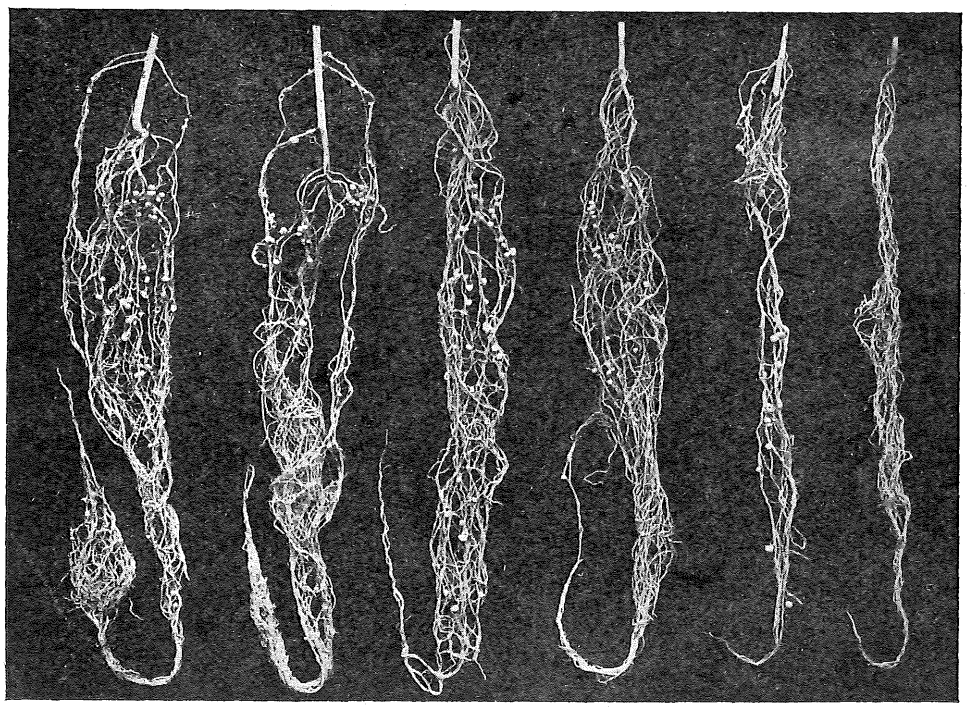

Fig. 3.
Nodulation produced by isolates from indigenous legumes. Isolates, from left to right, R.226, R.203, R.228, R.224, R.202, Control.

Jacksonia scoparia R.Br., *Mirbelia reticulata* Sm., *Platylobium formosum* Sm., *Acacia cunninghamii* Hook., and *Acacia granitica* Maid.

The isolates from indigenous legumes varied considerably in their nitrogen-fixing efficiency on *Vigna unguiculata*. The most efficient were isolated from the two species of *Acacia*. Compared with the most efficient isolates in this experiment, those from *Platylobium*, *Jacksonia* and *Mirbelia* were relatively inferior, but significantly exceeded the uninoculated controls. It is worthy of note that strain R.226 from *Acacia cunninghamii* produced the greatest mean number of nodules per plant in this experiment.

Of the four isolates tested in experiment 1, R.113 and R.114 have maintained an efficiency of the order originally exhibited, but R.2 and R.43 appear to have deteriorated in culture.

The addition of *Crotalaria usaramoensis* to the cowpea group was made by Allen and Allen (1936). The isolate from this species was of the same order of efficiency as the two isolates from *C. goreensis*.

The isolates from *Centrosema* sp. did not nodulate on *Vigna unguiculata*.

In sand culture experiments in a nitrogen-free medium, after the exhaustion of cotyledonary nitrogen the plant is dependent on its microsymbiote for nitrogen. Under the conditions obtaining in this experiment there does not appear to be any precise relationship between the efficiency of an isolate and the period required for the appearance of a green colour in the leaves. An isolate of moderate efficiency may be responsible for the appearance of a green leaf colour in as short a time as that taken by a strain of superior nitrogen-fixing efficiency.

Experiment 3

From the results of experiments 1 and 2, isolate R.113 was selected as an efficient nitrogen-fixing strain for the purpose of comparing the relative efficiency of six further isolates from various Queensland legumes.

Isolates from indigenous legumes were obtained from *Pultenaea villosa*, *P. myrtoides*, *Acacia cunninghamii*, and *Acacia* sp. (*implexa* or *maidenii*). The remaining isolates were from *Leucaena glauca* and *Lespedeza striata*. The origin of the isolates and their period in culture are shown in Table 5.

Table 5.

ORIGIN OF ISOLATES AND PERIOD IN CULTURE IN EXPERIMENT 3.

Isolate No.	Host from which Isolated	Origin	Months in Culture
R.113	*Vigna unguiculata*	Qld.	44
R.211	*Leucaena glauca*	Qld.	9
R.212	*Lespedeza striata*	Qld.	10
R.207	*Vigna unguiculata*	Qld.	9
R.223	*Pultenaea villosa*	Qld.	5
R.225	*Acacia* sp. (*implexa* or *maidenii*)	Qld.	5
R.227	*Pultenaea myrtoides*	Qld.	5
R.232	*Acacia cunninghamii*	Qld.	3

The experiment was terminated when the plants were 61 days old. At this time the plants symbiosing with the most effective strains were flowering and producing pods. The top growth and the root systems of some of the plants are shown in Figures 4 and 5, and the data obtained are summarized in Tables 6 and 7.

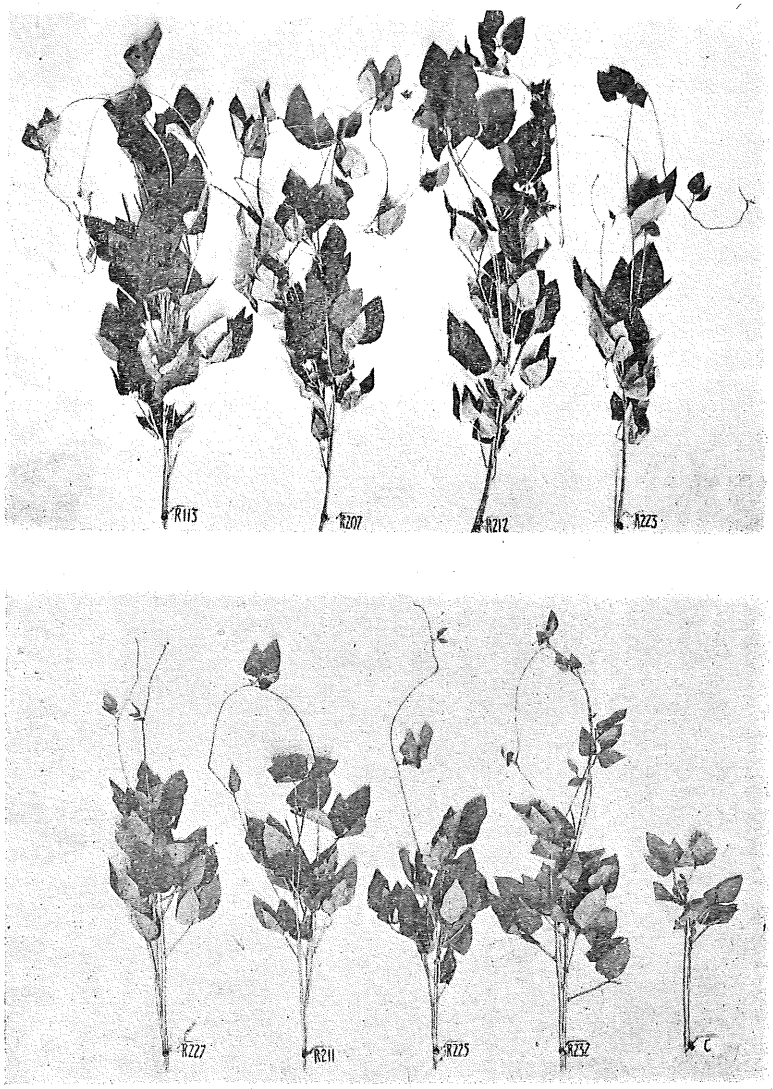

Fig. 4.
Top growth of various plants in Experiment 3.

The following tentative additions are made to the cowpea cross-inoculation group as a result of this experiment :—

Pultenaea villosa Willd. and *Pultenaea myrtoides* A.Cunn.

Table 6.

SUMMARY OF DATA FROM TOPS—EXPERIMENT 3.

Isolate No.	Legume from which isolated	Leaf colour	Mean height	No. of pods per plant	No. of flowers per plant	Mean green weight of tops	Nitrogen in dry tops	Green weight differences	
								At 1% level significantly exceeds	At 5% level significantly exceeds
			in.			gm.	%		
R.113	*Vigna unguiculata*	Green	46.5	4.3	2.25	67.34	2.80	R.207 *et seq.*	R.212 *et seq.*
R.212	*Lespedeza striata*	"	48.5	2.1	2.0	54.82	3.01	R.227 "	—
R.207	*Vigna unguiculata*	"	58.5	2.25	1.5	52.20	2.86	R.227 "	—
R.227	*Pultenaea myrtoides*	Light green	28.5	0	0.5	18.88	2.58	—	—
R.232	*Acacia cunninghamii*	V. light green	21.0	0	0.25	19.72	1.67	—	—
R.223	*Pultenaea villosa*	Light green	29.0	0.1	0.3	19.50	2.37	—	—
R.211	*Leucaena glauca*	"	25.0	0.1	0.25	14.91	2.20	—	—
R.225	*Acacia* sp. (*implexa* or *maidenii*)	V. light green	15.0	0	0.1	14.42	2.35	—	—
C	Not inoculated	Yellow	16.0	0	0	10.59	1.22	—	—

Result of Analysis :—

F for strains = **36.43**, which is highly significant.
s.e. strain mean = ± 3.522 = 11.5% G.M.
Necessary difference for significance = 10.3 (5% level).
14.0 (1% level).

Table 7.

SUMMARY OF DATA FROM ROOTS—EXPERIMENT 3.

Isolate No.	Legume from which isolated	Mean No. nodules per plant	Mean dry weight per block	Nitrogen in dried roots	Green weight differences	
					At 1% level significantly exceeds	At 5% level significantly exceeds
			gm.	%		
R.113	*Vigna unguiculata*	69	1.97	2.41	R.232 *et seq.*	R.227 *et seq.*
R.212	*Lespedeza striata*	43	1.59	2.26	R.225 "	R.232 "
R.207	*Vigna unguiculata*	58	1.40	2.61	C "	R.225 "
R.227	*Pultenaea myrtoides*	8	1.17	1.80	R.211 "	R.225 "
R.232	*Acacia cunninghamii*	4	0.74	1.12	—	—
R.225	*Acacia* sp. (*implexa* or *maidenii*)	6	0.69	1.63	—	—
R.223	*Pultenaea villosa*	5	0.47	1.85	—	—
R.211	*Leucaena glauca*	6	0.44	1.83	—	—
C	Not inoculated	0	0.57	1.25	—	—

Result of analysis :—

F. for strains = 8.165, which is highly significant.
s.e. for strain mean = ± .194 = 18.83% G.M.
Necessary difference for significance = .569 (5% level).
.773 (1% level).

The nodulation produced by strains R.113, R.207, and R.212 conforms to the orthodox "ideal," with the nodules occurring in the main on the upper region of the root system on or near the tap root (Fig. 5). *Leucaena glauca* was placed in the cowpea group by Allen and Allen (1936). The isolate from this species formed some of the largest individual nodules in the experiment, but the isolate was relatively ineffective. In contrast to the relatively or totally ineffective isolates from *Lespedeza* spp. reported in the previous experiments, the isolate from *L. striata* in this test was an efficient nitrogen-fixing strain.

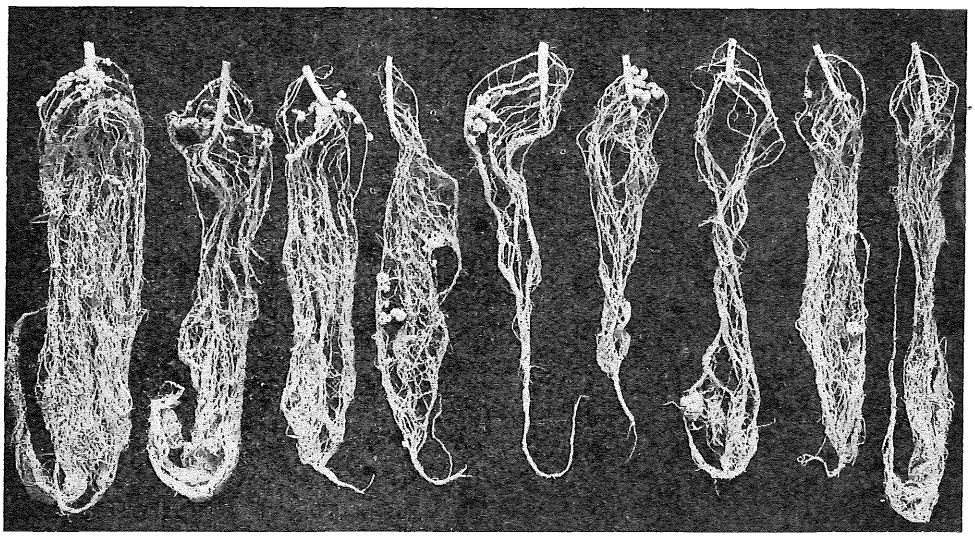

Fig. 5.
Root systems of various plants in Experiment 3. Isolate numbers, from left to right, R.113, R.207, R.212, R.227, R.223, R.211, R.232, R.225, Control.

CONCLUSIONS

Under greenhouse conditions in sand culture tests, 41 isolates of *Rhizobium* proved to be of varying efficiency in association with cowpea. One isolate (R.113) performed consistently well and a response to inoculation with this isolate has since been obtained under field conditions.

The role of indigenous legumes in furnishing strains of *Rhizobium* capable of symbiosing with cultivated legumes appears to have received scant attention in Australia. The indigenous legumes examined were found to be naturally well equipped with nitrogen nodules in the podsolized and poor sandy soils of coastal Queensland, where they form an important section of the flora. The isolates obtained from these sources have been shown to be capable of fixing nitrogen in association with cowpea. Pending the demonstration of the reciprocal cross-inoculation, these species are suggested for inclusion in the cowpea cross-inoculation group. There can be little doubt that the genus *Rhizobium* associated

with such indigenous legumes plays a significant role in the nitrogen economy of virgin soils. Subsequently an agriculturally important role is assumed by the organism when soils are cultivated and an association occurs with introduced legumes of the cowpea cross-inoculation group.

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