HERITABLE VARIATION IN NODULATION OF *CENTROSEMA PUBESCENS* BENTH.

By G. D. Bowen, B.Sc.* and Margaret M. Kennedy, B.Sc.†

SUMMARY

Field and glasshouse observations revealed extreme variation in nodulation and plant growth within several commercial seed lots of *Centrosema pubescens* Benth. inoculated with each of many Rhizobium strains.

Inoculation of vegetative propagations of plant selections and of seed from these showed that commercial seed contained stable sparsely nodulating and profusely nodulating lines.

Following inoculation with most Rhizobium strains, plants of the sparsely nodulating lines were greatly inferior to others in nodulation and yield in a nitrogen-deficient substrate. However, certain Rhizobium strains were found to nodulate these lines satisfactorily, good plant growth ensuing.

Profusely nodulating lines produced a greater number of nodules than average plants in commercial seed lots, but total nodule weight per plant and plant weight were unaltered by the profuse nodulation.

I. INTRODUCTION

The legume *Centrosema pubescens* Benth. has long been used extensively in tropical and subtropical regions as a green manure and cover crop (Whyte, Nilsson-Leissner, and Trumble 1953) and more recently in Queensland as a legume component of pastures. It has been shown previously (Bowen 1959*b*) that the number of nodules formed, the total nodule weight per plant, and plant yield of this legume vary with the Rhizobium strain used for inoculation. However, during nodulation studies (Bowen 1959*a*, 1959*b*) considerable variation of number and position of nodules, and of plant growth, was observed between adjacent plants growing in a uniform environment and inoculated with pure cultures of Rhizobium. This study investigates the hereditary nature of this variation.

II. MATERIALS AND METHODS

Seed.—Initial observations and plant selections were made on plants grown from *C. pubescens* seed of Malayan origin commercially available in Queensland.

* Formerly Pathologist, Queensland Department of Agriculture and Stock.

† Pathologist, Queensland Department of Agriculture and Stock.

Surface Sterilization of Seed and Plant Material.—Seed was surface-sterilized with either 0·1 per cent. mercuric chloride for 5 min or equal parts of ethyl alcohol and 20 volume hydrogen peroxide for 10 min. Plant material used for vegetative propagation was lightly surface-sterilized by wiping thoroughly with cotton wool soaked with 7 per cent. calcium hypochlorite filtrate. All surface sterilization was followed by washing the material in sterile water.

Vegetative Propagation.—In some instances surface-sterilized stolons were rooted directly in the testing units; in other cases the stolons were rooted in sterile sand before severance from the main plant and transfer to the testing units.

Inoculation and Growth of Plants.—Plants were grown in a closed glass-house in sterile washed river sand in testing units of a modified Leonard type (Leonard 1943), using a plant nutrient solution with nitrogen omitted (C. S. Andrew, personal communication). The test organism was introduced by inoculation of the seed with a high concentration of rhizobia or by application of 1 ml of a suspension of the rhizobia to the base of each seedling. Details of the performances of strains QA523, QA549 and QA837 in glasshouse studies have been published previously (Bowen 1959*b*).

III. RESULTS

(a) Variation in Nodule Numbers

Considerable variation in numbers of nodules on plants grown from commercial seed was observed both in sand culture in the glasshouse (Figure 1) and in the field (Figure 2). These large variations were recorded in the field with several Rhizobium strains and with commercial seed from three Queensland and five overseas sources.

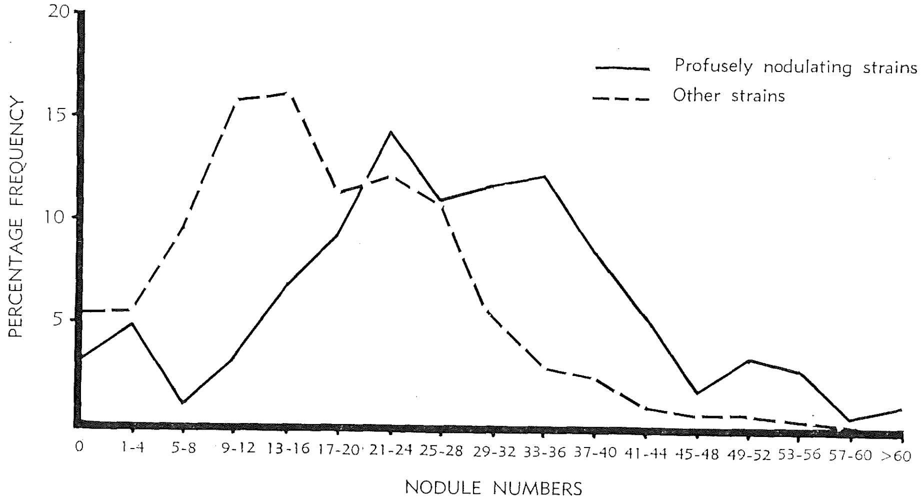

Fig. 1.—Distribution of nodule numbers per plant of *Centrosema pubescens*. Commercial seed inoculated in the glasshouse with profusely nodulating and poorly nodulating Rhizobium strains (Bowen 1959*b*). Data obtained from approximately 200 plants in each category.

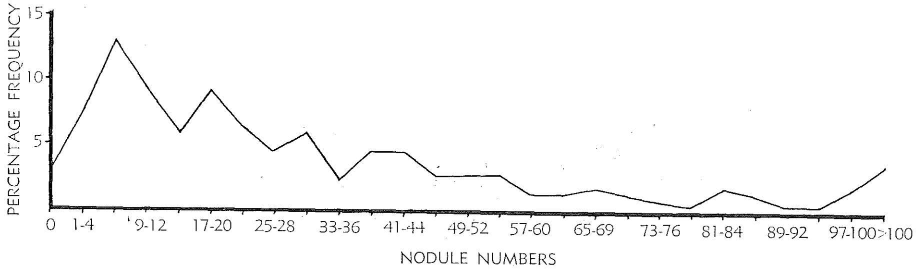

Fig. 2.—Distribution of nodule numbers per plant of *Centrosema pubescens.* Inoculated commercial seed planted in wallum-heath soil, February 1957. Plants 14 weeks old when examined.

In each case some plants were devoid of nodules or only sparsely nodulated and others were profusely nodulated compared with the normal nodule number. In both the glasshouse and the field, 3-5 per cent. of the plants did not form nodules.

The distribution of nodule numbers shown in Figure 1 renders the definition of sparse nodulation somewhat arbitary, but by taking less than one-third of the mean number of nodules per plant as a measure of sparse nodulation, 10-12 per cent. of plants in glasshouse sand culture and 30 per cent. of those in the field would be placed in this category. The higher proportion of apparently sparse nodulation in the field may result from the differences in growth conditions between glasshouse and field and the experimental errors inherent in field trials. In some soils where growing conditions tended to be adverse to nodulation, the proportion of poorly nodulated plants was higher than the above.

A tendency was observed in field studies for plants with sparsely nodulated main roots to also have sparsely nodulated stoloniferous roots.

Under conditions of nitrogen deficiency sparsely nodulated plants were yellow and noticeably smaller than adjacent well-nodulated plants (Figure 3). Some of the sparsely nodulating plants were also nodulated late (Figure 4).

(b) Nodulation of Vegetatively Propagated Plant Selections

Two series of vegetative propagations from field selections of profusely nodulated and non-nodulated plants were inoculated with Rhizobium strain QA549. This strain was a proven good strain and was available for commercial inoculation of *C. pubescens.* Nodule counts after eight weeks (Table 1) indicated that the clones maintained the selected character. However, since some plants of the clones from non-nodulated parent material produced nodules—these always being few in number—the "non-nodulating" character is more accurately described as sparsely nodulating.

Fig. 3.—Sparsely nodulating plant (yellow) of *Centrosema pubescens* growing among normal plants (green and vigorous).

TABLE 1

NODULATION OF VEGETATIVELY PROPAGATED SELECTIONS

Experiment No.	Non-nodulating Selections			Profusely Nodulating Selections		
	No. of Plants	No. of Nodulated Plants	Nodule Numbers/ Plant	No. of Plants	No. of Nodulated Plants	Nodule Numbers/ Plant
1 ..	10	6	0·6	17	17	16·3
2 ..	18	14	4·3	12	12	14·1

(c) Nodulation of Seedlings from Plant Selections

Seed was produced from five profusely nodulating and six sparsely nodulating field selections (subsequently referred to as P and S selections respectively). The seed and commercial seed were surface-sterilized and inoculated with Rhizobium strains QA523a and QA549. The results shown in Table 2 and Figures 5 and 6 were obtained after nine weeks and are derived from four replications of six plants per testing unit.

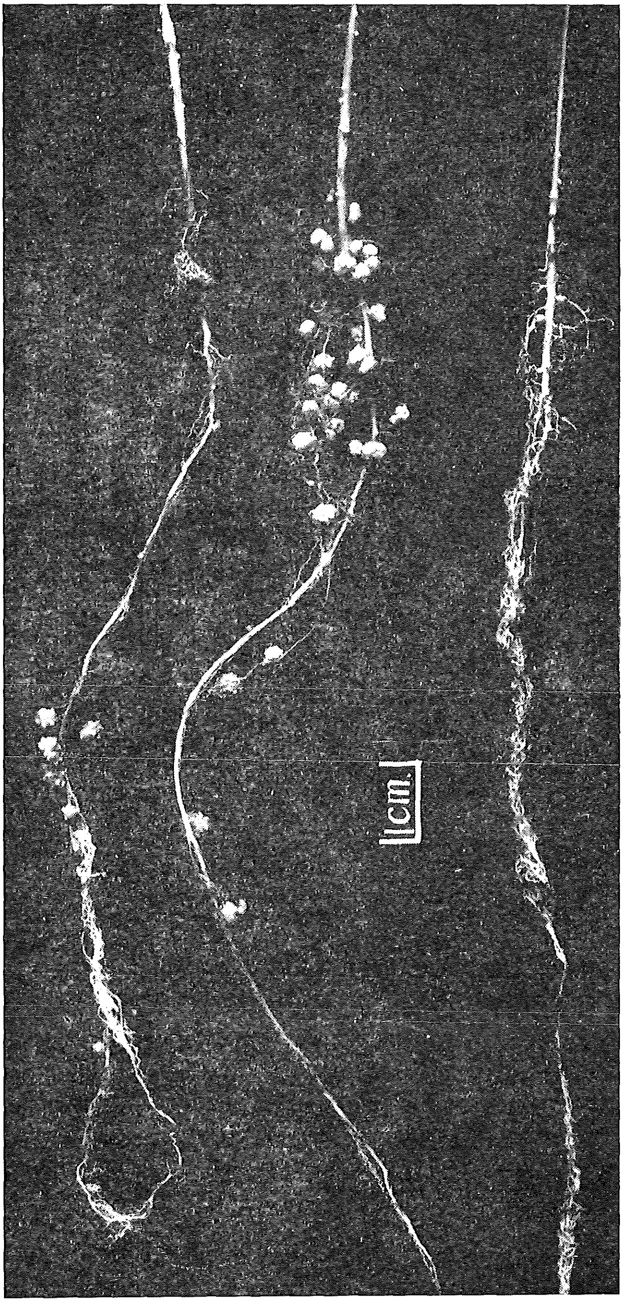

Fig. 4.—Roots of glasshouse plants all inoculated with Rhizobium Strain QA549 and growing in the same pot. Left, sparse and late nodulation. Centre, normal plant. Right, nodules absent.

TABLE 2

NODULATION AND GROWTH OF SEEDLINGS FROM PLANT SELECTIONS

Plant Selection	Mean No. of Nodules/Plant		Mean Oven-dry Weight of Nodules/Plant (mg)		Mean Oven-dry Weight of Plant Top (mg)	
	Inoculum		Inoculum		Inoculum	
	QA523a	QA549	QA523a	QA549	QA523a	QA549
(*a*) Sparsely nodulating*						
1	2·2	3·3	1·5	4·2	68·5	160·6
2	4·5	4·0	4·1	3·1	102	90·3
3	0·1	0·7	0·2	0·8	40	42
4	0·4	0·3	1·5	0·7	59·7	47
5	0·04	0·	0·2	0·	57	46
6	1·3	3·3	3·1	5·6	76	98·5
(*b*) Commercial seed						
7	6·5	9·5	14·2	16·2	240·5	258
(*c*) Profusely nodulating						
8	16·9	17·1	18·0	18·9	233	230
9	17·1	13·6	16·8	11·5	203	163
10	15·9	11·1	18·9	15·0	237	190
11	11·8	17·3	20·0	19·5	256	248
12	13·4	12·3	21·2	10·9	266	177
	8, 9, 10, 11, 12>>7 8>12		No significant differences		No significant differences	
Uninoculated seed ..	0		0		78	

* Because of the obviously highly significant differences between poorly nodulating selections and the rest, data from the former were not included in the statistical analysis.

The large differences between seedlings from S selections and other seed lots strikingly confirm that the amount of successful rhizobial infection (nodule formation) is determined by heritable characters of the plant, and that there exist in commercial seed separate stable lines with differences in nodulation potential. The greater nodule number of P lines than of normal plants from commercial seed was accompanied by a reduction in mean nodule size. This resulted in total nodule weight and plant response being the same in both cases. Plants of Selection 8 produced more nodules than those of Selection 12.

(d) Nodulation by Selected Rhizobium Strains

In earlier studies (Bowen 1959*b*), a few Rhizobium strains appeared to nodulate all plants. One of these was QA837. In some instances S selections nodulated profusely over a short period after some months in the glasshouse. Nodules from such material were used for isolation of strains QA869 and QA870. The Rhizobium strains QA549, QA837, QA869 and QA870 were inoculated to S lines, P lines and unselected material. In two of the five replicates (each with four plants) surface-sterilized seed was inoculated before planting, and in the remaining three seedling inoculation was carried out.

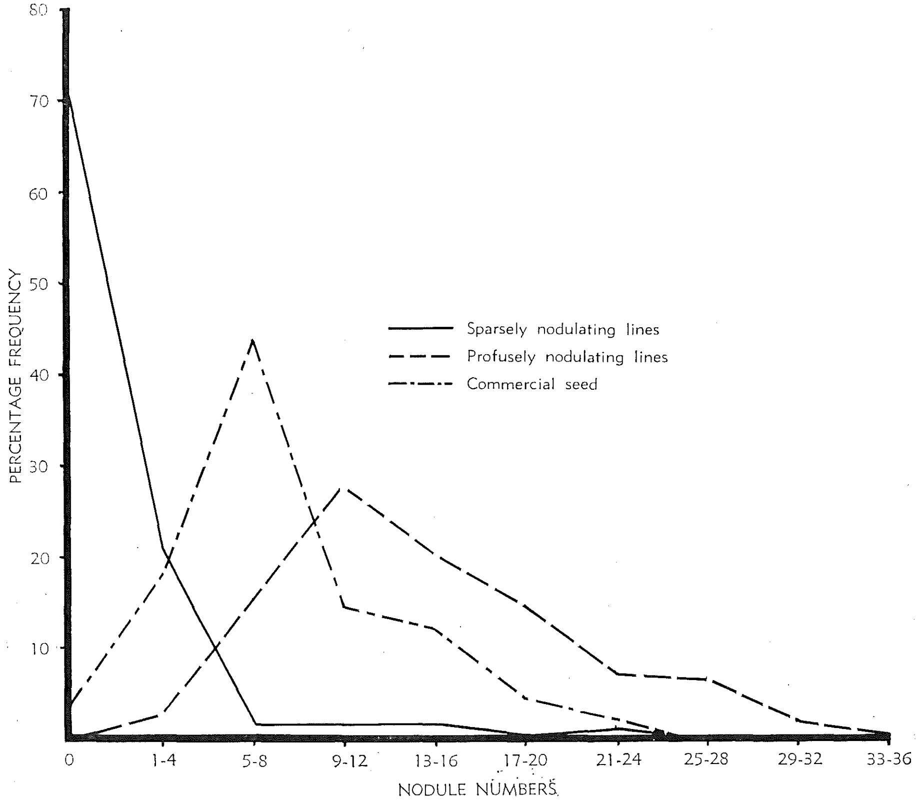

Fig 5.—Distribution of nodule numbers per plant with sparsely and profusely nodulating selections of *Centrosema pubescens*.

TABLE 3

NODULATION OF PLANT SELECTIONS BY FOUR RHIZOBIUM STRAINS

Rhizobium Strain	Sparsely Nodulating Selection		Commercial Seed		Profusely Nodulating Selection	
	Seed Inoculated	Seedling Inoculated	Seed Inoculated	Seedling Inoculated	Seed Inoculated	Seedling Inoculated
	(a) Mean Number of Nodules per Plant					
QA549	3·3	1·2	11·6	12·2	22·5	14·7
QA837	11·0	7·5	13·2	11·2	15·5	15·8
QA869	8·5	6·3	6·0	6·5	17·7	7·7
QA870	9·2	8·5	7·4	8·8	9·9	7·0
Uninoculated			0			
	(b) Mean Oven-dry Weight/Plant (mg)					
QA549	26	38	182	98	125	96
QA837	150	58	205	102	172	141
QA869	157	61	162	70	314	(4
QA870	77	74	156	71	214	71
Uninoculated			41			

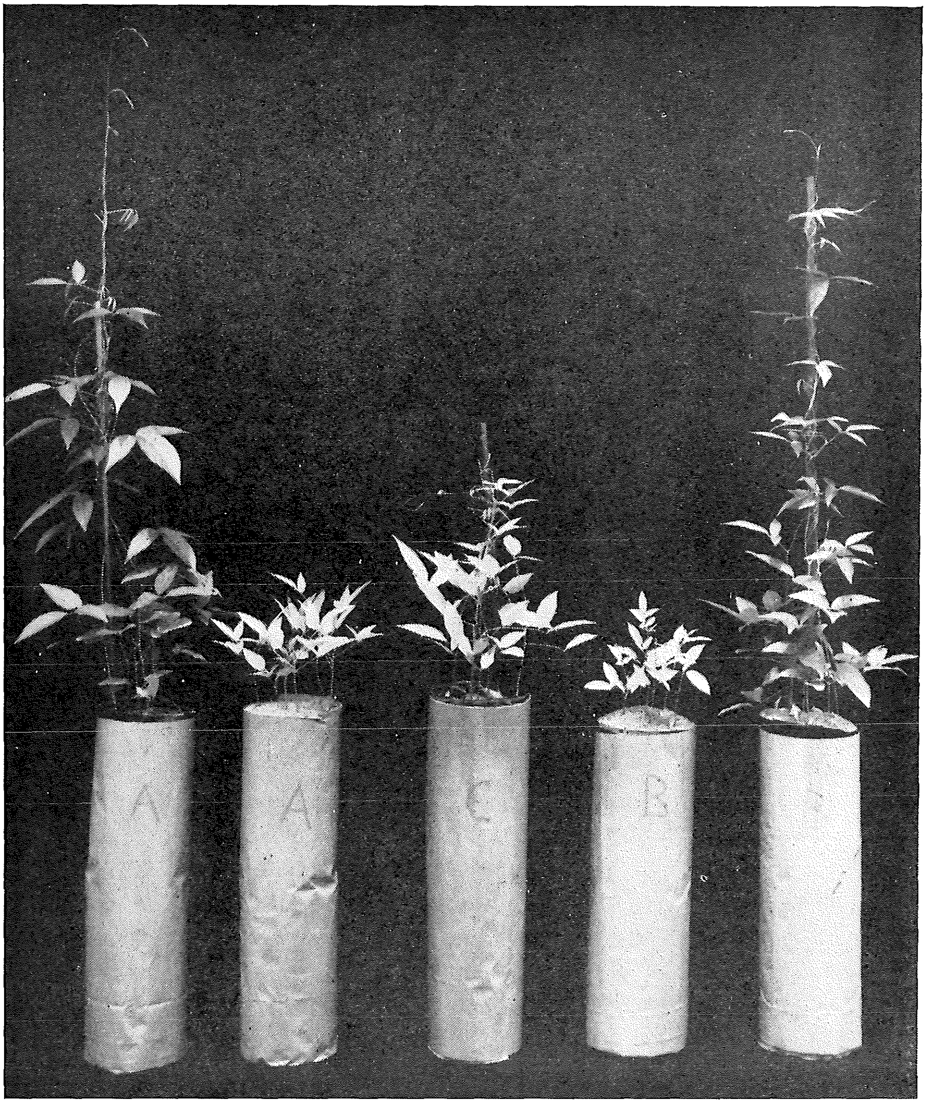

Fig. 6.—Growth of seedlings from profusely and sparsely nodulating selections of *Centrosema pubescens*. Left to right: (1) Profusely nodulating selections inoculated with QA523a. (2) Sparsely nodulating selections inoculated with QA523a. (3) Uninoculated. (4) Sparsely nodulating selections inoculated with QA549. (5) Profusely nodulating selections inoculated with QA549.

Despite variability due to uneven germination, the results after eight weeks (Table 3) showed conclusively that the S lines could be nodulated successfully by the use of Rhizobium strains QA837, QA869 and QA870. These nodulated all plants of all seed lots. In contrast, QA549 produced little nodulation and growth of S lines. These findings have been confirmed in other (unpublished) experiments by the authors.

There was an indication that with some Rhizobium strains growth of P lines may be greater than that of normal plants in commercial seed lots and successfully nodulated S lines.

IV. DISCUSSION

The results showed that marked variation in nodule numbers on *C. pubescens* in the field and glasshouse can be explained at least in part by the presence of sparsely and profusely nodulating lines in commercial seed lots. Evidence for a genetic factor in legume nodulation was advanced by Wilson, Burton, and Bond (1937); Nutman (1946) first demonstrated its importance in a series of intensive studies on red clover. *C. pubescens* is almost exclusively self-pollinated (Hutton 1960) and plant selections from within commercial seed breed almost completely true. It is probable that within commercial seed of this legume there are a number of stable lines with different nodulation responses. A significant difference in numbers of nodules formed by the P lines was shown.

Earlier studies (Bowen 1959*b*) have shown that the numbers of nodules formed is also a property of the Rhizobium strain. While most Rhizobium strains for *C. pubescens* nodulate the S lines only sparsely, the present studies demonstrate that some rhizobia can nodulate these reasonably well. There may yet be a tendency for the S lines to nodulate less than other lines and possibly produce less growth in a nitrogen-deficient medium but this needs further study.

While the experiment with the two Rhizobium strains QA523a and QA549 showed that P lines (profusely nodulating) produced no more growth than the normal plants of a commercial seed lot despite production of more nodules, the last experiment gave some indication that with other Rhizobium strains profuse nodulation may result in greater plant growth. Plant response in nitrogen-deficient substrate depends not on the number of nodules alone but on the total volume of actively fixing nodule tissue (Bowen 1959*b*). The interaction between nodule number and nodule size is complex and profusion of nodules may not always infer a decline in nodule size and loss of nitrogen-fixing activity per nodule. This is being studied further.

V. ACKNOWLEDGEMENTS

We wish to thank officers of the Biometrics Section for statistical analyses, the Ayr Regional Experiment Station for production of seed from the plant selections and the Photographic Section for the photography. The last portion of the study was made while one of us (G.D.B.) was employed by the Division of Soils, C.S.I.R.O., Adelaide.

REFERENCES

BOWEN, G. D. (1959*a*).—Field studies on nodulation and growth of *Centrosema pubescens* Benth. *Qd J. Agric. Sci.* 16:253-66.

BOWEN, G. D. (1959*b*).—Specificity and nitrogen fixation in the Rhizobium symbiosis of *Centrosema pubescens* Benth. *Qd J. Agric. Sci.* 16:267-82.

HUTTON, E. M. (1960).—Flowering and pollination in *Indigofera spicata, Phaseolus lathyroides, Desmodium uncinatum* and some other tropical pasture legumes. *Emp. J. Exp. Agric.* 28:235-43.

LEONARD, L. T. (1943).—A simple assembly for use in the testing of cultures of rhizobia. *J. Bact.* 45:523-5.

NUTMAN, P. S. (1946).—Genetical factors concerned in the symbiosis of clover and nodule bacteria. *Nature, Lond.* 157:436-5.

WHYTE, R. O., NILSSON-LEISSNER, G., and TRUMBLE, H. C. (1953).—Legumes in Agriculture. F.A.O. Agric. Stud. No. 21.

WILSON, P. W., BURTON, J. C., and BOND, V. S. (1937).—Effect of species of host plant on nitrogen fixation in *Melilotus. J. Agric. Res.* 55:619-29.

(Received for publication January 6, 1961)