FIELD STUDIES ON NODULATION AND GROWTH OF *CENTROSEMA PUBESCENS* BENTH.

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SUMMARY.

Field growth and nodulation of Centrosema pubescens in northern and southern Queensland have been studied.

Plant growth has been considered in terms of yield, growth rates, net assimilation rates, and leaf area indices. The yields followed a sigmoid curve. Growth rate and net assimilation rate increased to a maximum at the time of stolon production. While the growth rate was maintained at this level until winter retardation and recession of growth, net assimilation rate continually declined following stolon production. Net assimilation rates in northern and southern Queensland were similar. Winter retardation of growth occurred when minimum screen temperatures were below 55 deg. F. and maximum screen temperatures below 78 deg. F.

A periodicity in nodulation was observed. Nodulation continued throughout growth. Following stolon formation nodulation occurred on stoloniferous roots, while there was a progressive elimination of older nodules on the tap-root system. There was no indication of a progressive elimination of smaller nodules. Nodulation and nodule senescence are considered to be governed by vegetative growth of the plant rather than other plant factors such as flower production.

Nitrogen analyses showed that only a small proportion of the plant nitrogen is contained in the nodules and only 4-20 per cent. in the underground parts.

Cutting to simulate heavy grazing resulted in a loss of two-thirds of the plant roots by weight and inactivation and sloughing of a major proportion of the nodules.

I. INTRODUCTION.

Centrosema pubescens Benth., a legume native to South America, has been used in many tropical and subtropical environments as a cover crop (Whyte, Nilsson-Leissner and Trumble 1953). 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 in areas with an average annual rainfall greater than 45 in. Sloan (1957) recorded a doubling of carrying capacity in trials with a centro-para grass mixture compared with

para grass alone. Bowen (1959) indicated the specificity of this legume with respect to nodulation and also recorded the nitrogen-fixing efficiency of naturally occurring strains of Rhizobium on this legume. This paper deals with the course of nodulation with respect to plant growth in the field and the effect of management on these. Most reports to date on growth and nodulation of tropical legumes have been of a general nature only.

Although micro-environment and plant habit may be different in a pasture mixture than in a pure stand, the present investigations were carried out to form a basis for later work on the legume in association with grass.

II. FIELD STUDIES OF SEASONAL EFFECT ON PLANT GROWTH AND NODULATION.

In order to study nodulation with respect to plant development, plant growth studies were carried out at Ormiston (latitude 27 deg. S.) near Brisbane in southern Queensland and at Ayr (latitude 19·5 deg. S.) in North Queensland. The trial at the latter centre was made possible by the co-operation of the officers of the Ayr Regional Experiment Station. The detailed studies were supported by observations in other areas.

(1) Method.

At both sites inoculated seed was sown in rows 4 ft. apart in mid-November, 1956. The area available for the experiments was such that inclusion of uninoculated and applied nitrogen controls was impracticable. The sites were fertilized to make the soils complete in all nutrients other than nitrogen.

At regular sampling dates, eight separate 12 ft. lengths of row were selected according to a random plan and completely harvested. Because of practical difficulties in handling the large bulk of material at Ormiston, this was reduced later in the season to four rows. At Ayr, one-half of the area was cut at flowering and regrowth studied. Buffer portions were left when sampling. Plant stand at Ormiston was approximately 29 per 12 ft. row, and at Ayr the plants were thinned to five per 12 ft. row.

Data obtained from Ormiston were plant weight, root weight, nodule numbers and weight, and nodule activity. Stem/leaf ratios and nitrogen analyses were obtained on selected samples. Although losses of nodules when sampling were low, soil from around the plant roots was collected when sampling for recovery of nodules and roots which might have been torn off during plant removal. Observations were made also of the distribution of nodules on roots.

Difficulty was experienced in complete removal of roots and nodules at Ayr. Because of this, nodule data from Ayr were considered little better than qualitative and were used only to confirm results from Ormiston.

In both trials soil moisture was adequate for most of the growing season.

Table 1.

FIELD GROWTH AND NODULATION OF *C. pubescens*—ORMISTON, 1956–57.

Date of sampling	6–12–56.	20–12–56.	2–1–57.	15–1–57.	4–2–57.	18–2–57.	11–3–57.	8–4–57.	7–5–57.	26–6–57.
Days after planting	21	35	48	61	81	95	116	144	173	223
Stem and leaf yield	·407*	6·29	13·6	62·3	146·2	368	840	1,047	1,416	730
Stem/leaf ratio	..	..	·16	..	·77	..	·99	..	1·28	3·31
Nitrogen percentage (oven-dry weight)	..	..	3	..	..	..	2·4	..	1·5	1·2
Root weight	·133	1·021	2·640	9·169	15·86	37·25	48·1	71·5	112·7	139·4
Root nitrogen percentage (oven-dry weight)	..	..	1·7	..	..	..	..	..	1·3	1·4
Nodule weight	..	·012	·063	·203	1·066	2·678	4·909	8·350	16·50	11·17
Nodule numbers (including shells)	4·9	18·6	35·2	78	268	315	640	1,030	2,157	1,203
Number of shells	0	0	0	0	0	·5	1·2	37	108	457
Percentage of shells	0	0	0	0	0	·16	·19	3·6	5	38
Mean nodule diameter (m.m.)	..	1·4	2·2	2·4	2·8	3·7	3·5	3·5	3·5	3·7
Percentage of active tap-root nodules—										
0–2 mm. diameter	100	100	100	100	89·6	95	70	92·5	(90)	0
3–5 mm. ,,	..	..	..	..	..	91·2	76	64	66·7	5·5
5 mm. ,,	..	..	..	..	..	60	34·8	35·5	15·6	0
Stage of plant growth	..	..	..	..	Running	Nodal rooting	..	Commencement of flowering	..	Frosted
Average minimum screen temperature since previous sampling (°F.)	56·2	59·8	67·2	65·4	65·6	66·2	66·6	60·4	59·8	50·9
Average maximum screen temperature since previous sampling (°F.)	76·8	79·2	88·7	85·0	81·5	82·5	80·4	80·7	79·9	72·5

* All weights are grams oven-dry, per 12 ft. row. Nodule data are on a 12 ft. row basis.

Table 2.

FIELD GROWTH AND NODULATION OF *C. pubescens*—AYR, 1956-57

Date of Sampling	24–12–56.	21–1–57.	18–2–57.	18–3–57.	15–4–57	14–5–57.	11–6–57.	10–7–57.	8–8–57.	18–9–57.	21–10–57.
Days after Planting	41	69	97	125	153	182	210	239	268	309	342
Uncut (1)					flowering						
Stem and leaf yield	6·25*	40·8	213	398	618	690	898	904	924	894	971
Stem/leaf ratio	..	0·54	0·76	0·975	1·10	1·35	2·84	2·08	2·45	0·85	0·54
Percentage nitrogen of aboveground parts (oven-dry)	..	..	2·8	..	..	1·9	..	1·3	..	..	2·0
Percentage of active nodules (1–5 mm.)	100	100	100	74	55·8	52·3	16·7	Trace	Trace	100	..
(2) *Regrowth after cutting at 154 days*											
Stem and leaf yield	..	..	..	..	..	21·5	323	326	346	411	421
Stem/leaf ratio	..	..	..	..	..	1·87	3·49	2·97	2·10	..	·81
Percentage nitrogen of aboveground parts	..	..	..	..	..	1·6	..	..	..	..	2·1
Percentage of active nodules (1–5 mm.)	..	..	..	..	..	47·4	22·9	Trace	..	..	..
Average minimum sreen temperature since previous sampling (°F.)	69·9	72·5	71·4	72·4	65·9	62·4	56·8	54·8	56·5	55·1	59·7
Average mean screen temperature since previous sampling (°F.)	77·9	80·6	79·2	75·3	73·3	70·8	64·5	66·2	64·7	65·8	70·3
Average maximum screen temperature (°F.)	89·9	88·5	88·6	86·1	84·7	84·9	78·3	76·8	76·8	80·8	85·4

* All weights are grams oven-dry, per 12 ft. row. Nodule data are on a 12 ft. row basis.

(2) Results.

The results of the investigations at Ormiston and Ayr are summarised in Tables 1 and 2 respectively. Nitrogen analyses of nodules are given in Table 3.

Table 3.

NITROGEN ANALYSES OF NODULES.

Nodule State.	Nitrogen Percentage. (Oven-dry Weight).
Active (pink)	5·0–5·8
Inactive (green and firm)	5·3
Degenerating (green-brown) ..	4·0
Advanced decay	1·9

(a) Plant Growth.

The oven-dry yields of aboveground parts are shown in Fig. 1 and the growth rates ($\frac{dw}{dt}$) for various intervals in Fig. 2. The growth rate was derived graphically from the growth curve.
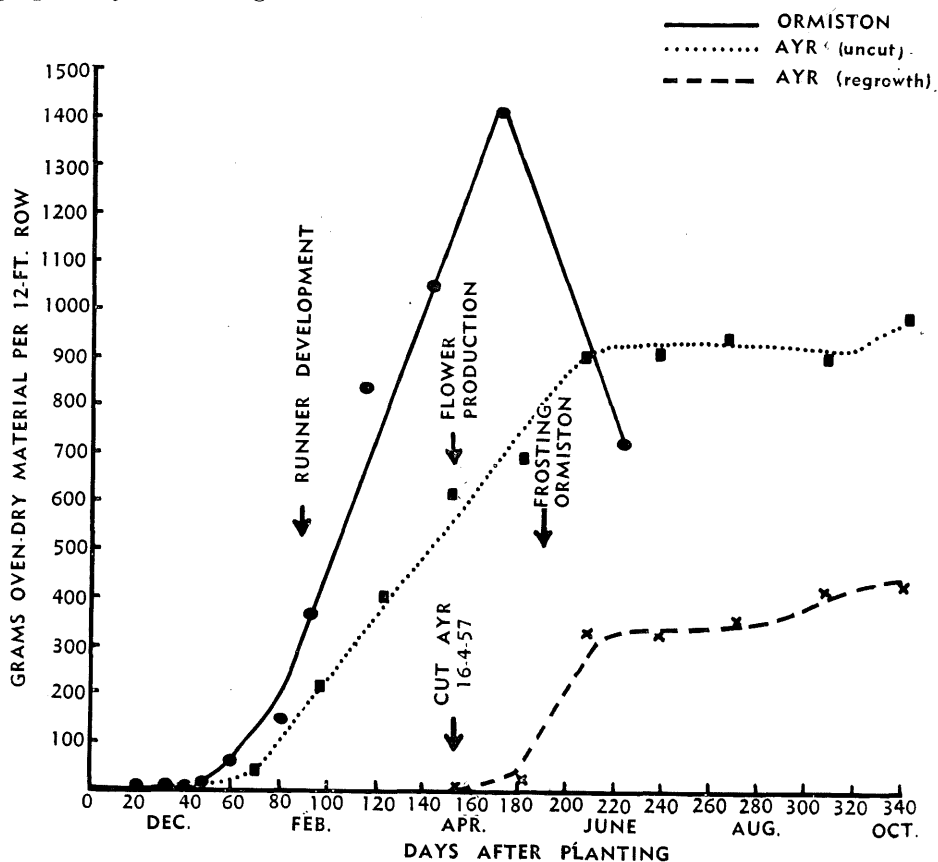
Fig. 1.

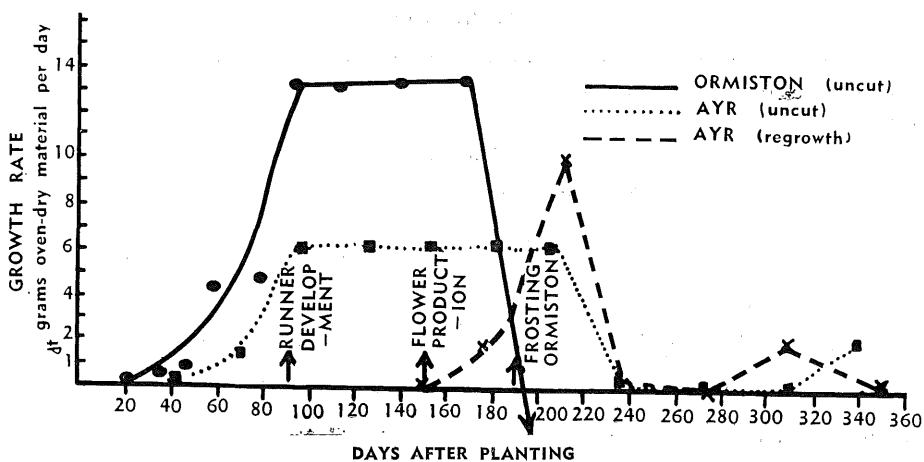Plant Yields (aboveground parts) for *C. pubescens* at Ormiston and Ayr, 1956-57.

Fig. 2.

Growth Rate Curves for *C. pubescens* at Ormiston and Ayr, 1956-57. The curve
for Ormiston reached —15 at 223 days.

The growth curve is sigmoid, yield increasing until early winter to
mid-winter. Growth rate increased to a maximum at the time of runner
formation (90 days at both sites), after which this maximum level was maintained
until early winter to mid-winter, when a decline in growth rate to zero occurred
until regrowth commenced in spring. This decline and cessation of growth was
associated at both sites with minimum screen temperatures of approximately
55 deg. F. and maximum screen temperatures of approximately 78 deg. F.
Growth occurred at Ayr for approximately five weeks longer than at Ormiston.
During the winter period, partial or complete defoliation is reflected in increased
stem/leaf ratio and lowered nitrogen content of aboveground parts. Defoliation
at Ormiston following frosting resulted in a recording of negative growth rate
during the 173-223 days period. At Ayr an increased growth rate ensured
cutting at flower formation.

Flower production at both sites occurred at 140–150 days. This indicates
the absence of a photoperiodic effect.

Net assimilation rates (N.A.R.) have been examined in these studies
rather than relative growth rate (R.G.R.) in order to include a
photosynthetic factor in the expression of growth. Various workers (e.g.,
Wilson 1940) have found a relation between photosynthesis, C/N ratio
and nodulation. Because of the difficulty of complete recovery of roots at Ayr,
N.A.R. at both Ormiston and Ayr was calculated on the aboveground parts alone
and not on the whole plant. The general shape of the N.A.R. curve was not
altered by this treatment. During the Ormiston trial the percentage of the
total weight contributed by the roots varied from 24·8 in the seedling stage
(21 days) to 7·4 at the period of maximum yield (173 days).

N.A.R. was calculated by the formula:

$$E_W = \frac{W_2 - W_1}{L_{W_2} - L_{W_1}} \quad 2{\cdot}303 \quad \frac{(\log_{10} L_{W_2} - \log_{10} L_{W_1})}{t_2 - t_1}$$

where E_W = N.A.R. on the basis of leaf weight,

$\quad\quad$ W = Oven-dry weight of the aboveground parts of the plants,

$\quad\quad L_W$ = Oven-dry leaf weight,

$\quad\quad$ t = Time in days.

The N.A.R. for various intervals is shown in Fig. 3.

Despite the maintenance of the high growth rate, N.A.R. declined considerably at both sites from the 80–100 days period (runner formation) until it was quite low at 170–180 days. Regrowth in spring at Ayr is reflected by increasing N.A.R. The N.A.R. curve for both sites is of a similar shape and
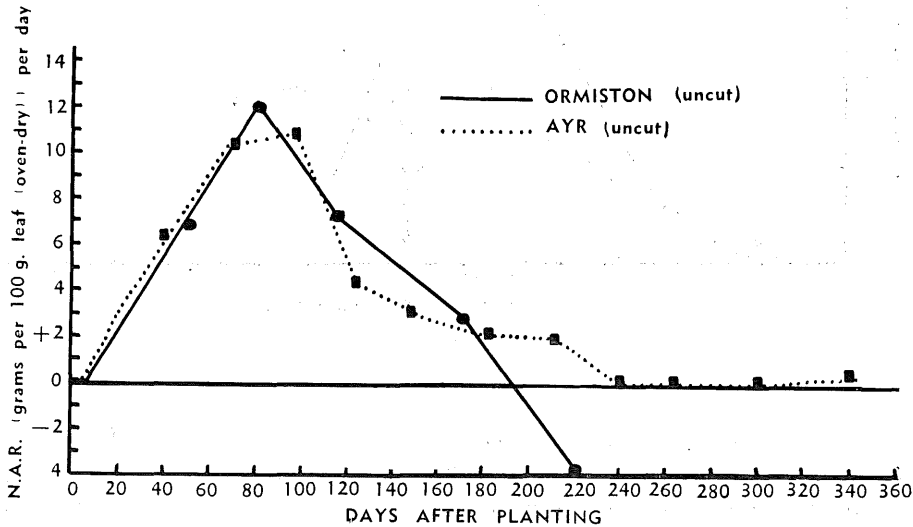
Fig. 3.

Net Assimilation Rates (N.A.R.) for *C. pubescens*, 1956-57.

order. At both sites R.G.R. was also almost identical and declined from 21 days (the earliest observation). Greater yield and growth rate at Ormiston than at Ayr was undoubtedly due to the denser plant stand at Ormiston. This is also reflected in the leaf area indices.

Leaf area indices (L.A.I.) are shown in Fig. 4. These were calculated as the ratio of the area of leaf surface to the ground area.

The shape of the L.A.I. curves closely follows that for yield. Since the leaves of *C. pubescens* are more or less horizontally disposed, one would expect complete light interception at an L.A.I. of 4–8. An L.A.I. of 4 did not occur at Ayr until the second season and at Ormiston until 5–6 months after planting. Closer spacings than the usual 4 ft. might be advantageous in producing higher yields in

the first season by means of more effective interception of light. The highest L.A.I. for a pure stand of *C. pubescens* observed by the author has been 12. This was in an ungrazed stand 15 months old in southern Queensland. The climbing habit of *C. pubescens* may lead to much higher L.A.I. in a mixed pasture.

Root growth increased throughout the period of the trial at Ormiston. The production of stoloniferous roots from the runners is dependent to a great extent on the presence of adequate surface soil moisture. In the Ormiston trial, stoloniferous roots made up 16 per cent. of the total root weight after 173 days. A considerable portion of the total root weight is made up of old taproot,
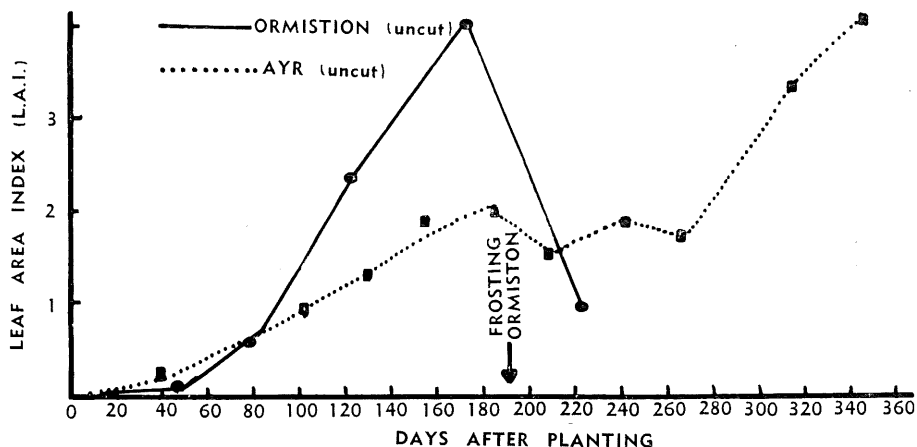

Fig. 4.

Leaf Area Indices (L.A.I.) for *C. pubescens*, 1956-57.

so the shallow, finer, stoloniferous roots probably represent far greater than 16 per cent. of the active feeding roots. This stoloniferous rooting habit enhances efficient exploitation of soil nutrients by the plant and may be an important reason for the ability of some tropical legumes to grow vigorously in soils with a relatively low nutrient status. The advent of stoloniferous rooting makes the plants more independent of the taproot system, except perhaps in times of moisture stress.

In a mixed pasture the contact of runners with the ground is somewhat less and therefore a decrease in stoloniferous rooting could be expected.

Observations at various sites show that smaller roots are lost in mid-winter after plant growth almost ceases. The course of the Ormiston investigation did not allow data on this to be quantitatively assessed. It is probably of the same order as roots lost on cutting (see next section).

(b) Nodulation.

Nodulation continues throughout the active growing period of the plant. During the dormant or slowly growing winter phase, trace infection takes place, the nodules remaining small but active.

Total nodule weight also increases up to the time of cessation of plant growth. Average nodule size (derived from mean nodule weight) increases up to 100 days. This coincides with stolon formation and stoloniferous rooting.

Nodule activity, judged by the presence or absence of haemoglobin on samples of nodules of different sizes, indicates that the larger nodules (greater than 5 mm. diameter) progressively become inactive, while a high percentage of the smaller nodules remain active until plant growth ceases. The data on activity and size of nodules at Ormiston on various dates in no way suggests a progressive elimination of smaller nodules at any stage of plant growth as recorded by Pate (1958a) for *Pisum arvense* L.

After runner formation and stoloniferous rooting the first-formed taproot nodules decay. Nitrogen fixation is increasingly aided by nodules forming on stoloniferous roots, so plant growth from the runners resembles·several almost independent plants. With the slowing of growth rate, the percentage of defunct nodules increases also. Cessation of plant growth causes inactivation of almost all nodules. In these studies estimates were not made of the amount of active nitrogen-fixing tissue at each date for possible correlation with plant growth. The author (Bowen 1959) has reported that in nitrogen-free sand culture in glasshouse investigations on *C. pubescens* a highly significant correlation was found between plant yield and weight of nitrogen-fixing tissue.

III. EFFECT OF CUTTING ON NODULATION.

While the investigations reported above gave data on plant and nodule behaviour under unmolested growth conditions, which would obtain when growing plants as a cover crop, no quantitative data were recorded on the effect on nodulation of cutting or damaging the plants. In another study rows of *C. pubescens* were cut to simulate the effects of heavy grazing.

Eight 13 ft. rows of *C. pubescens* planted on Jan. 30, 1957, in the grey-sandy soils of the wallum-heath at Coolum (latitude 26·5°S) were selected. In early May half of each row was cut back to 1 in. above ground level. Eight days and 30 days after cutting, four rows were dug and root weight, nodule numbers and nodule activity recorded.

The results are presented in Table 4.

(1) Plant Growth.

Regrowth occurred after cutting but was small. The most outstanding result of cutting is the marked loss of approximately two-thirds (by weight) of the root system at the 30-day sampling. Plant root loss after cutting was also observed in the growth trial at Ormiston.

Table 4.

EFFECT ON NODULATION OF CUTTING *C. pubescens.*

A. Mean Percentage Active Nodules 8 Days after Cutting.

Nodule Diameter. (mm.).				Plants Cut.	Plants Uncut.
2	..	..	..	23·2	71·6
3	..	..	..	15·8	74·5
4	..	..	..	9·2	54·1
5	..	..	..	4·6	25·8

B. Mean Number of Nodules per Plant after Cutting.

Time (days).				Plants Cut.	Plants Uncut.
8	..	..	..	27·9	34·3
28	..	..	..	7·36	40·5

C. Mean Root Weight per Plant (oven-dry) 28 days after Cutting.

Cut	..	..	..	..	..	..	..	.. 31 g.
Uncut	..	..	..	..	..	..	..	.. 90·7 g.

(2) Nodulation.

One week after cutting, a very large percentage of nodules in all size categories became defunct. These were missing at the 30-day sampling. By this time some nodulation was taking place, as was plant regrowth. Most of the nodules were small and were new infections, as evidenced by 60–70 per cent. active nodules in the 1–2 mm. category, compared with 23 per cent. eight days after cutting. Wilson (1942) recorded loss of nodules by a number of legumes on cutting.

IV. DISCUSSION.

A marked periodicity in nodulation of *C. pubescens* is obvious. This has been recorded for other legumes (Allen and Allen 1958; Beeley 1940).

Allen and Allen (1958) noted various causes for nodule loss. One such cause is plant fruition. In recent studies, Pate (1958a, 1958b) noted a great loss of nodules of field pea (*Pisum arvense* L.) and vetch (*Vicia sativa* L.) at flowering. This loss could be offset by prolonging the vegetative phase of growth by disbudding. In the present investigations on *C. pubescens* nodule inactivation commenced 30–60 days before flower production, in the period of declining N.A.R., and reached a maximum at the time of minimum plant growth (also minimum N.A.R.). This was approximately 60–80 days after flowering. Nodule inactivation in this species and possibly other legumes is more fundamentally correlated with plant growth rates or with N.A.R. than with

flowering itself. Inactivation and subsequent loss of nodules seems to result
from vegetative growth checks, whether these are due to environmental or
physiological causes or to direct damage, rather than to these factors directly.

Pate (1958a, 1958b) found that nodule initiation of field pea and vetch
ceases well before flowering. This did not occur with *C. pubescens* in the present
studies. The production of nodules paralleled the production of roots. Both
nodule and root production continued during and after flowering. The
differences from Pate's results may be due to the stoloniferous rooting habit
of *C. pubescens*. With the production of stoloniferous roots, nodulation becomes
confined very largely to these—little more takes place on the taproot system
in that season. Growth from stolons is partially independent of the taproot
system. Under the conditions of this investigation, maximum growth per unit
area was not attained in the first season. Closer planting would have resulted
in increased growth, stoloniferous rooting and nodulation, and hence nitrogen
fixation, per unit of land. At the commencement of the second season the stolons
are already formed, and maximum growth may occur early in the season. Under
these conditions nodulation may reach an early peak and not extend over a
considerable period, as in the first season.

The close correlation between course of plant growth and nodule activity
indicates that increase of plant growth either directly or by extending the
growing season, through the agencies of plant breeding or crop management,
should increase nodulation and nitrogen fixation. The course of plant growth
determined nodule initiation and longevity and not *vice versa*.

These studies have given an indication that growth of *C. pubescens*
declines or ceases when the minimum screen temperature is below 55 deg. F.
and the maximum screen temperature less than 78 deg. F. The author has
observed that spring regrowth occurs when these temperatures are exceeded.
However, in Queensland surface soil moisture is often low in spring, and
although plant regrowth occurs the low surface soil moisture impedes nodulation
of stoloniferous roots and presumably restricts nitrogen fixation. The present
studies suggest that where irrigation facilities are available application of
water when seasonal temperatures are below those mentioned may not give a
yield increase, but a liberal application when these temperatures are approached
in spring will result in unimpeded nodulation of stoloniferous roots and
enhanced nitrogen fixation.

The absence of uninoculated plots, and of a comparative series of *C.
pubescens* with nitrogenous fertilizers supplied at various levels, prevents
conclusions being made on the amount of nitrogen fixed in the growth trials at
Ormiston and Ayr. Very few data of this nature have been forthcoming for
tropical legumes growing in the field. Akhurst (1954) claimed that, compared
with bare ground, the nitrogen increase in the top 7 in. of soil under a cover of
C. pubescens in one year in Malaya was the equivalent of one ton of sulphate of
ammonia per acre (4 cwt. N per acre). Watson (1957) from pot work claimed
fixation equivalent to 210 lb. of nitrogen per acre in five months.

Calculations based on nitrogen analyses (Tables 1, 2 and 3) show that after four months' growth the nitrogen content of plant parts at Ormiston were: aboveground parts 40·5 lb. N per acre; roots 1·2 lb. N per acre; and nodules 0·53 lb. N per acre. At six months these were 43·4 lb., 3·1 lb. and 1·6 lb. respectively. At Ayr after four and six months the content of nitrogen per acre in the aboveground parts was 19·1 lb. and 24·3 lb. respectively. In a 15-months-old stand on a nitrogen-deficient wallum-heath soil north of Brisbane, the nitrogen content of tops and roots was 134 lb. and 35·6 lb. per acre respectively. (In this case some nitrogen would have been lost from seasonal loss of plant parts prior to sampling.) Allen and Allen (1958) stated: ''Conservative estimates of annual fixation of nitrogen by all legumes average 80 to 100 lb. nitrogen per acre per annum.'' Remembering that climax growth was not attained in the first season in the present investigations because of wide row spacing and, at Ayr, low plant density, the data above indicate that fixation of nitrogen by *C. pubescens* would be approximately of the order stated by Allen and Allen. According to data presented by Allison (1954), fixation supplying, for example, 100 lb. of nitrogen to the plant may be equivalent to 125–250 lb. of nitrogen applied to soil as an inorganic fertilizer. The difference in general growth and environmental conditions between Malaya and the sites used in these studies may account for the large discrepancy between results reported here and those of Akhurst (1954) and Watson (1957).

Provided no direct excretion of nitrogen occurs from nodules and good growth conditions are maintained, the data here indicate that in an ungrazed state organic nitrogen will be donated to the soil (and thence to associated plants) in small quantities shortly after runner formation. This will come partly from loss of nodules but mainly from loss of fine roots. The amounts will initially be only small, but they will increase to a maximum at approximately one month after cessation of plant growth in winter. (At this stage leaf drop will also be at a maximum.) The rates at which nitrogen will be made available to associated plants will depend on the effect of existing soil conditions on decomposition and nitrification of the organic matter.

Under a cutting or grazing system, organic nitrogen return to the soil by loss of roots and nodules can be induced at will.

However, the roots and nodules together contain only approximately 4–20 per cent. of the total nitrogen content of the plant, so the major return of nitrogen to the soil will be from return of aboveground parts either directly by leaf drop or indirectly by way of the grazing animal. While nitrogen will be added to the soil more quickly under a grazing system, the total amount of nitrogen fixed in a season may vary from the ungrazed, depending on growth as influenced by management and stocking rate. Further studies are needed on nitrogen fixation in relation to grazing management.

The very small amounts of nitrogen contained in the nodules compared with other plant parts are consistent with the now accepted theory of continual

translocation of fixed nitrogen from the nodule to the plant. The finding of very small amounts of nitrogen in the nodules is in direct contrast to the estimate of Butler and Bathurst (1957, pp. 168-178) of 72 lb. N/acre/annum being available through nodule decomposition in perennial clover and ryegrass swards, but corroborates the findings of Pate (1958a). Nodule decomposition releases only very small amounts of nitrogen to the soil. Roots contribute much more.

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