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# THE LIMITED NUMBERS OF NODULES PRODUCED ON LEGUMES BY DIFFERENT STRAINS OF *RHIZOBIUM*

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In the field a legume crop usually obtains its nodules from a mixed population of nodule bacteria including a variety of strains doubtless varying in their effectiveness towards the host plant. It is therefore of practical importance to determine what are the factors that determine which strains will infect the plant and in what proportions. Nicol & Thornton (1941) found that an important factor controlling this was the competition that took place between bacteria of different strains *outside* the roots of the host plant. Where one was markedly dominant in competition, this became the determining factor controlling infection. But otherwise the relative infectivity of the strains determined the proportion of the total nodules contributed by each of them.

The numbers of nodules produced on a legume by a given strain of *Rhizobium* will clearly be conditioned by a number of factors, some arising in the root surroundings, others from the general physiology of the host plants, and others more specifically related to the strain of invading bacterium. It is with the latter more specific relationships that the present paper deals. Obvious differences exist between strains of *Rhizobium* not only as regards the mean size but also the mean number of nodules characteristically produced by them under conditions of host-plant cultivation rendered as carefully standardized as possible. These differences might be due to different rates of successful infection,[1] or to the establishment of a limiting equilibrium due to ability of a given strain to produce only a limited number of nodules on a given mass of the root system. If such a limiting equilibrium exists, the number of nodules per gram of root should attain a constant level characteristic of each strain. This number should remain constant on a growing root

[1] A large part of the root hair infections must fail to result in nodules (see McCoy, 1932). The term 'successful infection' is here used to designate infections that result in the formation of nodules.

system though the absolute number of nodules increases with the growth of the roots. On a root system that makes its growth over a short period, the further production of nodules should stop when the roots cease growing or when the specific limiting number of nodules per gram of root has been reached, whichever occurs last.

On this latter type of root system, if the limiting number of nodules per gram of root is quickly reached, the nodules on the young plant will comprise a large fraction of the number finally possible and hence will greatly reduce the formation of further nodules by the same or by a different strain. A number of authors have recorded such an inhibiting effect of early nodules. In particular, Dunham & Baldwin (1931) found that early nodules produced by one strain could entirely inhibit the formation of nodules by a different strain. Nicol & Thornton (1941), however, found that with peas and soy beans the inhibiting effect of the early nodules acted with equal intensity against the same as against a different strain. In this work which was designed to study competition between strains, the second strain was applied to sand already populated with the first, so that the results were complicated by competition between strains outside the plant. To measure the effect of the early nodules upon subsequent infection it is necessary to remove the bacteria, derived from the first inoculum, that remain in the root surroundings, and to transplant the roots into a medium populated only by the second inoculum. Experiments of this kind were made with the object of determining whether the nodule numbers per unit mass of roots attained a limiting equilibrium and how the establishment of this equilibrium affected subsequent infection by the same and by a different strain. These experiments were made with clover, whose root system continues its growth over a long period, and with soy beans, whose roots make most of their growth during early stages of culture.

## Experiments with red clover, 1939

In this experiment two strains of clover *Rhizobium* were used—the efficient strain 205 obtained from Wisconsin[1] and the inefficient Coryn strain, whose nodules were described by Chen & Thornton (1940). The rates of nodule appearance due to these strains on clover seedlings were found by Nicol & Thornton (1941) to be markedly different, while their

[1] The author's thanks are due to the staff of the Wisconsin Agricultural Experiment Station for supplying cultures of this strain and of the four strains of soy-bean nodule bacteria used in the second experiment, described below.

experiment in which clover was grown in sterilized sand showed that the absolute number of nodules produced in three months' growth by the two strains also differed characteristically.

In the present experiment, Montgomery red clover was sown in wide test-tubes on slopes of agar medium of the following composition:

| | | | | | |
|---|---|---|---|---|---|
| $K_2HPO_4$ | 0·5 g. | NaCl | 0·1 g. | $FeCl_3$ | 0·01 g. |
| $KH_2PO_4$ | 0·5 g. | $Ca_3(PO_4)_2$ | 2·0 g. | Agar | 10 g. |
| $MgSO_47H_2O$ | 0·2 g. | $FePO_4$ | 0·5 g. | Water | 1 l. |

The tubes of media were sterilized in the autoclave. Twenty replicates were left sterile, twenty supplied with strain 205 and twenty with the Coryn strain. The bacteria were mixed with the melted agar cooled to 42° C. before making the slopes. Two seeds, externally sterilized by immersion for 3 min. in absolute alcohol and for 3 min. in 0·2% $HgCl_2$ and washed with sterile water, were sown at the top of each slope. The seeds were sown on 13 February, and on 27 March the seedlings were removed from the tubes and their nodules counted. They were then replanted in small pots each containing 3 kg. of nitrogen-deficient sand, sterilized by blowing superheated steam through each pot for half an hour. 175 ml. of the following sterilized food solution was added to each pot:

| | | | |
|---|---|---|---|
| $K_2SO_4$ | 0·9 g. | $FeCl_3$ | 0·02 g. |
| $K_2HPO_4$ | 0·5 g. | Boric acid | 0·02 g. |
| $CaH_2(PO_4)_2.4H_2O$ | 0·5 g. | $MnSO_4$ | 0·02 g. |
| $MgSO_4.7H_2O$ | 0·5 g. | Tap water | 990 ml. |
| NaCl | 0·5 g. | Lucerne root extract | 10 ml. |

Twenty replicate pots were supplied with a heavy inoculum of strain 205 and twenty with one of the Coryn strain, the bacteria being mixed with the food solution before addition. One seedling bearing strain 205 nodules, one bearing Coryn nodules and two plants without nodules were planted in each pot, each plant being separately labelled. On 10 July the roots were washed, the nodules on each plant were counted and the dry weights of individual root taken. The results are shown in Table 1.[1] The experiment was so designed as to test whether any of the following factors had any effect upon the final nodule numbers per gram of root:

[1] The nodules per gram of root were separately calculated for each plant and the means of the figures so obtained are those shown in the table. They differ from those derivable from the mean nodule numbers (column 5) and the mean root weights (column 6). The same process was followed for the corresponding figures in Table 3.

(1) time at which the bacteria were first applied; (2) size of the root system as modified by the efficient strain applied at seeding time; (3) a possible inhibiting action of the early nodules against the same or a different strain.

Table 1. *Effect of strain of* Rhizobium *on nodule numbers in red clover*

| Set | Strain applied: At sowing time | Strain applied: After trans-planting | Mean nodule numbers per plant: When transplanted | Mean nodule numbers per plant: At end | Final root dry wt. mg. Means per plant | Final nodules per g. root | $n$ |
|---|---|---|---|---|---|---|---|
| 1 | — | Coryn | — | 357·2 ± 42·8 | 118 | 3402·4 ± 475·8 | 17 |
| 2 | Coryn | Coryn | 38·7 ± 4·4 | 385·5 ± 74·0 | 163 | 2770·9 ± 558·3 | 10 |
| 3 | 205 | Coryn | 7·3 ± 0·8 | 904·1 ± 181·4 | 381 | 2273·7 ± 360·9 | 14 |
| 4 | — | 205 | — | 182·1 ± 30·8 | 355 | 544·8 ± 56·8 | 15 |
| 5 | Coryn | 205 | 50·0 ± 1·4 | 169·3 ± 38·4 | 239 | 694·5 ± 101·3 | 10 |
| 6 | 205 | 205 | 9·1 ± 1·5 | 331·1 ± 50·7 | 573 | 652·6 ± 121·3 | 14 |

At the time of planting out, seedlings that had grown for 6 weeks on agar already showed differences in nodule numbers characteristic of the strain supplied at seeding time (column 4). When removed from the agar the seedling root system showed no differences in size according to the culture supplied—the efficient nodules not having had time to produce increased growth.

After transplanting into the pots some plants died. The numbers surviving can be deduced from the degrees of freedom, $n$, shown in the last column. These made considerable growth before harvest with a large increase in nodule numbers. The final root weights shown in column 6 were much increased where 205 nodules, effective in nitrogen fixation, had developed on the seedling while growing in agar. This appears in comparing set 1 with 3 and set 4 with 6.

The absolute number of nodules at the end of the experiment (column 5) were not significantly affected by the presence of Coryn nodules on the seedlings but the presence of 205 nodules at the time of transplanting greatly increased subsequent nodule formation by either strain. This effect was in fact due to the enlargement of the root system resulting from nitrogen fixation by the early-formed efficient strain 205 nodules.

The nodules per gram of root (column 7) show no significant differences between sets receiving different treatments in their early growth but later grown in pots supplied with the same strain. Thus the time at which the bacteria were first supplied to the roots was without final effect on the nodules per gram of root. Nor were there very large differences in size of the root system produced by the early formed 205 nodules. There were, on the other hand, large differences in the mean number nodules per gram of root according to the strain in the sand which was in contact with the root system during the period when it made most of its growth. This mean number reached a definite limit characteristic of the strain present in the sand. This limit was apparently attained quite early in the plant's growth. The mean figure for plants grown in pots containing Coryn bacteria was 2816, and that for plants in pots containing strain 205, only 631 nodules per gram of root. These figures are in the ratio of 4·6 : 1. This ratio can be compared with that between the actual nodule numbers developed by the two strains in agar (column 4), because during this early period the size of the root systems was similar in all sets. The Coryn strain developed a mean of 44·4 nodules per seedling, and strain 205 a mean of 8·2, at the time of transplanting. These figures are in the ratio of 5·4 : 1. So that the two strains produced nodules whose numbers per unit of the root system were in approximately the same ratio both on seedling roots grown in agar and subsequently on plants grown in pots of sand. Thus the limit of infection for a root system of given size is characteristic of each strain and is quickly reached. But the absolute nodule numbers of nodules increased *pari passu* within the growth of the root system, which in clover continues over a long period. This explains why the presence of nodules on the seedling did not stop further nodule formation, which took place on a growing root system, and why the final number of nodules per gram of root was that characteristic of the second applied strain, which was in contact with the root system while this was making most of its growth.

The following experiment, similar in general design to the first, was made with soy beans, whose root system makes most of its growth when the plant is quite young. It was designed to determine what specific limits of nodule numbers per gram of root were possessed by four strains of soy bean *Rhizobium* and to test the influence of the early nodules upon later infection by the same and by different strains.

## Experiment with soy beans, 1939

Soy beans were grown in glazed earthenware pots each containing 12 kg. of sand and 1 l. of food solution similar in composition to that used in the first experiment. Five seeds externally sterilized were sown in each pot on 21 June. Eight replicate pots were left uninoculated and eight each were supplied with each of the following strains of *Rhizobium*:

| | | |
|---|---|---|
| Wisconsin | 501 | Effective |
| ,, | 505 | |
| ,, | 502 | Ineffective |
| ,, | 507 | |

The plants were grown for 9 weeks and their roots were thoroughly washed and the nodules counted. They were then replanted in the pots in such a way that each pot whose sand contained an effective strain (501 or 505) received one plant bearing nodules produced by the same strain, one plant bearing nodules produced by each of the ineffective strains and one uninoculated plant. Similarly each pot whose sand contained an ineffective strain (502 or 507) received one plant bearing nodules produced by the same strain, one plant bearing nodules produced by each of the effective strains and one plant without nodules. Each plant was separately labelled. The scheme of transplanting is shown in Table 2. After a further 14 weeks' growth the nodules were recounted

Table 2. *Soy-bean experiment, scheme of transplanting*

| Plants with nodules, when transplanted, of strain | Transplanted into pots whose sand contained strains 501 Set | 502 Set | 505 Set | 507 Set |
|---|---|---|---|---|
| 501 | 1 | 2 | — | 3 |
| 502 | 4 | 5 | 6 | — |
| 505 | — | 7 | 8 | 9 |
| 507 | 10 | — | 11 | 12 |
| No nodules | 13 | 14 | 15 | 16 |

and dry weights of the roots were taken. The results are shown in Table 3. The plants which bore nodules produced by strains 501, 502 or 505 before transplanting did not show any significant increase in nodule numbers after transplanting (sets 1–9, columns 4 and 5). Thus the limit of nodule numbers attainable on the root systems in these sets had been reached within the first 9 weeks' growth. The number of nodules per gram of root was specific to the strain of Rhizobia (column 7). The mean number of nodules per gram of root in sets 4, 5 and 6 which bore nodules produced by strain 502, was 284·7, a figure significantly higher than the mean numbers, 191·7 and 163·4 of the sets whose nodules were produced by strains 501 and 505 respectively (sets 1–3 and 7–9).

The plants without nodules at the time of transplanting made considerably greater root growth during the second growth period, probably

Table 3. *Effect of strain of* Rhizobium *on nodule numbers in soy beans*

| Set | Strain applied: At sowing time | Strain applied: After transplanting | Mean nodule numbers per plant: When transplanted | Mean nodule numbers per plant: At end | Final root dry wt. mg. Mean per plant | Final nodules per g. root | n |
|---|---|---|---|---|---|---|---|
| 1 | 501 | 501 | 17·7 | 16·1 ± 2·7 | 94 | 206·3 ± 30·2 | 9 |
| 2 | 501 | 502 | 17·2 | 18·5 ± 1·5 | 162 | 149·8 ± 38·6 | 4 |
| 3 | 501 | 507 | 22·4 | 22·6 ± 3·4 | 144 | 219·1 ± 68·4 | 6 |
| | 501 | Mean | | | | 191·7 ± 25·9 | 21 |
| 4 | 502 | 501 | 29·1 | 29·4 ± 4·8 | 123 | 269·1 ± 44·7 | 8 |
| 5 | 502 | 502 | 35·9 | 37·8 ± 4·4 | 158 | 284·3 ± 38·1 | 7 |
| 6 | 502 | 505 | 36·3 | 36·5 ± 6·1 | 131 | 300·8 ± 46·9 | 9 |
| | 502 | Mean | | | | 284·7 ± 24·6 | 26 |
| 7 | 505 | 502 | 18·9 | 21·0 ± 1·7 | 170 | 134·4 ± 20·9 | 6 |
| 8 | 505 | 505 | 22·5 | 19·6 ± 2·0 | 114 | 184·1 ± 29·9 | 7 |
| 9 | 505 | 507 | 18·4 | 19·3 ± 2·0 | 185 | 171·7 ± 50·6 | 6 |
| | 505 | Mean | | | | 163·4 ± 19·6 | 21 |
| 10 | 507 | 501 | 28·0 | 34·6 ± 4·7 | 77 | 451·0 ± 56·5 | 6 |
| 11 | 507 | 505 | 29·6 | 44·6 ± 8·9 | 101 | 446·0 ± 49·4 | 7 |
| 12 | 507 | 507 | 32·1 | 56·6 ± 10·1 | 141 | 436·2 ± 53·5 | 8 |
| | 507 | Mean | | | | 444·4 ± 29·4 | 23 |
| 13 | — | 501 | — | 30·9 ± 7·4 | 179 | 211·4 ± 26·0 | 7 |
| 14 | — | 502 | — | 70·3 ± 13·4 | 303 | 233·0 ± 39·7 | 8 |
| 15 | — | 505 | — | 43·0 ± 8·8 | 244 | 186·4 ± 27·4 | 7 |
| 16 | — | 507 | — | 57·0 ± 13·4 | 253 | 225·3 ± 43·6 | 7 |

because they were smaller at the time of transplanting and suffered less check. These plants in sets 13, 14 and 15, planted in sand containing bacteria of strains 501, 502 and 505 respectively, developed nodules whose numbers per gram of root did not differ significantly from those on plants that had received the corresponding strain at the time of sowing (compare sets 1 and 13, 5 and 14, 8 and 15). Thus the specific limit of nodules per unit mass of root system was attained regardless of the total mass of the root system, which varied widely, or the time at which the infection took place. This latter point shows that the number of nodules is determined by the size of the root system and not vice versa, since most of the root growth in sets 13, 14 and 15 took place before the plants had developed any nodules. Strain 507 has a much higher level of nodule numbers than the other three strains. The mean final nodule numbers per gram of root for sets 10, 11 and 12 was 444·4, a figure significantly higher than that for any other set or group of sets. This high figure was not reached during the period of 14 weeks' growth in set 16 which first received the bacteria at the time of transplanting. Nor was the full number of nodules reached during the first 9 weeks of seedling growth in sets 10, 11 and 12, which developed more nodules after transplanting. In sets 10 and 11 these additional nodules were in fact produced by the strains 501 and 505 respectively as was shown by examining the nodules.[1] These later-formed nodules were comparatively few and the number of nodules per gram of root finally reached was that characteristic of strain 507. The figures for sets 10 and 11, 451 and 446, do not differ significantly from that of 436·2 for set 12 which received strain 507 both at sowing time and after transplanting.

## Discussion

In the experiments described above the number of nodules $n$ divided by the dry weight of the roots $m$ was found to reach a limiting figure that was constant and specific for each strain of *Rhizobium*,

$$n = mk.$$

If a plant's roots are exposed in succession to pure cultures of two strains of *Rhizobium* having the limiting constants $k_1$ and $k_2$ and if each strain is allowed time to reach its limit, the number of nodules $n_1$ produced by the first strain will be $m_1 k_1$ where $m_1$ is the mass of the roots developed while in contact with this strain. On the simplest supposition, the number $n_2$ produced by the second strain will be $m_2 k_2$ where $m_2$ is the additional mass of roots developed in contact with it. The total nodules developed by the two strains will therefore be

$$n_1 + n_2 = m_1 k_1 + m_2 k_2.$$

In the experiment with clover, nearly all the root growth took place after transplanting so that $m_2$ was very large relatively to $m_1$. Consequently the number of nodules was determined by $k_2$ and was that characteristic of the second-applied strain. In the soy bean experiment, all or nearly all the root growth took place in the presence of the first-applied strain, whose specific constant, $k_1$, determined the limit of nodule numbers reached.

It would be interesting to investigate the condition where the host is removed into the presence of the second strain before the first strain had reached its limit of infection for a given mass of roots, and to discover to what extent the first strain can then impose its specific limit on further nodule formation in these same roots by the second strain. Thus if the number of nodules produced by the first strain, $n_1 = m_1 k_1 - x$, would the number $x$, produced by the second strain, be determined by

[1] Nodules produced by the effective strains 501 and 505 have soft reddish centres easily distinguishable in hand sections from the hard whitish centres of the ineffective nodules produced by strain 507 (see Nicol & Thornton, 1941).

the constant $k_1$ or by a constant $k_2$ specific to the second strain? The answer to this question might throw light on the mechanism of nodule limitation. The evidence from sets 10 and 11 in the soy bean experiment suggests the continued operation of the constant $k_1$ specific to the first strain, but this evidence is insufficient to form any basis for discussion.

## SUMMARY AND ABSTRACT

Pot experiments were made with red clover and with soy beans to determine how far the number of nodules developed was a specific character of the strain of *Rhizobium* supplied.

The number of nodules per gram of root was found to reach a limit specific to each strain. This limiting equilibrium was attained regardless of the size of the root system or the age of plant at which the culture was first supplied, provided enough time were allowed for the limit to be reached.

When two different strains were applied to the root surroundings in succession, the final number of nodules was determined by the limit specific to the strain in contact with the roots while these were making most of their growth. In clover this was the second and in soy beans the first applied strain.

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## NOTE CONCERNING AUTHORSHIP

The work described in this paper was carried out by Dr Chen shortly before his departure for Central China. Some difficulty in communication due to wars has made it necessary for the undernamed to write the paper from Dr Chen's notes and data without his having the opportunity to see it before publication. The writer thinks he has drawn conclusions from the data in agreement with Dr Chen's opinions, but he accepts full responsibility for these conclusions and for the actual writing, although credit for the work is due solely to Dr Chen.

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