RESTRICTION OF INFECTION THREADS IN NODULATION OF CLOVER AND LUCERNE

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Summary

Clover and lucerne roots from plants grown in tube culture were examined for infection thread formation and nodule number. The number of infection threads was about equal to the number of nodules in *Trifolium pratense* L.; this relation was shown to hold for abundantly and sparsely nodulating plants and for bacterial inoculants producing large and small numbers of nodules.

With *T. subterraneum* L., *T. repens* L., *T. incarnatum* L., and *Medicago sativa* L. infection threads were much more profuse than would be expected if each gave rise to a nodule.

Multiple infections were observed for a small proportion of nodules. Several adjacent hairs may be infected, all strands apparently contributing to formation of the one nodule; two infection strands per hair were occasionally noted.

I. Introduction

Nutman (1956) has referred to the variety of evidence bearing on the ratio of infection thread to nodule formation. It seems that inter- and intra- cross-inoculation group compatibilities might operate either by some response between bacterium and root hair wall, or, after invasion of the root hair and cortical cells, because of failure to stimulate the "nodule primordia".

Plants inoculated in pure culture normally have their roots covered with a dense rhizosphere population (Purchase and Nutman 1957), but only a small percentage of root hairs are ever infected (Thornton 1929; McCoy 1932). Some restriction must therefore always operate at the first stage of infection. Nevertheless, in some plants this small proportion of infected root hairs much exceeds the number of nodules formed (McCoy 1932), suggesting that in these cases the second stage is more significant in determining the particular nodule complement of a root.

In the present paper the infection thread–nodule relationship has been described for several associations of red clover in tube culture; observations on other clovers and on lucerne are included for comparison.

II. Methods

Roots taken from water culture or agar slope culture were stored in 70 per cent. alcohol. For examination they were placed on a slide, covered with a No. 0 coverslip, and the projecting hairs on either side of the root carefully examined for curling and root hair infection. Roots were stained with 10 per cent. Loeffler's alkaline methylene blue, which stains root hairs pale blue and infection threads dark blue.

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TABLE 1

COUNTS OF NODULES, INFECTED, AND CURLED ROOT HAIRS ON CLOVER ROOTS

Species	Strain	Age (days)	Total No. of Hairs per Root	No. of Curled Hairs per Root	Curled Hairs (%)	Estimated No. of Infected Hairs per Root (I)	Infected Hairs (%)	No. of Nodules	Ratio I/N
T. pratense	SU281	46	175,000	9,700	5·6	0	0	1	0
		60	124,000	2,100	1·7	4	0·003	2	2
	NA30	46	21,000	800	3·8	4	0·02	5	0·8
		60	86,000	1,200	1·4	4	0·01	16	0·2
T. repens	SU281	46	28,000	300	1·0	8	0·03	19	0·4
		67	20,000	200	1·2	24	0·12	19	1·3
	NA30	46	62,000	1,100	1·8	72	0·12	24	3·0
		67	59,000	1,800	3·1	188	0·32	7	26·9
T. incarnatum	SU281	46	63,000	900	1·4	84	0·13	19	4·4
		74	88,000	2,100	2·3	132	0·15	12	11·0
	NA30	46	Not counted	Not counted	—	8	—	5	1·6
		74	64,000	2,400	3·7	144	0·23	16	9·0
T. subterraneum	SU281	46	123,000	5,300	4·3	24	0·02	15	1·6
		81	187,000	9,900	5·3	724	0·39	54	13·4
	NA30	46	144,000	10,800	7·5	160	0·11	18	8·9
		81	186,000	28,000	15·0	976	0·52	60	16·3

In order to examine the relative numbers of curled to total root hairs, and the ratio of infection threads to nodules forming on the same roots, counting procedures were standardized as follows:

Curled hair counts.—Rough scale diagrams were made of the roots, which were examined with a 16-mm objective at intervals 0·5–1 cm apart by using stage co-ordinates. The field diameters varied from 1·5 to 2·1 mm according to the ocular used. The length of the roots was measured from the diagrams and estimates of the curled and total hairs were determined for the whole length of the root.

Infected hair counts.—For counts of infected hairs a 4-mm objective and 10 × ocular were used. High-power examination is necessary to differentiate between true infection threads and thread-like coagulation of the protoplasm which is occasionally very prevalent. Also the crossing of two hairs often gives the appearance of an infection thread under low power.

A typical root has about 10–12 rows of epidermal cells across the upper and lower surfaces of the preparation, the hairs on which could not be examined, and about 4–5 rows on either side of the root which were examined. Thus it is likely that approximately a quarter of the root hairs were available for examination and a factor of 4 was applied to the counts to give the total number of root hairs in any category.

III. Results

Certain generalizations could be made as to the distribution of root hairs along the root edge:

(1) Roots were free from root hairs in the zone 0·3–3 cm immediately behind the root tip. Older portions of the root showed a somewhat irregular distribution of root hairs, but frequently a thick mass of root hairs was observed just below the crown. The piliferous layer is not usually sloughed off under these conditions. Curling and infection of root hairs was not found to be zoned.

(2) It is necessary to examine the whole root, including laterals, to obtain reliable sampling of infected root hairs, because of their small number and irregular distribution. Curled hairs were relatively frequent and sample counts provided an estimate of their proportion.

Observations of exceptional behaviour may be mentioned:

(1) Only one case of infection in a straight root hair was noted. Single hairs on relatively isolated positions on the roots may be infected, and in a preliminary experiment one plant had only 39 root hairs, all curled and infected. Two or more infection threads may be observed on a single developing nodule. As many as nine infected hairs were once noted in 2 mm of a subterranean clover root.

(2) In one or two instances the infection thread was seen to be divided and rejoined within the root hair, giving a small loop. On several occasions two infection threads could be observed within the one root hair but it was not certain whether division of the thread had occurred from near the point of entry, as sometimes happens. They apparently resembled those described by Bieberdorf (1938) in soybeans, and by Fahraeus (1957) in white clover.

(a) *Observation on Clover in Water Culture*

The data shown in Table 1 were obtained from examination of a few roots of commercial lines of *Trifolium pratense* L. (red clover), *T. subterraneum* L. (subterranean clover); *T. repens* L. (white clover), and *T. incarnatum* L. (crimson clover) grown in seedling salts solution.

It will be seen that the number of curled root hairs per root is very variable and usually far exceeds the number of infections. Subterranean clover has a higher percentage of curled root hairs than the other species, as well as a larger total of root hairs.

In red clover the ratio of infections to nodules is about 1, but in white, crimson, and subterranean clovers the ratios are 2, 3, and 5 respectively in younger plants, and 14, 10, and 15 in older plants. These values clearly indicate the restriction in red clover as compared with the other species examined, which all show increased susceptibility to infection with age.

Uninoculated plants of all species had no infection threads and virtually no curled root hairs.

(b) *Infection in Selected Lines of* T. pratense

At the suggestion of Dr. P. S. Nutman, who supplied the material, information was obtained on the relation between infection and nodulation with associations of red clover plants bearing characteristically different numbers of nodules. The plants were grown on agar slope cultures and results are shown in Table 2, which shows the counts of infected hairs per root (observed $\times 4$), together with the number of nodules per plant.

As anticipated, nodule counts increased with time to a much greater extent with the ineffective compared with the effective strain. Nodule numbers were highest with host lines selected for abundant nodulation and inoculated with the ineffective strain, and lowest with sparse selections effectively nodulated, the other two groups producing intermediate numbers of nodules. Over this wide range of nodulation capacity the ratio of nodule numbers to infected hairs remained of the same order. The number of infection threads may be expressed as a fraction or percentage of the nodules on each plant. An analysis of variance performed on the 28- and 48-day percentages after angular transformation shows no significant difference between the relationships of infection threads and nodules for the different associations.

Effective strains have more infection threads per nodule than ineffective, but this is not significant. Between different plant lines the relationship between infection threads and nodules is closely similar. It seems, therefore, that the limitation of sparse, compared with abundantly nodulating lines, or effective, compared with ineffective strains, comes into operation as early in the association as infection thread development.

With these, as with the preliminary counts, the mean ratio of infected hairs to nodules was about 1; hence it seems possible that with red clover each infection thread results in nodulation. Higher ratios (3·5–4) were noted in three out of the 26 cases cited in Table 2.

Three roots of a resistant line of red clover were examined without any infection threads being discovered.

It seems clear that for red clover the first stage of infection—the root hair entry stage—in some way defines the number of nodules formed. Whether any hairs are penetrated by bacteria which fail to form infection threads is not yet known.

Table 2

NUMBERS OF NODULES AND INFECTED HAIRS ON DIFFERENT ASSOCIATIONS OF RED CLOVER

Age (days)	Abundant Clover Line				Sparse Clover Line			
	Effective Strain		Ineffective Strain		Effective Strain		Ineffective Strain	
	No. of Infected Hairs (I)	No. of Nodules (N)	No. of Infected Hairs (I)	No. of Nodules (N)	No. of Infected Hairs (I)	No. of Nodules (N)	No. of Infected Hairs (I)	No. of Nodules (N)
28	40	26	44	51	16	10	12	8
	48	12	8	43	0	5	12	15
	16	21	48	53	8	8	4	7
	4	5	52	15			16	26
Totals	108	64	152	162	24	23	44	56
Ratio I/N	1·69		0·94		1·04		0·79	
48	28	24	108	167	12	16	16	60
	16	38	36	137	24	6	108	118
	4	27	144	180			64	103
Totals	48	89	288	484	36	22	188	281
Ratio I/N	0·54		0·59		1·64		0·67	

(c) *Counts on* Medicago sativa *L.*

A few counts on effectively nodulated plants of lucerne, taken from agar slope cultures are presented in Table 3. Some lucerne plants were found, unlike red clover, to have a marked excess of infections compared with nodules.

These results differ markedly from the report made by McCoy in 1932, where a great excess of infected hairs was observed, in one case with a ratio of 68 to 1 of infected hairs to nodules. However, the difference in the two counts may be related to the method of growing the plants, since McCoy records only 0·25 per cent. infection in lucerne grown in tube culture, compared with 2·5 per cent. in sterilized sand. It seems not unlikely that abrasions to the roots may facilitate entry in the latter case.

IV. Discussion

These results show that the primary infection of the root of a number of species of clover and of lucerne is no more, or only a little more, than sufficient to produce the number of nodules found thereon.

Some evidence has been produced that nodulation on red clover occurs at discrete foci on the roots (Purchase and Nutman 1957). Such foci may be the disomatic cells described in the elegant studies of Wipf and Cooper (1940).

We may postulate that, with red clover, invasion can only occur in a root hair close to a disomatic "centre" of activity. In the other species examined, entry is clearly more promiscuous, and may indeed correspond to the behaviour of pea and vetch noted by Wipf and Cooper (1940), who observed that "an infection thread may pass through nearly all the cortical cells and cause no stimulation whatsoever. In such cases there are no evidences of either a disomatic cell with its larger nucleus or a group of such cells."

Table 3

COUNTS OF NODULES AND INFECTED ROOT HAIRS ON LUCERNE

Age (days)	No. of Nodules (N)	Estimated No. of Infected Hairs (I)	Ratio I/N
28	9	28	3·1
	6	20	3·3
	2	0	0
48	11	0	0
	12	4	0·3
	7	8	1·1
	7	4	0·6
	14	132	9·4
Totals	68	196	Mean 2·9

More detailed cytological studies might help to present a clearer picture.

Mixtures of strains have been isolated from a single nodule in rather rare instances (Vincent 1954). The double infections, or, more especially, the occurrence of two or more infected hairs per nodule may account for such cases. It still seems possible that entry may be effected by a single bacterium.

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