The relation between root hair infection by *Rhizobium* and nodulation in *Trifolium* and *Vicia*

By P. S. Nutman

Soil Microbiology Department, Rothamsted Experimental Station, Harpenden, Herts.

The infection of the root hairs of young seedlings of twelve species of *Trifolium* and of *Vicia hirsuta* was examined. The amount of infection (numbers of hairs containing infection threads) at 2 weeks varied much between species of host and was less affected by bacterial strain; host and strain differences were independent. In most hosts a high proportion of infections did not result in nodule formation.

The relative rate of increase in numbers of infected hairs was constant before nodulation began. The duration of this pre-nodulation phase of exponential increase in infection, but not its rate, differed between species. Nodulation (and lateral root formation) caused an abrupt lowering of the initial rate of infection. Post-nodulation infection also increased exponentially. Low concentrations of nitrate nitrogen delayed nodulation and increased the number of hairs infected.

Infected hairs were not randomly distributed along the root, infection beginning at a few well-separated points. Later infections occurred near these primary foci to give zones of infection which then spread up and down the root. The positions of nodules or lateral roots were not related to the primary foci of hair infection.

Introduction

Because of observational difficulties, detailed investigation of the infection of legume root hairs by nodule bacteria, commonly the first stage in the process leading to nodule formation, has lagged behind studies on actual nodulation, particularly in its genetic and physiological aspects (Nutman 1958, 1959*a*).

Fåhraeus's (1957) description of a method that allows the infection of root hairs to be continuously observed has stimulated renewed research in this subject. Fåhraeus showed that initiation and development of the infection thread was associated with the near presence of the host nucleus, and that hair infection does not always lead to nodule formation. Nutman (1959*b*) described host and strain differences in intensity and type of infection with agar-grown plants and Lim (1961) with soil grown plants. Purchase (1958) confirmed Fåhraeus's report that more infections abort in white clover than in red clover and later (private communication) substantiated Thornton's (1929) finding that combined nitrogen in the medium decreases the number of hairs infected.

A series of stages intervene between the colonization of the rhizosphere by nodule bacteria and nodule formation. The object of this work is the study of the infection process as a whole; the rate of infection and its distribution in the young seedling, and in particular the influence of the presence of nodules on hair infection.

[122]

METHODS AND MATERIALS

Plants were grown on an agar medium in small glass chambers constructed on microscope slides. These were examined frequently under the microscope and the positions of infections, nodules, root tips, etc., noted on the stage verniers so that scale maps of roots could be drawn and the progress of infection related to root growth, etc. For a full description of method, media and bacterial strains and plant material used, see Nutman (1959*b*).

The slide cultures were observed from the upper side only. Separate experiments in specially constructed cells showed that two-thirds to four-fifths of all infections were seen from one side of the root; no allowance for unobserved infections is made in the figures or tables.

The plant cultures were grown in cabinets, either (A) at 20 °C in a 12 h day of natural daylight supplemented with fluorescent lighting, or (B) at 20 °C day and 15 °C night temperatures in wholly artificial illumination of 1000 f.c. supplied by fluorescent and incandescent lamps for 12 h daily (Gibson 1959).

RESULTS

(1) *Variation in the number of infected root hairs*

(a) *Host differences*

The progress of infection during the first 3 weeks' growth was followed in twelve small-seeded species of *Trifolium* inoculated with *Rhizobium trifolii* strain ClF (Read 1953) and in *Vicia hirsuta* L., inoculated with *Rhizobium leguminosarum*, strain VI. Table 1 gives the detailed results and figures 1 and 2 show the logarithm

TABLE 1

host	mean seed wt. (mg)	no. of plants examined	geometric mean no. of infected hairs at 11 days	5% fiducial limits	mean root length at 11 days (mm)
Trifolium parviflorum Ehrh.	0·45	4	82·2	41–164	20·6
T. fragiferum L.	1·46	5	78·6	42–146	19·3
T. scabrum L.	0·85	4	46·9	23–94	16·8
T. glomeratum L.	0·42	4	29·8	15–60	15·1
T. nigrescens Viv.	0·25	4	20·9	9–47	11·5
T. arvense L.	0·38	4	16·4	8–31	10·7
T. repens L.	0·36	7	10·6	6–18	11·7
T. patens Schreb.	0·56	4	5·6	2–12	20·9
T. dubium Sibth.	0·25	4	1·9	0·5–5	12·6
T. angustifolium L.	0·07	3	1·2	0–4	17·5
T. ornithopodioides L.	0·38	9	1·0	0·3–2	16·9
T. procumbens (*T. campestre* Schreb.)	0·66	5	0·8	0·1–2	18·6
Vicia hirsuta L.	—	4	16·9	8–34	21·6

of number of infected root hairs plotted against seedling age. Four to six plants were examined for each species; each curve refers to an individual plant. Some of the results were obtained by replicating species between experiments. Most plants
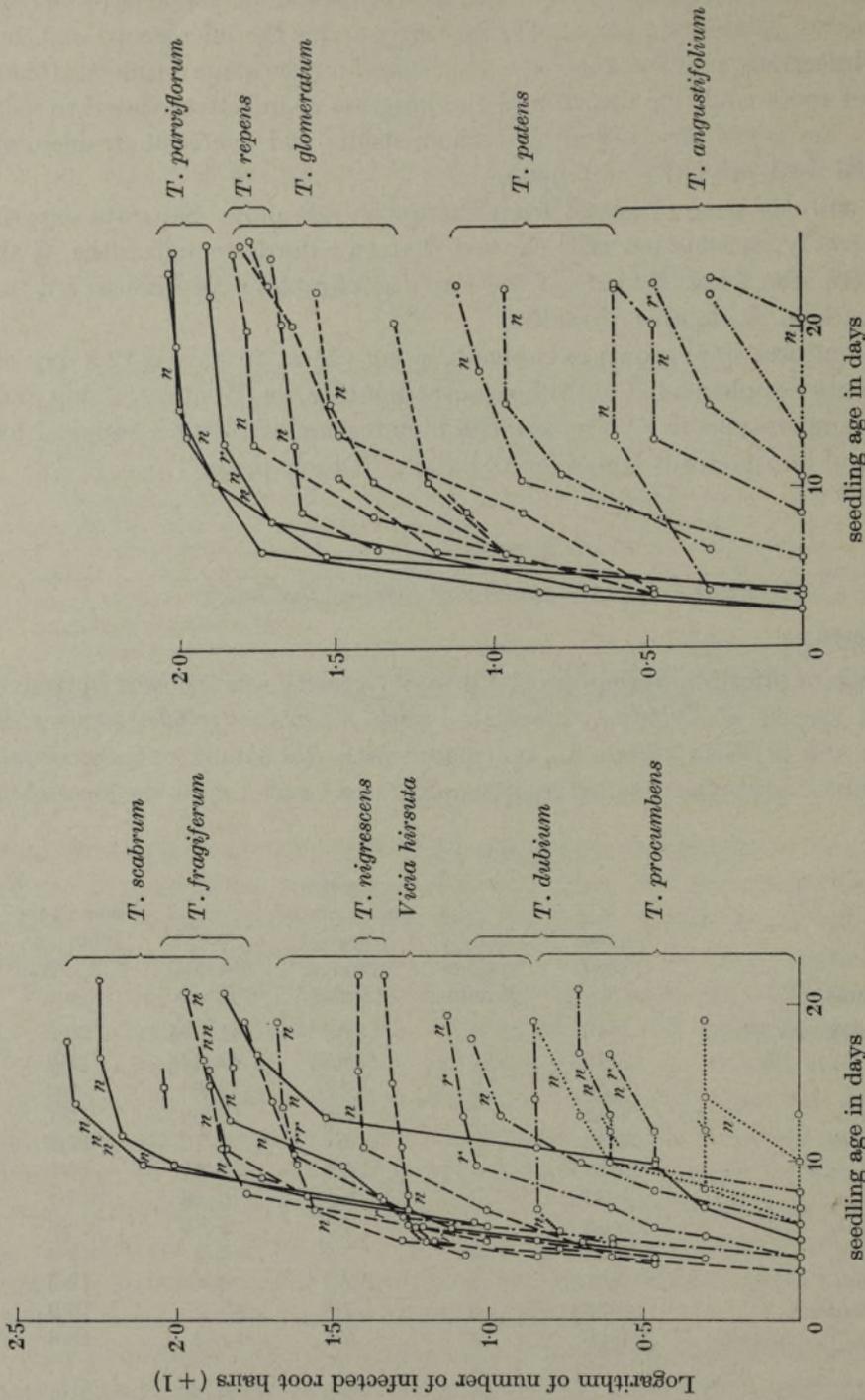
FIGURES 1 AND 2. The progress of root hair infection on species of *Trifolium* and *Vicia*.
'*n*' and '*r*' show times of appearance of nodules and lateral roots, respectively.
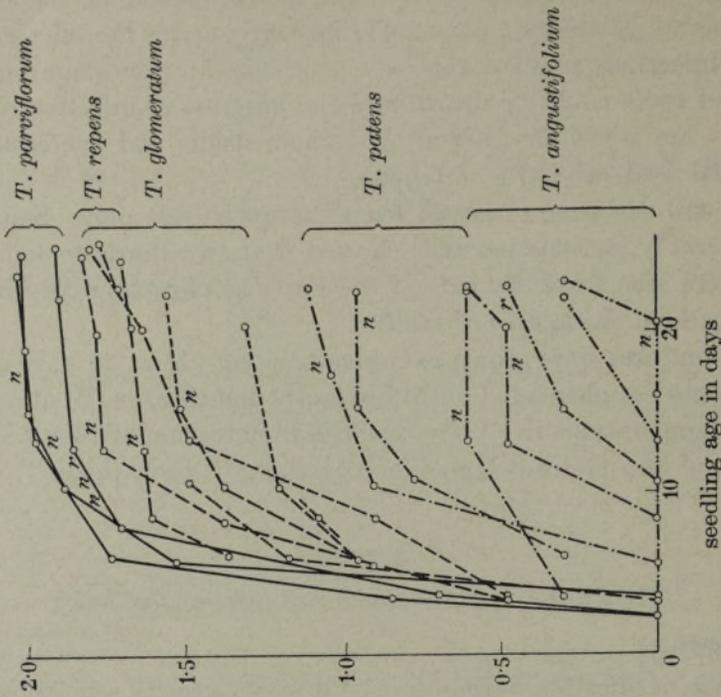
were examined at short intervals. Some were examined once only and these observations, appearing in the figures as separate points, show that disturbance from repeated examination did not affect the course of infection. The letters '*n*' and '*r*' placed against the curves show the times when nodules and lateral roots were first seen.

In most species some tens or hundreds of infections occur before any of these lead to nodule formation. The course of infection is similar in all species and consists of two distinct phases. In the first phase hairs are infected at a regularly increasing rate. This phase is succeeded by a short period during which the rate of infection falls to a much lower value characteristic of the second phase. In both phases the rates of increase are approximately exponential as shown by the linear relationship between log number of infections and time. The rates do not differ much as between species and it is clear that the large differences in amount of infection at the end of the experiments are due to the differing durations of the first phase.

These results also show (1) that infections tend to be formed more abundantly on hosts infected early, and (2) that the change in relative rate of infection occurs at or a little before nodules and lateral roots first appear. The connexion between this change in rate and primary nodule formation is discussed below.

The number of infections arising on the seedling root is thus determined by two factors: the time the root first becomes susceptible and the time the first phase ends. These times define the periods over which infections multiply at the two approximately constant rates. In *Trifolium fragiferum*, for example, the first phase lasts for about 6 days, and in *T. nigrescens* for about 3 days.

The number of abortive infections in *T. dubium*, *T. angustifolium* and *T. procumbens* is so small that the two phases become hard to distinguish and the first phase tends to disappear. As in red clover (Purchase 1958) these species have few or no infections surplus to those leading to nodulation, so that the rates of infection and nodule formation are about the same (allowing for infections not seen on the reverse side of the root). Other work has shown that the numbers of nodules do not increase exponentially with time in this species—or in red clover, white clover or lucerne (Nutman 1948, 1957)—but arise less and less frequently as the seedling ages.

The two-phase mode of infection thus appears to be correlated with the occurrence of surplus infections, both in the first and second phases. Surplus infections continue to form after nodules arise, in spite of a fall in relative infection rate, because the absolute rate is high.

Figures 1 and 2 illustrate variation between individual plants and between species. This is further elaborated in table 1 which gives the average number of infections on all hosts at eleven days, viz. after the end of the first phase. The table includes information on root length. The results are from nine separate experiments made under identical conditions (see (A) above) with replicates distributed partly between experiments. Although replication was unavoidably sparse and standard errors large, differences in susceptibility between species were large and significant.

The *T. procumbens* results relate to material obtained from the Plant Breeding Station, Aberystwyth. In another test *T. procumbens* from Aberystwyth,

Copenhagen, Rothamsted and Zurich were compared (under conditions (B) above) The mean number of infections at 15 days were respectively 16·0, 14·0, 11·4 and 5·5, the Zurich and Aberystwyth strains differing from one another significantly. The English strain 'S100' of white clover and the Swedish strain 'Morso' were also compared with one series of counts at 17 days (under conditions (B)). Mean number of infections for 'S100' was 43 and for 'Morso' 64; this difference was not significant.

Susceptibility is unrelated to the density or type of root hair development (see Nutman 1959b, table II). Thus *T. ornithopodioides* and *T. procumbens* are equally sparsely infected although the root of one is clothed thickly in long hairs and the other scantily with short hairs. The hairs of *T. scabrum* and *T. parviflorum* differ in density and length although both are abundantly infected.

Table 1 also shows that host differences in susceptibility are independent both of seed size, which partly controls the size and surface area of root, and of root length. *T. parviflorum* and *T. procumbens* which differ most in susceptibility are alike in having long roots, whereas *T. dubium* and *T. procumbens* have few infections but differ in root length. There is a correlation between root length and number of infected hairs for species having ten or more infections at 11 days. It is to be expected, discounting other factors, that a longer root will have more infections, but this correlation does not account for more than a small part of the differences between host species. The root of *T. parviflorum* is twice the length of *T. arvense* at 11 days but has nearly six times as many infections.

As noted above, sparsely infected species do not show the first phase of infection, which may account for length and susceptibility not being correlated because root growth in such species is considerable before infection begins.

Most species responded similarly under the two sets of controlled environmental conditions employed, but there were exceptions: *T. scabrum* was more abundantly infected and *T. procumbens* somewhat less infected under conditions (A) than (B). Susceptibility was not related to the actual number of nodules formed on the young seedlings or subsequently.

(b) Influence of bacterial strain on infection

The above host comparisons were made with a single strain of nodule bacteria. Experiments were next done to compare the number of infections produced by three bacterial strains in *T. fragiferum*, *T. glomeratum* and *T. procumbens*. The full results for *T. glomeratum* (figure 3) show that ClF is the most and SU 297 (Vincent & Waters 1954) the least infective strain. With each strain infection occurs in two phases, bacterial strain affecting both the rate in the first phase and the time of transition from one phase to another. ClF and Coryn (Chen & Thornton 1940) infect at about the same rate in the first phase which ends earlier for Coryn. Strain SU 297 infects at a lower initial rate which lasts longer than for the other two strains.

Table 2 compares numbers of infections formed by all strains on all hosts on day 12; results are given as geometric means. ClF produces most infections in each host and SU 297 the fewest. Assuming independence of host and strain effects, the

expected numbers of infections have been calculated from the marginal totals. Figure 3 shows that the expected and observed numbers do not differ significantly and that within this material strain invasiveness and host susceptibility are independent.
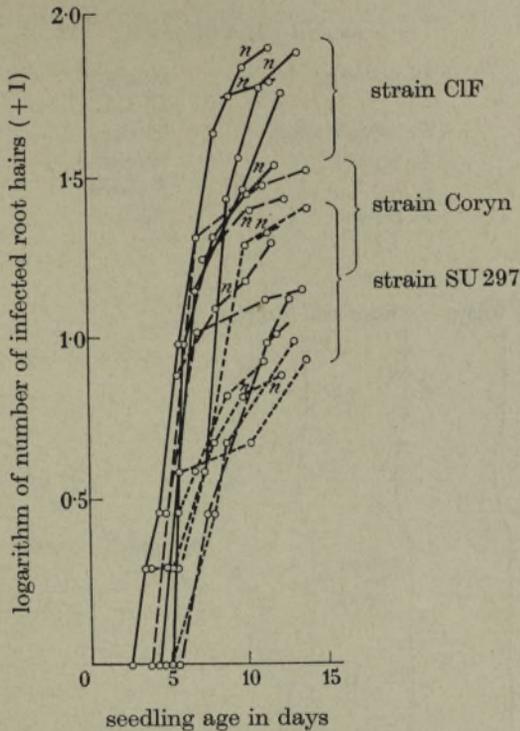

FIGURE 3. Infection of *Trifolium glomeratum* by three strains of nodule bacteria.

TABLE 2. THE INTERRELATION BETWEEN STRAIN VIRULENCE
AND HOST SUSCEPTIBILITY

Observed (and calculated) numbers of infected root hairs

host species	bacterial strains		
	ClF	Coryn	SU 297
Trifolium parviflorum	132 (129)	60 (54)	22 (31)
T. fragiferum	104 (119)	45 (48)	38 (27)
T. glomeratum	56 (49)	17 (21)	9 (12)

No difference between observed and calculated numbers significant at 5 % level.

A comparison of the infective virulence of strain ClF and SU 297 upon *T. nigrescens* showed similar trends. At 15 days ClF and SU 297 formed 38·9 and 28·5 infections, respectively.

Five other strains were compared with ClF on *T. fragiferum* using four plants for each strain, and table 3 shows geometric mean numbers of infections on day 12 with 5 % fiducial limits. ClF is again the most infective strain, and the others differ little from one another despite their very different origins.

Although differences in infective virulence between strains are appreciable they are much overshadowed by those of host origin.

TABLE 3. STRAIN DIFFERENCES IN INFECTIVE VIRULENCE ON
TRIFOLIUM FRAGIFERUM

strain	host and place of origin		mean no. infected hairs	5 % fiducial limits
C1F	*T. pratense*	England	114	64–204
2057	?	U.S.A.	47	26–85
CC229	*T. ochroleucum*	Turkey	37	20–67
Haldon 1	*T. repens*	England	36	20–66
A 121111	?	Sweden	36	20–66
Hannay 7	*T. repens*	Wales	25	14–46

root at 3 days   5 days   8 days   and 16 days

centre
line
of root

apex on
3rd day

$n,7$

$n,7$

—3

—5

—7

—10

—13

0   5mm

—16

—5
$n,12$

—8

16

—16

FIGURE 4   FIGURE 5

—3

$n,18$ —5

—7

—11

—14
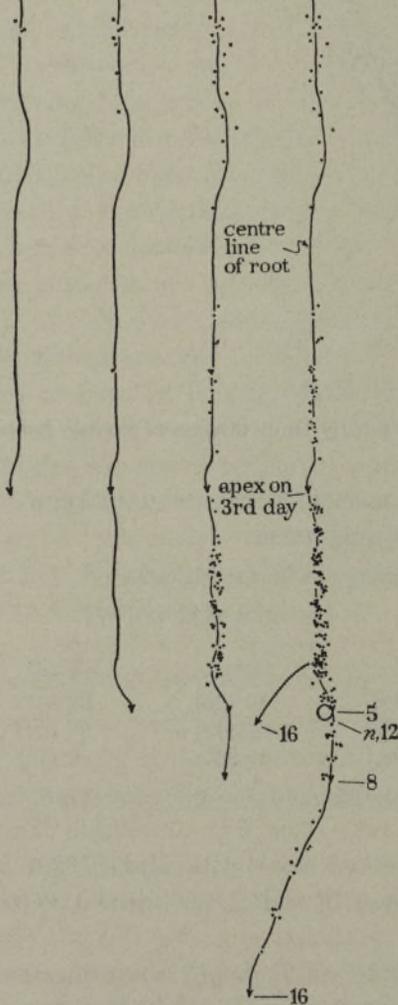
—18
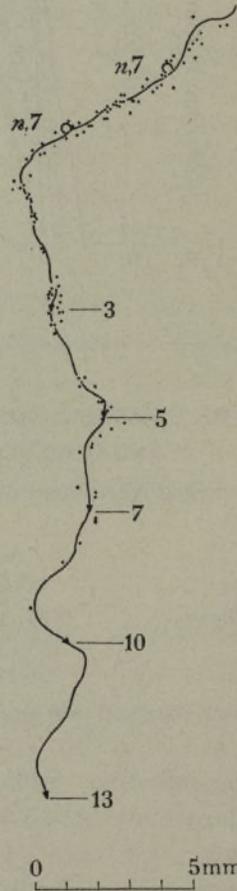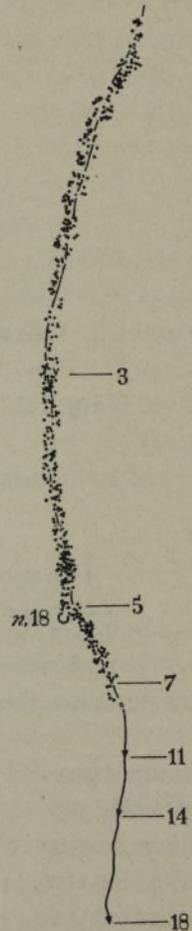
FIGURE 6

FIGURES 4 to 6. Root maps of *Trifolium fragiferum* showing positions of infected hairs, nodules and root apices at the times shown; figure 5 observed after 13 days, figure 6 after 18 days.

(c) *The distribution of infection along the root*

The positions of all infected hairs were noted on the microscope stage verniers and drawings made for later identification. This was a necessary check on the validity of each observation, particularly those referring to very young infections.
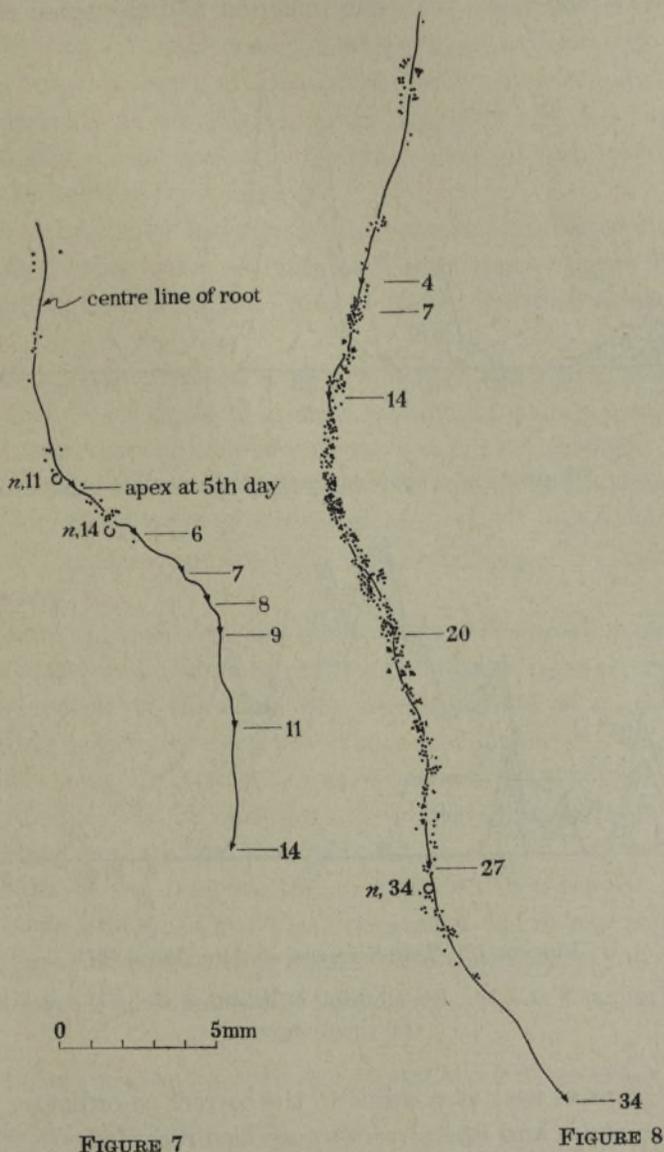

FIGURE 7

FIGURE 8

FIGURES 7 and 8. Root maps of *Trifolium repens* showing infected hairs, nodules and positions of root apices; figure 7 after 14 days, figure 8 after 34 days.

It also enabled infection to be shown on root maps and on distribution diagrams, as in figures 4 to 10.

Repeated examination of many roots provided recurring evidence that the spatial pattern of infections on an abundantly infected species such as *T. fragiferum* is very complex. Not only is infection restricted at first to a few broad zones

along the root but within such zones areas of very dense and less dense infection occur. The co-ordinate unit used (0·1 mm) to locate infection was too large to investigate the fine details of this pattern, for this new methods are being developed; in this section the broad zoning only will be briefly described, because this is relevant to the discussion of data presented in §(2). The root maps are drawn to a ×4 scale and where more than one infection had the same reading they are
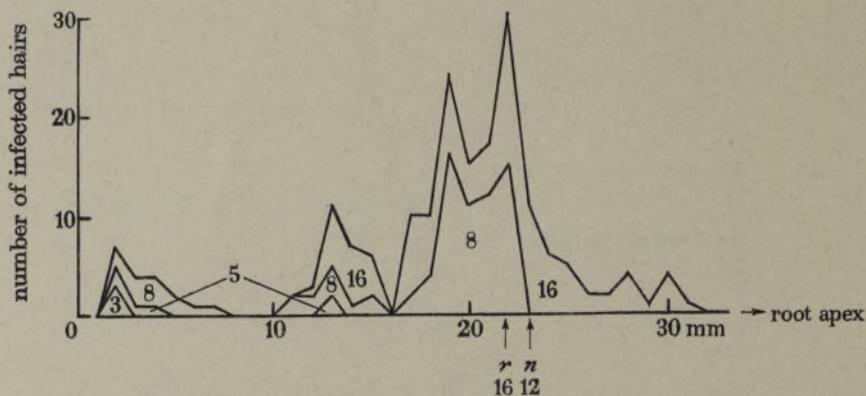

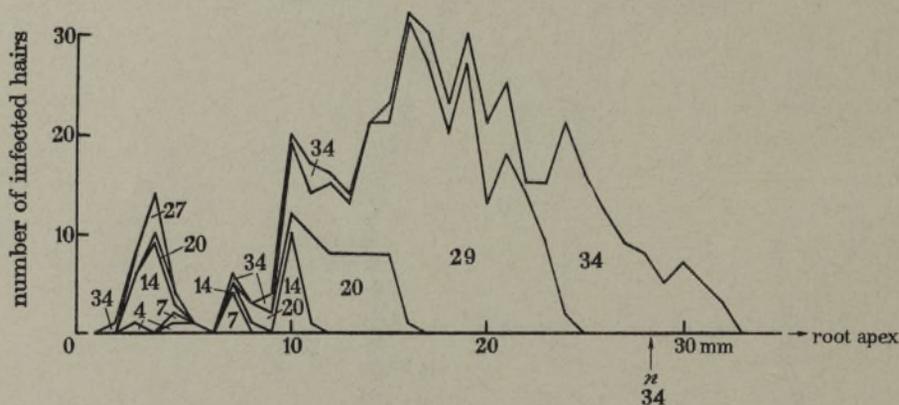FIGURE 9. *Trifolium fragiferum* inoculated ClF

FIGURE 10. *Trifolium repens*, inoculated ClF

FIGURES 9 and 10. Distribution of infection along the root at the times shown

entered on the maps as near as possible to the correct co-ordinates. The locations of root apices, nodules and lateral roots were also recorded. In the distribution diagrams the frequency of infection is given per mm actual length of root measured from the point where the first root hair is formed. The distribution for each time of observation after the first is shown by cumulative totals.

Figures 4 to 6 show the roots of *T. fragiferum* inoculated with strain ClF and observed at intervals after germination as indicated. Figures 4 and 5 show three zones with many more infections than elsewhere. In figure 4, which gives a map for each time the root was examined, about 1 cm separate the two top zones and several millimetres the second and third, with most infections occurring

n the third zone. In figure 5 where the zones are more evenly spaced, the first has most infections. On both roots infections within the zones are distributed unevenly and each zone has several regions of local concentration. The root shown in figure 5 first nodulated at 7 days and had fewest infections; the root in figure 4 nodulated at 12 days and bore an average number of infections for this host and strain.

The root shown in figure 6 had an unusually large number of infections and nodulation was delayed. A clear zonation was evident at first, but this became obscured by later infections until all parts of the root except the distal few milli-metres became infected. As with the roots in figures 4 and 5, small regions of unusually high density of infection were observed although even within such zones by no means all the hairs became infected.

Figures 7 and 8 show similar features for the pattern of infection on the roots of *T. repens*, nodulation being either early and infection sparse (figure 7) or late and infection abundant (figure 8). As in *T. fragiferum* the first infections formed high on the root.

Excepting figure 4, these maps do not show changes with time apart from root length. After an early rapid phase of growth, root length usually increased linearly with time, but in some roots this constant rate was not established.

The distribution diagrams in figures 9 and 10 show the changing pattern of infection with time. They refer to a typical root of *T. fragiferum* and a late-nodulating root of *T. repens*, respectively, inoculated with ClF; figures 8 and 10 refer to the same root.

Four features should be noted: (1) the focal points of the broad zones of infection noted on the root maps are initiated as single infected root hairs or small groups of such hairs widely spaced on the roots, (2) later infections first arise near these initial sites and then over wider stretches of root until in the extreme case (where nodulation is much delayed) most of the root becomes susceptible, (3) infections spread from initial sites both up and down the root, (4) the places where nodules arise bear no relation to the initial sites of infection.

The infected hairs of the root are thus distributed in a regular pattern with respect to both time and position; the exponential phase of early increase in number of infections coincides with a regular spread of susceptibility from a few original centres, and ends when nodules form.

(2) *The influence of nodule and root formation on the infection rate*

(a) *Analysis of natural variation*

Except for the few hosts and strains that form very few infections, the number of infected hairs increases exponentially with time, rapidly for the first few days and more slowly later, with the rate of infection changing at or a little before nodules first appear on the roots. This may reflect a causal relationship or merely be coincidental with changes normal to the root at this time affecting infection and nodulation independently.

This question was examined first by comparing infection upon individual plants nodulating naturally at widely different times. Most variation in nodulating time

9-2

was seen in *T. repens*, and figure 11 gives the results for one test with this species. Of five plants, one nodulated abnormally late, on about the thirty-fourth day, and infection on this plant continued at the high initial rate for much longer than on the others. The change in rate again slightly preceded nodulation. Although this relation is clear for large differences in the time the first nodule appears, it is

seedling age in days
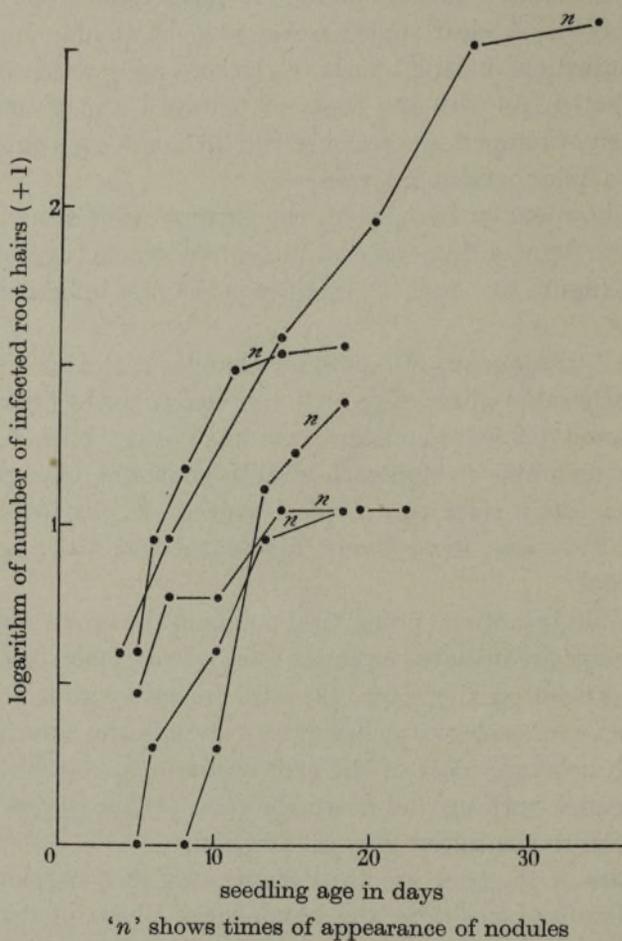
'*n*' shows times of appearance of nodules

FIGURE 11. Relation between infection and nodulation in *Trifolium repens*.

not so evident for smaller time differences. To investigate this relation further a number of experiments were analyzed to determine the significance of the correlation between change of rate of infection and nodulation time.

If t_1 is the time at which infection begins, and k_1 and k_2 the rates of exponential increase in the two phases, and t_2 the time at which the rate changes (neglecting the transition period), the number of infections (i) at time t is given by:

$$\log_{10}(i+1)^* = k_1(t-t_1) \quad \text{for values of } t < t_2,\tag{1}$$

or
$$\log_{10}(i+1) = k_1(t_2-t_1)+k_2(t-t_2) \quad \text{for } t > t_2.\tag{2}$$

The description of infection given by (2) emphasizes the direct connexion between t_2 (the time at which the rate changes) and $\log_{10}(i+1)$ whereas attention

* One is added to the actual number of infections to accommodate zero readings.

s more usefully drawn to the apparent inhibitory influence of the nodule formed at about time t_2 (at t_n) upon later infection. For this reason it is better to consider the inverse relation between infection and t_n. The inverse relation is more convenient because it is independent of t, whereas the direct inhibitory effect is related to $(t-t_n)$; it can also be summed for more than one nodule or none.

In the analysis the correlation of $\log_{10}(i+1)$ with $1/t_n$ for the first-formed nodule only, and with $S1/t_n$ for all nodules were first determined. Inspection of the results suggested that lateral roots (formed at t_r) may also inhibit infection so that correlations involving $1/t_r$ were considered. The lag period t_1, which affects the time for multiplication of infections in the first phase, was also incorporated into the analysis. Finally, because the change in infection rate is often sharply defined, the squares of some of these functions were examined.

TABLE 4. THE INFLUENCE OF NODULE AND ROOT FORMATION UPON INFECTION IN *TRIFOLIUM FRAGIFERUM* (INOCULATED STRAIN ClF)

Correlation coefficients of $\log_{10}$ infection number $(+1)$ at time t (in days) against various functions of the times at which first infection (t_1), nodulation (t_n), and lateral root formation (t_r) occurs.

function	$t=3$	4	5	6	9	13	18
	-0.980^{**}	-0.907^{**}	-0.608	-0.528	-0.470	-0.338	-0.304
$1/t_{n_1}$ for 1st nodule only	-0.333	-0.183	-0.325	-0.554	-0.507	-0.643^{*}	-0.592
$1/t_{r_1}$ for 1st lateral root only	-0.099	$+0.102$	-0.034	-0.417	-0.326	-0.426	-0.508
$1/t_{n_1}+1/t_{r_1}$	-0.171	$+0.044$	-0.111	-0.505	-0.415	-0.538	-0.597
$(1/t_n$ for all nodules$)$	$+0.021$	-0.034	-0.242	-0.261	-0.237	-0.333	-0.334
$(1/t_r$ for all roots$)$	-0.193	$+0.100$	$+0.195$	-0.267	-0.597	-0.700^{*}	-0.592
$(1/t_n)+S(1/t_r)$	-0.185	$+0.088$	$+0.118$	-0.346	-0.665^{*}	-0.797^{**}	-0.867^{**}
$(1/(t_n-t_1))$	-0.185	-0.199	-0.383	-0.445	-0.391	-0.486	-0.487
$(1/(t_r-t_1))$	-0.281	-0.002	$+0.107$	-0.344	-0.648^{*}	-0.750^{*}	-0.616^{*}
$(1/(t_n-t_1))+S(1/(t_r-t_1))$	-0.315	-0.052	$+0.007$	-0.440	-0.719^{*}	-0.834^{**}	-0.914^{**}
$(1/(t_n-t_1)^2)+S(1/(t_r-t_1)^2)$	-0.391	-0.136	-0.036	-0.468	-0.720^{*}	-0.831^{**}	-0.906^{**}

$* \ P=0.05$, $** \ P=0.01$.

All correlation coefficients were calculated over a range of values of t. Table 4 gives the analysis for an experiment with ten seedlings of *T. fragiferum* inoculated with ClF. The top row of correlation coefficients refer to t_1, the lag period. The highly significant inverse correlation of infection with t_1 at low values of t steadily becomes smaller as t increases and is no longer significant by the fifth day.

For older plants (at 13 or 18 days) infection is inversely related to $1/t_{n_1}$ and $1/t_{r_1}$ (for the first-formed nodules and roots) and to $\{S1/t_n$ and $S1/t_{r_1}\}$ but the corresponding coefficients only reach significance for $1/t_{n_1}$ and $S1/t_r$. However, when the influence of nodules and roots are combined these coefficients become increasingly negative as t increases and are usually significant after the sixth day. Half the plants formed only one nodule or root during the experiment so that the contribution from additional nodules and roots was small but nevertheless important.

These correlations are improved by taking into account the effect of the lag period in shortening the first infection phase, $S1/(t_n-t_1)+S1/(t_r-t_1)$, but are not made more significant by squaring the time components. The best fit may lie

between the direct and squared values. Other relations, such as $t_1 + S(1/t_n + 1/t_r)$, etc., were less satisfactory and are not shown.

As t increases the density of infection is increasingly affected by nodule and root formation, but below about 8 days this influence is not statistically significant. This time is appreciably earlier than the first macroscopic appearance of nodules and laterals at 10·4 and 12·8 days, respectively, suggesting that the influence of the nodule and root may begin at the primordial stage.

Although the experiment recorded in table 4 provided most data for this analysis, several other experiments were similarly examined. These were confirmatory whenever variation in times of nodule and root formation were large within an experiment. The effect of the lag period decreased and that of root formation increased with seedling age, and, as in the first analysis, lateral roots contributed largely to inhibition.

(b) *The influence of low levels of nitrogen on hair infection*

The results of the above analysis of the influence of nodule formation and rooting on infection are very dependent on chance variation in experiments of necessarily small size,

TABLE 5. EFFECT OF LOW LEVELS OF NITRATE ON INFECTION AND ON
NODULE AND LATERAL INITIATION IN WHITE CLOVER

	expt. 1			expt. 2		
	0	N 1	N 2	0	N 1	N 2
seedling age in days at:						
(1) initial nodulation	9·0	12·0	12·8	8·0	9·0	11·0
(2) initial lateral root formation*	12·4	15·6	16·2	12·5	13·5	12·0
geometric mean no. infections at 17 days	32·4	94·9	65·9	13·0	67·7	37·1

* Some plants had no laterals at 17 days; these are given an arbitrary value of 18 in calculating means.

Gibson & Nutman (1960) showed that very low levels of nitrate ions significantly delay nodule formation. This fact has been used to obtain independent confirmation of the inhibitory effect of nodule inception on infection rate in white clover. To increase replication, infections were counted on plants fixed in formalin at 17 days; no intermediate counts were made. The seedlings were grown at zero and two levels of potassium nitrate (equivalent to 10 and 25 μg N per plant), in two experiments, one in the constant environment cabinet (expt. 1) and the other in the glasshouse (expt. 2). Daily observations were made of nodule and lateral root formation. The results in table 5 confirm those of Gibson in showing that nodulation is delayed by small amounts of nitrate ions in the medium; lateral rooting was also slightly delayed in expt. 1. The number of infections at 17 days is larger in the nitrate treatments of both experiments, particularly with 10 μg N (significant at P, 0·05 and 0·01, respectively). This result does not necessarily conflict with the work of Thornton and Purchase on the decrease of infections by nitrate because these authors used much more nitrate. The stimulation of infection by low

nitrate associated with delay in nodule and possibly also lateral root initiation is presumably caused by the prolongation of the first phase of the infection process.

As in the earlier experiments, infection on individual plants was inversely related to the times of appearance of nodules and roots, but correlation coefficients relating $\log_{10}(i+1)$ to $S1/t_n + S1/t_r$ within treatments did not reach significance. (Expt. 1 $r = -0.494$, expt. 2 $r = -0.380$; 5 % pt. 0.532). These results therefore support the general conclusion that the change in rate of infection and the appearance of nodules and lateral roots are causally connected, infection thread initiation being inhibited in some way by the formation of any lateral organ on the root.

DISCUSSION

This work not only confirms earlier work in showing that the number of root hairs infected in clover usually exceeds the number of nodules formed, but also shows the orderly ways in which the surplus infections arise on the root. The number of such infections is determined partly by intrinsic host factors, in that they differ between plant species, partly by bacterial strain differences, and partly by the time of actual nodule initiation, which is also presumably host controlled. The intrinsic host differences predominate: about equal numbers of host species and bacterial strains were examined, both of very diverse origin; host differences covered more than a 100-fold range in number of infections, those from bacterial strain less than a 10-fold range. These large effects were not related to any morphological or physiological property of host or strain, nor was their ecological significance apparent. Thus, on the one hand, bacterial strains isolated from different host species in widely separated habitats may be indistinguishable in the number of infections which they produce (table 3) and, on the other, bacterial strains of divergent infectiveness may be found in the same soil sample (Lim 1961).

Physiologically the interest of the results lies in the large number of abortive infections, in the exponential nature of the infecting process and in the remarkable influence of nodules and lateral roots on the course of infection. The multiplication of points of infection before nodules form may be related to the way in which infection is distributed in the root. Hairs in different parts of the root are not uniformly susceptible to infection. Infection begins at a few well-separated places along the root, these then become the centres from which infection spreads to other parts, both up and down the root, the distal spread usually being more rapid. Although infection broadly conforms to this description in all hosts and with all strains, its detailed pattern is more complex and will be the subject of further work.

The formation of a lateral meristem in the root, whether of a nodule or lateral rootlet, greatly slows the rate of infection. Analysis showed that this slowing is closely related to the times of origin of all laterals and nodules formed, but that the first-formed lateral organ has the greatest effect. The rate of nodule formation in the young agar-grown seedling progressively declines (Nutman 1948); this accentuates the inhibitory effect of early nodule formation and infection consequently tends to occur in two distinct phases, the second also exponential in form.

This description of infection raises the question of the mechanism of host control, both in its inducing and inhibiting aspects. In terms of infection-thread development, three stages can be distinguished: (1) *infection-thread initiation*, controlled in some way by the host-cell nucleus; (2) *growth of infection thread* in hair cell and cortex, associated with migration of host nucleus; (3) *activation of the tetraploid centres* in the cortex which give rise to the young nodule (Wipf & Cooper 1938, and others). The last stage is distinguished by a very large extension of the infection thread system in the rapidly dividing cells of the primordial nodule and by strong inhibition of new infections in the root.

The first problem raised by this work is the large number of abortive infections often found. Thread growth can be stopped at any time up to the stage of nodule initiation. Previous results showed a high correlation between early arrest of thread growth in the root hair and a lengthening of the first period of exponential increase in infection number (Nutman 1959b). It may be that during this first period the host nucleus tends to move more rapidly away from the tip of the infection thread or that the process of thread growth is slower in plants which have many infections; the former is more likely. Once the tetraploid cell is penetrated, thread development seems not to be arrested and nodule and coordinated thread development proceed to completion. The first two stages above are concerned with the abnormal deposition of cell wall material and the third with stimulated host cell division. The stimulated production of cell wall substances in infection threads may predispose the invaded tissue, particularly the tetraploid cells to divide.

The second question is why the activation of a nodule or lateral root initial immediately affects the susceptibility of the whole root to infection. A possible explanation would be diversion of material for synthesis of cell walls to the newly developing meristems. The normal 'resistance' to infection of the distal parts of the root, and lessened susceptibility after lateral organs are formed may be regulated by a mechanism of this kind. An alternative theory might propose the production of substances in the growing meristems which directly inhibit infection.

No explanation can be advanced for the differing liabilities for infection of the different zones of the root, or how infection 'spreads' from a few clearly predisposed centres. These features may suggest the movement within the root of a stimulatory principle, but whatever hypothesis is put forward it will have to account for the activation and migration of the host cell nucleus, about which nothing appears to be known. There is no evidence that the pattern of infection is related to variation in the distribution of bacteria in the rhizosphere. Recently Fåhraeus & Ljunggren (1959), Ljunggren & Fåhraeus (1959) and Ljunggren (1960) have shown that the rhizosphere of legumes contains small amounts of polygalacturonase when inoculated with nodule bacteria able to infect them, but not when inoculated with incompatible bacteria. The occurrence of this enzyme in these circumstances is highly suggestive; it may be associated with penetration of the primary cell wall of the root hair or with infection by invagination, as suggested by Nutman (1956). Further work on the function of this enzyme may elucidate the problem of distribution of infection along the root.

References

Chen, H. K. & Thornton, H. G. 1940 The structure of ineffective nodules and its influence on nitrogen fixation. *Proc. Roy. Soc.* B, **129**, 208–229.

Fåhraeus, G. 1957 The infection of clover root hairs by nodule bacteria studied by a simple glass slide technique. *J. Gen. Microbiol.* **16**, 374–381.

Fåhraeus, G. & Ljunggren, H. 1959 The possible significance of pectic enzymes in root hair infection by nodule bacteria. *Physiol. Plant.* **12**, 145–154.

Gibson, A. H. 1959 A study of the physiology and cytology of nodule formation on members of the family Leguminosae. Ph.D. Thesis, University of London.

Gibson, A. H. & Nutman, P. S. 1960 Studies on the physiology of nodule formation. VII. A reappraisal of the effect of preplanting. *Ann. Bot.* (N.S.) **24**, 420–433.

Lim, G. 1961 Microbiological factors influencing infection of clover by nodule bacteria. Ph.D. Thesis, University of London.

Ljunggren, H. & Fåhraeus, G. 1959 Effect of *Rhizobium* polysaccharide on the formation of polygalacturonase in lucerne and clover. *Nature, Lond.* **184**, 1578–1579.

Ljunggren, H. 1960 Transformation in *Rhizobium trifolii*. *Rep. Roth. Expt. Sta. for* 1960, p. 85.

Nutman, P. S. 1948 Physiological studies on nodule formation. 1. The relation between nodulation and lateral root formation in red clover. *Ann. Bot.* (N.S.) **12**, 81–96.

Nutman, P. S. 1956 The influence of the legume in root nodule symbiosis. *Biol. Rev.* **31**, 109–151.

Nutman, P. S. 1957 Studies on the physiology of nodule formation. V. Further experiments on the stimulating and inhibitory effects of root secretions. *Ann. Bot.* (N.S.), **21**, 322–337.

Nutman, P. S. 1958 The physiology of nodule formation. *Nutrition of the legumes*. Ed. E. G. Hallsworth, London: Butterworth, pp. 87–107.

Nutman, P. S. 1959 *a*. Sources of incompatibility affecting nitrogen fixation in legume symbiosis. *Symp. Soc. Exp. Biol.* **22**, 42–58.

Nutman, P. S. 1959 *b* Some observations on root-hair infection by nodule bacteria. *J. Exp. Bot.* **10**, 250–263.

Purchase, H. F. 1958 Restriction of infection threads in nodulation of clover and lucerne. *Aust. J. Biol. Sci.* **11**, 155–161.

Read, M. P. 1953 The establishment of serologically identifiable strains of *Rhizobium trifolii* in field soils in competition with native microflora. *J. Gen. Microbiol.* **9**, 1–4.

Thornton, H. G. 1929 The role of the young lucerne plant in determining the infection of the root by the nodule forming bacteria. *Proc. Roy. Soc.* B, **104**, 480–492.

Vincent, J. M. & Waters, L. M. 1954 Survival and success of inocula in laboratory trials. *Aust. J. Agric. Res.* **5**, 61.

Wipf, L. & Cooper, D. C. 1938 Chromosome numbers in nodules and roots of red clover, common vetch and garden pea. *Proc. Nat. Acad. Sci., Wash.*, **24**, 87–91.