HOST SPECIFICITY IN THE ROOT HAIR "CURLING FACTOR" OF *RHIZOBIUM* SPP.

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[*Manuscript received October* 17, 1968]

Summary

Thirty-eight cultures of rhizobia and 10 non-rhizobia growing in the root zone of clover (*Trifolium glomeratum* L.), 5 rhizobia and 3 non-rhizobia in that of lucerne (*Medicago sativa* L.), and 8 rhizobia in that of Siratro (*Phaseolus atropurpureus* DC.) revealed a specific relationship between bacteria and host that determined the kind and degree of deformation of the root hairs.

With all hosts the markedly curled condition was practically restricted to the host plant associated with virulent homologous rhizobia. Notable exceptions with *T. glomeratum* were the two cultures of *Rhizobium leguminosarum* which caused marked curling of the root hairs, and a culture of *R. lupini* which produced a less marked but similar result. The two less drastic degrees of deformation ("moderately curled" and "branched") were generally most frequent with homologous associations but were found in some degree with most of the rhizobia tested on *T. glomeratum*, including avirulent *R. trifolii*. Four rhizobia (all cowpea) and generally all the non-rhizobia (including agrobacteria) were without effect on this host. Tests with *M. sativa* and *P. atropurpureus* confirmed the evidence of specificity favouring the homologous association. The several agrobacteria were again without effect when tested on *M. sativa*.

Concurrent or prior growth of plants with their homologous rhizobia did not affect the kind or degree of root-hair response to the heterologous rhizobia. Bacteria-free filtrates of *R. trifolii* and *R. meliloti* were able to cause branching and the moderate type of curling, but failed to produce the markedly curled condition. The effect of filtrate prepared from a suspension of the homologous strains of rhizobia was greater than that from heterologous strains.

I. INTRODUCTION

It is well known that the root hairs of legumes are likely to be deformed when grown with their rhizobia (McCoy 1932; Thornton 1936), but the phenomenon has generally been regarded as relatively non-specific in that it has been reported for several combinations of rhizobium and legume that do not lead to the formation of nodules (McCoy 1932; Sahlman and Fåhraeus 1962). However, closer inspection of these reports reveals that relatively few rhizobial strains and hosts have been investigated and that root hairs classed simply as "deformed" include various degrees of curling or branching. McCoy (1932) did indeed retain distinction between "bent" and "curled" for experiments involving the homologous association between host and bacterium, but the two criteria were pooled in the one experiment that provided quantitative data for a heterologous situation (pea growing with *Rhizobium meliloti*).

A degree of deformation, generally not well characterized but recorded on one occasion as less marked than with the rhizobia themselves (Thornton 1936), was also

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reported for bacteria-free filtrates prepared from bacteria that had been grown separately (McCoy 1932). This effect of filtrate, also regarded as having a low order of specificity, has often been attributed to β-indolylacetic acid. Sahlman and Fåhraeus (1962) have, however, produced some cogent arguments against attributing this role to an auxin known to be produced by many bacteria that have no corresponding effect on the root hairs of legumes. The same authors do not exclude an invasion-promoting role for β-indolylacetic acid, but their evidence seems to rule out responsibility for curling of root hairs.

When we attempted to re-examine the question, particularly to repeat the work of Sahlman and Fåhraeus (1962), it became apparent that grades of deformation had to be defined clearly in a way that would permit the condition of root hairs to be recorded objectively. It was also clear that before any attempt could be made to study the nature of a "curling factor" it would be necessary to accumulate sufficient critical data to define the biological parameters of the problem. Haack (1964) has in fact adopted a similar approach, and both investigations show clearly a high order of specificity between bacterium and host so far as the deformation of root hairs is concerned.

II. Materials and Methods

(a) *Plant Culture*

The Fåhraeus slide method (Fåhraeus 1957), as modified by Nutman (1959), was used for direct observation of the growing plants of clover (*Trifolium glomeratum* L.). The same method was also made to serve for lucerne (*Medicago sativa* L.) and Siratro (*Phaseolus atropurpureus* DC.) by replacing the coverslip with a second slide and increasing the space to provide room for the larger roots. The seedling agar had the percentage composition (w/v): $CaCl_2$, 0·01; $MgSO_4.7H_2O$, 0·012; KH_2PO_4, 0·01; $Na_2HPO_4.12H_2O$, 0·015; $FeSO_4.7H_2O$, 0·003; agar (Oxoid "Ionagar"), 0·23. In experiments involving *M. sativa* the agar was increased to 0·6% so as to provide better adhesion between the base and coverslide.

(b) *Bacteria*

The following bacterial strains and substrains were used:

R. trifolii	
Virulent	TA1 (SU329), SU94L, SU36, SU111, SU297/31, SU297/32, SU160, WU290 (SU688), UNZ29 (SU495), WA67 (SU432), substrains of A121111 (ex Rothamsted): SU436/1, SU436/2, SU436/3
Avirulent	SU64a, SU64b/1, SU64a/4N, substrains from Rothamsted: avirulent Bart A (SU434/1, SU434/2), A11 (SU435/1, SU435/2)
R. leguminosarum	TA101 (SU567), SU391
R. meliloti	SU47, U45 (SU496)
R. phaseoli	CC511 (SU330)
Lotus rhizobia	SU343, CC829 (SU503)
R. lupini	W72, A13 (SU502), Ld83, Ld84
R. japonicum	CB1809 (SU697)
"Cowpea" rhizobia	CB756 (SU421), CB1103 (SU696), CB627 (SU370), CB376 (SU452), NGR8 (SU474)
Agrobacterium radiobacter	SU583, SU589
A. tumefaciens	SU582, SU585

The following bacteria were also used: *Pseudomonas aeruginosa*, *Ps. syringae*, *Escherichia coli*, *Aerobacter* (*Klebsiella*) *aerogenes*, contaminant I, contaminant II. These were routine lines except for the two unidentified non-rhizobial contaminants, which were encountered in some uninoculated control plants early in this work.

The rhizobia were almost all from the collection maintained at Sydney University (SU numbers) and had recently been tested for their ability to nodulate an appropriate host. Substrains represented reselected colonial types and the suffix N indicates a re-isolate (still serologically typical) from an occasionally formed nodule on *T. glomeratum*. The agrobacteria and *R. lupini*, Ld83 and Ld84, were supplied by Dr. P. H. Graham of the University of Sydney.

(*c*) *Incorporation of Bacteria*

The bacteria were incorporated in the melted seedling agar (cooled to 40°C) at the time of setting up the slide. Uninoculated controls were checked microscopically and by sampling on agar media for freedom from residual bacteria and contaminants.

(*d*) *Bacterial Filtrates*

Filtrates were prepared from a non-gummy strain of *R. trifolii* (SU297/32) and from *R. meliloti* (SU47) by suspending cells harvested from yeast–mannitol–agar in distilled water (approx. 5×10^9 rhizobia/ml) for an hour, depositing most of the bacteria by centrifugation, and passing the supernatant through a grade 5 sterile sintered-glass filter [Baird & Tatlock (London) Ltd.]. No growth was detected in 2-ml samples taken into yeast–mannitol solution. The bacteria-free filtrate was added aseptically, together with suitably concentrated seedling solution to the Fåhraeus assemblies. At this stage it was diluted about threefold, but this was not critical in that 12-fold dilution gave a similar result.

(*e*) *Observations and Grading*

The whole root of the clover, all of the lucerne root under the slide, and 1 cm of Siratro 1 cm from the top were scanned systematically in order to classify all root hairs that could be clearly seen in the median optical plane. Hairs were recorded as deformed in the following categories:

(1) "Branched"—having a definite branch but without curling.
(2) "Moderately curled"—having the tip curled through an angle of rotation of at least 90° but less than 360°.
(3) "Markedly curled"—having the tip curled to at least 360°; generally markedly deformed and tightly curled as a consequence.

III. Results

(*a*) *Bacteria with* T. glomeratum

Results with the larger collection of bacteria tested on *T. glomeratum* are summarized in Table 1. The response (in each category) can be usefully characterized in terms of whether the number of deformed hairs is:

High (not less than the reference culture, TA1);

Low (not greater than the uninoculated control);

Medium (greater than uninoculated control but less than TA1).

TABLE 1

RESPONSE OF ROOT HAIRS OF *T. GLOMERATUM* TO ASSOCIATED BACTERIAL GROWTH

Number of replicate experiments given in parentheses. All other values are antilogarithms of transformed values. H, high; M, medium; L, low [see Section III(*a*)]

Bacterium	No. of Hairs per 12 Plants: Branched	Moderately Curled	Markedly Curled
Homologous rhizobia			
Virulent *R. trifolii*			
TA1(8)	127 H	65 H	45 H
SU94L(2)	157 H	32 H	54 H
SU36	127 H	60 H	40 H
SU111	122 H	34 H	56 H
SU297/31(3)	119 H	41 H	25 H
SU297/32	153 H	43 H	73 H
SU160	103 H	64 H	48 H
WU290	90 H	40 H	50 H
WA67	133 H	88 H	42 H
SU436/1	147 H	82 H	64 H
SU436/2	199 H	66 H	37 H
SU436/3	89 H	73 H	41 H
UNZ29	143 H	25 M	8 M
Avirulent *R. trifolii*			
SU64a	15 L	12 M	0 L
SU64b/1	2 L	18 M	0 L
SU64b/2	2 L	22 M	0 L
SU64a/4N	8 L	0 L	0 L
SU434/1	16 L	22 M	0 L
SU434/2	0 L	18 M	0 L
SU435/1	14 L	24 M	0 L
SU435/2	4 L	24 M	0 L
A. radiobacter			
SU583	44 L	9 L	0 L
SU589	10 L	1 L	0 L
A. tumefaciens			
SU582	13 L	9 L	0 L
SU585	5 L	5 L	0 L
Uninoculated controls (10)	5	4	0

Bacterium	No. of Hairs per 12 Plants: Branched	Moderately Curled	Markedly Curled
Heterologous rhizobia			
R. leguminosarum			
TA101	301 H	18 M	49 H
SU391(2)	106 H	35 H	28 H
R. lupini			
W72(2)	28 L	65 H	9 M*
A13	61 L	19 M	0 L
Ld83	35 L	15 M	0 L
Ld84	21 L	17 M	0 L
R. meliloti			
SU47(2)	10 L	14 M	0·2 L
U45	4 L	22 M	0 L
Lotus rhizobia			
SU343	76 H	2 L	1 L
CC829	68 H	2 L	0 L
R. phaseoli			
CC511(2)	64 H	16 M	0·2 L
R. japonicum			
CB1809	150 H	6 L	0 L
Cowpea rhizobia			
CB756(3)	10 L	9 L	0 L
CB1103	1 L	9 L	0 L
CB627	71 H	8 L	0 L
CB376	12 L	9 L	0 L
NGR8	17 L	7 L	0 L
Ps. syringae	1 L	3 L	0 L
Ps. aeruginosa	5 L	0 L	0 L
E. coli	4 L	10 L	0 L
A. aerogenes	0 L	22 M	3 M*
Contaminant I	7 L	6 L	0 L
Contaminant II	4 L	5 L	0 L

* In these cases the condition was not typical of the tightly convoluted condition otherwise graded in this category.

For the values in Table 1, the number of hairs per 12 plants in each of the three categories was designated high, medium, or low as follows:

	High	Medium	Low
Branched	>62	Not applicable	$\leqslant 62$
Moderately curled	>30	12–30	<12
Markedly curled	>16	2–16	<2

These values were based on the 5% fiducial limits established for TA1 and the uninoculated control, using a $\log(x+1)$ transformation.

The virulent strains of *R. trifolii* were generally responsible for a high response, except that for strain UNZ29 it was only medium in both curled categories. The avirulent *R. trifolii* caused very little deformation (7 out of 8 graded as medium in the moderately curled category).

TABLE 2

RESPONSE OF ROOT HAIRS OF *M. SATIVA* TO ASSOCIATED BACTERIAL GROWTH

Bacterium	No. of Hairs per 12 Plants:		
	Branched	Moderately Curled	Markedly Curled
Homologous rhizobia			
R. meliloti, SU47	270	219	15
R. meliloti, U45	672	179	14
Heterologous rhizobia			
R. trifolii, TA1	39	18	0
R. trifolii, SU94L	11	9	0
Cowpea rhizobium, NGR8	18	8	0
A. radiobacter, SU583	4	7	0
A. radiobacter, SU589	3	6	0
A. tumefaciens, SU582	3	9	0
Uninoculated control	0	7	0

Apart from most of the cowpea cultures, the heterologous rhizobia were responsible for slight but definite response in the branched or moderately curled categories or both. The two *R. leguminosarum* and one of the four *R. lupini* were exceptional in that they each caused a significant degree of marked curling, a response otherwise restricted to the virulent homologous rhizobia (with the exception of a borderline case with the culture of *Aerobacter aerogenes*). The latter, like the one case of *R. lupini*, produced a few cases of curling through 360° though not in the tight form typical of the homologous condition.

Most of the cowpea strains ranked as poorly as the non-rhizobia in being generally without effect. It is noteworthy that none of the four agrobacteria was any better than other non-rhizobia.

(*b*) *Bacteria with* M. sativa *and* P. atropurpureus

Representative homologous and heterologous rhizobia were also tested on *M. sativa* (Table 2) and *P. atropurpureus* (Table 3). Agrobacteria were included in the case of *M. sativa*.

The results confirmed the fact that the homologous combinations resulted in greater curling generally and were almost solely responsible for the markedly curled condition.

TABLE 3

RESPONSE OF ROOT HAIRS OF *P. ATROPURPUREUS* TO ASSOCIATED ROOT GROWTH

Bacterium	No. of Hairs per 12 Plants:*		
	Branched	Moderately Curled	Markedly Curled
Homologous rhizobia†			
Cowpea rhizobium, CB756	213	1262	86
Cowpea rhizobium, CB627	196	1236	78
Cowpea rhizobium, CB376	260	694	22
Cowpea rhizobium, NGR8	102	378	29
R. lupini, W72	173	879	170
R. japonicum, CC1809	160	847	45
Heterologous rhizobia			
R. trifolii, TA1	7	45	2
R. meliloti, SU47	111	57	1
Uninoculated control	48	29	0

* Counts restricted to second centimetre of root.

† In the sense of being able to nodulate the test plant freely.

(c) *Simultaneous Growth of Homologous and Heterologous Hosts*

If the specificity of marked curling depended on the capacity of a particular bacterium to utilize a host-specific substrate for the production of an otherwise

TABLE 4

RESPONSE OF ROOT HAIRS OF JOINTLY GROWN *T. GLOMERATUM* AND *M. SATIVA* TO *R. TRIFOLII* AND *R. MELILOTI*

Bacterium	No. of Hairs per 12 *T. glomeratum* Plants:			No. of Hairs per 12 *M. sativa* Plants:		
	Branched	Moderately Curled	Markedly Curled	Branched	Moderately Curled	Markedly Curled
R. trifolii, TA1	117	87	118	20	8	2
R. meliloti, SU47	140*	24	1	118	93	24
Uninoculated	8	6	0	0	0	0

* Almost entirely due to two plants which accounted for 106 cases, whereas, in the case of *R. trifolii*, the branched condition was evenly spread.

non-specific factor, one would expect that a heterologous host would be affected by propinquity to rhizobia and a homologous host.

To test this, *T. glomeratum* and *M. sativa* plants were set up on the one slide sufficiently close for the root hairs to touch often. These assemblies were inoculated with *R. trifolii* (strain TA1) or *R. meliloti* (strain SU47), or left uninoculated. Results given in Table 4 showed that even in the presence of the markedly deformed homologous host, the heterologous plant showed only the usual lower-order, less-specific response.

(*d*) *Effect of Preplanting with Homologously Inoculated Host*

It seemed possible that the failure of the heterologous host to respond to a product from the associated homologously inoculated host might have been due to failure of a specific factor to diffuse as far as the associated plant. To overcome this objection the medium (0·6% agar) was first heavily preplanted with seedlings (inoculated or uninoculated) and then used for the slide assembly, after removing the plants and heating briefly at 100°C to melt the agar, and for 30 min at 80°C to destroy the rhizobia. Table 5 shows the combinations of treatment and results.

TABLE 5

INFLUENCE OF PREPLANTING ON ROOT-HAIR RESPONSE IN *T. GLOMERATUM*

Preplanting Treatment	Final Inoculum	No. of Hairs per 12 Plants:		
		Branched	Moderately Curled	Markedly Curled
A. Nil	Uninoculated control	6	2	0
	R. trifolii, TA1	252	58	127
	R. meliloti, SU47	156	14	0
B. Clover without inoculum	Uninoculated control	38	18	0
	R. trifolii, TA1	374	106	104
	R. meliloti, SU47	233*	100*	1
C. Clover inoculated with *R. trifolii*, TA1	Uninoculated control	716	48	2
	R. trifolii, TA1	164	91	89
	R. meliloti, SU47	320	69	5
D. Clover inoculated with *R. meliloti*, SU47	Uninoculated control	8	28	0
	R. trifolii, TA1	146	82	103
	R. meliloti, SU47	52	25	0

* Almost all due to a high result with one to two plants.

Without preplanting (series *A*) there was the usual response to homologous (TA1) and heterologous (SU47) inoculation. This persisted in the preplanted series (*B–D*). Any response to the prior presence of the clover plant itself (uninoculated controls in series *B* cf. series *A*) was small compared with that of host and clover rhizobia together (uninoculated control in series *C*). The presence of heterologous rhizobia with the preplanted host (uninoculated control in series *D*) caused no additional stimulation. It follows that any soluble and heat-stable factor produced as a result of the growth of rhizobia in the vicinity of the roots of preplanted clover

is specific in its ability to deform the root hairs when that host is replanted in the same medium.

(e) *Bacterial Filtrates*

Tables 6 and 7 confirmed the effect of bacteria-free filtrate prepared from homologous rhizobia (grown on a solid medium in the absence of host plant), and

TABLE 6

RESPONSE OF ROOT HAIRS OF *T. GLOMERATUM* TO HOMOLOGOUS AND HETEROLOGOUS RHIZOBIA AND THEIR BACTERIA-FREE FILTRATES

Bacterium or Filtrate*	No. of Hairs per 12 Plants:		
	Branched	Moderately Curled	Markedly Curled
Experiment 1			
Homologous bacterium (*R. trifolii*, SU297/32)	358	45	129
Filtrate, 8 ml	240	133	3
Filtrate, 4 ml	263	69	0
Filtrate, 2 ml	387	72	3
Untreated	37	6	0
Experiment 2			
Homologous bacterium (*R. trifolii*, SU297/32)	521	62	117
Filtrate, 8 ml	318	13	0
Heterologous bacterium (*R. meliloti*, SU47)	351	25	0
Filtrate, 8 ml	31	8	1
Untreated	67	2	0

* In 25 ml of final seedling solution.

TABLE 7

RESPONSE OF ROOT HAIRS OF *M. SATIVA* TO HOMOLOGOUS AND HETEROLOGOUS RHIZOBIA AND THEIR BACTERIA-FREE FILTRATES

Bacterium or Filtrate*	No. of Hairs per 12 Plants:			No. of Hairs per 12 Plants:		
	Branched	Moderately Curled	Markedly Curled	Branched	Moderately Curled	Markedly Curled
	Slide Culture Method			Agar Slope Culture Method		
Homologous bacterium (*R. meliloti*, SU47)	109	556	139	256	634	286
Filtrate	219	21	0	64	29	0
Heterologous bacterium (*R. trifolii*, SU297/32)	15	1	0	180	84	2
Filtrate	9	14	0	26	18	0
Uninoculated control	3	2	0	22	20	0

* Thirteen ml in 40 ml of final seedling solution.

showed that this was much more marked than for the filtrate of the heterologous bacterium. Filtrate effects were, however, almost entirely restricted to the branched and moderately curled categories.

(*f*) *Avirulent Rhizobia Combined with Filtrates of a Virulent Strain*

Two avirulent cultures of *R. trifolii* (SU434/1 and SU435/1) were tested on *T. glomeratum*, separately and each together with the filtrate of the virulent *R. trifolii*, SU436/1 (Table 8). There appeared to be a simple additive effect in the case of the branched and moderately curled category (treatments 4 cf. 2 and 3, and 6 cf. 2 and 5) but no complementary effect able to cause any significant increase in the few

TABLE 8

TEST FOR COMPLEMENTARY EFFECT BETWEEN AVIRULENT *R. TRIFOLII* AND FILTRATE OF VIRULENT *R. TRIFOLII* ON *T. GLOMERATUM*

Values in parentheses are totals of separate bacterial and filtrate values

Treatment	No. of Hairs per 12 Plants:		
	Branched	Moderately Curled	Markedly Curled
1. Virulent *R. trifolii*, SU436/1	579	76	110
2. Filtrate of SU436/1	375	31	0
3. Avirulent *R. trifolii*, SU434/1	6	102	2*
4. Avirulent SU434/1 + filtrate of SU436/1	371	159	5*
	(381)	(133)	(2)
5. Avirulent *R. trifolii*, SU435/1	32	62	4*
6. Avirulent SU435/1 + filtrate of SU436/1	426	82	8
	(407)	(93)	(4)
7. Uninoculated control	30	6	0

* Characteristically loosely curled.

cases classed as markedly curled. One point was observed, however: the combined treatment resulted in root-hair deformation in the fields of view nearest the top of the root, a condition regularly seen in the presence of the virulent rhizobia but not with the avirulent, nor with the filtrate of the virulent bacteria.

IV. DISCUSSION

Throughout this work, as with that of Haack (1964), the markedly curled condition of root hairs was almost entirely restricted to the leguminous host associated with those rhizobia able to form nodules with it. The failure of avirulent homologous rhizobia to produce this form of curling, the fact that practically all infection threads were observed in markedly deformed hairs, and the observation by Munns (1968) that curling and nodulation were coincidentally sensitive to acid conditions, strongly suggest that this extreme response is a prerequisite to invasion, or results from the action of a substance or circumstance which is itself a prerequisite to this event.

The marked curling of the root hairs of *T. glomeratum* which we observed with *R. leguminosarum*, and those of pea with *R. trifolii* (Haack 1964), conformed to other evidence of close relationship between those two rhizobial "species". Our positive, though ambiguous, result between one strain of *R. lupini* (W72) and *T. glomeratum*, and Haack's (1964) interaction between pea and an isolate from *Onobrychis* were

less expected. The fact that marked curling with heterologous associations were not, at least in our cases, accompanied by root-hair invasion is a reminder that, although one prerequisite had been met, others were not. Nutman (1949) reported that a non-nodulating ("resistant") line of red clover had as many deformed hairs in the presence of virulent *R. trifolii* as were found with the fully invadable host line. The relatively weak deforming property of *R. trifolii*, strain UNZ29 (Table 1) is interesting in view of indications of its poor performance under field conditions (Brockwell and Dudman 1968; Ireland and Vincent 1968).

The recognition of categories which we class as moderately curled or branched, like the less marked deformation recognized by Haack (1964), meant that plants inoculated with rhizobia (homologous or heterologous) were generally distinguishable from those that were uninoculated or were growing in the presence of non-rhizobia. Haack (1964) found little root-hair reaction to several rhizobial strains unable to nodulate *Pisum* and virtually no reaction with heterologous rhizobia in the case of *Ornithopus*. It is of interest that the exception in the case of the second host was with an isolate from *Anthyllis*, a finding in agreement with cross-inoculation relationships (Jensen 1967). Exceptions to a recognizable response by root hairs to heterologous rhizobia in our work were restricted to four out of five cowpea rhizobia on *T. glomeratum* and the one clover rhizobium tested on *P. atropurpureus*. The failure of the four agrobacteria to exert any effect on the root hairs of either *T. glomeratum* or *M. sativa* is one, but not a conclusive, point of evidence against close relationship between agrobacteria and rhizobia, a proposition currently occupying the minds of those concerned with the taxonomy of the two genera (e.g. Moffett and Colwell 1968).

Other workers (McCoy 1932; Thornton 1936; Thornton and Nicol 1936; Sahlman and Fåhraeus 1962) have reported the deformation of root hairs by bacteria-free filtrates prepared from suspended rhizobia. In some cases this has been recorded as a non-specific phenomenon (McCoy 1932; Sahlman and Fåhraeus 1962), but these reports failed to define the criteria or to present the data quantitatively, or both. The results we have reported in Tables 5–7 show that the markedly curled condition is seldom seen with a diffusible factor on its own (see also Thornton 1936). Such a diffusible factor can, however, cause a significant number of branched and moderately curled hairs, with a degree of specificity between rhizobia and host that was not previously apparent.

An accepted lack of specificity in the deformation of root hairs has evidently been largely responsible for little interest being shown in this aspect of the association between rhizobium and legume. However, the marked degree of specificity that has now become apparent, and the particular dependence of the markedly curled condition on the actual presence of specific bacteria, seem to justify much more attention being given to the responsible factor (or factors). It remains yet to be determined whether more than one factor is involved in causing the forms or degrees of response that have been observed.

The most likely separation could be between factors concerned with root-hair branching and those causing curling. There is evidence that these effects can operate independently as in the cases of several heterologous bacteria (lotus rhizobia, *R. phaseoli*, *R. japonicum*, and the cowpea strain, CB627) on *T. glomeratum* (Table 1); *R. meliloti* compared with homologous rhizobia on *P. atropurpureus* (Table 3); in

a comparison between *R. meliloti* and its filtrate on *M. sativa* (Table 7); in a lack of relationship between the number of branched and curled hairs in Table 8; and in the independently additive effect of each character when an avirulent rhizobium was combined with filtrate from a virulent strain (Table 8).

The two categories of curling could be more closely related in that the markedly curled condition (requiring the presence of suitable, generally homologous invasive bacteria) might be attributed to localized concentration of a factor that would be provided more diffusely in the case of a filtrate. The data in Table 1 (including the particular case of UNZ29) generally reveal a parallel trend between the moderately and markedly curled categories. The same is true of the comparisons between the homologous and heterologous bacterium: plant associations in Tables 2, 3, 6, and 7. Filtrates generally produced less curled hairs than the bacteria themselves. Exceptionally (most concentrated filtrate in experiment 1 of Table 6) the total curled hairs was almost as great with the filtrate as with the corresponding rhizobia but were practically all in the "moderate", not "marked", category. Combination of filtrate from an invasive strain with the non-invasive bacteria (Table 8) was able to lift the curling capacity of the treatment significantly; the fact that this was practically all in the moderately curled category could still represent failure to reach localized concentrations obtained in the vicinity of virulent bacteria.

V. Acknowledgments

This work has been supported by the Australian Research Grants Committee, the Wheat Industry Research Council, and the Rural Credits Division of the Reserve Bank of Australia. The authors are happy to acknowledge helpful advice from Dr. P. S. Nutman at the commencement of the work.

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