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Relationship between Timing of Infection and Nodulation Competitiveness of *Rhizobium meliloti*

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Abstract

Experiments were conducted to investigate whether the timing of successful infection of legume plants by rhizobia was related to the ability of the strains to compete for nodule occupancy. The time needed for 16 strains of *Rhizobium meliloti* in stationary growth phase to initiate nodulation of lucerne plants (*Medicago sativa* L.) grown in nutrient solution was estimated by evaluating the interval between inoculation of plants and initiation of infection leading to the uppermost tap root nodule. The competitive ability of each strain was evaluated in 33 paired-strain nodulation experiments. The results from each pair were compared to the time to infection by the constituent strains. Strains that infected plants significantly faster ($P<0.05$) were more competitive in 10 of 15 pairs. However, when the strains showed no difference in time to infection, they had similar nodulation competitiveness in only two of 18 pairs, probably because other factors were then affecting competitive ability. The effects of growth phase of rhizobia and incubation in luteolin solution on timing of infection and nodulation competitiveness also were investigated. Exponential-phase cells of strain PDDCC1320 initiated infection more quickly than did stationary-phase cells. This

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experimental treatment reduced the time to infection of strain PDDCC1320 to equal that of the otherwise faster strain PDDCC2752. Luteolin had no significant effect on the timing of nodule initiation by the strains. Comparison of competitive ability with timing of infection revealed that significantly faster strains were always (4 of 4 strain pairs) associated with significantly greater competitive ability. In those treatments where the strains were not significantly different in periods to infection, similar nodulation competitiveness between the strains was observed in only one of three strain pairs. These results indicate that it is possible to rank strains of rhizobia for nodulation competitiveness by screening them for time taken to infect plants.

Keywords: Competition, growth-phase, *Medicago sativa*, luteolin, *Rhizobium*, speed of infection, tertrahydoxyflavone.

1. Introduction

Competition for nodule occupancy between inoculant strains and the often less effective indigenous strains in the soil is frequently a major barrier to increasing yields of legumes by inoculation (Streeter, 1994). One approach to resolve this problem is to use strains of rhizobia that are not only effective in fixing nitrogen, but also competitive in nodule formation. Methods currently used for evaluating strains for nodulation competitiveness rely on competition experiments involving identification of inoculant strains after isolation from the nodule (Schmidt et al., 1968; Kishinevsky and Bar-Joseph, 1978; Schwinghamer and Dudman, 1973). Simpler methods include use of ineffective strains (Amager, 1981) and of strains which produce nodules that can be distinguished macroscopically, e.g., dark nodules (Ayanaba et al., 1986) or blue nodules (Wilson, 1995).

Because the molecular and physiological mechanisms associated with competitiveness are still unknown (Triplett, 1990), screening strains for competitiveness in single-strain inoculation experiments remains difficult. Several workers have reported that the competitive ability of a strain of *Rhizobium* spp. is associated with its ability to initiate infection rapidly and effectively (Labandera and Vincent, 1975; Olivares et al., 1980; van Rensburg and Strijdom, 1982; Stephens and Cooper, 1988; Oliviera and Graham, 1990). The location of a nodule on the legume root provides an indication of the relative time after inoculation that it was initiated, because infectibility of host-root cells by rhizobia is developmentally restricted and transient (Bhuvaneswari et al., 1981). For example, in lucerne, cowpea and soybean, the average distance of the uppermost nodule on the tap root from a mark made at

the root tip at the time of inoculation has been shown to change in a linear manner as inoculation is delayed (Bhuvaneswari et al., 1981).

Mulligan and Long (1985) showed that the common nodulation genes, nodABC, of *R. meliloti* were inducible by plant exudate. The first signal compound to be isolated from lucerne seeds was luteolin (3',4',5',7-tetrahydroxyflavone) (Peters et al., 1986). Subsequently, other flavanoids and betaines have been identified in lucerne seeds and roots and shown to induce transcription of nodulation genes (Dharmatilake and Bauer, 1992; Phillips et al., 1995). The effect of luteolin on nodulation and nitrogen fixation has been investigated (Kapulnik et al., 1987), but there are apparently no reports on its effects on the speed of infection and competitive ability of rhizobia.

In this study, the time after inoculation that the uppermost nodule had been initiated was estimated by relating its position on the root to the rate of root elongation. We then investigated the possibility that differences between strains of *R. meliloti* in periods to nodule initiation determined by this method could be related to their competitive abilities, thereby providing a method for screening rhizobia for competitive ability. We also tested the hypothesis that phase of growth of the rhizobia and the availability of luteolin could modify their speed of infection and competitive ability.

2. Materials and Methods

Rhizobia

The strains of *R. meliloti* used in these studies are listed in Table 1. Starter cultures were prepared from stock cultures held on yeast-extract mannitol agar (YMA) slopes (Vincent, 1970) by transferring a loopful of cells into 10 ml of sterile yeast-extract mannitol broth (YMB) (Vincent, 1970), and growing them at 30°C on a rotary shaker (100 rpm). After 3 days, when stationary growth phase had been reached at ca. 10^9 cells ml^{-1}, the starter cultures were diluted to 10^3 cells ml^{-1} and 0.2 ml added to 10 ml of YMB as inoculum. Inoculum cultures were grown for 3 days to the stationary growth phase as above. Viable cells were enumerated by the drop-plate count method (Somasegaran and Hoben, 1985) on YMA after incubation for 3 days at 30°C. During this period, the inoculum cultures were kept refrigerated. The inoculum cultures were diluted with sterile Ringer's solution to give 10^6 cells ml^{-1} based on the results of the drop-plate count.

Table 1. Strains of *R. meliloti* used in the investigation

Strain	Source
PDDCC2751	PDDCC[1] of DSIR[2], Auckland
PDDCC2752	PDDCC
PDDCC5374B	PDDCC
PDDCC1805	PDDCC
PDDCC5557	PDDCC
PDDCC4133	PDDCC
PDDCC2748	PDDCC
PDDCC1325	PDDCC
PDDCC1320	PDDCC
7P12	Nodule isolate from Canterbury (isolated by P.M. Stephens)
2751a–c	Mutant derived from strain 2751, resistant to 50 μg ampicillin ml^{-1} and 125 μg chloramphenicol ml^{-1}
2752rif	Mutant derived from strain 2752, resistant to 100 μg rifampicin ml^{-1}
7P12rif	Mutant derived from strain 7P12, resistant to 100 μg rifampicin ml^{-1}
5374Bstr	Mutant derived from strain 5374B, resistant to 300 μg streptomycin ml^{-1}
5557spc	Mutant derived from strain 5557, resistant to 300 μg spectinomycin ml^{-1}
2748spc	Mutant derived from strain 2748, resistant to 300 μg spectinomycin ml^{-1}

[1]Plant Diseases Division Culture Collection, now called International Collection of Microorganisms from Plants (ICMP). [2]Department of Scientific and Industrial Research. Note: The PDDCC strains are referred to by their numbers only hereafter.

Preparation of plants

Seeds of lucerne (*Medicago sativa* L.), cv. WL318, were surface-sterilised with 0.2% (w/v) mercuric chloride (Somasegaran and Hoben, 1985) and germinated on 1.5% (w/v) water agar in petri dishes for 48 h at 30°C.

The apparatus in which the tests were conducted was similar to that devised by Cooper (1978), except for the size of the test tube. The apparatus was made by rolling a 100 mm × 75 mm chromatography paper strip to form a cylinder 32 mm in diameter and 75 mm long. The cylinder was placed in a 32 mm diameter and 200 mm long test tube, which was capped with a cotton wool plug. The apparatus was autoclaved, and 10 ml of N-free sterile plant nutrient solution at pH 6.7 (Cooper, 1978) were added aseptically. After inoculation (see below), two seedlings were aseptically planted in each tube by inserting their radicles between the cylinder of chromatography paper and the side of

the test tube. The tubes were arranged in a completely randomized design in a growth room with 24-h light at 103 μmol photons m^{-2} s^{-1} illuminance and a 21–25°C temperature range. The 24-h light regime was adopted because the light intensity was low. The tubes were placed in growth cabinets which prevented exposure of roots to light.

Screening strains for periods to initiation of nodulation

Sixteen strains of *R. meliloti* were used to inoculate 140 sterile lucerne seedlings per strain. The seedlings were inoculated by dripping 20 μl of inoculum containing 10^6 cells ml^{-1} on each radicle, i.e., an inoculation rate of 2×10^4 cells per seedling. The position of the root tip for each seedling was marked on the surface of the test tube at the time of inoculation and the tubes were immediately transferred to the growth room.

Elongation of the root was measured 48 h and 120 h after inoculation. The distance of the uppermost tap root nodule from the root tip mark was measured 17 days after inoculation; the identity of each nodule was verified 13 days later. The time (h) required to initiate successful infection, i.e., infection leading to each uppermost tap root nodule, was calculated by dividing the distance (mm) of the nodule from the root-tip mark by the rate (mm h^{-1}) of root elongation. Very few nodules (less than 1%) formed above the root-tip mark and these were considered to have been formed at the time of inoculation, i.e., 0 h.

Rate of root elongation

Calculating the time to initiation of a nodule by dividing nodule distance by the rate of root elongation assumed a linear rate of root elongation. To examine the validity of this assumption, root lengths of 30 plants were measured every 24 h for 10 days. The rate of root elongation was linear and could be described as:

mm of root extension = 0.46 + 0.21 h ($R^2 = 0.998$).

Therefore, it was valid to assume linear rate of root elongation in calculating the period to initiation of nodulation.

Delayed inoculation experiment

To ascertain whether the calculated time to initiation of infection leading to the uppermost nodule was a true indication of the relative period to successful infection of each plant by *R. meliloti*, an experiment was conducted to

determine whether delay in inoculation would result in a shift of the location of the uppermost nodule on the tap root. Root tip positions of 70 seedlings were marked 6 hours before, or at the time of, inoculation. The roots of seedlings planted in test tubes as described above were inoculated with 20 µl of a suspension of strain 2751 adjusted to 10^6 cells ml^{-1}. Root elongation and the position of the uppermost nodule on each tap root were measured and the time needed for successful infection calculated as described above.

Competition experiments

The 16 strains were paired in 33 competition experiments. The constituent strains of some pairs were significantly different from each other in periods to nodule initiation whilst those of other pairs were not. Equal quantities (5 ml) of the broth cultures of the two constituent strains of a pair, each adjusted to 10^6 cells ml^{-1}, were mixed. Each seedling was then inoculated with 20 µl of the mixed inoculum, i.e., 10^4 cells of each strain per seedling. The actual ratio applied was determined by plate-counting the mixed inoculum on antibiotic-free and antibiotic-enriched YMA. Thirty plants per competition experiment were inoculated and grown as described above.

Nodules were typed 30 days after inoculation using antibiotic resistance. At least 60 nodules were randomly selected from each treatment for nodule typing. An antibiotic resistant mutant was used for nodule typing (Schwinghamer and Dudman, 1973) where it was one of a pair, otherwise intrinsic antibiotic resistance (Beynon and Josey, 1980) was used. In both cases, nodule extracts were first streaked on YMA. Colonies were then streaked on antibiotic-supplemented YMA to separate antibiotic-resistant strains from sensitive strains.

Effect of growth phase of rhizobia and incubation in luteolin

Incubation of rhizobia in luteolin. Inoculum cultures of strains 2752 and 1320 were grown for either 13.5 h (early exponential phase, ca. 10^5 cells ml^{-1}) or 73.5 h (stationary phase, ca. 10^9 cells ml^{-1}). These periods of incubation and their respective viable cell concentrations were determined from growth curves. The cultures were centrifuged for 10 minute at 8,000 g and the cells resuspended in sterile Ringer's solution to give a final concentration of ca. 10^6 cells ml^{-1}. Luteolin (Spectrum Manufacturing Corporation, Gardena, CA) was dissolved in methanol and filter-sterilized (0.2 µm pore size filter). The solvent was then allowed to evaporate as described by Peters and Long (1988) before inoculant cultures were added (to make 10 µM luteolin solution) for incubation. The cultures were incubated without shaking in either luteolin solution or Ringer's

solution (control) for 3 hours. After incubation, each strain was inoculated to 85 lucerne plants.

Competition experiments. Four competition experiments were conducted comparing both growth phases of strain 2752 against both growth phases of strain 1320, both in the presence and in the absence of luteolin. After incubation, equal amounts (3 ml) of appropriate cultures were mixed to make the two-strain inocula. The actual proportion of each strain in the inoculum was determined by plate-counting the inoculum on YMA and YMA containing 10 μg of ampicillin ml^{-1}. Strain 2752 was resistant and strain 1320 sensitive at this concentration. Seedlings were inoculated and grown as outlined above. Nodules were typed 30 days from planting on ampicillin-supplemented YMA.

Statistical analysis

The periods (h) to initiation of nodulation by plants inoculated with each strain were square-root transformed before performing analysis of variance. The means were separated by the least significant difference test. In competition experiments, the proportion of nodules occupied by each strain was analysed by the chi-squared test of goodness of fit to the proportion of the strain present in the inoculum (Little and Hills, 1978).

3. Results

Periods to nodule initiation

The strains exhibited considerable variation in their mean periods (h) to initiation of nodulation of plants (Fig. 1). The quickest and slowest strains were 7P12 (90 h) and 1320 (126 h), respectively.

All the strains, except for 1325 and 5374Bstr, which had few nodules (less than 4 per plant, on average), formed comparable numbers of nodules (5 to 8 per plant, on average) when inoculated individually.

The delayed inoculation experiment showed that a 6 hour delay in inoculation resulted in a statistically significant ($P<0.05$) and longer period to initiation of successful infection (Table 2). However, the distance of the nodule from the root-tip mark did not change significantly (Table 2). Therefore, taking the rate of root elongation into consideration when evaluating timing of infection by measuring the distance of the uppermost nodule from the root-tip mark increased the sensitivity of the method.

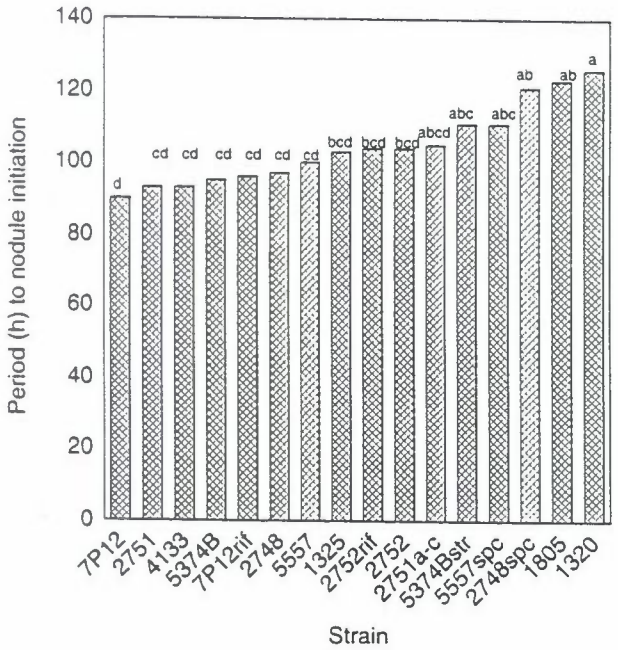

Figure 1. Periods to infection by strains of *R. meliloti*. The bars represent back-transformed means (obtained by squaring the transformed means and subtracting 1) of 140 plants per strain. Bars with the same letter are not significantly different at 5% level of significance.

Table 2. Effect of delayed inoculation on period to nodule initiation

Time of inoculation (h)	Period (h) to initiation of nodules[1]		Distance (mm) of uppermost nodule from root-tip mark[1]
	Transformed (sqrt[h+1])	Back-transformed[2]	
0	9.1 b[3]	81	19 a
6	10.5 a	110	22 a
L.S.D.	1.3		6

[1]Means of 59 plants at each time of inoculation. [2]These are weighted means obtained by squaring the transformed means and subtracting 1. [3]Means followed by the same letter are not significantly different at 5% level of significance.

Back-transformation of the periods to initiation of infection in Table 2 revealed that a 6 h delay in inoculation resulted in a 29 h delay in initiation of infection. This result may seem to contradict other findings that older and larger seedlings nodulate faster (Nazih and Weaver, 1994). However, a 6 h difference may be insignificant as regards physiological changes in seedlings compared to days or weeks. One possible reason for this disproportionate increase in our case could be the fact that plants in the delayed-inoculation treatment were disturbed twice, i.e., at the time of planting and, without much time to recover, at the time of inoculation. Consequently, root elongation, which was used in calculating time to infection, was retarded more for plants in this treatment than those in the control treatment.

Competitive ability

Tables 3a and 3b show that only four pairs of strains (pairs 11 and 15 in Table 3a and pairs 1 and 2 in Table 3b) were not significantly different from each other in competitive ability. In the experiments which included strains 1325 and 5374Bstr (pair 3 in Table 3a and pairs 1, 4 and 12 in Table 3b), the competitive abilities of both strains could be explained in part by the significantly fewer nodules these strains formed, in comparison to their competitors, even in single-strain inoculation experiments.

Relationship between timing of infection and competitive ability

Table 3a shows that when there were differences in periods to infection between the strains compared, timing of infection was a good indicator of competitive ability in 10 (pairs 1 to 10) of 15 strain pairs. Thus, the shorter the time to infection the greater the competitive ability of a strain. Interestingly, in four (pairs 11 to 14) of the five cases where there was no agreement between timing of infection and competitive ability, strain 1805 was involved. Table 3b also shows that when there were no differences in periods to infection between competing strains, competitive ability was correctly predicted in only two pairs (pairs 1 and 2) of eighteen.

Effect of luteolin and growth phase

The mean periods to nodule initiation of the plants by the strains at different growth phases and luteolin treatment are given in Fig. 2. The results show that growth phase or treatment with luteolin had no significant effect ($P>0.05$) on the period to nodule initiation by strain 2752. On the other hand,

Table 3a. Competitive abilities of the strains and how they related to periods to nodule initiation when competing strains had significantly ($P<0.05$) different periods to nodule initiation

No.	Strain pair		Quicker strain at initiating nodules	Nodule occupancy (expected numbers in parentheses)		More competitive strain
	A	B		A	B	
1	7P12	1320	A	62(30)	0(32)	A
2	7P12	2748spc	A	90(48)	1(43)	A
3	7P12	5374Bstr	A	44(28)	13(29)	A
4	2751	1320	A	39(15)	17(41)	A
5	2751	2748spc	A	65(34)	0(31)	A
6	4133	1320	A	38(13)	13(38)	A
7	5374B	1320	A	40(30)	7(17)	A
8	5374B	2748spc	A	61(35)	1(27)	A
9	2752	1320	A	30(17)	31(44)	A
10	1320	7P12rif	B	26(37)	31(20)	B
11	2751	1805	A	21(21)	13(13)	Neither
12	2748	1805	A	2(30)	49(21)	B
13	1805	7P12rif	B	45(25)	12(32)	A
14	1805	5557	B	45(18)	1(28)	A
15	5557	2748spc	A	20(18)	21(23)	Neither

exponential-phase cells of strain 1320 initiated nodules significantly quicker ($P<0.05$) than stationary-phase cells.

Relationship between periods to nodule initiation and competitive ability in luteolin experiments

Results of two sets of eight competition experiments, carried out at different times, are presented in Table 4. Although strain 2752 remained more competitive than strain 2752 in seven of the eight experiments, exponential-phase cells improved the competitive ability of strain 1320 to equal that of strain 2752 in stationary phase (pair 5 in Table 4).

Table 3b. Competitive abilities of the strains and how they related to periods to nodule initiation when there were no significant differences ($P<0.05$) between competing strains in periods to nodule initiation

No.	Strain pair		Quicker strain at initiating nodules	Nodule occupancy (expected numbers in parentheses)		More competitive strain
	A	B		A	B	
1	1805	5374Bstr	Neither	27(24)	38(41)	Neither
2	2751	4133	Neither	29(33)	31(27)	Neither
3	2751	2752rif	Neither	106(68)	2(40)	A
4	2751	5374Bstr	Neither	50(34)	18(34)	A
5	2752	5557spc	Neither	42(24)	18(36)	A
6	2752	2752rif	Neither	40(27)	9(22)	A
7	5557	2752rif	Neither	7(25)	41(23)	B
8	4133	7P12rif	Neither	48(32)	36(52)	A
9	2751	2751a–c	Neither	60(43)	0(17)	A
10	1320	5557spc	Neither	30(38)	24(16)	B
11	2748	2752rif	Neither	6(13)	38(31)	B
12	1325	2751a–c	Neither	20(33)	31(18)	B
13	5374B	2751a–c	Neither	30(41)	30(19)	B
14	1805	5557spc	Neither	23(50)	62(35)	B
15	5557	2751a–c	Neither	7(46)	54(15)	B
16	1320	2748spc	Neither	7(19)	43(31)	B
17	2748	4133	Neither	0(19)	33(14)	B
18	5557	4133	Neither	6(28)	38(16)	B

Comparison of competitive ability with periods to nodule initiation showed that significantly faster infecting strains were always associated with significantly greater competitiveness (strain pairs 1 to 4). However, when the strains were not different in periods to nodule initiation, only in one case (pair 5) in four could competitive ability be correctly predicted.

4. Discussion

If all plants in a treatment were completely uniform in their rates of root elongation, measuring nodule distances alone would suffice as a measure of relative periods to infection. Even plants of a single cultivar usually differ in some characteristics, and outcrossing plants such as lucerne are even more heterogeneous. This is probably the main reason that relating nodule distances to the rates of root elongation of individual plants gave a better estimate, than

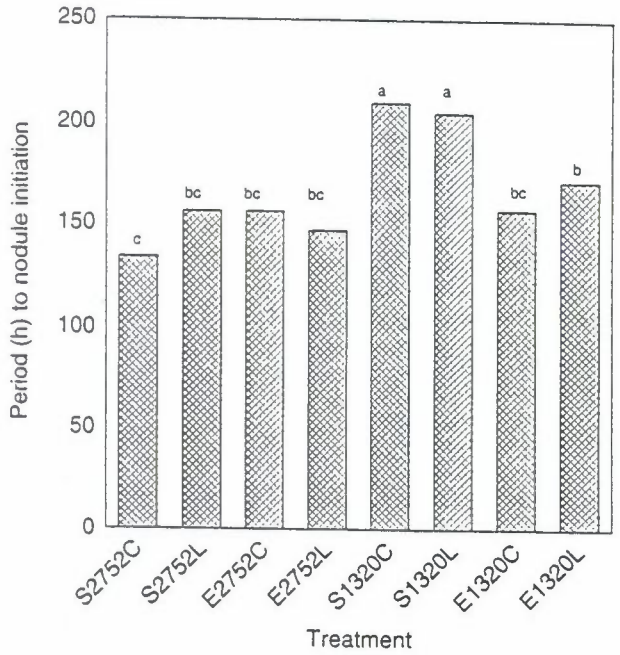

Figure 2. Effect of luteolin and growth phase of *R. meliloti* on periods to infection by plants. S = stationary growth phase, E = exponential growth phase, C = control, L = luteolin-treated. 2752 and 1320 are strains. The bars represent back-transformed means (obtained by squaring the transformed means and subtracting 1). Bars with the same letter are not significantly different at 5% level of significance.

nodule distances only, of the relative timing of nodulation by the strains (Table 2).

The relationship between time to nodule initiation and competitive ability (Table 3a) is in agreement with the reports cited in the introduction of this paper. But to our knowledge, the results reported here are the first involving so many strains and using nodule position in conjunction with rate of root elongation to screen strains for timing of infection.

There is no relationship between timing of infection and competitive ability of *B. japonicum* (Zdor and Pueppke, 1988; Fernandez-Flouret and Clayet-Marel, 1988; Smith and Wollum, 1989). The reason for these results is that in each case there were no differences in periods to infection between competing strains. This agrees with our results with paired strains with similar periods to infection (Table 3b). Montealegre et al. (1995) also reported no correlation bet-

Table 4. Effects of luteolin and growth phase on competitive abilities of *R. meliloti* strains

No.	Strain pair		Quicker strain at initiating nodules	Nodule occupancy (expected numbers in brackets) Experiment 1		Experiment 2		More competitive strain
	A	B		A	B	A	B	
(a) Strain pairs significantly different ($P<0.05$) in periods to nodule initiation								
1	S2752C[1]	S1320C	A	27(17)	32(42)	31(19)	37(49)	A
2	E2752C	S1320C	A	36(17)	18(37)	56(35)	6(27)	A
3	S2752L	S1320L	A	46(20)	19(41)	25(17)	20(28)	A
4	E2752L	S1320L	A	40(26)	17(31)	53(37)	2(18)	A
(b) Strain pairs not significantly different ($P>0.05$) in periods to nodule initiation								
5	S2752C	E1320C	Neither	29(25)	31(35)	31(30)	19(20)	Neither
6	E2752C	E1320C	Neither	39(25)	20(34)	34(28)	9(15)	A
7	S2752L	E1320L	Neither	32(19)	35(48)	22(15)	42(49)	A
	E2752L	E1320L	Neither	44(26)	20(38)	35(26)	15(24)	A

[1]S = Stationary growth phase; E = Exponential growth phase; C = Control; L = Luteolin-treated. 2752 and 1320 are strains.

ween number of nodules above the root-tip mark and nodule occupancy in *Phaseolus vulgaris*. However, the time to first appearance of nodules correlated with nodule occupancy, implying that counting of nodules above the root-tip mark was only a less sensitive method of evaluating speed of infection.

The results on the effect of growth phase on timing of infection (Fig. 2) are in agreement with those of Bhagwat and Thomas (1983), who reported that exponential-phase cells of *Bradyrhizobium* sp. infected cowpea plants 7.5 h earlier than stationary-phase cells. If the decrease in time to infection by rhizobia incubated in root exudate (Bhagwat and Thomas, 1982; Halverson and Stacey, 1984) is due to induction of nodulation genes (Mulligan and Long, 1985), it could be expected that incubation of rhizobia in luteolin would decrease their periods to infection. However, luteolin had no effect on timing of infection (Fig. 2), but nodule number and shoot DM increased with luteolin treatment (data not presented). Therefore, there are probably other factors in root exudate which may decrease the periods to infection of plants by otherwise slow strains.

The results on the effect of growth phase and luteolin treatment of rhizobia on competitive ability (Table 4) confirmed earlier results (Table 3a) that

competitive ability can be predicted where there are significant differences between strains in their periods to initiation of nodulation. However, the response of competitive ability to changes in timing of nodule initiation could have been demonstrated more clearly if some of the treatments altered the timing of nodule initiation by strain 1320 to a significantly earlier time than strain 2752.

Where there were no differences in periods to infection between competing strains (Tables 3b and 4), the competitive abilities of the strains could not be predicted. This could imply that either the method we used for evaluating timing of infection was not sensitive enough or that factors other than timing of infection (e.g., antagonism between strains) were affecting nodulation competitiveness. Another possibility is that double nodule occupancy, which was not evaluated, affected competitive ability. This seems unlikely because in the competition experiments in which antibiotic-resistant mutants were used for nodule-typing, the mutants were in most cases less competitive. Therefore double occupancy would imply that some of the nodules occupied by the antibiotic resistant mutants were also occupied by the sensitive strains. As the sensitive strains were already found to have occupied more nodules, the ranking of the strains for competitive ability would not have changed.

The relationship between period to infection of plants and competitive ability indicates that timing of infection can be an important factor in nodulation competitiveness. Thus, when competing strains differ in periods to infection, there are prospects for ranking strains of rhizobia for nodulation competitiveness by screening them for promptness of infection in single-strain inoculations, i.e., before they are put into competition with other strains.

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