Quantitative Study of Nodulation Competitiveness in *Rhizobium* Strains

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We compared the nodulation competitiveness of three strains of *Rhizobium leguminosarum* by counting the number of nodules formed on faba bean plants after the application at sowing time of different concentrations of the strains to soils already containing *Rhizobium* strains of the same species. A relationship of type \( y = ax^n \) was found to exist between the ratio of the nodules formed by the applied inoculum strain to the nodules formed by the soil strains and the ratio of *Rhizobium* cells in the inoculum to the cells in the soil. This relationship was also confirmed in another competition experiment in which two *R. melliloti* strains of identical competitiveness were mixed in various proportions. The relationship can also be applied to the majority of results reported in the literature. Should it prove to be more widely applicable, it could be used to estimate the relative competitiveness of *Rhizobium* strains and thus predict the performance of an inoculum in a given soil.

When several *Rhizobium* strains occur in the presence of the leguminous plant to which they are specific, certain strains form nodules in preference to others (14, 15, 17). *Rhizobium* strains differ from one another in ability to be selected by the plant host (nodulation competitiveness). The number of nodules formed by each strain present depends not only on the nodulation competitiveness of the various strains but also on their numbers. To predict the performance of one strain in relation to the performance of another, one must know the quantitative relationship between the proportion of the strain in the inoculum and the proportion of nodules formed.

When the relative proportion of a strain in a mixture is increased, the number of nodules formed by this strain also increases, but not in a directly proportional way (5). Ireland and Vincent (9) found that in a soil containing ineffective *Rhizobium trifolii*, the logarithm of the percentage of plants nodulated by effective strains is a direct function of the logarithm of the number of effective rhizobia applied on the seed and the logarithm of the number of rhizobia in the soil. In a study of soybean plants, Weaver and Frederick (19, 20) showed that the percentage of nodules formed by a strain applied at sowing time to a soil containing *R. japonicum* varies proportionally with the logarithm of the number of bacteria in the inoculum. An identical relationship has been found for faba bean plants (1). In both studies, the slopes of the regression lines were steepest for the most competitive strains.

The aim of this work was to see if this relationship is generally applicable and whether the slope of the lines obtained is a true indication of strain competitiveness. Two different systems were used: first, a system similar to those cited above, in which we compared the numbers of nodules produced in faba bean plants by various amounts of different strains added to soils containing *Rhizobium* strains of the same species, and second, a more precisely defined system in which various proportions of two *R. melliloti* strains were applied to lucerne tube cultures under controlled conditions.

**MATERIALS AND METHODS**

Faba bean plant experiment. Strains FH20S1, FSS1, and FH1635 of *R. leguminosarum* are spontaneous streptomycin-resistant mutants of strains effective on faba bean plants and isolated from French soils. These mutants are resistant to 500 mg of streptomycin sulfate per liter and have retained symbiotic effectiveness.

The bacteria were cultured in shake flasks containing Bergersen medium (3) with 0.2 g of yeast extract per liter for 3 days at 28°C. Bacterial counts were made at the time of inoculation.

Four different soils were used: CB, a clay loam with a pH of 8.2; CN, a clay loam rich in organic matter with a pH of 8.0; H, a highly calcareous loamy clay sand with a pH of 8.1; and P, a sandy soil with a pH of 5.9. The numbers of *R. leguminosarum* cells present in the soils were determined by plant infection counts (17), with vetches (*Vicia sativa*) as the trap hosts.

Three faba bean plants, *Vicia faba* var. Ascott, were grown in each 1-liter plastic pot filled with soil. Inoculation was carried out at sowing time by application around each seed of 1 ml of a suspension containing...
one *R. leguminosarum* strain. Four dilutions of each strain—the pure culture and dilutions in sterile water of 10⁻¹, 10⁻², and 10⁻³—were used, with three replicates of each treatment. The plants were kept in a greenhouse and subjected to supplemental lighting and daily watering. After 2 months, one of the replicates was unearthed, and the origin of 20 nodules was determined for each pot. For this procedure, the nodules were surface sterilized by being soaked for 3 min in 0.2 g of HgCl₂ per liter. They were then rinsed six times in sterile water, and each was crushed in 2 ml of sterile water. A single drop of suspension was then transferred with a multiple-point inoculator to agar medium with or without streptomycin sulfate (100 mg liter⁻¹). The other replicates were treated similarly 1 and 2 weeks later.

Lucerne experiment. The two *R. meliloti* strains used, 2011S2 and 2011N1, are spontaneous mutants of strain 2011.

Strain 2011S2 is resistant to 500 mg of streptomycin sulfate per liter and effective in symbiosis, whereas strain 2011N1 is ineffective. Both strains exhibit identical nodulation competitiveness (2).

We grew the lucernes, *Medicago sativa* cv. du Puits, with the roots enclosed within test tubes, by a slight modification of the Gibson technique (8). The tubes were placed in a controlled-environment chamber with a 16-h day period at 22°C and 220 μEinsteins of illumination m⁻² s⁻¹ and an 8-h night period at 18°C.

Two days after transplantation, the plants were inoculated with aqueous suspensions *Rhizobium* strains grown on yeast extract-mannitol agar (17) for 48 h. An initial count was made, and the suspensions were kept at 4°C for the duration of the 3-day period required for obtaining results. We prepared inocula by mixing the strains in the desired proportions and applying the suspensions immediately to the plants to give 10⁷ bacteria per tube. There were 10 replicates per treatment. The exact number of bacteria applied in the inoculum was determined from a count made at the time of inoculation.

After 18 days, the nodules on each plant were counted and marked, and the effectiveness of these nodules was determined visually after a further 10 days.

RESULTS AND DISCUSSION

The percentage of nodules formed on faba bean plants by each *Rhizobium* strain inoculated at four concentrations in four soils is given in Table 1. Variance analysis of the data subjected to arc sinus square root transformation revealed significant ($P < 0.01$) effects of strain, concentration, and soil. Effects of replicates and interactions were not significant ($P < 0.05$). Thus, each of the three strains had a different competitiveness. Although the number of bacteria applied was not absolutely identical, it was still possible to compare the strains. Strain FH20S1 was the most competitive: it formed the most nodules at each concentration and in each soil. Strain FSS1 was the next most competitive, and strain FS163S was the least.

The behavior of the soil strains differed considerably from one another. CB *Rhizobium* soil strains formed many more nodules than did H soil strains, although both soils contained approximately equal numbers of bacteria. CN and P soils also contained approximately equal numbers of bacteria but contained less than did the other two soils. CN and P soil strains produced similar numbers of nodules and produced more nodules than did soil H strains, despite the fact that the soil H strains occurred in greater numbers. The differences in soil strain behavior therefore cannot be explained by differences in number alone. Either strain competitiveness varied from one soil to another or the nature of the soil influenced the implantation of new strains.

In all cases, as the number of bacteria in the inoculum is increased, the number of nodules formed by the inoculum strain increases. In 9 of the 12 cases studied, the linear regression of the number of nodules formed by the added strain expressed as a function of the logarithm of the number of inoculum bacteria was significant ($P < 0.05$; Fig. 1). The slope of each line varied with the combination but was not an indication of strain competitiveness, as was thought in previous studies (1, 19). Thus, the lowest coefficient, which should indicate poor acceptance of the added strain, was obtained with strain FH20S1 in soil P, although this strain in fact formed 90% of the nodules.
Another way with relationship, Medicago truncatula strain competitiveness between each number of bacteria on the sativa, exists between CB 0.971a Rhizobium 0.904 0.938 P 0.05) regression lines.

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FIG. 1. Relationship between the percentage of nodules formed by the inoculum strain (NA) and the logarithm of the number of bacteria in the inoculum (IA) when faba bean plants inoculated with different Rhizobium strains were grown on soils containing indigenous rhizobia. Symbols: •, strain FH163S; ■, strain FSS1; ▼, strain FH20S1; -----, significant (P < 0.05) regression lines.

The possibility of expressing the results in another way so as to bring out differences in strain competitiveness was therefore investigated.

Marques Pinto et al. (12) found that a linear relationship, with a slope approximating 1 for Medicago truncatula and lower than 1 for M. sativa, exists between the logarithms of the ratio of the number of bacteria of each strain present on the root surface and the ratio of each strain in the inoculum. The number of nodules formed by each strain was linked by a similar function to the number of bacteria present on the root surface.

A linear relationship must therefore exist between the logarithm of the ratio of nodules formed by the added strain to the nodules formed by the soil strains (NA:NB) and the logarithm of the ratio of the number of bacteria in the inoculum to the number in the soil (IA:IB): log NA:NB = log C_AB + k log IA:IB.

In 8 of the 12 cases studied, the regression between log NA:NB and log IA:IB was significant at the 0.05 level (Table 2). The values of the regression coefficients (k) varied from 0.285 to 0.530 and were not significantly different. The estimated mean k value was 0.399 ± 0.055. The C_AB values increased with increases in the percentage of nodules formed by the added strain. These C_AB values might therefore be indicative of the interrelationship in competitiveness existing between the various strains present. If this is so, then for strains of equal competitiveness, for which C_AB = 1, the line should pass through the origin, and when the number of strain A cells in the inoculum is equal to the number of strain B cells, then the number of nodules formed by strain A should be equal to the number of nodules formed by strain B.

Our intention was to verify this by inoculating lucernes with various proportions of two strains (2011S2 and 2011N1) exhibiting equal competitiveness but different effectiveness and then determining the origin of each resulting nodule.

The linear regression relating the logarithm of the ratio of nodules formed by strains 2011S2 and 2011N1 and the ratio of the amount of these two strains in the inoculum was significant (P < 0.01) and could be considered to pass through the origin (Fig. 2). The regression coefficient (k = 0.471) was not very different from that found for the faba bean plant experiment.

It seems, therefore, that C_AB represented the competitiveness of strain A in relation to that of strain B. Its value would be equal to the ratio of the number of nodules formed by strain A to the number of nodules formed by strain B when the two strains are present in the inoculum in equal quantities.

To see if this relationship was applicable on a wider scale, we applied it to results from experiments of the same type that were reported

### TABLE 2. Correlation coefficient (r) and k and c values when the regressions of log NA:NB = log C + k log IA:IB were significant

<table>
<thead>
<tr>
<th>Soil</th>
<th>FSS1</th>
<th>FH20S1</th>
<th>FS163S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>k</td>
<td>c</td>
</tr>
<tr>
<td>CB</td>
<td>0.971a</td>
<td>0.472</td>
<td>0.064</td>
</tr>
<tr>
<td>H</td>
<td>0.980a</td>
<td>0.344</td>
<td>1.78</td>
</tr>
<tr>
<td>CN</td>
<td>0.980a</td>
<td>0.457</td>
<td>0.23</td>
</tr>
<tr>
<td>P</td>
<td>0.904</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Significant at the 0.05 level.

b Significant at the 0.01 level.
earlier and in which at least four $I_A:I_B$ values were available (Table 3).

Robinson (13) mixed together five pairs of $R$. trifolii strains in various proportions and determined the number of nodules formed by each strain. The regression in four of the five combinations was significant, and the $C_{AB}$ values showed the same directional trend as that shown by the percentage of nodules formed by strain A. The $k$ coefficients did not differ significantly, and the estimated mean $k$ value was $0.370 \pm 0.057$, which is not different from that determined for the faba bean plants in our experiment.

The same finding was observed for experiments carried out by Weaver and Frederick (20), in which the estimated mean $k$ value ($0.318 \pm 0.082$) was not significantly different from our values.

Two $k$ values were found to differ widely from the others: 1.258, determined from the experiment by Škrdleťa and Karimová (16) with soybean plants, and 0.038, determined by Russell and Jones (15) for clover.

![Graph A](image1)

**FIG. 2.** Relationship between the logarithm of the ratio of nodules formed on $M. sativa$ by strains 2011S2 and 2011N1 ($N_A:N_B$) and the logarithm of the ratio of the numbers of cells of the two strains in the inoculum ($I_A:I_B$).

![Graph B](image2)

**FIG. 3.** Representation of the proportion of nodules formed by a strain $[N_A:(N_A + N_B)]$ as a function of the logarithm of the ratio of the numbers of cells of the two strains in the inoculum ($I_A:I_B$) for different $C_{AB}$ values when $k = 0.4$ (A) and for different $k$ values when $C_{AB} = 1$ (B).

When these results are taken as a whole, the relationship seems to be more or less generally applicable, so that: $N_A:N_B = C_{AB} (I_A:I_B)^k$.

Graphic representations of the proportion of nodules formed by a strain $(N_A:N_A + N_B)$ as a function of $\log I_A:I_B$ are given for various values of $C_{AB}$ when $k = 0.4$ (Fig. 3A) and for various values of $k$ when $C = 1$ (Fig. 3B).

The data obtained in the faba bean plant and lucerne experiments, fitted to a theoretical curve

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plant</th>
<th>Strains in competition</th>
<th>$r$ value</th>
<th>$k$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Faba bean</td>
<td>FH20S1, soil strain</td>
<td>0.982&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.352</td>
</tr>
<tr>
<td>4</td>
<td>Soybean</td>
<td>110, soil strain</td>
<td>0.959&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.794</td>
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<tr>
<td>10</td>
<td>Soybean</td>
<td>138, soil strain</td>
<td>0.969&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.154</td>
</tr>
<tr>
<td>13</td>
<td>Trifolium</td>
<td>TA1, CC265</td>
<td>0.901</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Trifolium</td>
<td>CC2480a, CC224</td>
<td>0.984&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.329</td>
</tr>
<tr>
<td>13</td>
<td>Trifolium</td>
<td>TA1, CC224</td>
<td>0.974&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.339</td>
</tr>
<tr>
<td>13</td>
<td>Trifolium</td>
<td>UN229, CC224</td>
<td>0.984&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.527</td>
</tr>
<tr>
<td>13</td>
<td>Trifolium</td>
<td>TA1, NA30</td>
<td>0.985&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.284</td>
</tr>
<tr>
<td>15</td>
<td>Trifolium</td>
<td>A, B</td>
<td>0.917&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.038</td>
</tr>
<tr>
<td>16</td>
<td>Soybean</td>
<td>D216, D234</td>
<td>0.996&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.218</td>
</tr>
<tr>
<td>20</td>
<td>Soybean</td>
<td>138, soil 1 strain</td>
<td>0.970&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.349</td>
</tr>
<tr>
<td>20</td>
<td>Soybean</td>
<td>138, soil 3 strain</td>
<td>0.968&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.340</td>
</tr>
<tr>
<td>20</td>
<td>Soybean</td>
<td>138, soil 6 strain</td>
<td>0.970&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.265</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant at the 0.05 level.

<sup>b</sup> Significant at the 0.01 level.
in which \( k = 0.4 \), are presented in Fig. 4 and 5. It is evident that should this interrelationship prove generally applicable, then it should be feasible, in relatively simple experiments, to express in a quantitative manner the competitiveness of a given strain in relation to the competitiveness of others and in relation to the competitiveness of soil strains. Thus, the number of cells of a given strain that would have to be added to a soil to guarantee that the strain forms a certain percentage of nodules could also be predicted.

In comparison with the competitiveness index defined by Marques Pinto et al. (12) and used by Labandera and Vincent (11) and Franco and Vincent (7), this new competitiveness index is easier to measure because it does not involve a representation of the strains at root level. \( C_{AB} \) would in fact be a global assessment, including possible differences in multiplicity among the strains after inoculation as well as differences in host selectivity, the only difference measured by the competitiveness index of Marques Pinto et al.

It is also interesting that the above relationship corresponds to the interrelationship, taken point by point, between two Freundlich adsorption isotherms. The number of nodules formed by a strain therefore varies as a function of the number of bacteria in the medium in the same way that the amount of a substance that is adsorbed varies as a function of its concentration in a solution. By taking the analogy still further, it can be supposed that the root consists of a certain number of adsorption sites and that for each Rhizobium strain, there is a different isotherm corresponding to the affinity of the strain for such sites of adsorption. The differences observed in strain competitiveness would thus represent a selectivity in strain adsorption by the root. This hypothesis agrees with the microscopic observations made by Dazzo et al. (6) of clover. Dazzo et al. demonstrated that infective \( R. \) trifolii, when compared with noninfective \( R. \) trifolii or other Rhizobium species, was preferentially absorbed to clover root hairs.

**ACKNOWLEDGMENTS**

We thank M. Bours and G. Sommer for technical assistance.

**LITERATURE CITED**


**FIG. 4.** Percentage of nodules formed on faba bean plants by the inoculum strain as a logarithmic function of the ratio of the number of inoculum cells to the number of rhizobia in soil, as fitted to a theoretical curve \( \frac{N_A}{N_B} = C_{AB} (I_A; I_B)^k \) in which \( k = 0.4 \). Symbols: ○, inoculum strain FH163S; □, inoculum strain FSS1; ▽, inoculum strain FH20S1.

**FIG. 5.** Proportion of nodules formed on lucernes by strain 2011S2 as a function of the proportion of the strain in the inoculum, as fitted to a theoretical curve \( \frac{N_A}{N_B} = C_{AB} (I_A; I_B)^k \) in which \( k = 0.4 \).


