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Nodulation Suppression by *Rhizobium leguminosarum* bv. *phaseoli* in Bean Split-root Systems

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Abstract

The plant host influences the extent of nodule formation by autoregulation. In this study, nodulation suppression by the host was examined as a factor in inter-strain competition for nodulation of the common bean (*Phaseolus vulgaris* L.) cv. Bountiful using split-root systems. The pattern of suppression induced by *R. leguminosarum* bv. *phaseoli* TAL 182, a highly competitive and effective strain, was characterized in a time-course experiment. A primary inoculation was done on one side of a split-root at day zero, followed by a secondary inoculation on the opposite side with increasing delay (one to 14 days). The extent of suppression of secondary nodulation was time-dependent, becoming more pronounced as the delay period was increased. A 14-day delay in secondary inoculation resulted in 94% suppression of secondary nodulation. Six *R. leguminosarum* bv. *phaseoli* strains with varying competitive abilities were used as primary inoculants to compare their ability to induce nodulation suppression. The strains varied in their degree of suppressiveness. A highly competitive strain (TAL 1472) was also highly suppressive whereas a poorly competitive strain (CIAT 632) was less suppressive. Suppression ability of a strain was not

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related to the speed of nodule formation. While the mechanism(s) of nodule suppression has not yet been elucidated, our results suggest that suppressiveness may play a role in interstrain competition.

Keywords: Nodulation suppression (autoregulation), competition, *Rhizobium*, *Phaseolus vulgaris* , split roots

Abbreviations: YEM – yeast extract mannitol

1. Introduction

The ability of a rhizobial strain to dominate the nodules of legume plants is dependent on environmental factors as well as on the genetic compatibility between the plant host and the rhizobial partner (Dowling and Broughton, 1986). Host selection of the competing strain may constitute an important factor that affects the ability of rhizobia to compete and nodulate successfully. The influence of the host on the nodulating success of a strain is well documented in various legume-*Rhizobium* associations (Bromfield, 1984; Caldwell and Vest, 1968; Hardarson et al., 1982; Jones and Hardarson, 1979; Materon and Vincent, 1980; May and Bohlool, 1983; Vincent and Waters, 1953). However, only a few studies have addressed the role of the host as a variable that can significantly influence the outcome of competition (Sargent et al., 1987).

While the mechanism of host selection is still not known, it is clear that the development of nodules is under the regulation of the plant host (Pierce and Bauer, 1983). Autoregulation is thought to occur during nodulation development, allowing the plant to optimize nodulation and prevent excessive nodulation (Rolfe and Gresshoff, 1988). The systemic nature of autoregulation is seen across a split-root system where a suppression of nodulation is observed on one half of the system when the other half has been previously nodulated. Such a host response was reported in alfalfa (Caetano-Anolles and Bauer, 1988), clover (Sargent et al., 1977), siratro (Djordjevic et al., 1988), and soybean (Kosslak and Bohlool, 1984; Olsson et al., 1990). In this study, host nodulation suppression was examined as a factor in competition among strains of *Rhizobium leguminosarum* bv. *phaseoli* for nodulation of the common bean (*Phaseolus vulgaris* L. cv. Bountiful).

2. Materials and Methods

Bacterial strains and media

The six *R. leguminosarum* bv. *phaseoli* strains used in this study, their maintenance, and growth conditions were as described elsewhere (George and Robert, 1991).

Plant inoculation and growth conditions

The plant tests for all nodulation and nodulation suppression experiments were done using plastic growth pouches (Northrup King Seed Co., Minneapolis, MN) that contained nitrogen-free nutrient solution (Hoagland and Arnon, 1938). The solution was modified to one eighth-strength salts and one half-strength trace elements, and was sterilized by autoclaving.

The split-root plastic growth pouch used in the nodulation suppression experiments was prepared by cutting the paper wick into two and dividing the plastic pouch by heat sealing (Fig. 1). The paper trough in the middle was used to support the plant and plastic straws on both sides of the pouch were used to deliver nutrient solution. A 15 ml volume of nutrient solution was maintained on each side of the growth pouch by periodic watering.

The common bean (*Phaseolus vulgaris* L.) cv. Bountiful, provided by the NifTAL Project, University of Hawaii, was the host plant used in all assays. The seeds were selected for uniformity, surface-sterilized in 30% H_2O_2 for 20 min, rinsed five times with sterile water, and germinated on a sterile bed of a 3:1 (v/v) mixture of vermiculite-perlite. The split-root systems were generated by cutting off the root meristem of 2-day-old seedlings. Each seedling was placed on top of the paper trough with moist paper strips connecting the trough and the paper support in the two chambers, allowing equally vigorous lateral roots to develop in each chamber of the plastic pouch. After 3 days, extraneous lateral roots were severed, leaving two secondary roots of equal length on each side. The connecting paper strips were then removed to create a discontinuity between the two chambers, preventing cross-contamination. The cotyledons were excised at this time to minimize plant variability due to nitrogen reserves.

For all inoculations, each strain was grown in 10 ml of YEM broth at 28°C for 2 days and diluted in nutrient solution to approximately 10^6 cells ml^{-1}. Five days after germination, a primary inoculation, consisting of one ml of diluted culture, was done on one side of the split-root (E, denoting early inoculation). A secondary inoculation was done on the opposite side of the split-root after

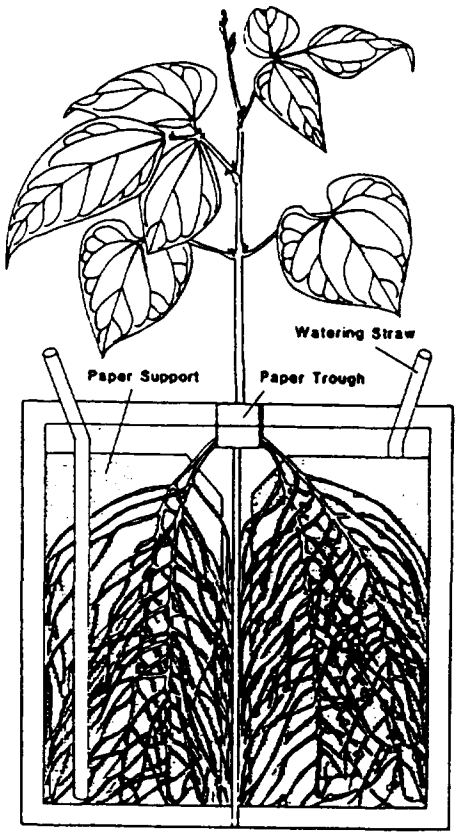

Figure 1. Diagram of a split-root plastic growth pouch used in the study.

a given delay period (D, denoting delayed inoculation). All experiments included uninoculated split-root plants as controls. The plants were grown in a temperature-controlled (25°C) room providing a 16 hr daylength and a light intensity of 500 microEinsteins $m^{-2}sec^{-1}$ at plant level.

In all experiments, the plans were harvested 4 weeks after the primary inoculation. Nodules on each side of the split-root were counted and collected, and nodule weights were determined after drying at 75°C for 2 days.

Nodulation suppression by TAL 182

Strain TAL 182, a highly effective and highly competitive strain (George and Robert, 1991), was the standard strain used in this study. The pattern of nodulation suppression induced by TAL 182 on common bean was characterized in a time-course experiment using five replicate split-root systems per

treatment. One side of a split-root system was inoculated at zero time, and the second side with increasing delay (E/D treatment). At zero time, the control consisted of early inoculation on both sides of the split-root (E/E control). The delay period consisted of 1-day increments for 10 days after the primary inoculation and a 14-day delay. For each delay period, the control consisted of a delayed inoculation only on one side (O/D control). In this experiment TAL 182 was both the primary (E) and secondary (D) inoculant. Inhibition of secondary nodulation was expressed as percent suppression, using the formula:

$$\% \text{ Suppression} = 100 - \% \text{ Nodulation}$$

The percent nodulation was derived by expressing the extent of secondary nodulation (D side of E/D treatment) as a percentage of the nodulating potential of that side of the split-root at the time of the delayed inoculation (D side of O/D control) at each delay period. By quantifying suppression this way, the analysis was standardized with the comparison of nodules at the same developmental stage.

The delay period of secondary inoculation with the model strain (TAL 182), which showed maximal suppression of nodulation, was used as the standard delay period in subsequent suppression experiments to evaluate the suppressiveness of other strains.

Nodulation suppression patterns of different strains

Each of the six *R. leguminosarum* bv. *phaseoli* strains was used as the primary inoculant on a split-root system to assess their ability to suppress strain TAL 182, which was used as the standard secondary inoculant on the delayed side 2 weeks after the primary inoculation. At zero time, the control was an E/E inoculation with each strain on both sides. At the delayed time, the control was an O/D inoculation with the standard strain. All treatments and controls consisted of five replicate split-root systems.

Statistical analysis was performed by using PC-SAS (Statistical Analysis System for Personal Computers, SAS Institute, Inc., Cary, NC).

Assessment of nodulation profiles

To compare the speed of nodule formation by the individual strains, bean seeds were surface-sterilized and germinated as previously described. After 2 days, the seedlings were transferred to unmodified plastic growth pouches (1 plant/pouch). After another 3 days, each of the six strains was inoculated individually on five replicate plants. The location of newly emerged nodules

was marked on the plastic pouch and their number was counted daily for 10 days after inoculation.

3. Results and Discussion

A split-root system was used to characterize nodulation suppression in the common bean and to examine whether differences in the competitiveness of *R. leguminosarum* bv. *phaseoli* strains could be explained by the extent of their ability to induce an autoregulatory response.

Time-course of nodulation suppression by TAL 182

Figure 2 shows the pattern of nodulation suppression induced by TAL 182 on the common bean as secondary inoculation was delayed for increasing periods of time. When a primary inoculation was done on one side of a split-root and was followed by a secondary inoculation on the opposite side with increasing

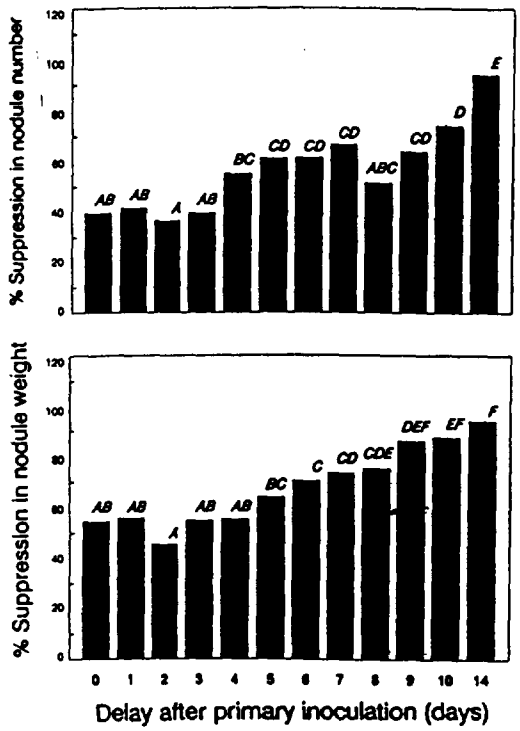

Figure 2. Time-course of nodulation suppression induced by *R. leguminosarum* bv. *phaseoli* TAL 182 on common bean *P. vulgaris* L.) cv. Bountiful. Bars with the same letters are not significantly different by LSD ($P \leq 0.05$).

delay, the suppression of nodulation on the side that received the delayed inoculation was time-dependent. As the delay period was increased, the extent of suppression became more pronounced. A 14-day delay in secondary inoculation resulted in the maximum suppression response, showing 94% suppression in nodule number and nodule weight. This gradual response in the common bean is in contrast to that observed by Kosslak and Bohlool (1984) in soybean, where this degree of suppression was achieved after a 4-day delay in secondary inoculation. The longer period of time required for the development of the suppression response in *P. vulgaris*, as compared to *Glycine max*, might be related to the differences in the nodulation characteristics of the two plants. In soybean, the nodules are concentrated on the tap root whereas in common bean, most nodules are distributed on the lateral roots.

Nodulation suppression by R. leguminosarum *bv.* phaseoli *strains*

The competitive abilities of six *R. leguminosarum* bv. *phaseoli* strains used in this study were previously characterized in Leonard jars, based on their average nodule occupancies in double-strain inoculations of two common bean cultivars (George and Robert, 1991), as highly competitive (TAL 1472 and TAL 182), moderately competitive (CIAT 899, Viking 1, and KIM-5), and poorly competitive (CIAT 632). The competitiveness of the model strain (TAL 182) was tested in growth pouches as well, and similar results were observed (data not shown). When these strains with varying competitive abilities were used as primary inoculants, all strains induced significant suppression (Fig. 3). Although the differences in the magnitude of suppression were modest, there were significant differences ($P < 0.001$) among the strains. Overall, a comparison of the nodule number and nodule weight resulting from the secondary inoculation on the delayed side of the E/D treatment showed that TAL 1472 and CIAT 899 were more suppressive than KIM-5 and CIAT 632. The two highly competitive strains (TAL 182 and TAL 1472), were among the most suppressive, while the poorest competitor (CIAT 632) was among the least suppressive.

Strain speed of nodulation

Several studies have provided evidence that the speed of a strain to form nodules can affect the outcome of competition (Kosslak et al., 1983; Kosslak and Bohlool, 1985; de Oliveira et al., 1990; Skrdleta, 1970; Stephens and Cooper, 1988). A comparison of the nodulation profiles of the six strains (Fig. 4) showed that neither suppressiveness nor competitiveness could be explained

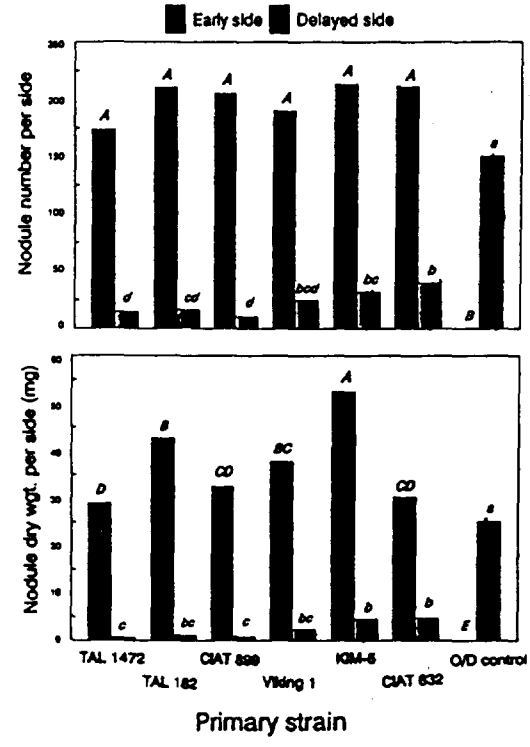

Figure 3. Nodulation suppression induced by six *R. leguminosarum* bv. *phaseoli* strains on common bean (*P. vulgaris* L.) cv. Bontiful. The early side of the E/D (Early/Delayed) treatment was inoculated with each of the primary strains at day zero while the delayed side was inoculated with a standard strain (TAL 182) at day 14. The O/D (Uninoculated/Delayed) control represents the nodulation potential of the plant at the delayed time in the absence of suppression. Bars with the same upper case or lower case letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

by a higher potential for nodule initiation. KIM-5, a moderately competitive strain, was the best nodulator in terms of speed (Fig. 4) but was less suppressive than some strains (Fig. 3). The poorly competitive and less suppressive strain (CIAT 632) initiated nodules at the same rate as a highly competitive and more suppressive strain (TAL 1472). In some studies, strains of *R. leguminosarum* bv. *trifolii* (Stephens and Cooper, 1988) and *R. leguminosarum* bv. *phaseoli* (de Oliveira and Graham, 1990) which nodulated their plant hosts rapidly, were more competitive than slower-nodulating strains. In other studies, competitiveness did not correlate with the speed with which strains produced nodules or with the number of nodules that they produced (Pinto et al., 1974; Smith and Wollum, 1989).

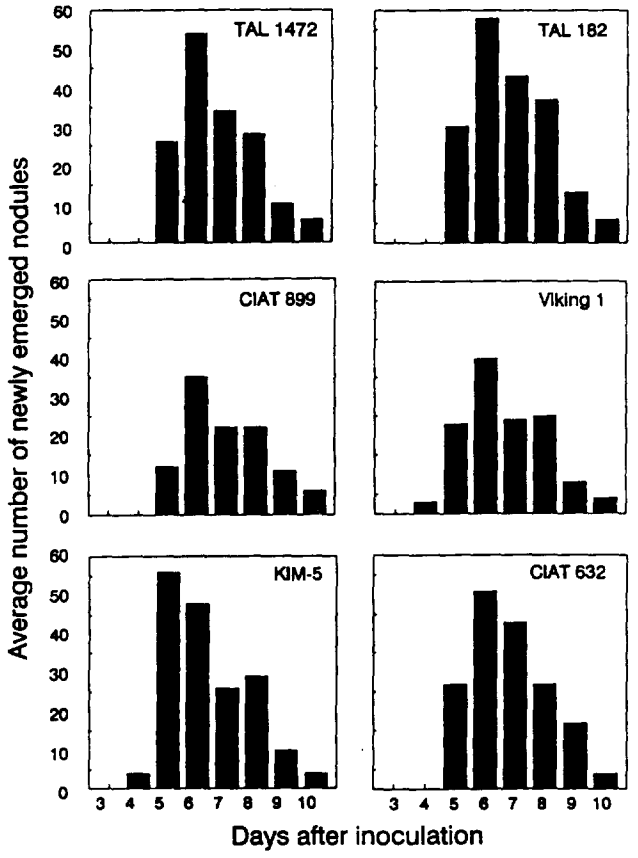

Figure 4. Speed of nodule formation of six *R. leguminosarum* bv. *phaseoli* strains on common bean (*P. vulgaris* L.) cv. Bountiful. The location of newly emerged nodules was marked on the growth pouch as they became visible, and their number was counted daily for 10 days after inoculation.

A possible relationship between suppressiveness and competitiveness was previously suggested by Sargent et al. (1987), who used Tn5 mutants slightly impaired in their nodulation and competitive abilities, to show an associated impairment in suppression in clover split-roots. In this study, nodulation-competent wild-type strains whose inherent competitive abilities were well-characterized (George and Robert, 1991), were used, thereby eliminating the complication of a nodulation defect in the competition process. The results of this study suggest the involvement of a postinfection event in interstrain competition and highlight a more prominent role of the host in the process. One may speculate that all nodulation-competent competing strains are able to infect their host, but when a strain fails to induce a strong suppression

response from the host, nodule primordia formed by the competing strain may continue to develop and eventually form the majority of the nodules.

Acknowledgements

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REFERENCES

Bromfield, E.S.P. 1984. Variation in preference for *Rhizobium meliloti* within and between *Medicago sativa* cultivars grown in soil. *Appl. Environ. Microbiol.* **48:** 1231–1236.

Caetano-Anolles, G. and Bauer, W.D. 1988. Feedback regulation of nodule formation in alfalfa. *Planta* **175:** 546–557.

Caldwell, B.E. and Vest, G. 1968. Nodulation interaction between soybean genotypes and serogroups of *Rhizobium japonicum*. *Crop Sci.* **8:** 680–682.

de Oliveira, L.A. and Graham, P.H. 1990. Speed of nodulation and competitive ability among strains of *Rhizobium leguminosarum* bv. *phaseoli* . *Arch. Microbiol.* **153:** 311–315.

Djordjevic, M.A., Ridge, R.W., Chen, H., Redmond, J.W., Batley, M., and Rolfe, B.G. 1988. Induction of pathogenic-like responses in the legume *Macroptilium atropurpureum* by a transposon-induced mutant of the fast-growing, broad-host-range *Rhizobium* strain NGR234. *J. Bacteriol.* **170:** 1848–1857.

Dowling, D.N. and Broughton, W.J. 1986. Competition for nodulation of legumes. *Annu. Rev. Microbiol.* **40:** 131–157.

George, M.L. and Robert, F.M. 1991. Competition among *Rhizobium leguminosarum* bv. *phaseoli* strains for nodulation of common bean. *Can. J. Microbiol.* (in press).

Hardarson, G., Heichel, G.H., Barnes, D.K., and Vance, C.P. 1982. Rhizobial strain preference of alfalfa populations selected for characteristics associated with N_2 fixation. *Crop Sci.* **22:** 55–58.

Hoagland, D.R. and Arnon, D.I. 1943. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Std. Circ.* No. 347.

Jones, D.G. and Hardarson, G. 1979. Variation within and between white clover varieties in their preference for strains of *Rhizobium trifolii*. *Ann. Appl. Biol.* **92:** 221–228.

Kosslak, R.M. and Bohlool, B.B. 1984. Suppression of nodule development of one side of a split-root system of soybeans caused by prior inoculation of the other side. *Plant Physiol.* **75:** 125–130.

Kosslak, R.M. and Bohlool, B.B. 1985. Influence of environmental factors on interstrain competition in *Rhizobium japonicum*. *Appl. Environ. Microbiol.* **49:** 1128–1133.

Kosslak, R.M., Bohlool, B.B., Dowdle, S., and Sadowsky, M.J. 1983. Competition of *Rhizobium japonicum* strains in early stages of soybean nodulation. *Appl. Environ. Microbiol.* **46:** 870–873.

Materon, L.A. and Vincent, J.M. 1980. Host specificity and interstrain competition with soybean rhizobia. *Field Crops Res.* **3:** 215–224.

May, S.N. and Bohlool, B.B. 1983. Competition among *Rhizobium leguminosarum* for nodulation of lentils *(Lens esculenta)*. *Appl. Environ. Microbiol.* **45:** 960–965.

Olsson, J.E., Nakao, P., Bohlool, B.B., and Gresshoff, P.M. 1989. Lack of systemic suppression of nodulation in split root systems of supernodulation soybean (*Glycine max* [L.] Merr.) mutants. *Plant Physiol.* **90:** 1347–1352.

Pierce, M. and Bauer, W.D. 1983. A rapid regulatory response governing nodulation in soybean. *Plant Physiol.* **73:** 286–290.

Pinto, C.M., Yao, P.Y., and Vincent, J.M. 1974. Nodulating competitiveness amongst strains of *Rhizobium meliloti* and *R. trifolii*. *Aust. J. Agric. Res.* **25:** 317–319.

Rolfe, B.G. and Gresshoff, P.M. 1988. Genetic analysis of legume nodulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39:** 297–319.

Sargent, L., Huang, S.Z., Rolfe, B.G., and Djordjevic, M.A. 1987. Split-root assays using *Trifolium subterraneum* show that *Rhizobium* infection induces a systemic response that can inhibit nodulation of another invasive *Rhizobium* strain. *Appl. Environ. Microbiol.* **53:** 1611–1619.

Skrdleta, V. 1970. Competition for nodule sites between two inoculum strains of *Rhizobium japonicum* as affected by delayed inoculation. *Soil. Biol. Biochem.* **2:** 167–171.

Smith, G.B. and Wollum II, A.G. 1989. Nodulation of *Glycine max* by six *Bradyrhizobium japonicum* strains with different competitive abilities. *Appl. Environ. Microbiol.* **55:** 1957–1962.

Stephens, P.M. and Cooper, J.E. 1988. Variation in speed of infection of "no root hair zone" of white clover and nodulating competitiveness among strains of *Rhizobium trifolii*. *Soil Biol. Biochem.* **20:** 465–470.

Vincent, J.M. and Waters, L.M. 1953. The influence of the host on competition amongst clover root nodule-bacteria. *J. Gen. Microbiol.* **9:** 357–370.