

## INFLUENCE OF ACID SOIL ON NODULATION AND INTERSTRAIN COMPETITIVENESS IN RELATION TO TANNIN CONCENTRATIONS IN SEEDS AND ROOTS OF *PHASEOLUS VULGARIS*

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**Summary**—The relationship between acid soils and (1) early nodulation in common beans, (2) interstrain competitiveness of *Rhizobium leguminosarum* bv. *phaseoli* strains and (3) tannin concentration in roots, were evaluated in two experiments. Ten *Phaseolus vulgaris* cultivars of different seed tannin content were grown in pots in a greenhouse in soil at pH 4.5 or 5.5, inoculated with equal amounts of strains CIAT 899 and KIM5. At pH 5.5, on all cultivars strain KIM5 occupied a greater proportion of nodules than strain CIAT 899. This was reversed at pH 4.5. Nodule number and nodule weight, 21 days after inoculation, were strongly reduced but root tannin concentrations doubled at pH 4.5. Nodule numbers were negatively correlated with tannin concentration in roots at both pH values. Seed tannin content was negatively correlated with early nodulation, but this was statistically significant only at pH 5.5. Nodulation of cultivars with high seed tannin contents was generally more acid tolerant than of those with low seed tannin content. In a second experiment, six *Phaseolus vulgaris* cultivars were planted at two field sites with acid soil and one site with neutral soil. In the field, as in the pots, nodule numbers at early stages of plant development were reduced by 30-50% in the acid soils compared to the neutral soil. In contrast, root tannin content in acid soils was twice that in the neutral soil. There were significant cultivar and cultivar by location effects.

Strains of rhizobia were tested for tolerance to bean seed tannin extracts, to seed diffusates and for survival on seed surfaces. The *Rhizobium* strains were sensitive to tannin extracts, with obvious strain differences. Sensitivity of most strains was greater at acidic pH. Seed diffusates, in contrast, did not inhibit growth of most strains.

### INTRODUCTION

Various types of common bean are the main sources of vegetable protein for people in Latin America. They are cultivated mainly by small-scale farmers on marginal, frequently acid soils (Vargas and Graham, 1989). An increased understanding of the mechanisms involved in pH tolerance of plants could help in breeding or selecting plants and rhizobia that are better adapted to acid soils. The formation of nitrogen-fixing nodules on roots of legumes is influenced by many environmental factors (Kosslak and Bohlool, 1985; Abaidoo *et al.*, 1990). In particular, nodule formation is sensitive to low soil pH (and associated factors, e.g. Al and Mn toxicity, Ca deficiency) and high soil temperatures. The precise effects of pH and the other stress factors on nodulation of legumes, however, remain poorly understood. Bean seeds contain components commonly known as antinutritional factors, one of the most important being tannins. These polyphenolic substances repel predators, inhibit enzymes, form insoluble, indigestible complexes with proteins, carbohydrates, Ca, Mg and Fe. They also inhibit growth of rhizobia and

nodulation of groundnuts and common beans (for a review see Muthukumar *et al.*, 1985). Tannin production in plants can be stimulated by environmental stress, such as low soil fertility (Pankhurst and Jones, 1979) which is common in the tropics.

We have investigated the effect of acidity on early nodulation, interstrain competitiveness, plant growth and tannin concentration in the roots of different bean cultivars. Correlations between either (a) early nodulation or (b) inoculant strain competitiveness and tannin content of seeds or roots are demonstrated. To test the hypothesis that the reduction of nodule numbers at high tannin concentrations in roots is due to their toxicity for rhizobia, the response of *R. leguminosarum* bv. *phaseoli* strains to tannin extracts, to seed diffusates and on seed surfaces was observed.

### MATERIALS AND METHODS

#### *Inoculum strains and seed sources*

*Rhizobium leguminosarum* bv. *phaseoli* strains and *Phaseolus vulgaris* cultivars were obtained from the strain and germplasm collections of NifTAL-Project and the Centro Internacional de Agricultura Tropical, (CIAT), Cali, Colombia.

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### Site characteristics and amendments

Experiments were conducted at three field sites at elevations of 110, 290 and 660 in on the island of Maui, Hawaii and are referred to herein as Hamakuapoko, Kuiaha and Haleakala, respectively. The soil at the Hamakuapoko site was a fine, Kaolinitic, isohyperthermic, Typic Haplustoll (annual rainfall: 1130 mm; neutral, highly fertile, large amounts of soil N). The soil at the Kuiaha site was a clayey, ferritic, isohyperthermic Humoxic Trophohumult (annual rainfall: 2110 mm; pH 4.5, moderate soil N) and that at the Haleakala site was a clayey, oxidic, isohyperthermic Humoxic Trophohumult (annual rainfall: 1979 mm; pH 3.9-4.8; moderate soil N). Except for Hamakuapoko, the sites were free of indigenous bean infecting rhizobia (Woomer *et al.*, 1988). To minimize differences in soil environment, soils at all sites were amended before the start of experiments by applying nutrients (except N) in recommended amounts (George *et al.*, 1987). Nutrients added (kg ha<sup>-1</sup>) were 300P; 200K; 50Mg; IOZn; 0.5B; 0.5Mo.

For pot experiments soil from the Kuiaha site was sieved (< 5 mm). Half of the soil was amended with Ca(OH)<sub>2</sub> to raise the initial pH from 4.5 to 5.5. Soils were mixed with fertilizers (mg kg<sup>-1</sup>) 200P; 25Mg; 300K and micro-nutrients 0.2Mo; 1.75B; 2.5Zn; 7.5Fe.

### Inoculum preparation

Peat-based inocula of *R. leguminosarum* bv. *phaseoli* strains KIM5 (Ta1943), CIAT 632 (Tal 1383) and CIAT 899 (Tal 1797) were prepared as described by Somasegaran and Bohlool (1990).

### Experimental design

Field experiments (plot size 40 m<sup>2</sup>) were made using a complete random block design, with four replications, at Hamakuapoko, Kuiaha and Haleakala. Six *P. vulgaris* cultivars (APN 84, BAT 271, Chingo, DOR 42, RAB 39, XAN 40) were planted (20 seeds m<sup>-1</sup> row) and each seed was inoculated at planting time with an aqueous peat-inoculant suspension of the *R. leguminosarum* bv. *phaseoli* strains CIAT 899 + CIAT 632 (1:1 mixture with 10<sup>8</sup> rhizobia per seed). Plants were harvested after 23 days and shoot dry weight, root dry weight, nodule numbers and root tannin content were determined.

Pot experiments were done in the greenhouse at the Haleakala research station. Plastic pots (3 l.) were used for the experiments and each pot contained 2.6 kg of soil at field capacity (320 g H<sub>2</sub>O kg<sup>-1</sup> soil) at either pH 4.5 or 5.5. Ten bean cultivars (APN 42, Bolita 42; BAT 1449; Cuarentemo de Honduras; Aqua Caliente 92; Guerrero 924; MCD 2004; Red Cloud; Rio Tibagi; Talamanca) were planted with six replications each and inoculated with a double strain mixture of *R. leguminosarum* bv. *phaseoli* strains CIAT 899 and KIM5 as described above. Uninoculated controls were also included.

Plants were harvested at 22 days and shoot dry weight, root dry weight, nodule numbers, nodule weight, root tannins and per cent nodule occupancy by strain CIAT 899 or KIM5 or double strain infection were analyzed.

### Strain identification

Nodule occupancy by inoculant strain was determined by indirect enzyme-linked immunosorbent assays (Wolff *et al.*, 1991).

### Tannin assay

Tannins in seeds and roots were determined following the method of Deshpande and Cheryan (1985). Dry seeds or roots were ground and a 100 mg sample was extracted with 1.5 ml 1% HCl in methanol for 10 min. The samples were then centrifuged at 1500g and aliquots were immediately analyzed for tannin using the Vanillin Assay (Broadhurst and Jones, 1978).

### Reaction of *Rhizobium* strains to bean tannins

Tannins were extracted in methanol-water (70:30 v : v), with 1 % formic acid, from the testa of bean seeds and the sensitivity of *R. leguminosarum* bv. *phaseoli* strains to the crude bean tannin extracts was assayed as growth inhibition on agar plates at pH 5, 6 and 7 (Wolff *et al.*, 1991).

### Toxicity of seed exudates

Toxicity of bean seed exudates on *R. leguminosarum* bv. *phaseoli* strains was tested by the method of Materon and Weaver (1984). Cultures were grown in 20E medium (Streit *et al.*, 1991) on a rotary shaker. Log phase cultures were used to inoculate melted 20E agar that had been cooled to 45°C. The agar contained approx. 5 x 10<sup>4</sup> cells ml<sup>-1</sup> and 15 ml was added to standard plastic Petri dishes and allowed to solidify. Seeds of *P. vulgaris* cultivars were autoclaved and then placed onto the surface of the solidified agar and incubated at 28°C. The degree of toxicity was measured after 1-3 days as the diameter of the zone of growth inhibition around each seed.

### Survival of strains on the seed surface

Seeds were surface sterilized for 1 min in 70% ethanol, followed by a 5 min exposure to 3% sodium hypochlorite and then four rinses in sterile water. The surface sterilized seeds were soaked in wash cultures of *R. leguminosarum* bv. *phaseoli* strains (10<sup>6</sup> cells ml<sup>-1</sup>) for 10 min. After this inoculation, the seeds were placed on water agar in Petri dishes and kept at 20°C in the dark. Three replicate samples (10 seeds each) were taken. Counts of *Rhizobium* on seeds were done by plating after serial dilution and expressed as colony forming units per seed (cfu seed<sup>-1</sup>).

### Statistical analysis

Analysis of variance, Duncan multiple range tests and regression analysis were done by using

STATGRAPHICS program from STSC, Inc.,  
Rockville, Md, U.S.A.

## RESULTS

The nodule numbers, shoot and root dry weights, root tannin content and nodule occupancy by inoculant strains of 10 bean cultivars grown in pots of soil amended to two different pH values, are presented in Table 1. There was a significant ( $P < 0.05$ ) effect of pH on all symbiotic variables tested, such as nodule numbers, nodule weight and inoculant strain competitiveness. Nodule weight at pH 4.5 was reduced to half that at pH 5.5, while nodule numbers were reduced by an average of 25%. In contrast, tannin content in roots of plants at low pH was nearly twice as high as in roots at the higher pH value. Interstrain competitiveness was also influenced by pH. At pH 5.5, strain KIM5 occupied about 70% of nodules and CIAT 899 only 15%. At pH 4.5 strain CIAT 899 (50%) was superior to KIM5 (35%). On most cultivars more nodules were occupied by both strains at pH 4.5 than at pH 5.5. Cultivar differences in nodule occupancy by inoculant strain were small but were more pronounced at pH 5.5.

At 21 days from planting there were no significant differences between inoculated and uninoculated control plants in shoot and root dry weights or root tannin content. No nodules were observed on the control plants.

In the pot experiments, in addition to the pH effect, there was also an effect of cultivar and a cultivar x pH interaction on most of the tested variables. Cultivars differed significantly in early nodulation. Differences between cultivars for root tannin content were small although the cultivars varied widely in seed tannin content (Fig. 1).

In the field experiments, significant location, cultivar and cultivar x location effects on all variables were found (Table 2). Highest nodule numbers, shoot and root dry weights but lowest root tannin concentration were found at the NifTAL field site at Hamakuapoko. Early nodulation (NN) measurements in the acid soils of Kuiaha was 50% and at Haleakala 30% of that in the neutral field site of Hamakuapoko. In contrast, root tannin content at Kuiaha and Haleakala was about twice as high as at Hamakuapoko. Beans at Haleakala had the highest tannin concentration but lowest nodule numbers (Fig. 2).

The bean cultivars used in this study varied widely in tannin content of the seeds (Fig. 1). There was no significant correlation between tannins in seeds and tannins in roots, either in the pot or the field experiments (Tables 1 and 2). Table 3 shows the correlations found between the tannins in seeds or roots and the symbiotic variables. Nodule number and nodule dry weights from the pot experiments were both negatively correlated with the tannin content of the roots. This tendency was partly confirmed by the field experiments, in which pooled data from the three

Table 1. Effect of soil pH on early nodulation, tannin concentrations in roots of 10 *P. vulgaris* cultivars and interstrain competitiveness of *R. leguminosarum* bv. *phaseoli* KIM5 and CIAT 899

Cultivar	pH 4.5							
	NN	NW	SDW	RDW	RT	% nodule occupancy		
						KIM5	CIAT 899	Double
APN 42	43.8 a	20.5abc	254 abc	112 ab	126.2 a	23.4 ab	55.6 ab	21.0 a
BAT 1449	51.3 ab	24.2 c	223 ab	96 a	118.7 a	45.5 cd	32.1 a	22.4 a
Bolita 42	58.7 bc	24.4 c	247 ab	116 ab	124.7 a	28.9 abc	53.3 ab	17.8 a
Caliente	41.8 a	15.3 ab	283 bc	110 ab	135.5 a	27.8 abc	58.9 b	13.3 a
Guerrero	59.3 bc	15.3 ab	200 a	120 abc	122.8 a	52.3 d	31.8 a	15.9 a
Honduras	68.8 cd	37.1 d	320 c	134 bc	120.0 a	44.5 bcd	31.3 a	24.2 a
MCD 2004	77.7 d	32.4 d	449 e	181 d	118.7 a	36.7 abcd	44.4 ab	18.9 a
Red Cloud	47.8 ab	13.2 a	600 f	222 e	132.0 a	24.8 abc	53.9 ab	21.3 a
Rio Tibag	53.7 ab	24.8 c	340 d	151 cd	131.7 a	30.0 abc	47.8 ab	22.2 a
Talamanca	45.3 bc	22.8 bc	278 bc	141 bc	132.0 a	22.7 a	59.2 b	18.1 a
Cultivar	pH 5.5							
	NN	NW	SDW	RDW	RT	% nodule occupancy		
						KIM5	CIAT 899	Double
APN 42	77.8 cd	45.8 b	358 ab	156 b	71.7 ab	71.1 bcd	15.6 abc	13.3 abcd
BAT 1449	62.0 ab	46.7 b	316 a	150 ab	65.3 ab	79.6 cd	9.8 abc	10.6 abcd
Bolita 42	83.2 cd	52.7 bc	286 a	142 ab	57.8 a	77.8 cd	7.8 ab	14.4 abcd
Caliente	51.8 a	29.3 a	300 a	118 a	76.3 b	60.0 ab	21.1 bcd	18.9 abcd
Guerrero	67.7 bc	27.3 a	280 a	163 b	80.5 b	54.4 ab	23.2 bcd	11.4 cd
Honduras	70.5 bc	45.7 b	317 a	149 ab	72.0 ab	86.8 d	4.5 ab	8.7 ab
MCD 2004	78.7 d	54.8 bc	449 c	195 c	195 c	73.5 ab	88.9 d	3.4 a
Red Cloud	95.7 e	57.4 c	697 d	259 d	58.0 a	43.1 a	33.7 d	23.2 d
Rio Tibag	83.8 d	49.8 bc	431 bc	207 c	65.5 ab	84.4 d	5.6 ab	10.0 abc
Talamanca	59.8 ab	47.5 b	353 ab	198 c	73.3 ab	53.6 ab	25.2 cd	21.2 bcd

NN = nodule numbers per plant; NW = nodule dry weight per plant; SDW = shoot dry weight ( $\text{mg plant}^{-1}$ ); RDW = root dry weight ( $\text{mg plant}^{-1}$ ); RT = root tannin concentration ( $\text{mg catechin equivalents } 100 \text{ g}^{-1}$  root dry weight).

Means in the same column followed by the same letter are not significantly different by Duncan's multiple range test ( $P < 0.05$ ).

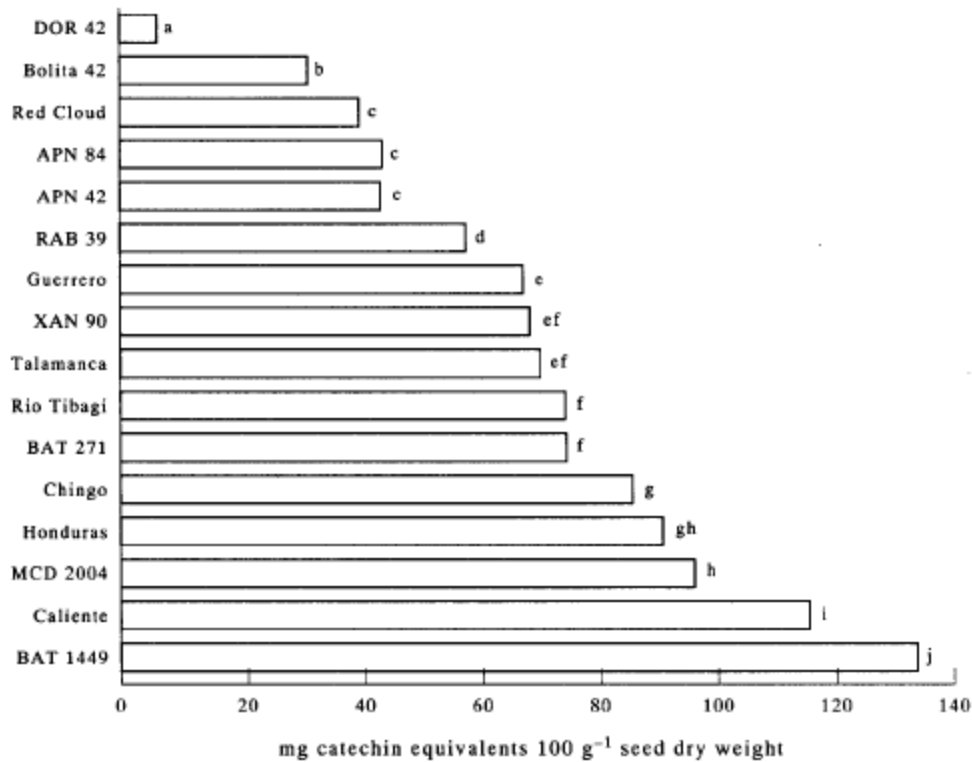


Fig. 1. Seed tannin concentration of *P. vulgaris* cultivars. Means followed by the same letter are not significantly different by Duncan's multiple range test ( $P < 0.05$ ).

locations showed that nodule numbers and root tannins were inversely related (Fig. 2). However, there was no significant correlation between nodule numbers and root tannins when the field sites were analyzed individually. Interstrain competitiveness

Table 2. Effect of field site on early nodulation and tannin concentration in roots of six *P. vulgaris* cultivars

Cultivar	Haleakala			
	NN	SDW	RDW	RT
APN 84	33.6 c	587 a	137 b	114.5 a
BAT 271	24.6 c	548 a	156 b	108.0 a
CHINGO	19.9 bc	489 a	105 a	119.0 a
DOR 42	12.7 ab	535 a	144 b	105.7 a
RAB 39	22.3 c	533 a	152 b	112.8 a
XAN 90	8.4 a	483 a	145 b	108.0 a
Cultivar	Kuiaha			
	NN	SDW	RDW	RT
APN 84	20.9 a	266 a	112 a	105.3 bc
BAT 271	46.3 b	426 b	171 b	102.2 abc
CHINGO	35.7 b	297 a	145 ab	115.5 c
DOR 42	17.4 b	208 a	112 a	88.8 ab
RAB 39	41.9 b	418 b	167 b	102.3 abc
XAN 90	24.7 a	249 a	122 a	86.0 a
Cultivar	Hamakuaopoko			
	NN	SDW	RDW	RT
APN 84	76.9 c	1762 bc	321 b	56.0 bc
BAT 271	54.2 ab	1856 c	233 a	42.0 a
CHINGO	68.2 bc	1369 abc	196 a	62.3 c
DOR 42	42.8 a	1260 ab	257 a	36.5 a
RAB 39	58.9 b	1646 abc	249 a	53.3 b
XAN 90	67.9 bc	1233 a	224 a	50.5 b

Variables as in Table 1.

was also related to root tannin content (NO, Table 3), but was significant only at pH 4.5 in pots.

Seed tannin content was also negatively correlated with nodule numbers, but this correlation was significant only at pH 5.5 in pots (Table 3).

Sensitivity of the rhizobial strains to bean tannin extract increased at low pH [Table 4(a)]. There were obvious strain differences in relation to the pH effect. While the strains H2C and KIM5 were strongly inhibited at all pH values, the sensitivity of strains CIAT 151 and CIAT 899 increased markedly at pH 5. Of all the *R. leguminosarum* bv. *phaseoli* strains KIM5 and H2C were the most sensitive at all pH values tested. Strain CIAT 899 was relatively tolerant of tannins.

The effects of seed diffusates on the *Rhizobium* strains is shown in Table 4. Strain H2C was sensitive to the bean seed diffusates. None of the other strains tested was inhibited by seed diffusates from any cultivar at that time. Growth of strains CIAT 899 and CIAT 151 was in many cases promoted in a zone around the seeds. The effect was strongest for strain CIAT 899.

To investigate the effect of seed tannins on the survival of *R. leguminosarum* bv. *phaseoli* strains *in vivo*, seeds from cultivars of different tannin content were selected. Although there was a significant inhibition by the tannins on the growth of the *R. leguminosarum* bv. *phaseoli* strains *in vitro*, no inhibition of growth on the seed surface was detected

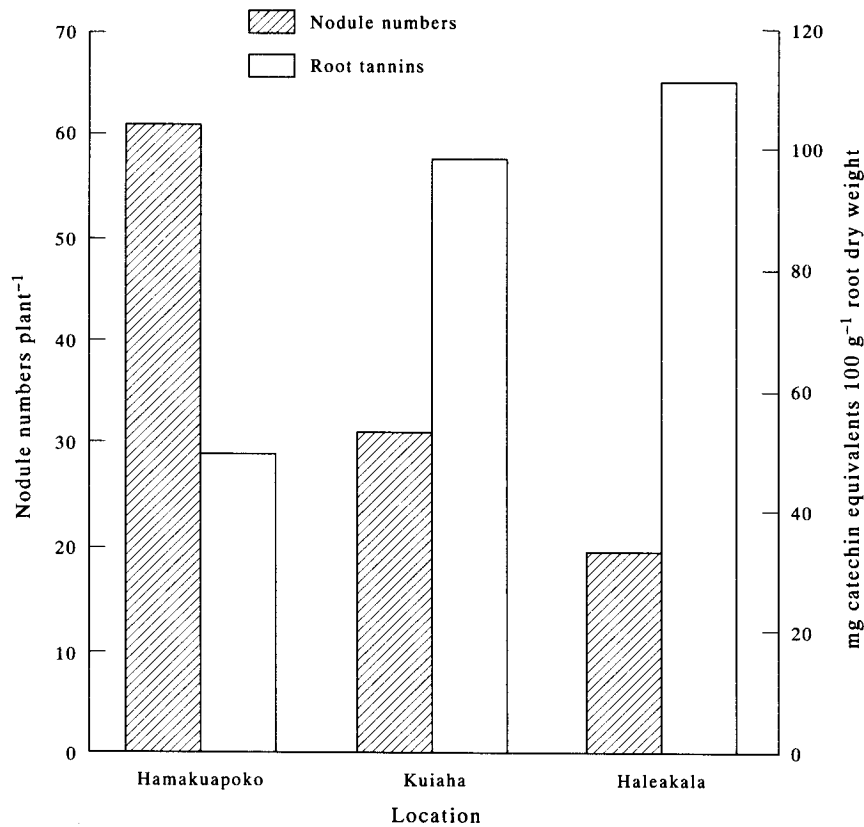


Fig. 2. Field site effect on early nodulation and root tannin concentration. Mean of six bean cultivars. For each parameter the histograms are significantly ( $P < 0.05$ ) different.

[Table 4(c)]. Cell number per seed increased approximately by 1 log unit over a period of 4 days and the trend was similar for all cultivars. No significant strain differences were observed.

#### DISCUSSION

High nodule numbers at early stages of plant development have been shown to be positively correlated with total plant N late in the growth cycle and

hence it could be considered as an important factor in bean breeding programs for enhanced biological nitrogen fixation (BNF) (CIAT, 1989). The world's main bean producing areas are in the tropics, where a large proportion of arable land is acidic, a major factor limiting nodulation and N<sub>2</sub> fixation in common beans (Vargas and Graham, 1989). Additionally, symbiotic legumes are often more sensitive to unfavorable soil conditions than their nitrogen fertilized counterparts (Piha and Munns, 1987). Although an understanding of the plant-microbe interaction under stress is of great economical importance, knowledge at the physiological and genetic level is very limited.

In our pot culture trials and field experiments, early nodulation was strongly affected by soil pH and cultivar (cf. Vargas and Graham, 1989). Cultivars differed widely in acid tolerance with significant pH x cultivar and cultivar x location interactions. None of the cultivars proved to be most successful in early nodulation at all locations or all pH values tested (cf. CIAT, 1989).

Acid sensitivity of nodulation was not related to reductions in either shoot or root dry weight. Interestingly, nodule weight was more affected by soil pH than were nodule numbers for all cultivars. This confirms the observations of Evans *et al.* (1980) who found that nodulation can be 10 times more sensitive

Table 3. Linear correlations between bean tannin concentration in roots and seeds and early nodulation and interstrain competitiveness of *R. leguminosarum* strain KIM5 and CIAT 899 at two pH levels in the greenhouse

	pH	Root tannins	Seed tannins
		<i>r</i> (significance level)	
NN	4.5	-0.73*	NS
	5.5	-0.67*	-0.69*
NW	4.5	-0.62*	NS
	5.5	-0.75*	NS
NO KIM	4.5	-0.72**	NS
	5.5	NS	NS
NO 899	4.5	+0.79**	NS
	5.5	NS	NS
NO Double	4.5	NS	NS
	5.5	NS	NS

NN = nodule numbers per plant; NW = nodule weight per plant; NO = nodule occupancy by inoculant strain or double infection.

Table 4. Sensitivity of six *R. leguminosarum* bv. *phaseoli* strains to bean tannin extract (cv. APN 84) at three pH values; to seed diffusates from six *P. vulgaris* cultivars and growth on seed surfaces of four bean cultivars

Strain	CE3	H2C	KIM5	151	895	899
<b>(a) Growth inhibition by bean tannin extract (mm)</b>						
<i>pH</i>						
5	1 ab	15 h	13 g	9 f	3 cd	4d
6	1 ab	8 f	9 f	2 bc	1 ab	1 ab
7	0 a	4 d	6 e	1 ab	1 ab	1 ab
<b>(b) Sensitivity to seed diffusates</b>						
<i>Bean cultivar</i>						
APN 42	-	+	-	-*	-	-*
APN 84	-	+	-	-*	-	-*
BAT 1449	-	+	-	-*	-	-*
Guerrero	-	+	-	-	-	-*
Red Cloud	-	+	-	-	-	-*
Talamanca	-	+	-	-	-	-*
<b>(c) Growth on seed surfaces</b>						
<i>Bean cultivar</i>						
Bat 1449	g	ND	g	ND	g	g
Guerrero	g	ND	g	ND	g	g
Red Cloud	g	ND	g	ND	g	g
Talamanca	g	ND	g	ND	g	g

Means followed by the same letter are not significantly different by Duncan's multiple range test ( $P < 0.05$ ).  
s = sensitive; n = not sensitive; \* = growth promoted by seed diffusates; g = growth; ND = no determination.

to acidity than the growth of *Rhizobium* or the legume root alone.

Nodule occupancy by strain KIM5 or CIAT 899 was influenced by bean cultivar. Such effects in the greenhouse have already been described by DeOliveira and Graham (1990). In field experiments, however, cultivar effects on nodule occupancy by inoculant strain is often less pronounced (Wolff *et al.*, 1991). Interstrain competitiveness is known to be a stable factor of a given strain (Kosslak and Bohlool, 1985). Strain KIM5 is known to be more competitive than CIAT 899 under various conditions (Somasegaran and Bohlool, 1990). In a low pH soil, however, per cent nodule occupancy by CIAT 899 was higher than by KIM5. A few reports for *R. leguminosarum* bv. *phaseoli* show that interstrain competitiveness can be altered by abiotic stress, e.g. low soil pH or high soil temperatures. Effects of acidity were described between the *R. leguminosarum* bv. *phaseoli* subgroups I and II (subgroup II is now classified as *R. tropici*). Under moderate pH conditions, *R. tropici* strains are less competitive than type I strains (Martinez-Romero *et al.*, 1991). Whether the enhanced inter-strain competitiveness at low pH is a general feature of *R. tropici* strains needs further investigation. *R. tropici* strains are known to be acid tolerant *in vitro* (Martinez-Romero *et al.*, 1991). In the double-strain inoculants used in this study, strain CIAT 899 was acid tolerant while KIM5 was more sensitive (Wolff *et al.*, 1991). Under the controlled conditions of the pot experiment, the change in interstrain competitiveness at pH 4.5 could be due to their differential sensitivity to low pH. However no significant differences were found for strain survival rates in an acid

soil (Streit *et al.*, 1991). Also in the presence of an indigenous *Rhizobium* population, competitiveness of both strains decreased to the same extent in acid soils (Wolff *et al.*, 1991). Vargas and Graham (1989) found no significant differences in the number of the *R. tropici* strain CIAT 899 and the *R. leguminosarum* bv. *phaseoli* group I strain CIAT 632 (acid sensitive) in the rhizosphere of beans at pH 4.5. Therefore, it appears doubtful whether differences in acid tolerance of *Rhizobium* strains *in vitro* can predict *in vivo* symbiotic response.

Tannins are known to inhibit nodulation in beans and other legumes when applied to the soil (Blum and Rice, 1969; Muthukumar *et al.*, 1985), but there are no known reports on the effect of tannins in the seed coat or roots on nodulation in beans. Our results show that tannins of both origins can affect nodule numbers during early stages of plant development. Nodule occupancy by the tannin-sensitive strain KIM5 was negatively correlated to root tannin content of the cultivars. With CIAT 899 (tannin-tolerant) the correlation was positive. Tannins are more actively bound at low pH (Haslem, 1989) and the sensitivity of the *Rhizobium* strains to tannins under acid conditions may be related to binding.

Nodulation of cultivars whose root tannin content increased strongly when grown in acid soils, tended to be acid sensitive. Nodulation of cultivars with high seed tannin content was in general more tolerant of low pH. The correlations between tannin content and nodule numbers/strain competitiveness suggest the possibility that plant defense mechanisms, triggered in response to abiotic stress, may be partly responsible for the poor nodulation in acid soils in the tropics.

The mechanism by which tannins influence nodulation is unknown, but it can be assumed that inhibition of growth of *Rhizobia* (Pankhurst and Jones, 1979) may be important. We also found growth inhibition of the tested strains by tannin extracts *in vitro*. But our experiments fail to show inhibition on seed surfaces. Other unknown factors may have also enhanced the effects of seed tannins in this experiment and confound the difficulty in correlating *in vivo* behavior of a strain and its tannin tolerance shown *in vitro*. Therefore the mechanism by which tannins lead to a reduction in nodulation remains unclear. However it is noteworthy that strains which were tolerant to tannins were also tolerant to acidity (Wolff *et al.*, 1991).

Environmental stress limits the *RhizobiumPhaseolus* symbiosis by reducing nodulation and nitrogen fixation and increasing nodule senescence. Broad ranges of cultivar and strain responses to abiotic stress factors have been described. For a better understanding of these processes more studies, at the physiological or genetic level, of host-strain interaction under stress conditions are necessary.

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