



Addressing edaphic constraints to bean production: the Bean/Cowpea CRSP project in perspective

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Abstract

Edaphic factors constrain bean production in most areas where this crop is grown. They include nutrient constraints particularly N and P deficiency, soil acidity including Al and manganese toxicity, and drought. The Bean/Cowpea Collaborative Research Support Program (CRSP) has supported research on edaphic constraints to bean production since its inception, the major partnerships involving the University of Wisconsin and EMBRAPA, Brazil (1981–1989) and the University of Minnesota and INIAP, Ecuador (1989–2002). Research over this period has emphasized cultivar and strain variation in nodulation and nitrogen (N₂) fixation, host strain interaction and coevolution, host and strain tolerance of soil acidity, and nitrogen fixation under phosphorus limited conditions. This paper reviews recent developments in these areas of nitrogen fixation research, but does so from the perspective of the Bean/Cowpea CRSP project.

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1. Introduction

In Latin America and much of Africa, the production of beans (*Phaseolus vulgaris* L.) is concentrated on small, near subsistence holdings, using minimal technical inputs (Graham, 1981; Oliveira et al., 1998). Yields are low, in part because of disease and insect pressures, but also because of edaphic constraints that include soil nitrogen and phosphorus deficiencies

(Graham, 1981; Thung, 1991; Oliveira et al., 1998), soil acidity (Graham, 1992), and manganese and iron toxicities (Giller et al., 1992). Wortmann et al. (1998) reported N deficiency in 93 of 95 bean soils from East Africa, with P also limiting to plant growth in all but 11 of these sites. Giller and Cadisch (1995) and Franzluebbers et al. (1998) note that small-holders in Africa commonly apply less N and P as fertilizer than is removed in the grain. Stoorvogel et al. (1993), and later Sanchez (2002) suggest average annual depletion rates across 37 countries in the region of 22 kg N, 2.5 kg P and 15 kg K ha⁻¹. According to Sanchez, the inability of genetically improved materials to express

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their potential under conditions of nutrient limitation is a major factor in relatively low adoption rates for such cultivars in Africa. Similar problems have been reported for bean production in Latin America (Fasbender, 1967; Malavolta, 1981; Bazan, 1975; Thung, 1991). The limited future availability of phosphorus fertilizers (Vance et al., 2000), and the poor root health of beans in both Latin America and Eastern Africa, only exacerbate these problems.

The majority of soils in East Africa also have pH limitations for bean growth, with 52% of soils in this region and 42% of soils in southern African having a pH of 5.2 or less (Wortmann et al., 1998). In addition to P adsorption, problems for bean production in such soils can include Mo, Ca, K and S deficiencies, and Al and Mn toxicity. Again, the problems in Latin America are similar (Cochrane, 1979), although Spehar (1995) points to key breakthroughs that have allowed significant increases in soybean production in the Cerrados of Brazil. These include minimum tillage to maintain soil organic matter (Boddey et al., 1997), with the lime needed for pH amendment often surface applied. Zinc and other micronutrient deficiencies are surprisingly common constraints to bean production on some of the more alkaline soils of Andean South America (Flor et al., 1975).

The Bean/Cowpea Collaborative Research Support Program (CRSP) has supported research on edaphic constraints to bean production, and particularly on symbiotic nitrogen (N_2) fixation, since its inception. The first of these projects (1981–1989), between the University of Wisconsin and the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) Bean Center in Goiania, Brazil, included Bliss and Dazzo as principal investigators in the USA and Araujo as the principal investigator in Brazil. Henson worked for the University of Wisconsin at the EMBRAPA Bean Center during much of this project, and also played a significant role in research activities there. The second project (1989–2002), between the University of Minnesota and the Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP), Quito, Ecuador, included Graham as US-PI, and Estevez de Jensen, Bernal and Peralta as principal investigators in Ecuador. Edaphic concerns were also an important component of the CRSP projects in Honduras and Mexico. In each of these programs the emphasis has been on *P. vulgaris*. This was in part because cowpea was perceived as a

highly promiscuous and high N_2 -fixing host, not often benefited by inoculation (Burton, 1967); and a species well adapted to high, temperature, drought and acid soil stress. This paper will attempt to highlight the research contributions of the different CRSP projects, within the overall advances made recently in this field.

2. Cultivar variation in bean nodulation and N_2 fixation

Phaseolus beans have a reputation for weakness in the ability to nodulate and fix N_2 in symbiosis (Graham and Halliday, 1977; Graham, 1981). Contributing factors are thought to include the poor soil conditions under which the crop is commonly grown; intermittent or terminal drought; the short growing season of many bean cultivars; limited energy supply to the nodule; and, in some areas, the effects of N fertilization on the inoculated legume. N fertilization is likely to be a major factor in a decline in the percent N derived from fixation in beans from 44% prior to 1987, to only 37% since (Van Kessel and Hartley, 2000). Unkovich and Pate (2000) suggest rates of N_2 fixation in the bean/*Rhizobium* symbiosis of from 0 to 150 kg ha⁻¹, with an average accumulation of 55 kg shoot N ha⁻¹. With a shoot to root ratio in beans of 2.8, this is equivalent to an average total N_2 fixation of 85 kg ha⁻¹. This is low compared with fixation rates in other major grain and pasture legumes.

While many commercial cultivars are relatively weak in the ability to fix N_2 in symbiosis, wide variation in this trait has been reported. Lines identified as superior in N_2 -fixing symbiosis include Puebla 152 (Graham and Rosas, 1977; Pereira et al., 1993), Bayocel (Castellanos et al., 1996), Carioca (Araujo et al., 1996), Italian Barlotti (Buttery et al., 1997), BAT271 (Buttery et al., 1997), ICA20667 and ICA21573 (Redden and Herridge, 1999), Capixaba Precoce (Vargas et al., 1991), and RIZ21 (Elisondo Barron et al., 1999).

Traits identified as correlated with active nodulation and high N_2 fixation in symbiosis, and that might have potential in a selection program for these traits in beans include:

- nodule number (Wolyn et al., 1989; Pereira et al., 1993), nodule mass (Dobereiner, 1966; Graham

- and Rosas, 1977; Rosas and Bliss, 1986; Wolyn et al., 1989) and nodulation score (Rosas and Bliss, 1986),
- acetylene reduction assay at 50% bloom (Rosas and Bliss, 1986) or seasonal estimates of C₂H₂ reduction based on multiple sampling throughout the growing season (Graham and Halliday, 1977; Graham and Rosas, 1977),
- duration of the fixation period as affected by speed in nodulation (Kipe-Nolt and Giller, 1983; Chaverra and Graham, 1992); prolonged nodule formation (Kipe-Nolt and Giller, 1983), and delayed nodule senescence (Espinosa-Victoria et al., 2000),
- shoot biomass and shoot N (Rennie and Kemp, 1983; Wolyn et al., 1989; Miranda and Bliss, 1991; Elisondo Barron et al., 1999).

Some comments on these traits are warranted:

Nutman (1967) for *Trifolium*, points out that inefficiently nodulated plants can have high nodule number, and that nodule number and weight per nodule are usually inversely related. Similar data are reported for beans by Pereira et al. (1993). Because of this, we have preferred to use nodule mass instead of nodule number as a selection criterion.

Attewell and Bliss (1985) noted significant N accumulation during pod fill, and suggested that lateral root nodules could be active sites of N₂ fixation during the reproductive period. In a subsequent study Wolyn et al. (1989) distinguished crown and lateral root nodulation in beans, and showed shoot N accumulation by the R7 growth stage to be highly correlated with lateral root nodulation and nodule mass. Sampling so as to best represent these two pools of nodules can be difficult.

Miranda and Bliss (1991) selected lines active in symbiotic nitrogen fixation on the basis of total seed N. They justified this approach on the relatively high N harvest index figures (0.74–0.90) in their materials. In contrast, Elisondo Barron et al. (1999) noted that when selection was based on seed yield alone, seed yield and seed N increased but shoot mass and shoot N in the selected plants was less. They concluded that selection for seed yield under low soil N conditions would favor lines with high harvest index, and limit gains due to enhanced symbiotic N₂ fixation. While the relationship between seed yield and biomass yield is likely to vary with the parents, Elisondo Barron et al. (1999) chose to select on the basis of seed yield and plant biomass at physiological maturity. In these

studies, a near infrared reflectance spectrometry system (Mündel and Schaalje, 1988), calibrated using semimicro Kjeldahl analysis was used to estimate plant nitrogen.

A number of other traits have been correlated with N₂ fixation in *P. vulgaris* or other legumes, and are likely to have value in the identification of promising parental lines. They include allantoin, phosphoenolpyruvate carboxylase (PEPC) and leghemoglobin concentrations (Ceccatto et al., 1998; see also Herridge and Peoples, 1990; Degenhart et al., 1992). These traits are likely to be either too time consuming or too expensive for use in the routine evaluation of breeding populations.

Approaches used in studying cultivar variation in traits affecting N₂ fixation in beans, have usually worked well when applied to soybean, and vice versa (Pazdernik et al., 1996, 1997). Thus an area warranting further attention in beans is that of cultivar variation in root-nodule senescence. Significant cultivar variation in this trait has been reported for soybean (Espinosa-Victoria et al., 2000), with some evidence of variation also evident in beans (Hungria and Franco, 1988). However, more work is needed.

3. Breeding for enhanced N₂ fixation in *P. vulgaris*

Surprisingly, given the variation demonstrated, there are few studies in which breeding for enhanced N₂ fixation in *P. vulgaris* has been reported. However, indirect selection for this trait is probable wherever breeding nurseries are grown at low N. The BILFA nurseries (Bean improvement for low fertility soils in Africa, Wortmann et al., 1995; Lunze et al., 2002) exemplify this approach. Bliss (1993) discusses factors influencing choice of method in breeding for enhanced N₂ fixation. Studies undertaken at Wisconsin used the backcross inbred method of population development (McFerson et al., 1982) with Puebla 152 as the donor parent, and Sanilac, Ex Rico 23, ICA Pijao, Porrillo Sintetico and Jamapa as recurrent parents. Sixty backcross inbred families were generated from each cross, then evaluated using a number of approaches (McFerson et al., 1982; St. Clair et al., 1988; Miranda and Bliss, 1991). St. Clair et al. (1988) identified lines from the cross of Sanilac × Puebla 152

with the agronomic traits of Sanilac, but similar to Puebla 152 in ability to fix N_2 in symbiosis. When four of these inbred backcross lines were intercrossed 50% of the resultant F_3 families were superior to Sanilac in symbiotic N_2 fixation, and one family exceeded Puebla 152 (St. Clair and Bliss, 1991). Five lines from the ICA Pijao \times Puebla 152 cross have been released as improved germplasm lines (Bliss et al., 1989); 'Ouro Negro' was also released in Brazil as a bean line with high potential for symbiotic N_2 fixation (Henson et al., 1993). The populations developed above have also been used in studies carried out under low soil P (Pereira and Bliss, 1989, see p. 7).

In related studies, Pereira et al. (1993) compared nodule number per plant in 10 lines of black bean, including Puebla 152, then used two cycles of recurrent selection to generate field populations that were evaluated for shoot N and grain yield. Substantial gain per cycle was achieved in nodule number and weight per plant, but individual nodule weight declined.

Elisondo Barron et al. (1999) evaluated 36 lines from the studies of Acosta Gallegos (1988), Castellanos et al. (1988), Pereira et al. (1989) and Chaverra and Graham (1992) for differences in traits contributing to better overall N_2 fixation. They identified Puebla 152, BAT271 and RIZ21 as superior in N_2 fixation in symbiosis, and apparently different in the traits contributing to this ability, then mounted a recurrent selection program for enhanced nitrogen fixation in common bean. Selection based on seed yield and biomass at physiological maturity, measured under conditions of low soil N, was used to select indirectly for N_2 fixation. Because lines with high N_2 -fixing ability are often delayed in maturity, only progeny maturing in 97 days or less were considered for selection, and crossing, selfing, seed multiplication and field testing phases were completed in a single year. Average increase per cycle in seed yield, seed N and total shoot dry weight at physiological maturity were significant, but days to first flowering and maturity were not affected. Three lines (EL 2, 19 and 34) were markedly superior to the best parent and to elite check varieties in each of the traits specified above.

Heritability estimates for N_2 fixation in bean have only rarely been obtained. Rosas and Robledo (1995) grew progeny from the cross of RAB201 \times Puebla 152 (low and high N_2 fixing lines) in a field plot labeled with ^{15}N , then used population variances and

parent-offspring regression to obtain heritability estimates. Estimates of broad sense heritability for shoot dry weight, shoot total N, total fixed N and seed yield were intermediate in value (0.36–0.53), and narrow sense heritability estimates low to intermediate (0.22–0.41). Additive gene effects contributed significantly to the differences observed among genotypes.

Molecular markers for traits associated with nodulation and nitrogen fixation have not been employed in any Bean/Cowpea CRSP activity, but are now needed. A number of recombinant inbred lines featuring parents differing in nodulation and N_2 fixation ability have been developed at CIAT, and could be useful in such studies. Advantage also needs to be taken of work underway in the *Medicago* and *Lotus* (Barker et al., 1990; Handberg and Stougaard, 1992) genome projects. In the only BNF-marker studies reported for beans, Tsai et al. (1998) analyzed 70 F_2 derived F_3 families from the cross of BAT93 \times Jalo EEP558 under low N conditions, and identified seven linked markers affecting nodule number. In subsequent studies with recombinant inbred lines from the same cross, Souza et al. (2000) found 15 markers distributed over seven linkage groups associated with nodule number at low N. Four of these markers were associated with both nodule number and resistance to *Xanthomonas axonopodis* pv. *phaseoli*.

4. *Rhizobium* and host-rhizobium interaction

Domestication of beans is thought to have occurred on various occasions in Andean and Central America 4400 to 2500 years before the present (Kaplan and Lynch, 1999). In these centers of origin, *Rhizobium etli* is the principal microsymbiont (Souza et al., 1994; Eardly et al., 1995; Aguilar et al., 1998), though isolates of *R. tropici* (Graham et al., 1982; Martinez-Romero et al., 1991) and alleles from *R. leguminosarum* have been recovered in Colombia (Eardly et al., 1995). The presence together of host plants and *Rhizobium* in the different areas of Latin America where domestication occurred would have provided ample opportunity for coevolution between host and *Rhizobium* (see page 5).

Accelerator mass spectrometry (AMS) radiocarbon dating places beans in the north-eastern USA as early as 1300 AD (Hart and Scarry, 1999; Hart, 2000) but the spread of beans to other regions of the world postdates

Spanish colonization of Latin America. Such seed movement would have carried along some *R. etli* as seed borne contaminants (Perez-Ramirez et al., 1998; Herrera-Cervera et al., 1999), but the number and diversity of the rhizobia transported is likely to have been limited (B. Tlusty and P.H. Graham, unpublished data). In those regions where there were no compatible native rhizobia, initial nodulation and N₂ fixation would have been low, and founder effects likely. However, *Phaseolus* beans are promiscuous (Michiels et al., 1998), and also symbiose with *R. gallicum* and *R. giardinii* (Amarger et al., 1997) and rhizobia from alfalfa (Eardly et al., 1985), *Dalea* (Graham et al., 1999), *Onobrychis* (Laguere et al., 1997), and *Leucaena* (Martinez-Romero et al., 1991). Where soils to which beans were introduced already contained such indigenous rhizobia, nodulation and N₂ fixation of beans could have been enhanced and soil populations of these indigenous organisms benefited. The result appears to be that different organisms (or mixes of organisms) have assumed importance in the different parts of the world where beans are now grown. Thus in south-western Spain, Rodriguez-Navarro et al. (2000) found 73% of soil isolates to belong in the species *R. etli*, 10% in *R. gallicum* and 6% in *R. giardinii*. Only one isolate grouped with *R. leguminosarum* bv. *phaseoli*, and three with *Sinorhizobium fredii*. In contrast, in Senegal and Gambia, the isolates recovered from bean nodules belonged primarily to *R. etli* and *R. tropici* (Diouf et al., 2000), while for Central Minnesota (Bernal et al., pers. commun.) 71% of the bean isolates recovered fell within *R. leguminosarum* bv. *phaseoli*. These different patterns of species dominance invite further attention, with significant differences in the compatibility of particular host cultivar/microsymbiont combinations likely. A modified root-tip marking procedure (Bhuvanewari et al., 1981) is well suited to explore such differences (McDermott and Graham, 1990; Chaverra and Graham, 1992; Lupwayi et al., 1996), and in studies at the University of Minnesota has already shown significant differences in compatibility even for bean cultivars and *R. etli* strains. Bernal (1993) tested *R. etli* strains from Ecuador and Argentina against bean cultivars representative of the subgroups of *P. vulgaris* distinguished by Singh et al. (1991), and found Mesoamerican cultivars generally slower to nodulate with Andean bean rhizobia than with cultivars from the Andean

Table 1
Influence of origin of host cultivar on speed in nodulation for bean rhizobia from the Andean region of Ecuador and Argentina^a

Host cultivar ^b	Uppermost nodule position (mm above RTM) ^c
Algarrobo	3.4 a
Imbabelle	2.9 ab
Negro Argel	2.6 abcd
Nuna	2.4 abcd
Blanco Sabanero	2.3 abcd
Porrillo Sintetico	2.0 bcde
Ecuador 299	1.8 bcde
Bola 60	1.5 cde
Duran	1.4 cde
Puebla 152	0.9 de

^a Speed in nodulation was determined for root-tip marked (RTM) plants using a procedure modified from that of Bhuvanewari et al. (1981). Source: Bernal (1993).

^b Algarrobo, Imbabelle, Nuna, Blanco Sabanero and Bola 60 are from the Andean gene pool of *P. vulgaris*. Negro Argel, Porrillo Sintetico, Ecuador 299, Duran and Puebla 152 are from the mesoamerican gene pool (Singh et al., 1991).

^c The data presented is an average for 37 *Rhizobium* strains of which 21 were isolated in Ecuador, and 11 in Argentina. Two *R. etli* and three *R. tropici* were included as controls.

region (Table 1). Bernal (2001) and Bernal and Graham (2001) even found differences among the rhizobia from northern and southern Ecuador in phenotypic and genotypic characteristics, and in nodulation with local and introduced hosts. Differences with microsymbionts other than *R. etli* are likely to be even more significant. This is evident in the studies of Chaverra and Graham (1992), while in Spain, Rodriguez-Navarro et al. (2000) found recovery of non-*R. etli* isolates greater when Negro Jamapa was used as host. In Minnesota, where bean inoculation is only now becoming significant, we had anticipated that *Rhizobium* strains from the native prairie legumes *Dalea purpurea* and *D. candida* would be common in bean nodules. *Dalea* rhizobia are infective and effective on beans (Graham et al., 1999), and occur on bean seed, but have proven slow to nodulate beans, and so easily outcompeted by other rhizobia (B. Tlusty and P.H. Graham, pers. commun.).

The ability of some cultivars to select for particular strains of rhizobia in a multistrain environment has been documented in a number of legumes, including beans (Montealegre et al., 1995; Montealegre and Graham, 1996; Rosas et al., 1998). Preference in

nodulation has been viewed as one way to ensure nodule dominance by particular inoculant rhizobia, but a really effective preference system has yet to be developed. Mechanisms to explain preference in nodulation are lacking, and the genetic basis for this response is not known. Longer-term field studies that evaluate the impact of preference in nodulation for the systems already described are also needed.

With the background of promiscuity described above, it is surprising that yield responses to inoculation have occurred in the Andean center of origin of the crop (Pineda et al., 1994; Cordova et al., 1995; Acuña et al., 2001). A number of strains have also been identified as efficient in N₂ fixation in the studies undertaken in Spain, Morocco, etc. (Mhamdi et al., 1999; Rodriguez-Navarro et al., 2000). Further evaluation of such strains as potential inoculant-quality rhizobia should be done using host cultivars from both the Mesoamerican and Andean gene pools, and include studies on speed of nodulation.

5. Soil acidity and host–*Rhizobium* response

Acid soils constrain legume growth through effects on the host, the *Rhizobium* and the process of nodulation itself. In beans, effects of hydrogen ion concentration include death or marginalization of the microsymbiont (Anyango et al., 1995; Hungria et al., 1997; Hungria and Vargas, 2000) as well as effects on nodule formation (Vargas and Graham, 1988). Manganese and aluminum toxicity can also impact the microsymbiont, but more commonly have greater effects on the host (Graham, 1992).

Acid soil tolerance in some of the rhizobia that nodulate beans was shown by Graham et al. (1982), and led eventually to the description of the species *R. tropici* with the acid-tolerant CIAT899 (=UMR1899) as type strain (Martinez-Romero et al., 1991). This organism grows well even at pH 4.25 (Graham et al., 1994) and tolerates both 100 μM Al³⁺ and 200 μM Mn (Vargas and Graham, 1988). Further studies on the acid tolerance of *R. tropici* UMR1899 have been facilitated by the use of HOMOPIPES buffer (P.H. Graham, pers. commun.). This buffer (Research Organics, Cleveland) is effective in pH control from pH 3.9 to 5.1 and from 7.7 to 8.9. When exposed to acid pH, cells of UMR1899 produce acid shock

proteins, and increase cellular K⁺ levels (Aarons and Graham, 1991), regulate cytoplasmic pH, accumulate glutamate, and change cell membrane composition (Ballen et al., 1998). Tolerance to crystal violet (Graham et al., 1994), captan and streptomycin (B. Tlusty and P.H. Graham, pers. commun.) in this organism is further evidence of unusual cell-membrane composition. A proton translocating ATPase is also active under acid conditions (Ballen, 1998), while the calcium requirement of *R. tropici* UMR1899 at acid pH is seemingly less than for most other rhizobia (Howieson et al., 1992; Ballen et al., 1998). *R. tropici* UMR1899 is effective in N₂ fixation with beans, but at near-neutral pH is not particularly competitive in nodule formation (Martinez-Romero and Rosenblueth, 1990; Chaverra and Graham, 1992; Wolff et al., 1993). Competitive ability increases with soil acidity (Vargas and Graham, 1988; Frey and Blum, 1994), presumably the reason for the greater frequency with which *R. tropici* is recovered from acid soils (Vargas and Denardin, 1994; Giller et al., 1994; Hungria et al., 1997). Used in inoculants in the acid Cerrado soils of Brazil, the strains UMR1135 and UMR1899 increased yields by 900–1500 kg ha⁻¹ (Mendes et al., 1994). Marked host strain interaction was evident in this study, as was the poor performance of the acid-sensitive strain UMR1632 (Table 2). UMR1899 has now been used as an inoculant culture for beans in Brazil for a number of years (Araujo, 1994) with another acid-tolerant strain, PRF81, also recently approved for use (Hungria et al., 2000). Further stress tolerance and nodulation studies using a more diverse pool of *R. tropici* isolates should be undertaken. Both Andrade et al. (2002) and B. Tlusty and P.H. Graham (pers. commun.) have identified significant diversity within this species; the potential in this diversity needs to be further explored.

Because many soils now contain indigenous bean rhizobia that might limit gains from inoculation, an issue in Brazil is that of N fertilization of beans. With new cultivars and irrigation practices, farmers using heavy N fertilization rates can achieve yields >3000 kg ha⁻¹, some 400–700 kg ha⁻¹ more than that achieved with inoculation alone (Vargas et al., 2000). Long-term N fertilization can, however, have hidden costs. In central Minnesota where farmers have habitually used N fertilization in place of inoculation and N₂ fixation, marked soil acidification

Table 2
Response to inoculation with selected strains of bean rhizobia with two cultivars of bean under acid-soil conditions in Brazil^a

Strain/treatment	Nodule no. per plant	Nodule mass ^b per plant	Grain yield ^c (kg per ha)
UMR1020			
Capixaba ^b	64 ^c	105	1381
CNPAF 178	32	51	2354
UMR1024			
Capixaba	37	39	1661
CNPAF178	42	71	1257
UMR1135			
Capixaba	21	35	1627
CNPAF178	52	100	2526
UMR1632			
Capixaba	7	3	871
CNPAF178	35	68	1437
UMR1899			
Capixaba	18	19	1431
CNPAF178	69	52	2056
Not inoculated			
Capixaba	0	0	576
CNPAF178	0	0	751
100 kg N per ha			
Capixaba	1	3	1894
CNPAF178	1	1	1991

^a Source: Mendes et al. (1994).

^b The cultivars used were Capixaba Precoce and CNPAF178.

^c Nodule number and mass were determined 33 DAP for Capixaba Precoce and 39 DAP for CNPAF 178.

and increased incidence of root rots are now common, with concomitant decline in yield over the last decade (C. Estevez de Jensen, pers. commun.). A current focus in this region of the state is toward joint inoculation using acid-tolerant rhizobia and biocontrol agents effective against *Fusarium* and *Rhizoctonia*. It is intriguing in these studies that *R. tropici* appears to act synergistically with the biocontrol organism. While, both the positive and the potentially negative interaction of organisms in this dual inoculant have yet to be studied, this relatively low cost treatment warrants additional study across a range of soil environments, and especially for small landholder conditions in the tropics. Studies in which cultivar differences in growth under acid-soil conditions have been shown, commonly do not distinguish between the effects of pH, Al or Mn toxicity and

nutrient availability. Howeler (1991) reviewed studies undertaken by CIAT in Santander and Carimagua, Colombia, and noted that black beans generally performed better under acid-soil conditions than beans with other seed colors. Critical Al saturation values for *P. vulgaris* of only 8–10% were suggested, with most of the tolerant lines identified coming from Brazil. These include Iguacu, Jalo, Mulatinho Paulista, Roxo 760 (Malavolta et al., 1981), with the snap bean variety Dade (Foy et al., 1999) an exception. In the majority of these studies nodulation was not considered. Vargas and Graham (1988) identified three bean lines (Bico de Ouro, Preto 143 and Capixaba Precoce) that nodulated well in a pH4.5 irrigated sand system, but did not consider the effects of Al. Foy et al. (1999) make the point that shallow rooting in soils where the subsoil is low in pH, or high in aluminum, can increase the susceptibility of plants to drought. A similar problem could result from surface lime application, without incorporation. Effects on nodulation and nitrogen fixation due to higher soil temperature near the soil surface are also possible. Gonzalez and Lynch (1999) and others have identified a range of bean varieties with tolerance to manganese.

6. Nutrient stress and bean production

Studies on nutrient deficiencies within the Bean/Cowpea CRSP have emphasized beans, and concentrated on low P tolerance and zinc deficiency (see below). Molybdenum deficiency has not been a focus because such studies would have duplicated efforts in EMBRAPA and in Africa. There the importance of Mo for nitrogen fixation in beans, and the importance of Mo source in application to inoculated seed has been reemphasized, but additionally, both host genotypic differences in molybdenum accumulation (Brodrick and Giller, 1991) and the value of seed enrichment with molybdenum (Brodrick et al., 1992; Campo et al., 1999) shown.

6.1. N₂ fixation under low P conditions

Genetic differences among bean cultivars in growth at low P have been known for many years (Whiteaker et al., 1976). The mechanisms identified emphasize

improved efficiency in the uptake of phosphorus via changes in root hair density or length, or changes in root architecture via the formation of shallow basal or adventitious roots (Bonser et al., 1996; Yan and Lynch, 1998). Such mechanisms, again, expose the bean plant to drought, and the rhizobia and nodules to higher soil temperatures. Production of organic acids and phosphatases (Lynch and Beebe, 1995), and changes in the microbial population of the rhizosphere, have also been suggested as low-P tolerance mechanisms. Jones (1998) noted that the release of organic acids occurs in response to both Al toxicity and low-P stress; transgenic alfalfa plants modified in citrate production have been developed (Tesfaye et al., 2001), and show greater aluminum tolerance, enhanced P levels in tissue, and are more active in N₂ fixation than the wild-type (C.P. Vance, pers. commun.).

Few of these low P tolerance studies have considered the greater P need of the nodulated legume. The critical nature of P for essentially all parameters of nodule development and function is obvious in Table 3; similar data are provided by Pereira and Bliss (1987). In Table 3, nodules average almost 10% of the total plant P, perhaps a reflection of nodules as a strong sink for P, and their ability to absorb P directly from solution (Al-Niemi et al., 1998). Phosphorus efficient cultivars, which in addition partition a significant percentage of their P uptake to nodules, will be needed for improved bean N₂ fixation.

Pereira and Bliss (1989) suggest that it should be possible to improve both N₂ fixation and P utilization efficiency at the same time, and a number of bean lines have been reported as active in N₂ fixation at low to moderate P. They include Carioca (Ibijbjen et al.,

1996); Puebla 152 (Pereira and Bliss, 1987), Apore, Ouro Negro, BAT271, Carioca, Mulatinho Paulista and Puebla 152 (Araujo et al., 1997), APN18 and BAT271 (Vadez and Drevon, 2001) and ANT22 and E295 (Christiansen and Graham, 2002). Vadez and Drevon (2001) suggest that N accumulation in APN18 and BAT271 is linked to the partitioning of P, and propose the use of N:P accumulation as an indicator for the selection of plant genotypes active in N₂ fixation at low to moderate P. Further studies are needed in which these varieties are tested with P sources having different availability. Desirable genes affecting low P tolerance should be combined. Germplasm that combines low P and aluminum tolerance, with high N₂ fixing capacity (perhaps stemming from an ability to produce organic acids in the root and rhizosphere, as, for example, with the variety Dade (Foy et al., 1999)) should be identified. Results from the BILFA nurseries (Wortmann et al., 1995; Lunze et al., 2002) are, again, a positive step in this direction.

Phosphorus level and genotype can also influence *Rhizobium* representation in the nodules of their host (Table 4, I. Christiansen and P.H. Graham, pers. commun.). With P supplied as rock phosphate to provide 140 µM P in the soil solution, strains identified as *R. etli* (Clusters 1 and 2) occupied almost two thirds of the nodules, with no difference in strain nodule occupancy evident between the three genotypes. At 5 µM P strain distribution for the low-P tolerant cultivars ANT22 and E295 (Christiansen and Graham, 2002) did not differ significantly from that found at 140 µM P. However, for the P-sensitive cultivar G19833, Cluster 1 strains were reduced in nodule occupancy, with a corresponding increase in

Table 3
Influence of P supply on parameters of nodulation and nitrogen fixation in *P. vulgaris*^a (P.H. Graham and I. Christiansen, unpublished data)

Parameter measured	P supplied in nutrient solution (ppm)		
	1	4	16
Nodule dry weight (mg per plant)	70	212	354
P in nodule (%)	0.21	0.20	0.29
Plant P found in nodules (%)	9.0	10.9	9.7
Total plant N (mg)	45	108	126
Acetylene reduction (µM per plant h ⁻¹)	4.3	12.4	34.0
Specific nodule activity (µM C ₂ H ₄ g ⁻¹ nodules h ⁻¹)	0.061	0.058	0.096
Fixation per unit P (µM C ₂ H ₄ mg nodule P h ⁻¹)	28.5	26.9	33.7

^a The data provided is an average for four bean cultivars and four replications per cultivar. Plants were sampled 33 days after planting.

Table 4
Influence of P level and genotype on the recovery of specific clusters of rhizobia from bean nodules^{a,b}

Strain cluster	Phosphorus supplied (μM)					
	5			140		
	Genotype ($\chi^2 = 9.80^*$)			Genotype ($\chi^2 = 1.46$ ns)		
	Ant22	E295	G19833	Ant22	E295	G19833
1	48	47	29	55	50	56
2	52	48	50	49	55	46
3–9	50	55	71	43	45	48

^a 150 nodules were sampled for each P level and genotype, and were characterized using Box A1R-PCR (Versalovic et al., 1994).

^b Unpublished data of I. Christiansen and P.H. Graham (2001).

the representation of other rhizobia. This parallels the situation found with *R. tropici* in acid soils, and further suggests interaction between host and rhizobia in nodulation under stress conditions.

6.2. Micronutrient deficiency in Ecuador

While most of the CRSP edaphic studies have involved nitrogen fixation or phosphorus nutrition in beans, the high pH of the soils in Ecuador and their strong tendency to zinc deficiency (Table 5) have necessitated studies on this constraint.

At Pirampiro, Ecuador in 1993 yield increases up to 1272 kg ha⁻¹ were obtained with applications of 5–15 kg ha⁻¹ zinc soil applied (Cordova et al., 1995). In this study, and subsequently at Imbabura, differ-

ences in cultivar response were evident, with Imbabello and Je. Ma giving better yields at 0 or low rates of zinc application than was achieved with other varieties. Zinc chelates applied to the foliage at flowering and pod formation were also effective in overcoming zinc deficiency in beans. In a subsequent study using a DTPA-buffered nutrient solution with 1 μM Zn, AND684, E101, PVA773, LSA102, and SUG55 were tolerant of low zinc levels in solution (Gangotena et al., 1995). All were large seeded Andean genotypes with relatively high seed reserves of Zn (13–18 μg Zn per seed). PVA 773, LSA102 and SUG 55 have subsequently been released as new varieties in Ecuador.

7. Conclusion

Projects on edaphic constraints to production undertaken through the Bean/Cowpea CRSP have emphasized *P. vulgaris*. For this species they have identified host traits related to nitrogen fixation, and undertaken breeding programs to enhance rates of N₂ fixation. Progress has been substantial, but now needs to impact mainstream breeding programs, and to identify molecular markers that will facilitate inclusion of N₂-fixing ability as a goal in such programs. Even indirect selection by locating breeding nurseries on soils having low N levels is likely to have impact. We need to get away from the belief common in the American midwest, and perhaps amongst breeders, that beans do best when supplied with fertilizer N.

Marked symbiotic promiscuity in *P. vulgaris* is now evident. A new generation of strain selection studies

Table 5
Frequency of specific nutrient deficiencies (%) in soils from the northern-, central- and southern-Andean regions of bean production in Ecuador^a

	Northern	Central	Southern
No. of sites soil tested	25	47	128
Nutrient			
N	12	49	76
P	4	28	41
K	4	4	5
Ca	0	0	0
Mg	0	0	0
Cu	0	0	1
Fe	48	4	58
Zn	92	64	88
Mn	64	89	64

^a INIAP, Informe Annual, 1993.

are needed, but must pay greater attention to the host(s) with which rhizobia are to be used, and the possible importance and efficiency in nodulation of indigenous organisms. Soil phosphorus limitations are critical constraints to bean production in both Latin America and Africa, and affect plants dependent on N_2 fixation more than those supplied N fertilizer. Efforts to improve N_2 fixation and P utilization efficiency need to be undertaken concurrently, and should consider not only P uptake from soil, but also the ability to sequester different forms of P from soil, and to be efficient in its use. Such studies should also consider the possibility of mechanisms common to N_2 fixation, P uptake from soil, and pH or aluminum tolerance, and that change in soil microbial community structure could be important to the resolution of specific edaphic constraints.

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