

Chapter 3

THE IMPORTANCE OF NITROGEN FIXATION TO SOYBEAN CROPPING IN SOUTH AMERICA

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1. INTRODUCTION

Although soybean (*Glycine max* L. Merr.) is a relatively recent crop introduction to South America, the region currently accounts for about 45% of world soybean production. This paper provides a brief overview of both soybean production in this region and some of the problems that have been overcome in reaching current production and yield levels.

2. TAXONOMY, ORIGINS, AND IMPORTANCE OF SOYBEAN

Hymowitz and Newell (1980) divide the genus *Glycine* into the subgenera, *Glycine* and *Soja*. The former genus includes seven wild perennial species, most of which are indigenous to Australia (Brown *et al.*, 1985), whereas the latter includes the cultivated soybean, *Glycine max* (L.) Merr., and its annual wild counterpart, *G. soja* Sieb. and Zucc. *G. soja*, with twice the nucleotide diversity of *G. max* (Cregan *et al.*, 2002) occurs throughout northern, north-eastern and central China and in adjacent areas of the former USSR, Korea, Japan, and Taiwan. Hymowitz and Singh (1987) infer more than one domestication event in the Shang dynasty (ca. 1700-1100 BC) with the resulting emergence of soybean as a domesticated crop in central and northern China during the period from 1100-700BC. Movement of the crop into India, Nepal, Burma, Thailand, Indochina, Korea, Japan, Malaysia, Indonesia, and the Philippines occurred by the first millennium AD (Smartt and

Hymowitz, 1985) and was accompanied by change in several host genetic traits that affected symbiosis with rhizobia (Pulver *et al.*, 1982; Devine, 1984).

Europe first learned of soybean in 1712 through the writings of the German scientist, Englebert Kaempfer, with seeds sent by missionaries stationed in China and subsequently planted in the Jardin des Plantes, Paris, in 1740 (Probst and Judd, 1973). *Glycine max* was introduced into the USA in 1804, probably as a result of seed interchange between the USA and France (Hymowitz and Newell, 1980), and initially attracted more interest for its potential as a forage species (Probst and Judd, 1973). Significant production of soybean in the United States and Europe dates only from the beginning of the twentieth century (Piper and Morse, 1923; Gray, 1936; Morse, 1950; Hymowitz, 1970), with major germplasm collection and evaluation only initiated between 1927 and 1931 (Probst and Judd, 1973). Even today, the *Glycine* germplasm maintained in the USDA Germplasm Resource Information Network (<http://www.ars.grin.gov>) includes only 18,765 accessions of *G. max*, 1,117 of *G. soja*, and approximately 1,000 accessions of related *Glycine* spp. Also of concern is the very narrow genetic base used until recently in the majority of soybean-breeding programs. Thus, Delannay *et al.* (1983) noted that 10 introductions provided 80% of the northern US gene pool, whereas only 7 introductions contributed the same percentage to the southern US gene pool.

Soybean today is one of the most important and extensively grown crops in the world. It accounts for 29.7% of the world's processed vegetable oil and is a rich source of dietary protein both for the human diet and for the chicken and pork industries (Graham and Vance, 2003). Isoflavones from soybean may reduce the risks of cancer and lower serum cholesterol (Molteni *et al.*, 1995; Kennedy, 1995). Soybean is also used as a milk substitute in weaning foods and baking and in ink and biodiesel fuels (Anonymous, 2000; 2001; Graham and Vance, 2003).

Although soybean production continues to grow worldwide (Figure 1), with an estimated production in 2002/2003 of 183.28 Tg, a major part of this production comes from only four countries: the USA, Brazil, Argentina, and China (Figure 2). Mercosur, the common market agreement signed by Brazil, Argentina, Paraguay, and Uruguay in 1991, is today responsible for about 45% of world soybean production (Uruguay accounts for 0.03% of the production). Production has increased significantly over the last few years (Figure 3), even though that from the USA has declined from 47% of world production in 1999/2000 to 39% in 2001/2002 (CONAB, 2002a). Bolivia, a partner of Mercosur in the Andean region, now plants 680,000 ha annually with an estimated production of 1.3 Tg in 2002/2003 (USDA, 2002a). Export of soybean from South America continues to grow and to capture most of the increases in world soybean demand with the most dramatic increase being observed in Argentina (USDA, 2002b).

Soybean was probably introduced into Brazil in 1882, in the State of Bahia, as *Soja hispida* (*Glycine soja*) and *Soja ochroleuca* (D'utra, 1882; 1899). A few studies were subsequently undertaken at the experimental-station level, but it was not until the 1940s that soybean was grown commercially in the southern region of Brazil. A significant expansion of soybean production in this region occurred in the 1960s but, even as late as 1975, Brazil produced only 11.24 Tg of soybean annually. This situation started to change in the 1970s, with the expansion of crop production

Million tons

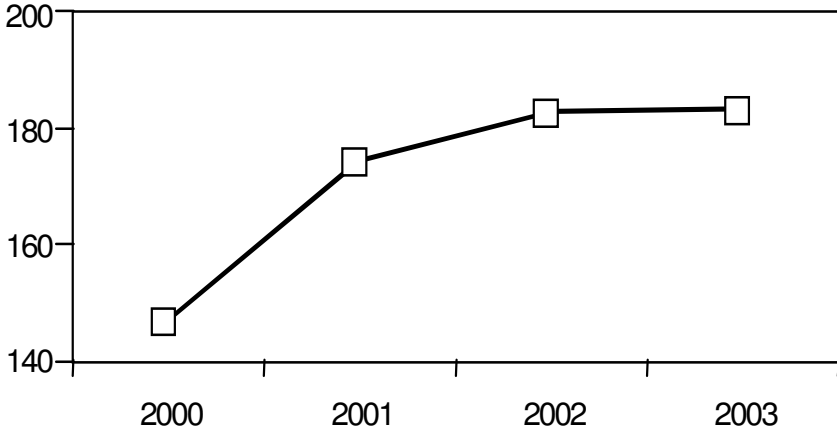


Figure 1. World soybean production in the last three years, with projection for 2002/2003 (CONAB, 2002a).

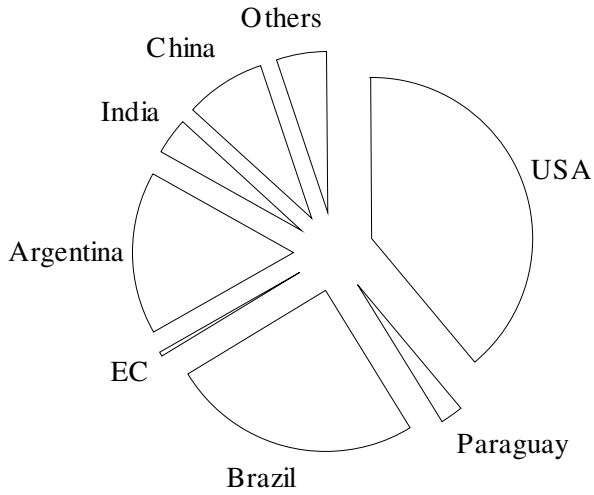


Figure 2. Contribution of different countries to world soybean production (estimated as 183.28 Tg) in 2002/2003 (CONAB, 2002a).

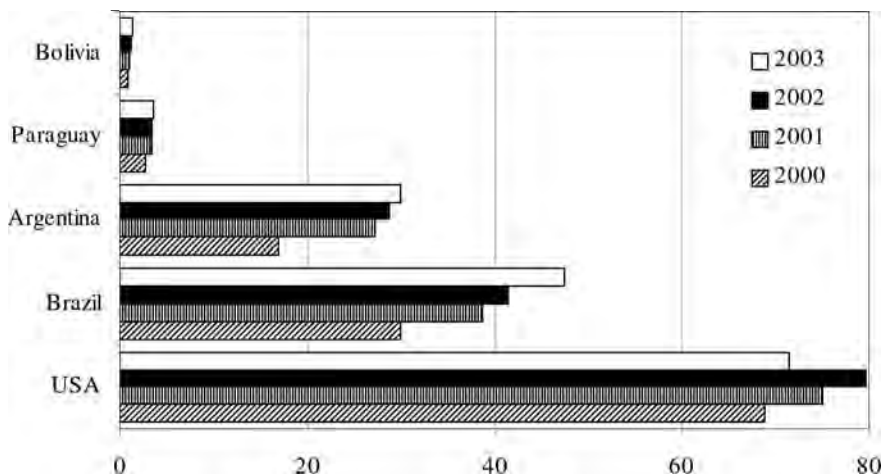


Figure 3. Annual soybean production (Tg) in the United States, and in the four main producers in South America: Brazil, Argentina, Paraguay and Bolivia (CONAB, 2002a; 2002c; 2003; USDA, 2002a).

into the "Cerrados", an edaphic savanna occupying 207 million ha and 25% of the land area of Brazil. The Cerrados are distinct in their soil chemical properties and environment (Goedert, 1985), necessitating the use of varieties that had longer juvenile periods, were aluminum tolerant, and were calcium-use efficient (Spehar, 1995). Initial yields were low but, as appropriate technologies were developed for the area, steadily improved. In 2002/2003, the three main states of this ecosystem, which are Mato Grosso, Mato Grosso do Sul, and Goiás (including the Federal District) cultivated 7.80 million ha and produced 23,329 Tg of soybean, with a yield average ($2,991 \text{ kg ha}^{-1}$) greater than the national mean ($2,765 \text{ kg ha}^{-1}$). A 10.6% increase in production area is expected in 2002/2003 (CONAB, 2003).

Soybean was known in Argentina in 1880, but the first field experiments there date from around 1908, and only 1,315 ha were planted in 1941/42. As in Brazil, significant expansion in the area cropped to soybean in Argentina dates to the mid 1970s with 169,400 ha cropped in 1972/73, 9.5 million ha in 1988/89, and 12.3 million ha in 2002 (Saumell, 1977; Miró, 1989; Anonymous, 2001). Soybean was introduced into Paraguay in the 1920s with seeds from the United States, Argentina, and Japan, but commercial expansion did not occur until the 1970s (Alvarez, 1989). Importation of Brazilian cultivars adapted to the tropics facilitated crop improvement and continues today (Oliveri *et al.*, 1981).

Breeding for improved productivity and for adaptation to the shorter day length, high temperature, and edaphic constraints common in the Cerrados have been a major part of soybean improvement in Brazil and have resulted in numerous varietal releases. Traditional breeding programs, which include both hybridization of selected parents in single, three-way or multiple combinations and selection by pedigree, mass selection or single/seed descent, have been used with both increased

crop yield and yield stability emphasized. Selection for a long juvenile period (Toledo *et al.*, 1994; Spehar, 1995) now allows soybean to be produced even in the state of Roraima, localized between 1°S and 2°N latitudes.

Another decision, made by the National Soybean Commission in the 1960s, was that biological nitrogen fixation (BNF) was an important trait that needed to be considered in breeding activities. Most Brazilian cultivars of this period were derived from North American genotypes, and differences between them in relation to their symbiotic performance had already been identified (Döbereiner and Arruda, 1967; Vargas *et al.*, 1982). Selection of parental lines active in symbiotic N₂ fixation with *Bradyrhizobium* was emphasized in the early breeding activities, but attention to this trait has declined in recent years. A consequence has been a decline in the symbiotic capacity of recently released cultivars (Bohrer and Hungria, 1998; Hungria and Bohrer, 2000). Nicolás *et al.* (2002) studied the genetics of nodulation and nitrogen fixation in Brazilian cultivars with contrasting symbiotic efficiency, and reported narrow-sense heritability (h_n^2) estimates of from 39% to 77% for nodulation and plant growth under low fixed-N soil conditions. These values are high compared to others reported in the literature. Simple sequence repeat (SSR) markers related to symbiotic performance in soybean have also been identified (Nicolás, 2001) and can now be used in the search for cultivars with higher biological N₂ fixation capacity.

3. BIOLOGICAL NITROGEN FIXATION

The area cropped with soybean in Brazil increased from 702,000 ha in 1940/41 to 17.95 million ha in 2002/2003. Soybean now accounts for 42% of national agricultural production and 16% of gross internal product (CONAB, 2002a; 2002b; 2003). The increase in area has been paralleled by a more than four-fold increase in national mean yield over the period from 1968 to the present (Figure 4). Argentina, Paraguay, and Bolivia report similar changes.

As a consequence of the higher yields, plant fixed-N demands have also more than doubled in the last 40 years. A program to select more efficient and competitive strains has been in operation in Brazil since the 1960s and continues to ensure that soybeans in Brazil can satisfy their fixed-N requirements through N₂ fixation (Vargas and Hungria, 1997; Hungria *et al.*, this volume). Bacterial strains are selected for use in commercial inoculant production only after extensive field testing in the main soybean-production areas. Four strains are currently recommended for use; *Bradyrhizobium elkanii* SEMIA 587 and SEMIA 5019 (=29w), since 1979, and *B. japonicum* SEMIA 5079 (=CPAC 15) and SEMIA 5080 (=CPAC 7), 1992 (Vargas and Hungria, 1997; Hungria and Vargas, 2000; Hungria *et al.*, this volume). Each of these strains can fulfill the crops need for fixed-N at yields greater than 4,000 kg ha⁻¹. They are provided free to inoculant producers, and all inoculants must carry two of the four strains. Their genetic relatedness with other well-studied *Bradyrhizobium* strains is shown on Figure 5.

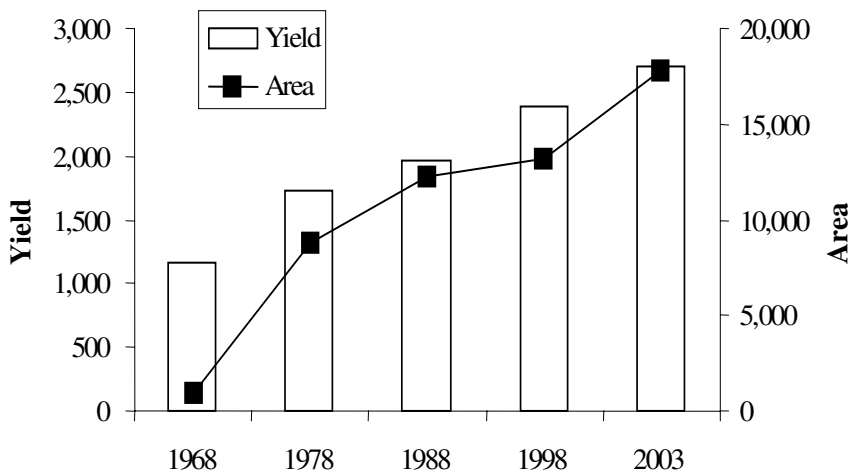


Figure 4. Change in mean yield (kg ha⁻¹) and area (x 1,000 ha) cropped with soybean in Brazil over the last 45 years (Anonymous, 2000; CONAB, 2002c; 2003).

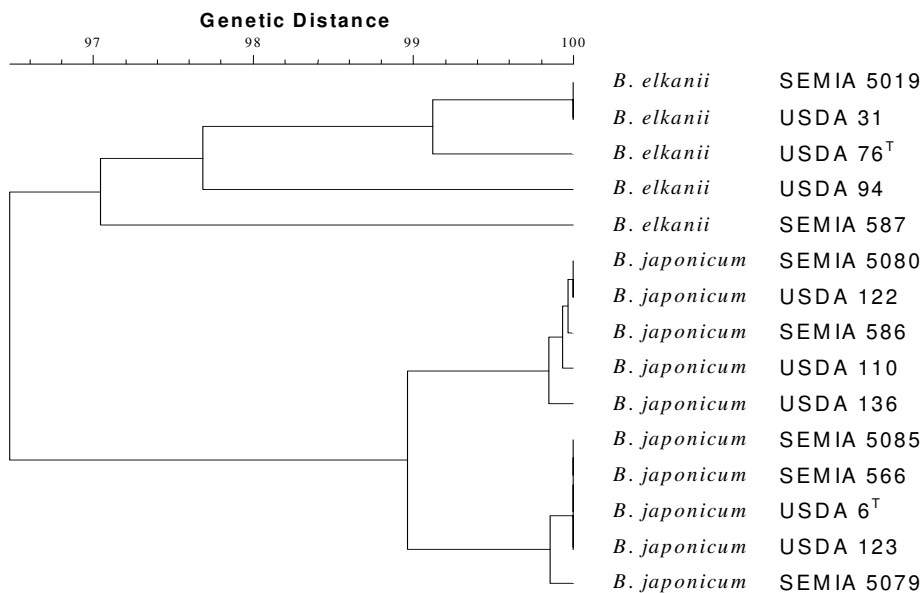


Figure 5. Genetic relatedness among soybean *Bradyrhizobium* strains recommended for use in commercial inoculants in Brazil (SEMIA 587, SEMIA 5019, SEMIA 5079, SEMIA 5080) and Argentina (SEMIA 5085). After Chueire et al. (2003).

Although more than 90% of the area cropped with soybean in Brazil today has been inoculated before, and generally contains naturalized rhizobia, yield increases averaging 4.5% have been obtained when soils containing 10^3 or more cells g^{-1} soil were reinoculated (Hungria *et al.*, this volume). Table 1 shows some of these results. This finding contrasts with those of studies in the USA (Thies *et al.*, 1991) and the difference in response warrants further detailed studies in both regions. Currently, re-inoculation is practiced by about 60% of farmers in Brazil (ANPI, Associação Nacional dos Produtores de Inoculante, personal communication) and by 50% of those in Argentina (Dr. Enrique R. Moretti, Laboratórios BIAGRO, Argentina, personal communication). As a result, the number of inoculant doses sold each year continues to increase in both countries (Figure 6). This situation is, again, in marked contrast to the USA, where the use of inoculants is a common practice in just 15% of the soybean-production area but, in Canada, about 60% of the farmers have adopted inoculation (Dr. R. Stewart Smith, Nitragin, USA, personal communication). In Argentina, the strain recommended by INTA (Instituto Nacional de Tecnología Agropecuaria) is *B. japonicum* E109 (=SEMIA 5085, =USDA 138), and its relatedness with other strains is shown in Figure 5.

Table 1. Effects of reinoculation and of addition of N-fertilizer on soybean yield ($kg\ ha^{-1}$) in soils with established populations of soybean bradyrhizobia ($> 10^3$ cells g^{-1} soil). After Hungria *et al.* (2001).

Treatment	South Region		Cerrados	
	Londrina	Ponta Grossa	Goiânia	Planaltina
Non-inoculated	3,836 a ¹	2,697 b	2,341 a	2,483 a
Non-inoculated + N-fertilizer ²	3,434 b	2,872 ab	2,432 a	2,660 a
Inoculated with efficient strains	4,025 a	2,912 a	2,462 a	3,119 a
CV (%)	9.9	8.1	8.8	7.0

¹Values followed by the same letter, in the column, do not show statistical difference (Duncan, $p \leq 0.05$).
²200 kg of N ha^{-1} as urea, split at sowing and at early flowering.

In contrast to their Mercosur neighbours, Paraguay has had no strain-selection program and just 15-20% of the soybean crop is inoculated. There is also no quality-control program in the country and locally produced and foreign inoculants used over the past two decades have often been of poor quality. Inoculants now sold in Paraguay come from Argentina, Brazil, and Uruguay. Mercosur legislation permits inoculants produced in one country to be sold in another, provided they carry strains recommended by local rhizobiologists. Despite the less-than-ideal situation, nodulation occurs without inoculation in most areas of Alto Paraná and Itapúa, where 80% of soybean production occurs (Figueredo, 1998). Symbiotic effectiveness varies from site to site but, in a recent survey, some isolates collected in the field were outstanding symbiotic performers, a first step for the identification of strains for use in commercial local inoculants (Chen *et al.*, 2002).

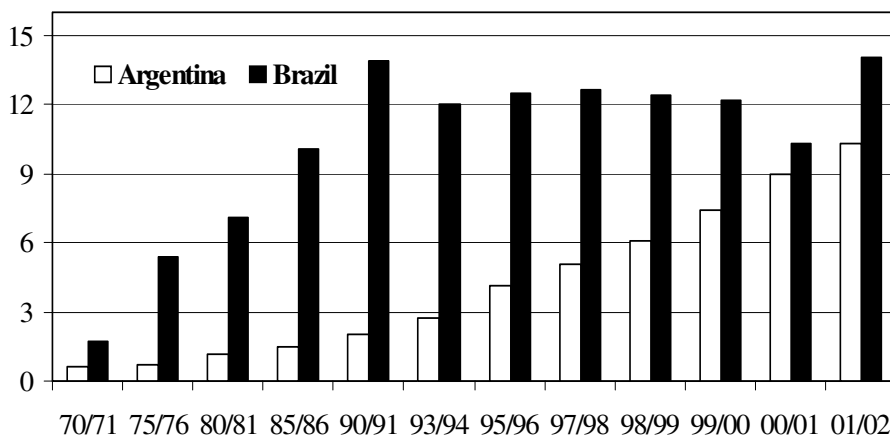


Figure 6. Units of soybean inoculant ($\times 10^6$) sold in Argentina and Brazil. (Dr. Enrique R. Moretti, Laboratórios BIAGRO, Dr. Solon Araújo, ANPI, and Dr. Laura Machado Ramos, Ministério da Agricultura, Pecuária e Abastecimento, personal communication).

4. ECONOMIC IMPORTANCE OF BIOLOGICAL NITROGEN FIXATION (BNF) IN SOUTH AMERICA

Economic returns from soybeans dependent on BNF in South America are outstanding. Brazil is used here as an example. For each 1,000 kg of soybean produced, approximately 80 kg of N (65 kg N allocated to seeds, which have *ca.* 40% protein, and 15 kg N left in roots, stems, and leaves) are required. When the national mean yield in Brazil in 2002/2003 of 2,765 kg ha⁻¹ is considered, the fixed-N requirement would be slightly more than 220 kg ha⁻¹. Where this is supplied through BNF, N losses are minimal. In contrast, the N-fertilizer-use efficiency of plants in the tropics is rarely as much as 60%, meaning that such plants would require *ca.* 360 kg N or 800 kg of urea (at 45% N) to achieve equivalent yields. At US\$0.20 kg⁻¹ of urea, the cost of applying N-fertilizer would be *ca.* US\$160 ha⁻¹, or in excess of US\$2.87 billion nationally (for 17.95 million ha). Because Brazilian transportation costs are very high, but total production costs are 40% lower than in the United States (CONAB, 2002a; 2002b), it is evident that BNF plays a key role in lowering Brazilian production costs. Both Unkovich and Pate (2000) and Giller (2001) suggest a potential for N₂ fixation by nodulated soybeans of 360–450 kg of N ha⁻¹. Thus, there is still significant room in Brazil for increased yield without N fertilization. The situation in the other countries in South America is similar.

The data available on the quantification of BNF under field conditions in South America is limited but the results from studies undertaken in Brazil are shown in Table 2. In these studies, the contribution of BNF to N accumulation among cultivars released in recent years is appreciably higher than was achieved in 33 commercial soybean fields in Australia. For these crops, with a mean % of N

derived from fixation estimated at 53%, the contribution of BNF to N accumulation was 178 kg N ha⁻¹ (Unkovich and Pate, 2000).

Table 2. Contribution of biological N₂ fixation to soybean production in some field experiments performed in Brazil.

Site	Condition ¹	Yield (kg ha ⁻¹)	kg of N ₂ fixed ha ⁻¹	%N from BNF	Method	Reference
Rio de Janeiro ²			250	84.6 (¹⁵ N) 88.8 (N-difference)	¹⁵ N isotope dilution, N-difference	Boddey <i>et al.</i> (1984)
Londrina, (Paraná)	CT	5,410	ca. 300	74.1	Ureides	Zotarelli (2000)
	NT	5,890	ca. 300	81.0		
Londrina	CT	4,380	251 (ureide) 242 (δ ¹⁵ N)	72.5 (ureide) 69.3 (δ ¹⁵ N)	Ureides and δ ¹⁵ N	Zotarelli (2000)
	NT	4,510	282 (ureide) 294 (δ ¹⁵ N)	80.4 (ureide) 84.3 (δ ¹⁵ N)		
Londrina	CT	4,128	292	87	Ureides	Hungria <i>et al.</i> (2003)
	NT	4,383	245	72		
Brasília (Cerrados)	CT	1,641	109	79.8	¹⁵ N isotope dilution	Boddey <i>et al.</i> (1990)
Uberlândia (Cerrados)	CT	3,482	234	88	Ureides	Reis <i>et al.</i> (2002)
	NT	3,293	208	94		

¹CT, conventional tillage; NT, no-tillage system.

²Plants grown in the field in concrete cylinders and evaluated 92 days after planting.

There is increasing pressure for farmers that grow high-profit crops to purchase N-fertilizer. However, as discussed by Hungria *et al.* (this volume), starter-N doses as low as 20-40 kg of N ha⁻¹ may decrease both nodulation and BNF under Brazilian conditions, with no benefits to yield (Crispino *et al.*, 2001; Mendes *et al.*, 2000; Hungria *et al.*, 2001). Indeed, in more than 50 experiments, where inoculation and fertilization with 200 kg of N ha⁻¹ have been compared (split application of N at sowing and flowering), no increase in yield due to N-fertilizer use has been reported. The results of one of these experiments are shown in Table 3. Similarly, there were no benefits when N-fertilizer was applied at a rate of 400 kg N ha⁻¹, split across ten application times (Nishi and Hungria, 1996; Hungria *et al.*, 1997, 2001; Crispino *et al.*, 2001; Loureiro *et al.*, 2001). It is important to emphasize that the application of only 30 kg of N ha⁻¹ would imply a cost of US\$13.3 ha⁻¹ to the farmer and of US\$239 million to the country per growing season, which would certainly decrease the competitiveness of the national product.

Table 3. Effects of inoculation and application of N fertilizer on nodule number (NN) and dry weight (NDW), root dry weight (RDW), yield and total seed N (TNG) of soybean, cultivar UFV-18. Experiment performed in Jaciara, Mato Grosso, in a soil with 10^5 soybean *bradyrhizobia* g^{-1} . Modified from Loureiro *et al.* (2001).

Treatment ¹	NN (n° pl ⁻¹)	NDW (mg pl ⁻¹)	RDW (g pl ⁻¹)	Yield (kg ha ⁻¹)	TNG (kg Nha ⁻¹)
Non-inoculated	38 c	1,22 bc	1,40 b	2,498 b	156 b
Non-inoculated + 200 kg N	40 bc	0,97 c	1,67 ab	2,668 ab	166 ab
SEMIA587	54 a	1,97 a	1,49 ab	2,640 ab	167 ab
SEMIA5080	49 ab	1,54 ab	1,54 ab	2,612 ab	169 ab
SEMIA587+5080	45 abc	1,55 ab	1,49 ab	2,781 a	179 a
SEMIA587+5080 + 30 kg N ha ⁻¹ at sowing	46 abc	1,24 bc	1,72 a	2,535 b	163 ab
SEMIA587+5080 + 50 kg N ha ⁻¹ at pre-flowering	50 ab	1,22 bc	1,50 ab	2,580 ab	168 ab
SEMIA587+5080 + 50 kg N ha ⁻¹ at pod filling	47 abc	1,60 ab	1,66 ab	2,527 b	169 ab
CV (%)	16.0	25.6	14.1	7.2	9.2

¹ Peat inoculants containing 10^8 cells g^{-1} were applied at a rate of 500 g 50 kg⁻¹ seeds, as a slurry with 300 ml of a 10% sucrose solution; N fertilizer applied as urea.

5. CROP MANAGEMENT IN SOUTH AMERICA

Soybean is grown in South America as a main summer cash crop. In Argentina, Paraguay, Uruguay and the southern region of Brazil, a rotation with a winter crop, usually wheat (*Triticum aestivum*), is also adopted by many farmers. Benefits to the succeeding crop are well documented, with yields after soybean considerably higher than after the other main summer crop, maize (*Zea mays*). Results from one such experiment in the state of Rio Grande do Sul, Brazil, are shown in Table 4. Most of the benefits are certainly due to BNF, which results in residues with a higher level of N for the following crop. As shown in Table 5, wheat yields in plots previously used for inoculated soybean were higher than those in plots with uninoculated soybean, with or without N-fertilizer (Nishi and Hungria, 1996; Hungria *et al.*, 1997, 2001). This increased yield and consequent profit should be considered in calculating economic benefits from the inoculation of soybean. Where rainfall is not a limiting factor in winter, other crops may be used in crop rotation with soybean, bringing benefits in terms of disease control and improvement of the soil's physical and chemical properties, among others. Unfortunately, many of these crops are not highly profitable in the short term, with farmers often reluctant to use them. Plant species successful in crop rotation with soybean include radish (*Raphanus sativus*),

blue lupin (*Lupinus angustifolium*), black oat (*Avena strigosa*), white oat (*Avena sativa*), crotalaria (*Crotalaria juncea*), millet (*Penisetum glaucum*), clovers (*Trifolium* spp.), common vetch (*Vicia sativa*), canola (*Brassica napus*), and linho (*Linum usitatissimum*) (Torres *et al.*, 1996, 2001; Gaudencio, 1999; Calegari, 2000; Gaudencio and Costa, 2001). Crop-rotation experiments have shown the benefits to soybean yield (Table 6; ranging from 12-64%), with the lowest yields usually from continuous double crop wheat-soybean or fallow-soybean. In the Brazilian Cerrados, where irregular rainfall limits activities to one crop per year, alternative crops, including millet and forage peanut (*Arachis pintoi*) have been evaluated, shown to grow well, and adopted by some farmers.

Table 4. Mean yield of wheat as a function of the previous crop.
After Wiethölter (2000).

Year	Previous crop		Difference kg ha ⁻¹
	Soybean	Maize	
	kg ha ⁻¹		
1993	3,039	2,744	295
1994	2,632	2,320	312
1995	3,418	2,995	423
1996	4,591	4,172	419
1997 ¹	1,998	1,062	936
1998	3,031	2,463	568
Mean ¹	3,342	2,939	403

¹Excess rainfall in 1997 decreased yield.

Table 5. Residual effects of soybean inoculation in the summer on wheat yield (kg ha⁻¹) in the winter. After Hungria *et al.* (2001).

Treatment	1994	1998
Non-inoculated	1,827 ab ¹	2,028 a
Non-inoculated + 200 kg N ha ⁻¹ , split at sowing and at early flowering stage	1,484 b	2,219 ab
Inoculated with efficient strains	2,000 a	2,449 b
CV (%)	12.7	9.3

¹Values followed by the same letter in a column do not show statistical difference (Duncan, $p \leq 0.05$).

Table 6. Compilation of data of experiments with crop-rotation systems in southern Brazil.

Nº of systems tested	Location	Best system ^a	Worst system ^a	Year	Yield Increase (%)	References ^b
12	Campo Mourão	Ca/Mz/Rd/Sb	Wt/Sb	2000/01	12	1
8	Londrina	Wt/Sb/Wt/Sb	Wt/Sb	1999/00	17	1
4	Londrina	Rd/Mz/Oa/Sb	Fallow/Sb	1985/86	24	2
4	Londrina	Wt/Sb/Wt/Sb	Fallow/Sb	1987/88	24	2
4	Londrina	Oa/Sb	Sb/Wt	1997/98	24	3
8	Campo Mourão	Sb/Oa/Mz-Mi	Wt/Sb	1997/98	32	3
9	Passo Fundo	Sb/Wt/Sb/Wt	Oa/Sb/Wt/Sb	1992	64	4
7	Guarapuava	Lp/Mz/Oa/Sb	Oa/Sb	1984/89	15	5
		Wt/Sb/Wt/Sb	Li/Sb/Cv/Mz			
		Oa+Cl/Sb/Oa+	Wt/Sb			
		Cv/Mz/Wt/Sb				

^aCa, canola; Mz, maize; Rd, radish; Sb, soybean; Wt, wheat; Oa, black oat; Mi, millet; Lp, lupin; Cl, clover; Cv, common vetch; Li, linho.

^b1, Gaudencio and Costa (2001); 2, Torres et al. (1996); 3, Gaudencio (1999); 4, Fontaneli et al. (2000); 5, Santos et al. (1998).

No-till (NT) soil management systems are particularly important in many areas of South America, where soils are commonly of low organic matter content, structurally fragile, and of low fertility. In NT, seeds are sown directly through the residue of the previous crop, so protecting the soil against erosion by water, maintaining soil structure, stability and moisture content, helping in the regulation of soil temperature and, with time, increasing soil organic matter content (Derpsch *et al.*, 1991). In 1999, the world area under NT was *ca.* 45.5 million ha (Table 7), but is increasing substantially. By 2002, the NT area in the USA was *ca.* 26.3 million ha, with 13 million ha of soybean (Anonymous, 2002), whereas the NT area in Brazil was 17.356 million ha (with 4.9 million of that in the Cerrados) in 2000/2001 (FEBRAPDP, 2003) and is *ca.* 20 million ha in 2002/2003, which represents half of the cropped area in Brazil. For BNF in soybean, NT results in higher number of bradyrhizobia in the soil; better nodulation, with nodulation extending lower in the soil profile; and higher BNF rates and yields in relation to conventional tillage (CT). Bradyrhizobial strain diversity in the soil is also enhanced (Ferreira *et al.*, 2000; Hungria, 2000; Hungria and Vargas, 2000). Without placing an economic value on biodiversity, Calegari (2000) estimated that a farmer adopting NT for a 50-ha area of soybean in southern Brazil would gain US\$8,902 as the result of higher yield and lower costs for maintenance, fuel, labor work, and fertilizers. This result is somewhat at variance with studies in the

northern USA, where cooler early-season soil temperatures and high soil-moisture content under conservation tillage can delay nodulation, result in enhanced root rot, and contribute to lowered flavonoid exudation from the root, limiting nodulation (Zhang *et al.*, 1996; 2002; Estevez de Jensen *et al.*, 2002).

Table 7. Estimates of world area under no-till management systems in 1998/99. After Derpsch (2000).

Country	Area (1,000 ha)
United States	19,347
Brazil	11,200
Argentina	7,270
Canada	4,080
Paraguay	790
Bolivia	200
Uruguay	50
Others	2,596
Total	45,533

In an 18-year field trial, Torres *et al.* (2001) found that CT (one disc plough and two light-disc harrow) soybean yield was higher for the first four years, but thereafter soybean yield was always higher under NT (Figure 7). Water and soil temperature conditions are always better under NT. In the first years under NT, some physical (*e.g.*, soil density), chemical, and biological (*e.g.*, lower nutrient

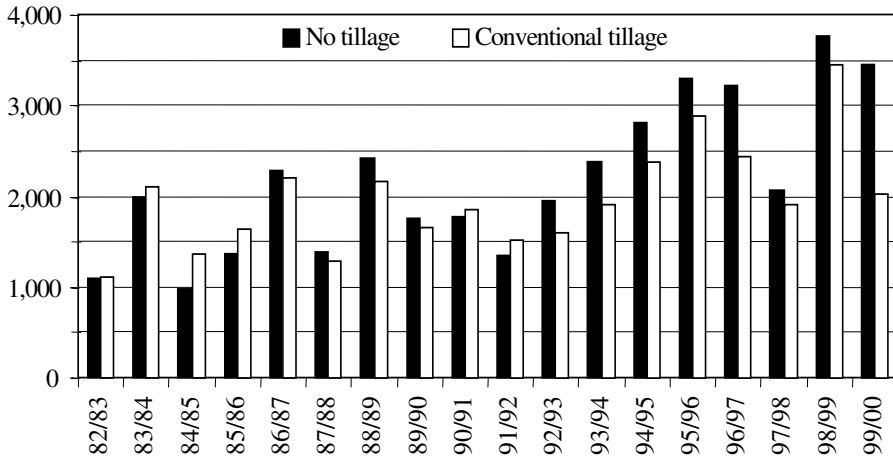


Figure 7. Soybean yield (kg ha⁻¹ on left axis) under no-tillage and conventional systems in different agricultural years. Field trial performed in an oxisol of Londrina, State of Paraná. After Torres *et al.* (2001).

content due to microbial immobilization) changes may result in lower yields (Derpsch *et al.*, 1991; Torres *et al.*, 1996, 2001). Later yields are higher because of both improvements in soil physical properties, which include soil aggregation, and increases in total soil C and labile soil C in the microbial biomass, so regulating mineral cycling (Hungria, 2000; Franchini *et al.*, 2002; Brandão-Junior *et al.*, 2002). Higher soybean yields after the first years under NT were also found in another 11-year study in southern Brazil, where soybean yield was sometimes twice that under non-conservation soil management systems (Figure 8).

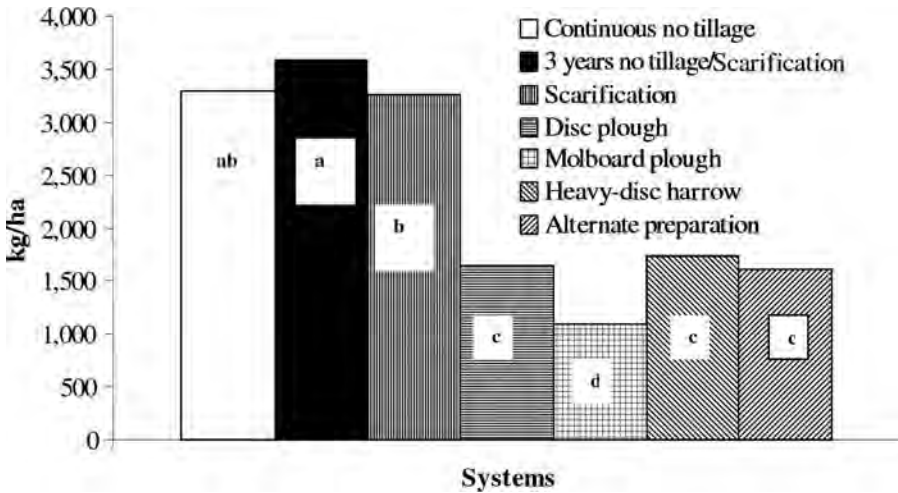


Figure 8. Soybean yields after 11 years under seven different systems of soil preparation. Experiment performed in Londrina, State of Paraná. Columns with the same letter are not statistically different (Duncan, $p \leq 0.05$). After Torres *et al.* (2001).

6. FINAL CONSIDERATIONS

Soybean production in South America has expanded enormously in recent years, both in area and in crop yield. In Brazil, improvements in crop yield and crop economics have been affected very markedly by a research and extension effort focused on BNF. Lines that are active in symbiosis with *Bradyrhizobium* have traditionally been favored in breeding. A strong and consistent effort is expected to select the most efficient inoculant strains and to market high quality inoculants. The result is that more than 50% of Brazilian farmers practice re-inoculation and quantification experiments in Brazil have shown that the crop derives 69-94% of its fixed-N need from symbiosis. In contrast, van Kessel and Hartley (2000) suggest that, for trials undertaken mainly in the USA and Australia, the percentage of fixed-N derived from BNF has declined since 1984 and is now only 54%; however, only

about 15% of farmers in the USA inoculate soybean. Some contrast between the South American and US systems of production is inevitable but any comparison should highlight problems to be avoided in South America. There is pressure to increase production in the South America by using fertilizer N. The consequences of such a change, however, need to be considered very carefully. For example, a recent commentary by Randall (2001) suggests that the more intensive corn and soybean production practiced in the mid-western USA is not sustainable. Randall points out several concerns: (i) a dependence of this production system on federal assistance; (ii) significant problems of soil erosion in the region; and (iii) declines in soil and water stewardship. Soybean growers in South America need both to maintain their focus on biological nitrogen fixation as an economic fixed-N source for this crop and to ensure that land quality is maintained (or improved) by continued study of soil quality and the factors affecting it.

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REFERENCES

- Alvarez, E. R. (1989). La soja en el Paraguay, retrospectiva y perspectiva. In *II Curso Internacional sobre Producción de Soja* (pp. 551-563). Encarnación, Brazil: Ministerio de Agricultura y Ganadería.
- Anonymous (2000). *Anuário Brasileiro da Soja 2000*. Santa Cruz do Sul, Brazil: Gazeta.
- Anonymous (2001). *Anuário Brasileiro da Soja 2001*. Santa Cruz do Sul, Brazil: Gazeta.
- Anonymous (2002). Agropecuária-Tele News at <http://orbita.starmedia.com/~telenews/agricultura.html>. Retrieved December 10, 2002.
- Boddey, R. M., Chalk, P. M., Victoria, R. L., and Matsui, E. (1984). Nitrogen fixation by nodulated soybean under tropical field conditions estimated by the ^{15}N isotope dilution technique. *Soil Biol. Biochem.*, 16, 583-588.
- Boddey, R. M., Urquiaga, S., and Neves, M. C. P. (1990). Quantification of the contribution of N_2 fixation to field-grown grain legumes - a strategy for the practical application of ^{15}N isotope dilution technique. *Soil Biol. Biochem.*, 22, 649-655.
- Bohrer, T. R. J., and Hungria M. (1998). Avaliação de cultivares de soja quanto à fixação biológica do nitrogênio, *Pesq Agropec Bras.*, 33, 937-953.
- Brandão Junior, O., Souza, R. A., Crispino, C. C., Franchini, J. C., Torres, E., and Hungria, M. (2002). Variação da biomassa microbiana em sistemas de manejo e rotação de culturas envolvendo a soja. In O.F. Saraiva and C.B. Hoffman-Campo (Eds.), *Resumos do II Congresso Brasileiro de Soja e Mercosoja* (p. 250). Londrina, Brazil: Embrapa Soja.
- Brown, A. H. D., Grant, J. E., Bhurdon, J. J., Grace, J. P., and Pullen, R. (1985). Collection and utilization of wild perennial *Glycine*. In R. Shibles (Ed.), *World Soybean Research Conference III* (pp. 345-352). Boulder, CO: Westview Press.
- Calegari, A. (2000). Plantas de cobertura e rotação de culturas no sistema plantio direto. In M. Veiga (Coord.), *Memorias de la V Reunión Bienal de la Red Latinoamericana de Agricultura Conservacionista* (CD ROM). Florianópolis, Brazil: EPAGRI/FAO.
- Chen, L. S., Figueredo, A., Villani, H., Michajluk, J., and Hungria, M. (2002). Diversity and symbiotic effectiveness of rhizobia isolated from field-grown soybean in Paraguay. *Biol. Fert. Soils*, 35, 448-457.

- Chueire, L. M. O., Bangel, E., Mostasso, F. L., Campo, R. J., Pedrosa, F. O., and Hungria, M. (2003). Classificação taxonômica das estirpes de rizóbio recomendadas para as culturas da soja e do feijoeiro baseada no sequenciamento do gene 16s RNA. *Rev. Bras. Ci. Solo*, 27, 833-840.
- CONAB (Companhia Nacional de Abastecimento) (2002a). Retrieved December 10, 2002, from http://www.conab.gov.br/politica_agricola/ConjunturaSemanal/Semana19a2308/Soja-19a2308.doc.
- CONAB (Companhia Nacional de Abastecimento) (2002b). Retrieved December 10, 2002, from http://www.conab.gov.br/politica_agricola/ConjunturaSemanal/Semana2810a0111/ConjunturaSemanaSoja13.doc.
- CONAB (Companhia Nacional de Abastecimento) (2002c). Retrieved December 10, 2002, from www.conab.gov.br/politica_agricola/Safra/Quadro9.xls.
- CONAB (Companhia Nacional de Abastecimento) (2003). Retrieved March 20, 2003, from www.conab.gov.br.
- Cregan, P., Zhu, Y., Song, Q., and Nelson, R. (2002). Sequence variation, haplotype analysis and linkage disequilibrium in cultivated and wild soybean populations. In *First International Conference on Legume Genomics and Genetics: Translation to Crop Improvement*, June 2-6 (p. 11). Minneapolis-St Paul, MN.
- Crispino, C. C., Franchini, J. C., Moraes, J. Z., Sibaldelle, R. N. R., Loureiro, M. F., Santos, E. N., et al. (2001). *Adução Nitrogenada na Cultura da Soja* (Comunicado Técnico, 75). Londrina, Brazil: Embrapa Soja.
- Delannay, X., Rodgers, D. M., and Palmer, R. G. (1983). Relative genetic contributions among ancestral lines to North American soybean cultivars. *Crop Sci.*, 23, 944-949.
- Derpsch, R. (2000). Expansión mundial de la siembra directa y avances tecnológicos. In M. Veiga (Coord.), *Memorias de la V Reunión Bial de la Red Latinoamericana de Agricultura Conservacionista* (CD ROM). Florianópolis, Brazil: EPAGRI/FAO.
- Derpsch, R., Roth, C. H., Sidiras, N., and Kopke, U. (1991). *Controle da erosão no Paraná, Brasil: Sistemas de cobertura do solo, plantio direto e preparo conservacionista do solo* (Sonderpublikation der GTZ, 245). Eschborn, Germany: GTZ.
- Devine, T. E. (1984). Genetics and breeding of nitrogen fixation. In M. Alexander (Ed.), *Biological Nitrogen Fixation: Ecology, Technology and Physiology* (pp. 127-154). New York, NY: Plenum Press.
- Döbereiner, J., and Arruda, N. B. (1967). Interrelações entre variedades e nutrição na nodulação e simbiose da soja. *Pesq. Agropec. Bras.*, 2, 475-487.
- D'utra, G. (1882). Soja. *J Agricultor* VII, 185-188.
- D'utra, G. (1899). Nova cultura experimental da soja. *Bol. Inst. Agron. Campinas*, 10, 582-587.
- Estevez de Jensen, C., Percich, J. A., and Graham, P. H. (2002). Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. *Field Crops Res.* 74, 107-115.
- FEBRAPDP (Federação Brasileira de Plantio Direto na Palha) (2003). Retrieved February 24, 2003, from http://www.febrapdp.org.br/pd_area_estados.htm.
- Ferreira, M. C., Andrade, D. S., Chueire, L. M. de O., Takemura, S. M., and Hungria, M. (2000). Effects of tillage method and crop rotation on the population sizes and diversity of bradyrhizobia nodulating soybean. *Soil Biol. Biochem.*, 32, 627-637.
- Figueredo, A. (1998). *Fijación Biológica de Nitrógeno*. Asunción, Paraguay: Universidad Nacional de Asunción. Dirección de Investigación, Postgrado y Relaciones Internacionales.
- Fontaneli, R. S., Santos, H. P., Voss, M. and Ambrosi, I. (2000). Rendimento e nodulação de soja em diferentes rotações de espécies anuais de inverno sob plantio direto. *Pesq. Agropec. Bras.*, 35, 349-355.
- Franchini, J. C., Crispino, C. C., Souza, R. A., Torres, E., and Hungria, M. (2002). Biomassa microbiana e emissão de CO₂ em sistemas de manejo do solo e rotação de culturas. In O. F. Saraiva and C. B. Hoffman-Campo (Eds.), *Resumos do II Congresso Brasileiro de Soja e Mercosoja* (pp. 133). Londrina, Brazil: Embrapa Soja.
- Gaudencio, C. A. (1999). Sistema de rotação de espécies perenes e anuais para recuperação biológica de Lassolos Roxos eutróficos e integração agropecuária, na Região Meridional. In *Resultados de Pesquisa da Embrapa Soja - 1998* (pp. 181-210; Documentos, 125). Londrina, Brazil: Embrapa Soja.
- Gaudencio, C. A., and Costa, J. M. (2001). Rotação de culturas anuais no Planalto Meridional Paranaense. In *Resultados de Pesquisa da Embrapa Soja -2000, Manejo do Solo e Plantas Daninhas* (pp. 19-25; Documentos, 161). Londrina, Brazil: Embrapa Soja.
- Giller, K. E. (2001). *Nitrogen fixation in tropical cropping systems*. Wallingford, UK: CABI Publishing.

- Goedert, W. J. (1985). *Solos dos Cerrados, Tecnologias e Estratégias de Manejo*. Planaltina, Brazil: Embrapa Cerrados.
- Graham, P. H., and Vance, C. P. (2003). Legumes: Importance and constraints to greater utilization. *Plant Physiol.*, *131*, 872-877.
- Gray, G. E. (1936). *All About the Soya Bean*. London, UK: John Bale.
- Hungria, M. (2000). Características biológicas em solos manejados sob plantio direto. In M. Veiga (Coord.), *Memórias de la V Reunión Bienal de la Red Latinoamericana de Agricultura Conservacionista* (CD ROM). Florianópolis, Brazil: EPAGRI/FAO.
- Hungria, M., and Bohrer, T. R. J. (2000). Variability of nodulation and dinitrogen fixation capacity among soybean cultivars. *Biol. Fert. Soils*, *31*, 45-52.
- Hungria, M., and Vargas, M. A. T. (2000) Environmental factors affecting N₂ fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Res.*, *65*, 151-164.
- Hungria, M., Campo, R. J., Franchini, J. C., Chueire, L. M. O., Mendes, I. C., Andrade, D. S., et al. (2003). Microbial quantitative and qualitative changes in soils under different crops and tillage management systems in Brazil. In *Annals of Technical Workshop on Biological Management of Soil Ecosystems for Sustainable Agriculture* (CD ROM). Londrina, Brazil: Embrapa Soja/FAO.
- Hungria, M., Campo, R. J., and Mendes, I. C. (2001). *Fixação Biológica do Nitrogênio na Cultura da Soja* (Circular Técnica, 13). Londrina, Brazil: Embrapa Soja/Embrapa Cerrados.
- Hungria, M., Loureiro, M. F., Mendes, I. C., Campo, R. J., and Graham, P. H. (2005). Inoculant preparation, production and application. This volume.
- Hungria, M., Vargas, M. A. T., Campo, R. J., and Galerani, P. R. (1997). *Adução Nitrogenada na Soja?* (Comunicado Técnico, 57). Londrina, Brazil: EMBRAPA-CNPSo.
- Hymowitz, T. (1970). On the domestication of soybean. *Econ. Bot.*, *24*, 408-421.
- Hymowitz, T., and Newell, C. A. (1980). Taxonomy, speciation, domestication, dissemination, germplasm resources and variation in the genus *Glycine*. In R.J. Summerfield and A.H. Bunting (Eds.), *Advances in Legume Science* (pp. 251-264). Kew, UK: Royal Botanic Gardens.
- Hymowitz, T., and Singh, R. J. (1987). Taxonomy and speciation. In J.R. Wilcox (Ed), *Soybeans, Improvement, Production and Uses*. 2nd Edit. (pp. 23-48). Madison, WI: Amer. Soc. of Agronomy.
- Kennedy, A. R. (1995). The evidence for soybean products as cancer preventative agents, *J. Nutr.*, *125*, S733-S743.
- Loureiro, M. F., Santos, E. N., Hungria, M., and Campo, R. J. (2001). *Efeito da Reinoculação e da Adução Nitrogenada no Rendimento da Soja em Mato Grosso* (Comunicado Técnico, 74). Londrina, Brazil: Embrapa Soja.
- Mendes, I. C., Hungria, M., and Vargas, M. A. T. (2000). *Resposta da Soja à Adução Nitrogenada na Semeadura, em Sistemas de Plantio Direto e Convencional na Região do Cerrado* (Boletim de Pesquisa, 12). Planaltina, Brazil: Embrapa Cerrados.
- Miró, D. A. (1989). La expansión del cultivo de soja y su impacto en el comercio exterior argentino. In *IV Conferência Mundial de Investigación en Soja* (pp. 18-27). Buenos Aires, Argentina: Asociación Argentina de la Soja.
- Molteni, A., Brizio-Molteni, L., and Persky, V. (1995). *In vitro* hormonal effects of soybean isoflavones, *J. Nutr.*, *125*, S751-S756.
- Morse W. J. (1950). History of soybean production. In K.L. Markley (Ed), *Soybeans and Soybean Products*, vol. 1 (pp. 3-59). New York, NY: Interscience Publ. Inc.
- Nicolás, M. F. (2001). *Fixação Biológica do Nitrogênio e Nodulação em Cultivares de Soja Brasileiras: Controle Genético e Mapeamento dos QTLs que Controlam esses Caracteres*. Curitiba, Brazil: Universidade Federal do Paraná (Ph.D. Thesis).
- Nicolás, M. F., Arias, C. A. A., and Hungria, M. (2002). Genetics of nodulation and nitrogen fixation in Brazilian soybean cultivars. *Biol. Fert. Soils*, *36*, 109-117.
- Nishi, C. Y. M., and Hungria, M. (1996). Efeito na reinoculação na soja [*Glycine max* (L.) Merrill] em um solo com população estabelecida de *Bradyrhizobium* com as estirpes SEMIA 566, 586, 587, 5019, 5079 e 5080. *Pesq. Agrop. Bras.*, *31*, 359-368.
- Oliveri, N. J., Perucca, C. E., and Morel, F. (1981). *Evolución de Cultivares de Soja (Glycine max L. Merr.) de Origen Brasileño*. Instituto Nacional de Tecnología Agropecuaria - Estación Experimental Agropecuaria INTA, Misiones, (Informe Técnico, 35).
- Piper, C. V., and Morse, W. J. (1923). *The Soybean*. New York, NY: McGraw-Hill.

- Probst, A. H., and Judd, R. W. (1973). Origin, history and develop, and world distribution. In B.E.Caldwell (Ed.) *Soybean, Improvement, Production and Uses* (pp. 1-15). Madison, WI: American Society of Agronomy.
- Pulver, E. L., Brockman, F., and Wein, H. C. (1982). Nodulation of soybean cultivars with *Rhizobium* spp. and their response to inoculation with *R. japonicum*. *Crop Sci.*, 22, 1065-1070.
- Randall, G. W. (2001). Intensive corn soybean production is not sustainable. Retrieved December 10, 2002, from www.extension.umn.edu/extensionnews/2001/IntensiveCornSoybeanAgriculture.html.
- Reis, E. H. S., Lara Cabezas, W. A. R., Alves, B. J. R., and Caballero, S. U. (2002). Suplementação de nitrogênio mineral na cultura da soja (*Glycine max*) estabelecida em sistema plantio direto e convencional. In O.F. Saraiva and C.B. Hoffman-Campo (Eds.), *Resumos do II Congresso Brasileiro de Soja e Mercosoja* (pp. 215). Londrina, Brazil: Embrapa Soja.
- Santos, H. P., Lhamby, J. C. B., and Wobeto, C. (1998). Efeito de culturas de inverno em plantio direto sobre a soja cultivada em rotação de culturas. *Pesq. Agropec. Bras.*, 33, 289-295.
- Saumell, H. (1977). *Soja, Información Técnica para su Mejor Conocimiento y Cultivo*. Buenos Aires, Argentina: Editorial Hemisferio Sur.
- Smartt, J., and Hymowitz, T. (1985). Domestication and evolution of grain legumes. In R.J. Summerfield and E.H. Roberts (Eds.), *Grain Legume Crops* (pp. 37-72). London, UK: Collins Professional and Technical Books.
- Spehar, C. R. (1995). Impact of strategic genes in soybean on agricultural development in the Brazilian tropical savannas. *Field Crops Res.*, 41, 141-146.
- Thies, J. E., Singleton, P. W., and Bohlool, B. B. (1991). Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Appl. Environ. Microbiol.*, 57, 19-28.
- Toledo, J. F. F., Almeida, L. A., Kiihl, R. A. S., Panizzi, M. C. C., Kaster, M., Miranda, L. C., and Menosso, O. G. (1994). Genetics and breeding. In *Tropical Soybean Improvement and Production* (pp. 19-36). Rome, Italy: FAO.
- Torres, E., Norman, N., and Garcia, A. (1996). Sucessão soja/aveia preta. In *Resultados de Pesquisa de Soja – 1990/91*, vol. 2, pp. 337-341 (Documentos 99). Londrina, Brazil: EMBRAPA-CNPSo.
- Torres, E., Saraiva, O. F., Gazziero, D. L. P., Pires, M. S. and Loni, D. A. (2001). Avaliação de sistemas de preparo do solo e manejo do plantio direto envolvendo sucessão e rotação de culturas. In *Resultados de Pesquisa da Embrapa Soja - 2000, Manejo do Solo e Plantas Daninhas* (pp. 9-14). Londrina, Brazil: Embrapa Soja.
- USDA (United States Department of Agriculture) (2002a). Retrieved December 10, 2002, from <http://ffas.usda.gov/wap/circular/2002/02-12/Wap%2012-02.pdf>.
- USDA (United States Department of Agriculture) (2002b). Retrieved December 10, 2002, from <http://ffas.usda.gov/oilseeds/circular/2001/01-12/full.pdf>.
- Unkovich, M. J., and Pate, J. S. (2000). An appraisal of recent field measurements of symbiotic N₂ fixation by annual legumes. *Field Crops Res.*, 65, 211-228.
- Van Kessel, C., and Hartley, C. (2000). Agricultural management of grain legumes; has it led to an increase in nitrogen fixation? *Field Crops Res.*, 65, 165-181.
- Vargas, M. A. T., and Hungria, M. (1997). Fixação biológica do N₂ na cultura da soja. In M. A. T. Vargas and M. Hungria (Eds.), *Biologia dos Solos de Cerrados* (pp. 297-360), Planaltina, Brazil: Embrapa-CPAC.
- Vargas, M. A. T., Peres, J. R. R., and Suhet, A. R. (1982). Fixação de nitrogênio atmosférico pela soja em solos de cerrado. *Inf. Agrop.*, 8, 20-23.
- Wiethölter, S. (2000). Características químicas de solo manejado no sistema plantio direto. In M. Veiga (Coord.), *Memorias de la V Reunión Bienal de la Red Latinoamericana de Agricultura Conservacionista* (CD ROM). Florianópolis, Brazil: EPAGRI/FAO.
- Zhang, F., Charles, T. C., Pan, B. and Smith, D. L. (1996). Inhibition of the expression of *Bradyrhizobium japonicum nod* genes at low temperatures. *Soil. Biol. Biochem.*, 28, 1579-1583.
- Zhang, H., Prithiviraj, B., Souliemanov, A., D'Aoust, F., Charles, T. C., Driscoll, B. T., and Smith, D. L. (2002). The effect of temperature and genistein concentration on lipo-chitoooligosaccharide (LCO) production by wild type and mutant strains of *Bradyrhizobium japonicum*. *Soil Biol. Biochem.*, 34, 1175-1180.
- Zotarelli, L. (2000). *Balço de Nitrogênio na Rotação de Culturas em Sistemas de Plantio Direto e Convencional na Região de Londrina, PR*. Seropédica, Brazil: Universidade Federal Rural do Rio de Janeiro (M.Sc. Thesis).