Chapter 2

NITROGEN FIXATION BY SOYBEAN IN NORTH AMERICA

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1. SOYBEAN: PATHWAYS TO NORTH AMERICA AND ESTABLISHMENT AS A CROP

The soybean, *Glycine max* (L.) Merr., is one of the world's most important and versatile crop species. Currently grown on 76 million hectares of land on five continents (FAO, 2003), soybeans are a staple part of the human diet, particularly in Asia, where they are consumed both as a vegetable and as a variety of processed food products. Large quantities of whole soybeans, soybean oil, and soybean protein products also are traded on world markets.

A member of the legume subfamily Papilionoideae, *G. max* is known only as a cultivated species and has never been found growing in the wild (Hymowitz, 1970; 1990). Its weedy uncultivated ancestor, *Glycine soja* Sieb and Zucc., is widely distributed in Asia, where it grows in fields and open areas. Soybean was domesticated from *G. soja* by farmers in northeastern China, who recognized its value as a food crop. The precise time frame is unknown, but soybean was likely brought into cultivation more than three millennia ago, during the Shang Dynasty (*ca*. 1700-1100 B.C.). It had spread to adjacent parts of China and Korea by the first century A.D. and then on to other parts of Asia, where it became an increasingly important component of the human diet.

An English seaman, Samuel Bowen, introduced soybean to North America in the decade just prior to the American Revolution (Hymowitz and Harlan, 1983). He had obtained a quantity of seeds from China *via* England, and he gave them to an acquaintance named Henry Yonge. Yonge was the Surveyor General of the Colony of Georgia, and beginning in 1765, he grew soybeans on his plantation near Savannah. Bowen had some of the harvested seeds processed into soy sauce and

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noodles and began experimenting with other manufacturing methods, but the undertaking ended with his death in 1777.

Soybeans were reintroduced to North America on a number of occasions after Bowen, and we know that American botanists and horticulturists planted and studied them during the first half of the nineteenth century (Hymowitz, 1990). It was the year 1851, though, that marked the rebirth of investigations into the potential of soybean as a North American crop plant. This time, the seeds came from Japan *via* San Francisco, where Dr. Benjamin Franklin Edwards had been pressed into service to examine a group of Japanese fishermen for contagious diseases (Hymowitz, 1990). The physician found the fishermen to be healthy and, in gratitude, they presented him with a package of soybean seeds. Dr. Edwards took this unusual gift with him when he returned home to Alton, Illinois. Fortuitously, this small city lies within a vast region of farmland that is well suited to soybean production. Edwards presented the seeds to a horticulturist friend, who immediately planted them and began to distribute succeeding crops of seeds through a network of horticulturists. Within just a few years, a loosely organized group of farmers and gardeners had evaluated soybean across the U. S. Midwest as a potential forage crop for animals. As was common then, they communicated their experiences with the new crop by means of notices and articles in the popular agricultural press.

The ability of legumes to fix nitrogen symbiotically had not yet been established and, if these early American soybean growers noticed root nodules, they could not have been aware of their agronomic significance. Symbiotic nitrogen fixation was initially documented by the experiments of Hellriegel and Willfarth in 1888 (Fred *et al.,* 1932) and the soybean root-nodule organism, *Bradyrhizobium japonicum*, was first isolated 7 years later (Kirchner, 1895). These scientific discoveries in Europe coincided with yet another introduction of soybean into the U.S., one that would firmly establish the species as a major agricultural crop.

This time, agricultural scientists deliberately brought the crop into the country, investigated its agronomic potential, and collaborated with other researchers to assure the success of the endeavor (Hymowitz, 1990). The first experiments were conducted by scientists from the New Jersey Agricultural Experiment Station at Rutgers College, who had brought seeds from Europe in 1878, multiplied them in the field, and soon distributed germplasm to agricultural experiment stations in other states. Seeds were also imported directly from Asia and, by the final decades of the nineteenth century, soybeans had become the subject of serious ongoing investigations to determine their suitability as an American crop.

The artificial inoculant industry dates back to 1895, when Nobbe and Hiltner were first granted patents on the use of pure cultures of rhizobia for legume seed treatments (Fred *et al.,* 1932). Even prior to this time, scientists had begun to determine the feasibility of ensuring nodulation and nitrogen fixation by transfer of small amounts of soil from fields in which soybeans had been grown to new fields. Cottrell and colleagues (1900) in Kansas and Lane (1900) in New Jersey were among the first to conduct such experiments with soybean in the United States and, as early as 1904, farmers had begun to offer “infected soil” for sale (Windish, 1981). Inoculation came into common practice during the early part of the twentieth century (Figure 1) and, as soybean began to establish itself commercially, individual states started to produce inoculants for farmers. In 1928 alone, Missouri and Wisconsin distributed 38,522 and 14,793 cultures, respectively, to soybean farmers (Fred *et al.,* 1932).

Figure 1. An early soybean inoculant produced by the Hazelmere Bacteria Company in Bowling Green, Indiana. Adapted from Fred et al., 1932.

2. SOYBEAN PRODUCTION IN NORTH AMERICA

Official records of soybean production in the United States did not appear until 1924 (Barnhart, 1954). Previously and for some time thereafter, the crop was grown on a relatively small scale, mostly as either a forage legume or a green manure to enhance soil fertility. Production initially was centered in the eastern part of the country, such that the leading states in 1919 were North Carolina, Kentucky, Mississippi, Virginia, and Alabama. Soybean production areas moved west rapidly over the ensuing five years, such that, by 1924, the leading states were Illinois, Indiana, Tennessee, North Carolina, and Missouri (Windish, 1981).

Soybean first became a significant American crop during World War II, when traditional sources of vegetable oils were disrupted by the conflict. Production of soybean for oil exceeded that for hay in 1941 (Barnhart, 1954) and, by 1942, the United States overtook Manchuria as the world's leading producer of the crop (Windish, 1981). The latter change is illustrated in Figure 2, which documents the growth and geographical redistribution of soybean production in the United States from 1927 to 2000. Soybean production was in its infancy in 1927 and scattered throughout the Midwest and southeast. By 1961, when soybean had become a major crop and was planted on 10.9 million hectares (FAO, 2003), production was concentrated in three Midwestern states; Illinois, Indiana, and Iowa. Smaller but significant acreages were also scattered in the south central and southeastern regions and in Minnesota, a state originally assumed to be too cold to sustain growth of crop (Cottrell *et al.,* 1900). Huge increases in soybean production are evident in the data from 2000, which show the strengthening and continued dominance of Illinois, Indiana, and Iowa. U. S. soybeans covered an area of 29.3 million hectares in 2000, when production exceeded 75 million metric tons for the first time (FAO, 2003).

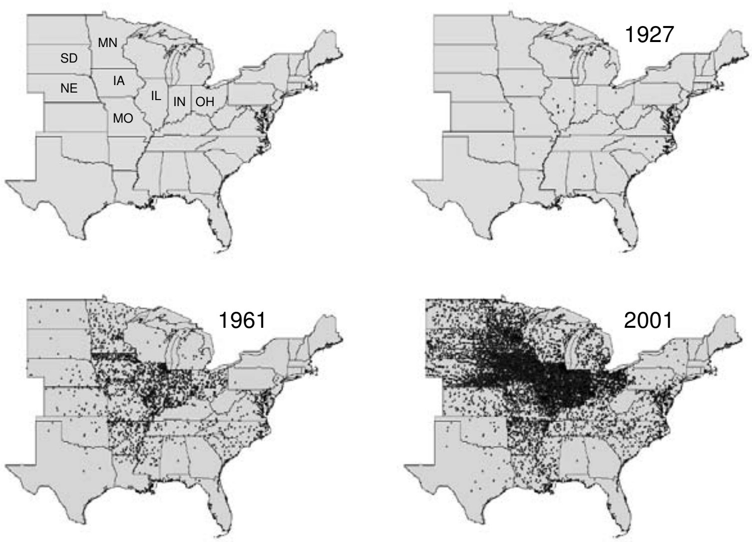

Figure 2. Changes in the magnitude and geographical distribution of United States soybean production from 1927 to 2001.
One dot is equivalent to 9,000 metric tons. The upper left hand map identifies the principal soybean-growing states of the Midwest: IA = Iowa, IL = Illinois, IN = Indiana, MN = Minnesota, MO = Missouri, NE = Nebraska, OH = Ohio, SD = South Dakota. Data Source: National Agricultural Statistics Service, United States Department of Agriculture.

Mexico and Canada produce relatively minor amounts of soybeans in comparison to the United States (Figure 3). Plantings in Mexico have fluctuated significantly over the past 40 years, and production has declined over the past decade. Soybean oil is the most important plant oil produced in Mexico, but domestic soybean production does not meet domestic needs. As recently as 1996-1997, imports from the United States accounted for nearly half of the soybeans crushed for oil in Mexico (Juarez, 1998).

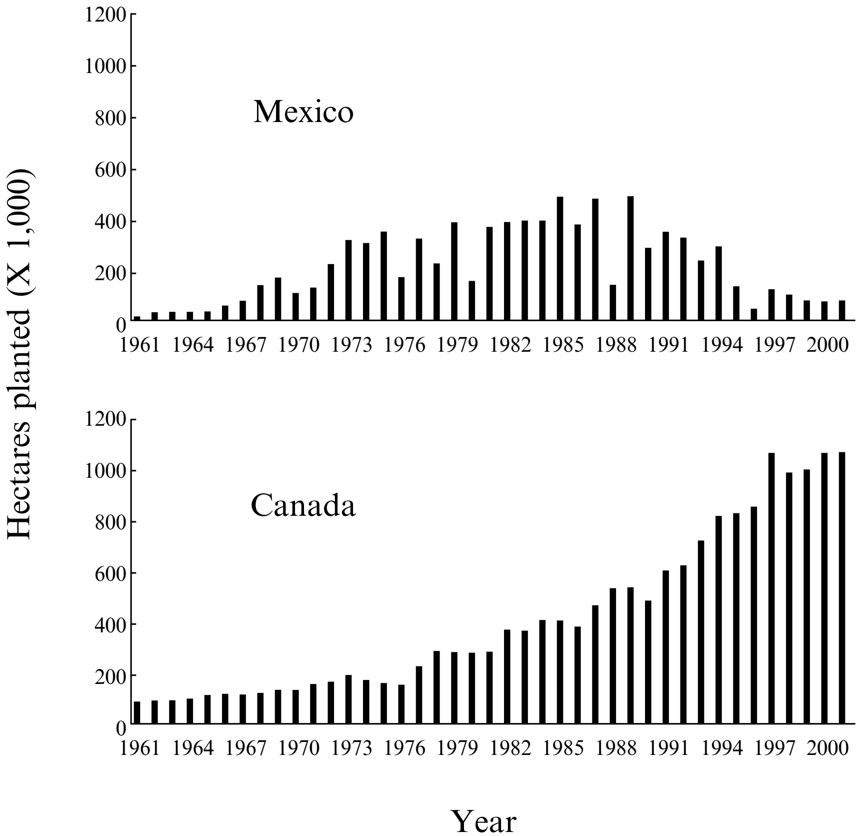

Figure 3. Soybean production in Mexico and Canada from 1961 to 2001.
Data Source: FAOSTAT Agricultural Database of the United Nations Food and Agricultural Organization.

Soybean was grown in Canada as early as 1939, when a total of 3,960 hectares was planted (Anonymous, 1939) and test fields were sown in Western Canada as early as 1941 (Rennie and Dubetz, 1984). But it was only during the past 25 years, as early maturing varieties were developed, that Canada became a significant soybean producer. The area planted to soybeans in Canada increased from 85,800 hectares in 1961 to 279,200 hectares in 1981 to 1.1 million hectares in 2001 (FAO, 2003). About 83% of Canada's current production areas lie in Ontario, primarily in the southern counties of the province along the north shore of the Great Lakes (Ontario Ministry of Agriculture and Food, 2003). Together, Mexican and Canadian soybean acreages are equivalent to less than 5% of the U. S. total.

3. MAJOR SOYBEAN CROPPING SYSTEMS

Soybean is normally grown in sequence with other crops throughout the major production areas of the American Midwest (Hoeft *et al.,* 2000). Crop rotations of this sort are common agricultural practices and they were in place prior to widespread introduction of soybean in the middle of the twentieth century. The appearance of soybean at this time coincided with and was facilitated by another fundamental change, the replacement of animal traction with machinery. Before widespread mechanization, the rotation cycle usually encompassed four years and included oats, two years of a legume, such as either alfalfa or clover, a year of corn, and then oats again. This sequence ensured adequate feed for farm animals as well as an abundant supply of fixed nitrogen, which was not yet available as commercial fertilizer.

Prior to World War II, it was possible to simply replace one of the forage legumes with soybeans for use as hay (Barnhart, 1954). With the advent of vast soybean acreages and the diminished need for animal feed, the rotation was simplified to today's typical corn-soybean sequence. About 80% of the total cropland in the Midwest is currently managed in this way (Hoeft *et al.,* 2000). The widespread acceptance of this practice is evident from the near identity of total corn and total soybean acreages in any given area and growing season. In 2001, for example, Illinois farmers planted 4.4 million hectares of corn and 4.3 million hectares of corn (Illinois Agricultural Statistics Service, 2002). In some of the more southern areas of the Midwest, corn often is replaced with either wheat or sorghum, but the two-year rotation with soybean is maintained (Hoeft *et al.,* 2000). Thus, regardless of the identity of the second crop and as a rule of thumb, farmers in the American Midwest generally plant soybean every other year.

4. BIOLOGICAL NITROGEN FIXATION BY SOYBEAN IN NORTH AMERICA

Biological nitrogen fixation has been assessed by a variety of protocols, each with its own inherent advantages and shortcomings (Bremner, 1977; Burris, 1974; Chalk, 1985; Danso, 1986; Danso *et al.,* 1993; Hardarson and Danso, 1993; Knowles, 1981). ^{15}N-based techniques have become the method of choice for legumes growing in the field (Unkovich and Pate, 2000), in part because they allow the calculation of nitrogen fixation as kg N/ha (Hardarson and Danso, 1993).

Two different ^{15}N-based protocols have been devised (Bergerson, 1980; Hardarson, 1990; Peoples *et al.,* 1989). The natural abundance method takes advantage of the fact that the heavy isotope is relatively more prevalent in the soil than in the atmosphere. Tissues from plants with direct access to N_2 from the atmosphere, thus, have lower ^{15}N enrichment than do plants that derive nitrogen exclusively from the soil, and the resulting differences in isotope abundances can be quantified. Isotope dilution-based techniques rely on enrichment of the soil supporting the growth of nitrogen-fixing legumes and nonfixing reference plants - often non-nodulating mutants - with ^{15}N-labeled fertilizers. Because the fixing plants capture gaseous N_2 from the air, the ratio of ^{15}N to ^{14}N in their tissues decreases relative to that in the non-fixing plants and this difference allows the percentage of nitrogen derived from the atmosphere to be calculated.

The literature on global nitrogen fixation is filled with uncertainty (Burns and Hardy, 1975; Hardy and Havelka, 1975; LaRue and Patterson, 1981; Smil, 2001; Sprent, 1986). This situation is partly due to differences of opinion about the most appropriate application of techniques to measure nitrogen fixation - and the interpretation of experimental results. But it also reflects a whole series of uncontrolled variables that are known to influence nitrogen fixation in the field. These include environmental factors, such as moisture, temperature, soil type, and nutrient status (especially that of mineral nitrogen), as well as plant genotype, adequacy and efficiency of nodulation (Brockwell and Bottomley, 1995), and a whole variety of agronomic practices.

LaRue and Patterson (1981) summarized a series of early experiments to determine nitrogen fixation by soybean and concluded that "the upper limit for average fixation by soybeans in American agriculture is 75 kg N/ha." This estimate is based on the assumption that soybeans growing in U.S. Midwestern fields accumulate a total of 150 kg N/ha and that 50% of the total is supplied by nitrogen fixation. This estimate agrees well with data from nine sets of field experiments, which were conducted in the 1970s and early 1980s. Three were based on isotope-dilution methods and were conducted on typical silty loam soils in the Midwest. The amounts of fixed nitrogen recorded in these experiments were reasonably uniform, given the observed variations in growth conditions, and ranged from 43-146 kg N/ha in eastern Nebraska (Deibert *et al.,* 1979) to 76-152 kg N/ha in south-central Minnesota (Ham and Cardwell, 1978) to 103 kg N/ha in east-central Illinois (Johnson *et al.,* 1975).

Unkovich and Pate (2000) revisited the issue of nitrogen fixation by legumes in the field and have summarized both a vast amount of experimental data as well as recent refinements in our understanding of measurement techniques. They conclude that average nitrogen fixation by dry land soybean supplies 100 kg N/ha on a worldwide basis and, if root biomass is included in the calculations, the amount increases to 142 kg N/ha. These figures are greater and likely more accurate than earlier estimations, but they remain subject to the considerable variability and uncertainty inherent in making generalizations about a crop cultivated under diverse agronomic regimes and across wide geographical areas. The practice of irrigation illustrates just one of these uncertainties. When soybean is grown under irrigation, levels of nitrogen fixation are elevated to 175 kg N/ha - with values approaching 250 kg N/ha if roots are included (Peoples *et al.,* 1995). Just one factor, assured optimal moisture can, therefore, nearly double nitrogen fixation in comparison to average field conditions.

5. PERSPECTIVES

If we accept the most recent figure of 142 kg N/ha, then we can estimate that, for 2001, soybeans growing in North America captured about 4.3 million metric tons of nitrogen from the atmosphere and cycled it into plant organic matter. This is a significant number but, as has been pointed out (Graham and Vance, 2000; Smil, 1997), the world's agriculture is relying less and less on biologically fixed nitrogen and more and more on synthetic fertilizer. Given the immense energy required to synthesize anhydrous ammonia (Smil, 2001), this substitution is likely not sustainable over time.

Dry mass accumulation - plant growth - is often viewed as a principle driver for biological nitrogen fixation, but it would be erroneous to conclude that, as soybean yields have increased in recent decades, nitrogen fixation has just simply increased in a corresponding fashion (Unkovich and Pate, 2000). Fertilizer use is rising in cropping systems, such as the corn-soybean rotation of the U. S. Midwest, and it is well known that nodulation and nitrogen fixation are directly inhibited by combined nitrogen (Streeter, 1988). New soybean varieties are being released and we continue to know very little about rhizobia in cropped soils. These complexities both confound our understanding of biological nitrogen fixation in soybean and offer fertile ground for future investigations.

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