AGRICULTURAL BIOTECHNOLOGY: BENEFITS OF TRANSGENIC SOYBEANS

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1. Introduction

Soybeans and other crops have been improved genetically for many decades through traditional crop breeding – a technique that requires that species be sexually compatible. With the development of biotechnology methods, scientists have the ability to transfer single genes from one living organism into another, regardless of species or sexual compatibility. Varieties that are developed through the transfer of genes between species that are not sexually compatible are referred to as "transgenic."

Transgenic soybean plants have been developed with a gene from a soil bacteria that allows the use of an herbicide that would normally kill soybeans. Transgenic soybean varieties have been adopted rapidly by U.S. growers (Table 1). Transgenic soybean acreage represents approximately 60 % of the total acreage planted to transgenic crops grown in the U.S. (Corn and cotton are the other major crops with transgenic variety plantings in the U.S.)

Farmers are planting transgenic soybeans in the U.S. because of the weed control benefits that result from the use of an herbicide that damages conventional varieties. This herbicide, glyphosate (trade name "Roundup") is a cost effective solution to many of the weed problems that U.S. soybean growers must overcome every year. The herbicide tolerant transgenic soybean is referred to as "Roundup Ready." The purpose of this report is to describe and quantify the weed control benefits provided on soybean acreage planted with the transgenic varieties in 1998.

TABLE 1: Herbicide Tolerant Transgenic Soybean Acreage: U.S.

Year	# of Acres (Millions)	% of U.S. Acreage
1996	1	2
1997	9	13
1998	27	37
1999	35	47

Source: Monsanto

2. U.S. Soybean Production

Soybean is one of the three largest crops grown in the U.S., grown on approximately 70 million acres in 1999 an area smaller than corn and comparable to wheat.

Thirty states have significant soybean acreage. Soybean production is centered in the Midwest where ten states account for 73% of U.S. acreage and production. The states of Illinois and Iowa each account for more than 10 million acres of soybeans [58]. The Delta states of Mississippi, Arkansas and Louisiana together account for 10% of U.S. acreage of soybeans.

Soybeans were grown primarily as forage crops in the U.S. through the 1930's. The production of soybeans increased in 1934 in response to the severe drought of that year in the upper Midwest. The performance of soybeans under drought conditions was better than corn, thereby enticing farmers to try this relatively new crop [100]. Soybean harvested for seed represented 40% of the planted acreage in 1939, indicating expanding acreage for processing.

Prior to World War II, the U.S. imported 40% of its edible fats and oils. At the advent of the War, this supply was cut, and processors turned to domestically produced soybean oil [1]. By 1944, 72% of the planted soybean acreage was harvested for seed [100].

World demand for cooking oil, salad oil and red meat increased substantially immediately after World War II. These demands stimulated the rapid expansion of soybean production in the U.S. [2]. In the 1950's, soybean meal became available as a low-cost, high-protein feed ingredient, triggering explosive growth in U.S. livestock and poultry production. U.S. soybean acreage increased from less than 20 million acres in the early 1950's to over 50 million acres in the 1970's and a record 72 million acres in 1998, representing 27% of the acreage planted to all crops in the U.S. Figure 1 charts the increase in U.S. soybean acreage from 1944 to 1999.

Total annual U.S. soybean production increased from about 500 million bushels in the 1950's to over 2.5 billion bushels (150 billion pounds) in 1997. Figure 2 charts U.S. soybean production 1944-1999. The increase in annual total U.S. production resulted not only from the expansion of acreage (Figure 1) but also from a steady rise in average U.S. soybean yield per acre. Figure 3 charts the increase in average U.S. soybean yields 1944-1999.

Two-thirds of the increase in soybean yields in the U.S. from 1943 to 1960 is attributed to the planting of improved varieties, with increased fertilizer use accounting for the remaining one-third of the increase [90].

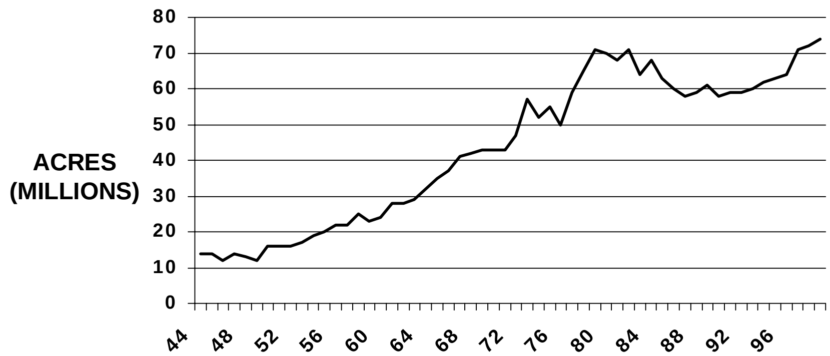
A statistical analysis of the change in soybean yields from 1965 to 1979 concluded that weed control provided by the use of herbicides accounted for 62 percent of the yield increase while further variety improvements accounted for 13 percent of the increase [73].

In the 1990's, U.S. soybean farmers received an average of $6 per bushel. However, prices dropped to $5/bushel in 1998 [1]. The total value of the soybean crop was $14 billion in 1998, representing approximately 15% of the value of all crops grown in the U.S.

The U.S. produces nearly half of the total world soybean crop. Other major producing countries include Brazil, China and Argentina. Competition in export markets comes from Brazil and Argentina as China is a net importer of soybeans. The U.S. accounted for 60% of world exports of soybeans in 1997/98.

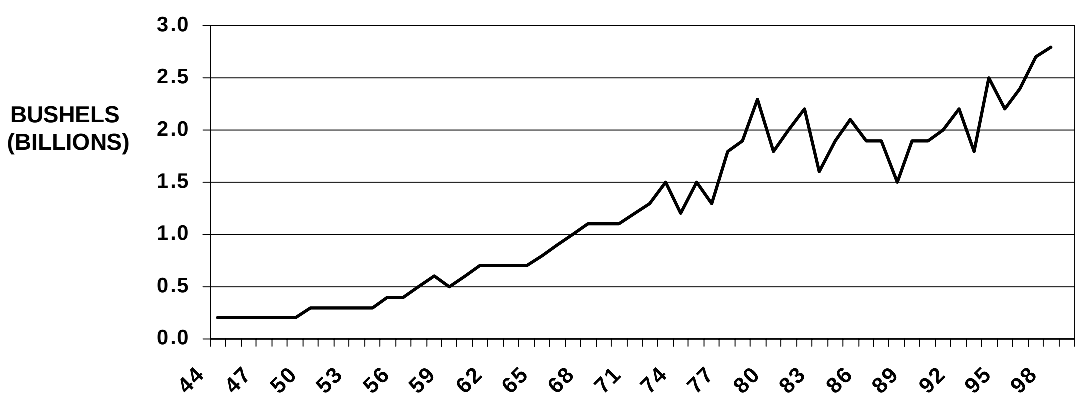
About 28% of U.S. soybeans (valued at $4.8 billion) were exported in 1998 [1]. The U.S. exported soybean meal and soybean oil valued at $1.6 billion and $0.9 billion, respectively, in 1998.

FIGURE 1
U.S. SOYBEAN AREA 1944-1999



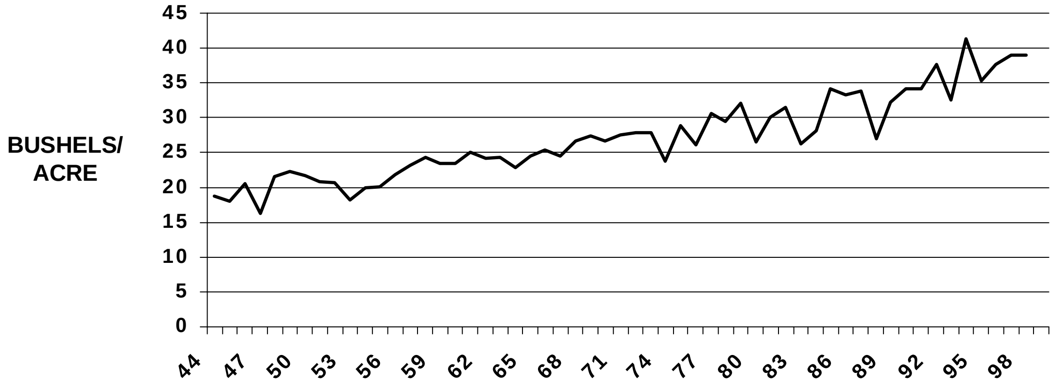
SOURCE: [58]

FIGURE 2
U.S. SOYBEAN PRODUCTION 1944-1999



SOURCE: [58]

FIGURE 3
U.S. SOYBEAN YIELD 1944-1999



SOURCE: [58]

3. **Soybean Products**

Approximately 74 billion pounds of soybean meal and 18 billion pounds of soybean oil were produced by U.S. processors in 1998 [1]. (See Appendix 1 for a description of soybean processing.) Ninety-six percent of the soy meal produced in the U.S. is used as an animal feed [74]. Three percent of the soybean meal is used in human food, and approximately 1% is used in industrial uses.

Soybean oil makes up about 75% of the oil used in U.S. shortening (baking and frying fats), margarine and salad/cooking oil. Table 2 shows the use of soybean oil in fats and oil products in 1998.

The large volume of soybean oil use has been attributed to at least three factors: 1) a plentiful and dependable supply; 2) competitive price; and 3) the improvements made in the flavor and oxidative stability of the oil [74].

Until 1980, cottonseed oil was the preferred oil for mayonnaise products. However, the cottonseed oil emulsion in mayonnaise is rather weak and sometimes breaks down at refrigerator temperatures. With improved flavor stability and stronger emulsion of soy oil, all mayonnaises produced commercially in the U.S. are now prepared exclusively with soybean oil as the key component. Similarly, soybean oil is now used almost exclusively in all prepared salad dressings and imitation mayonnaises sold in the U.S. [74].

Lecithin is a co-product of degumming soybean oil, that, after processing, has significant commercial value as an emulsifier in foods.

Soy lecithin is normally added to such food products as shortening, margarines, baked goods, chocolate confectionery coatings, peanut butter, powdered mixes and dietary food. Lecithin increases the stability of these products.

When added to margarine, lecithin prevents “bleeding” of the moisture present and reduces spattering during frying. In baked goods, lecithin functions as a useful emulsifier. It helps bring about rapid and intimate mixing of the shortening in the dough. In making chocolate, about 0.25-0.35% lecithin is added. It reduces viscosity of chocolate markedly, enables the manufacturer to apply a uniform coating, decreases the time for grinding and mixing the various ingredients and produces a more stable chocolate. Lecithin is used to keep the chocolate and cocoa butter in a candy bar from separating. In peanut butter, added lecithin, normally at 1-2% levels, gives a smoother, creamier spread that does not separate during wide temperature variations [74].

Approximately 45% of the soybean meal used as feed in the U.S. is for poultry feed while swine and beef feed represent 27% and 9%, respectively. Pet food, dairy animals, egg hatcheries and aquaculture make up the remainder. A typical broiler feed may contain 17-22% protein, of which more than 90% usually comes from soybean meal. The most important factor responsible for increasing the feed efficiency in poultry production has been the utilization of a high-protein diet containing dehulled and defatted soybean meal. The efficiency in broiler production increased from a gain of about 4 kg of feed per kg of bird in 1948 to the present rate of 2 kg of feed per kg of bird [97].

Soy protein products include defatted soy flakes, soy meal, soy flour and grits, soy concentrates, soy isolates, texturized soy proteins, full-fat soy flour and enzyme active soy flour [74].

Soy flour or grits are used as an ingredient in a variety of food products, including soups, stews, beverages, desserts, bakery goods, breakfast cereals and meat products [74].

Soy flour and concentrates may be processed further by thermoplastic extrusion to impart meat-like texture to these products. Soy protein ingredients are used extensively in meat products as extenders.

In bakery products, soy proteins are used as replacement for more expensive egg whites. Soybean proteins are used in whipped toppings, ice creams, coffee whiteners, imitation milk and cheese, frozen desserts and yogurts. Soy isolates are used in most of these products because of their high protein content and ability to impart desirable functionalities. Soy flours, concentrates and isolates are used as a source of protein in various infant foods based on cereals, vegetables and meats [74]. Soy flour use in some products limits fat absorption during deep frying, for example, in doughnuts.

Soy hulls are processed into fiber bran breads, cereals and snacks.

TABLE 2: Fats and Oils Used in Edible Products in the U.S.: 1997/1998[1]

	(Million LBS/YR)		% Soybean
Product	Total	Soybean Oil	Of Total
Shortening (Baking and Frying Fats)	5,183	4,247	82
Margarines	1,549	1,474	95
Salad and Cooking Oil	6,803	5,673	83
Other Edible	306	65	21

Source: [99]

[1] October 1997 – September 1998.

4. Soybean Physiology

Soybean is a valuable agricultural commodity due to its unique chemical composition. The soybean seed consists of lipids, proteins, carbohydrates and minerals. Other valuable components found in soybeans include phospholipids, vitamins and minerals. On average, oil and protein together consitute about 60% of dry soybeans. The remaining dry matter is componsed mainly of carbohydrates (35%) and ash (5%) [74].

Nine fatty acids make up the lipid content of soybeans [98]. During seed development, soybeans store their lipids, mainly in the form of triglycerides, in organelles known as oil bodies [74]. The soybean contains 20% oil, the second highest oil content among food legumes. The highest oil content is found in peanut, with about 48% oil; the third highest oil content is found in chickpeas, with about 5% [74]. Crude soy oil contains impurities such as phosphatides, free fatty acids, gummy substances, color bodies, hydrocarbons and a waxy fraction. Refining operations remove these impurities [74].

Nineteen amino acids make up the protein content of soybean flour, including the essential amino acids, lycine, isoleucine, leucine and valine [98]. Among cereal and other legume species, soybean has the highest protein content, about 40%. Other legumes have a protein content between 20 and 30%, whereas cereals have a protein content in the range of 8-15% [74].

Some of the proteins in soybeans are enzymes. The enzymes in soybeans have catalytic activity. Catalysts increase the rate at which chemical reactions take place, but they do not cause a reaction to take place [29]. They control the rate at which chemical reactions take place in the plant. Each enzyme is specialized in the reaction that it catalyzes [94]. For example, certain enzymes act as catalysts in the soybean plant's production of the essential amino acids.

A typical soybean plant cell contains at least 10,000 different enzymes [29]. As long as conditions are favorable, enzymes will catalyze repeatedly their respective chemical reactions.

5. Soybeans – Agronomic Factors

The bushy green soybean plant is a legume related to clover, peas and alfalfa. Each soybean plant produces 60 to 80 pods, each holding three to five pea-size beans.

Soybeans are generally planted in May and June. The soybean plant flowers and pod filling occurs in July and August. Harvest begins in September and is largely completed by mid-November [118].

Only 10% of U.S. soybean acreage is typically planted to continuous soybeans [144]. Fifty-eight percent of the nation's soybean acreage is in an annual rotation with corn while 20% is rotated annually with other row crops or small grains. Five percent of the soybean acreage is idle or fallow during the previous year. Seven percent of the nation's soybean acres is double cropped annually with soybeans being planted after winter wheat is harvested [144].

Soybeans are planted at a high per acre plant density with 84% of U.S. acreage planted with 75,000 or more plants per acre [16]. The average plant population on U.S. soybean acreage is 145,000 [16]. The average row spacing in U.S. soybean fields was 20 inches in 1994. Thirty-six percent of U.S. soybean acreage is planted in rows spaced less than 10 inches apart.

Conservation tillage programs, which reduce erosion from growers fields, are widely used by soybean growers. In 1994, 50% of soybean acreage was under conventional tillage and 50% was in conservation tillage programs. Conservation tillage programs where at least 30% of the soil surface covered with crop residue, include mulch till, ridge till and no-till systems. Twenty-four percent of the acreage was in mulch till, 1% was in ridge till and 25% was in no-till in 1994..

Typically, less than 1% of U.S. soybean acreage is treated with insecticides or fungicides [16]. Soybean plants have been bred with considerable insect and disease resistance. Another factor that reduces the impact of insect feeding is the ability of the soybean plant to compensate for a significant amount of defoliation.

The typical soybean farm operator in Central Illinois in 1998 grew 449 acres of soybeans and 455 acres of corn [145].

6. Soybean Genetic Improvements

A. Introduction

The soybean is a native of eastern Asia, where it is known to have been cultivated for over 4,000 years. Soybeans were first grown in the U.S. in about 1804 in the state of Pennsylvania, but they were still a very minor crop 100 years later [78].

No more than eight soybean cultivars were grown in the U.S. prior to 1898. From 1898 to 1923, more than 1,000 cultivars were introduced – most sent by research stations or grain merchants in Asia or brought in by agricultural explorers, diplomats or other travelers. As a result of the increasing success of soybeans, the USDA sent plant explorers to Asia, and from 1924 to 1933, 6,651 soybean accessions were introduced to the U.S. [76]. By 1947, over 10,000 soybean varieties had been introduced by the USDA.

Genetic improvement in soybean has resulted from the continual development and release of new cultivars with greater genetic yield potential than their predecessors. The amount of genetic improvement in yield that has been realized by hybridization and selection has been substantial [4]. For example, a 25 % increase in yield occurred between plant cultivars released before 1940 and cultivars released after 1970. [4] Other characteristics that have been the focus of traditional crop breeding include lodging resistance, reduced plant height, seed size, seed quality, oil quality, shattering resistance and resistance to insects, diseases and nematodes.

All soybean cultivars now grown in the U.S. were developed by artificial cross pollination.

Soybeans have a very limited tolerance to many of the herbicides that have been used to kill weeds in the crop. Rates of many herbicides are kept low because of the limited ability of the soybean plant to detoxify the chemicals if used at higher rates. Soybean plants are often damaged by herbicides under varying environmental conditions, such as higher than normal rainfall.

Soybean breeders traditionally have placed a relatively low priority on enhancing herbicide resistance.

In order for traditional breeding to produce herbicide resistant soybeans, genes for resistance must be available in crossing material that is compatible with soybeans. A lack of sufficient variability in resistance levels in soybeans has hindered breeding efforts. Soybean cultivars frequently show differences in the degree of injury caused by herbicides, but the occurrence of genotypes giving highly sensitive herbicide resistance has been rare [106]. This requirement has been the limiting factor for producing herbicide resistant soybeans with naturally occurring genes.

However, there has been limited work in genetically improving soybean cultivars for herbicide tolerance. Soybean cultivars have been "selected" for increased tolerance to the herbicide metribuzin. This selection procedure did not entail the creation of a new soybean cultivar; merely the selection among the populations of existing cultivars of a more tolerant plant. The procedure used to select soybeans for metribuzin tolerance is described in Section 10.

Two successful attempts to create genetically altered soybean plants with herbicide tolerance have been made: (1) the use of mutation breeding to develop soybean cultivars with increased tolerance to sulfonylurea herbicides and (2) the creation of transgenic soybean cultivars with tolerance for use of the herbicide glyphosate. These two genetic improvements are described further in this section.

Once the genetic change was accomplished in a single soybean cultivar, traditional crop breeding methods were used in order to increase the number of soybean cultivars with the tolerance to sulfonylureas and to glyphosate . Because of the importance of cross breeding in improving soybean cultivars, this section includes a discussion of these traditional methods. Since an understanding of crop breeding is dependent upon understanding the reproductive process of soybean plants, the next section of this report discusses that phenomenon.

B. Reproductive Process

The soybean flower is a perfect flower, meaning that both the male and female parts are present in the same flower. When the pollen is shed, it drops immediately on the female part of the same flower. This often happens before the flower opens (blooms). The pollen produced within the flower fertilizes the ovary of the same flower.

Because of the structure of the floral parts, soybeans are almost completely self-fertilized. Crossing has been estimated to be less than 0.5 % [77]. Soybean pollen is too heavy for wind transport but can be transported by insects [75].

Enclosed within the petals are ten anthers (male) arranged in a circle around a pistil (female). The pistil has a stigma at its tip that receives the male sex cells (pollen) from the anthers. Pollen germinates on the stigma, developing tubes that then grow into the stigma. Only a few pollen tubes reach the locule and compete for ovules to fertilize. Finally, the pollen tube grows into the ovule. Here the pollen tube tip bursts and releases two sperm cells. One sperm fuses with the egg and forms the first cell of the embryo while the other sperm fuses with the secondary nucleus forming the primary endosperm nucleus [88].

The time from pollination to fertilization varies from 8 to 10 hours. From the moment of fertilization, the ovary starts developing into the fruit.

C. Artificial Cross Breeding

The soybean cultivars grown by farmers in the U.S. up to the 1940's were introduced from Asia. Introductions were tested for agronomic performance, and the superior ones were released to farmers.

The best introductions were used as parents to develop superior cultivars, which are ancestors of current cultivars [75]. In the mid-1930's, soybean breeders began making two-way crosses between the best yielding introductions from Asia and produced progeny that were superior in yield to either parent. Subsequent matings among these selections generated a new round of superior cultivars released in the 1950's and 1960's [85].

The stigma is receptive to pollination at least one day before the anthers are sufficiently developed to shed pollen. This lag time between female and male development permits the breeder to introduce pollen from an outside source to obtain hybrid seed.

To prepare the flower for pollination, the five lobes of the calyx and the petals are removed to expose the female and male organs [75]. Emasculation is not necessary. Apparently an exposed stigma that is not promptly pollinated dries up and becomes non-receptive before anthers from the flower can shed pollen.

Flowers of the female parent are pollinated artificially the day before their male parts have matured enough to shed pollen. A floral bud at the appropriate stage is swollen and the corolla is visible. The five sepals of the calyx are removed with tweezers to expose the corolla. The male parts of a matured donor plant are removed by use of a pair of tweezers. The pollen is placed on a stigma by brushing the anthers against it [4]. If the pollination is successful, a pod will be visible in about seven days.

Breeding soybeans is tedious because the flower is very small. The soybean flower is one-quarter inch long. The number of crosses a person can make in a day ranges from less than 50, by inexperienced personnel, to about 150, by individuals with considerable experience [75]. One half or more of the pollinated flowers may abort.

Cross breeding is initiated by making a cross between two parents to produce a hybrid. The hybrid plant is crossed back to the parent that is being improved, called the recurrent parent because it recurs, or is used repeatedly for crossing. The other parent is the donor parent and contributes a desirable gene; however, the donor parent is not used in the backcrossing program. The purpose of crossing back to the recurrent parent is to recover all of its desirable genes. Each time a cross is made back to the recurrent parent, an additional 50 percent of its genes are recovered. The breeder will continue backcrossing until the desirable level of genes from the recurrent parent has been recovered [75].

Although more than 10,000 soybean strains have been introduced into the U.S. since the early 1900's, only a limited number of these introductions form the genetic base for cultivar development for the hybridization programs [85].

An analysis of the pedigrees of 136 soybean cultivars that originated from hybridization breeding programs during 1939 to 1981 revealed that only a limited number of ancestral introductions contributed germplasm. Only five introductions were the cytoplasm source for 121 of the 136 cultivars. The ancestry of the nuclear material in these 136 cultivars was narrow, with 12 introductions contributing about 88 percent of the germplasm. Traditional breeding procedures that emphasize the mating of elite strains are, in effect, continually recombining the genes contributed by a limited group of ancestral introductions [85].

The reason for such a narrow genetic base is that certain crosses of many of these ancestors were particularly productive in generating high-yielding cultivar releases, that, in turn, served as the parents in subsequent rounds of mating, resulting in still higher yielding releases [85].

Exotic soybean germplasm has not been used to a great extent in commercial breeding programs primarily because soybean breeders have been successful in using crosses of elite strains to generate high-yielding cultivars, and because crosses involving at least one unadapted parent have a low frequency of superior segregates in their progeny [85].

D. Mutation Breeding

Mutations are sudden changes in the hereditary material of an organism. Regarded broadly, they include all such changes that cannot be accounted for by the normal recombination of the units of heredity [104].

Mutations can result from the loss or rearrangement of a part of a chromosome or from the loss of entire chromosomes. Mutations also include the duplication of chromosome parts and the occurrence of additional chromosomes. Parts of chromosomes may change places. A piece of broken chromosome may rotate 180 degrees before rejoining the chromosome. Perhaps a broken piece is lost altogether along with a consequent loss of the genetic information that it carried [104].

Natural mutations have a number of causes, including cosmic rays, heat and aging, although in most cases the origin of a specific spontaneous mutation is unknown [104].

Mutations occur spontaneously in all living things. By changing the chemistry of the organism's genetic material or altering the structure of a chromosome, a mutation changes the structure or function of the organism and its offspring. Most often the mutations are harmful. Once in a while a mutation is beneficial, increasing the organism's chance to survive and reproduce or, in the case of plants that are directly useful to humans, increasing its value [104].

On this foundation, a number of plant breeders have ventured to induce mutations artificially and, thus, to take advantage of the beneficial ones. The principal mechanisms for inducing mutations is the irradiation of seeds, although mutations are also produced by irradiating entire plants and by treating seeds with mutagenic chemicals [104].

More than 1,000 varieties of induced-mutant crop plants – including wheat, rice, barley, oats, soybeans, fruit trees and ornamental plants – have been released to growers and are being grown by farmers on millions of acres throughout the world [104] [92]. A remarkable example has been the mutation breeding work of chrysanthemum in The Netherlands. Within a few years after an irradiation project had started, a whole range of floral color materials were obtained that quickly replaced other cultivars [92]. Swedish scientists were pioneers in the practical uses of induced mutations and developed a great deal of the present technology with mutation breeding of barley [91].

When breeders induce mutagenesis, they are likely to discover useful mutations that do not exist in naturally evolved populations [91]. However, the induction of a specific desirable mutation remains a random process. Even with the help of mutagens, any particular mutation occurs rarely [87].

A mutagen does not produce a specific mutation. What is achieved is an increase of the general mutation frequency, and, thereby, increased likelihood of an agronomically valuable mutation [104].

Ionizing radiations are available to plant breeders from a variety of sources: x-rays, gamma rays from radioactive isotopes (chiefly cobalt 60 and cesium 137) and neutrons from nuclear reactors [104].

Chemical mutagens are more readily available to plant breeders than radiation sources, and the ratio of mutational to undesirable modifications is somewhat better for chemical mutagens than for irradiation. Hence, chemical mutagens are becoming increasingly popular [91].

For practical plant breeding, the most interesting group of chemical mutagens are alkylating agents. These compounds bear one or more reactive alkyl groups capable of being transferred to other molecules at positions where the electron density is sufficiently high [91]. The alkylating agents react with DNA by alkylating the phosphate groups as well as the purine and pyrimidine bases. Alkylating agents produce unspecific effects, i.e., both small effects like point mutations and major chromosonal aberrations are induced [92]. Among the 30 to 40 chemical mutagens in the category, one of the most powerful and useful mutagens is ethyl methanesulfonate (EMS) [91]. The mutation rate with the use of EMS is approximately 50% [92].

A soybean seed contains a mature, arrested embryo. The embryo in a mature seed of soybeans has already initiated its first leaves. Mature seeds are metabolically quiescent. As long as the seed is relatively dehydrated, it remains dormant. It is alive, but all metabolic processes are occurring at very slow rates. Most of the common mutagens work by encouraging mistakes during DNA replication. Effective mutagenisis depends on applying the mutagenic agent at exactly the time when DNA is replicating. To activate the plant embryo, seeds need only to be saturated with water.

In the 1980's, the Dupont Company conducted a crop breeding program to enhance soybean tolerance for sulfonylurea herbicides. The molecular target of the sulfonylurea herbicides is the enzyme ALS, also known as acetohydroxyacid synthase (AHAS), that catalyzes the first common steps in the biosynthesis of the branched-chain amino acids isoleucine, valine and leucine in plants and microbes. This pathway is not present in animals [96].

Seed mutagenesis (using N-nitroso-N-methylurea and ethyl methanesulfonate) followed by selection for resistance to sulfonylurea herbicides yielded a soybean mutant with a high degree of resistance to applications of a variety of sulfonylurea herbicides [55].

Four hundred fifty thousand soybean seeds were presoaked in tapwater. The swollen seeds were then soaked in the mutagen. Treated seeds were washed and field planted.

Seeds of these plants were harvested and were screened for resistance to sulfonylureas by soaking them in a solution that included a sulfonylurea herbicide. These seeds were then planted and 75 mutants were selected based on their ability to form true leaves within several weeks. (This treatment severely retarded development of the parent soybean cultivars.) One mutant was significantly more tolerant of sulfonylurea treatments [55]. The mutants have an altered ALS that shows reduced sensitivity to inhibition by sulfonylureas [83]. Tolerance was conferred by a mutation in a single gene.

The sulfonylurea tolerant soybeans are designated by the trademark “STS.” In the late 1980’s, Dupont released the “STS” germplasm to private soybean seed companies to breed into their soybean lines [57]. STS soybeans were introduced commercially in 1992. Dupont markets “Synchrony STS” for use with STS soybeans only. Synchrony STS consists of chlorimuron and thifensulfuron.

Sulfonylurea-tolerant soybean cultivars are more tolerant of higher rates of the sulfonylurea herbicides chlorimuron and thifensulfuron. These higher rates are necessary for controlling difficult weeds, including lambsquarters, pigweeds, morningglories, sicklepod and velvetleaf [23].

Experiments in 1993 compared tolerance of a non-sulfonylurea tolerant soybean with an STS variety when treated with sulfonylurea herbicides. Injury to the non-STS variety ranged from 13 to 65% and averaged 3% to the STS variety with the same treatments [140].

E. <u>Transgenic Plants</u>

Advances in biotechnology have made it possible to transfer genes from organism to organism by means that bypass the normal sexual processes governing intraspecific inheritance. Isolated genes are moved into a crop plant in such a way that the genes are integrated into the chromosomes and expressed. A significant advantage is the fact that whole plants expressing a foreign gene can be regenerated from single transformed cells.

After being absorbed by plants, the herbicide glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate binds to EPSPS resulting in EPSPS's inhibition, causing the plant to starve for EPSP and the metabolic products derived from EPSP [101].

Research to develop glyphosate tolerant plants began at Monsanto in the early 1980's. Simple selection as whole plants or cell cultures either with or without mutagenesis agents proved to be largely unsuccessful. Attempts to identify glyphosate tolerant plant EPSPS as well as extensive random mutagenesis efforts failed to identify EPSPS with adequate glyphosate tolerance [141].

The ability to metabolize glyphosate is distributed widely among soil bacteria. Research demonstrated that EPSPS from a number of bacteria exhibited tolerance to glyphosate [101]. Monsanto scientists collected bacterial cultures from diverse sources and analyzed them for their tolerance to glyphosate. One type of bacteria represented heavily in the screening was glyphosate degrading bacteria. The rationale being that perhaps organisms that can grow in the presence of glyphosate and degrade the herbicide may also express naturally glyphosate-tolerant EPSPS. The EPSPS with the highest tolerance to glyphosate found in the screening was CP4 EPSPS from agrobacterium tumifacien, that demonstrated extremely high glyphosate tolerance [101].

The gene from CP4 EPSPS was cloned and introduced into soybeans by the particle acceleration method. Soybean cultivar A5403, a commercial variety developed by ASGROW Seed Company was used for the transformation [72].

The particle gun can accelerate microscopic projectiles to initial velocities of about 1,400 feet per second. At that speed the projectiles penetrate the cell walls of intact plant cells and carry DNA into the cell. With each bombardment, thousands of particles are accelerated at the same time, thereby delivering DNA into many cells simultaneously.

Gold beads approximately 1.3-3 microns in diameter were used. Gold is a dense chemically inert material, that is non-detrimental to the plant tissue [86]. Particle acceleration is capable of delivering biologically active foreign DNA into viable plant tissues at a frequency high enough to allow the recovery of stable transformations.

Immature soybean embryos were the targets of the rapidly accelerated DNA coated gold particles. Five to seven immature embryos were arranged on a plate and were subjected to particle acceleration [89]. Effective penetration was limited to a depth of five to seven cell layers. Treated embryos were chopped and plasmolized, and protoplasts were isolated. The embryonic axes were then plated on a germination and shoot multiplication medium and transformed plants were recovered.

The lead soybean line with a Roundup Ready gene is denoted 40-3-2, and expresses the CP4 EPSPS gene product. Upon glyphosate treatment the transgenic plant remains unaffected because the continued action of the introduced glyphosate-tolerant EPSPS enzyme meets the plant's need for aromatic amino acids. The endogenous EPSP is inhibited by glyphosate (upon glyphosate treatment), however, the plant relies on the introduced glyphosate-tolerant EPSPS for EPSP synthesis [101].

Line 40-3-2 and progeny from crosses between line 40-3-2 and other soybean varieties were yield tested under weed-free conditions in 1992 and 1993. Data from these experiments showed that there is no yield penalty observed upon glyphosate treatment of this line with Roundup even at rates as high as twice the level to control most weeds [71].

Line 40-3-2 has been used in various breeding programs to develop new cultivars with a Roundup Ready gene. (Roundup Ready is Monsanto's trademark for its genes conferring glyphosate tolerance.) As a single dominant gene, the glyphosate-tolerant gene can be used very effectively in breeding programs.

Over 150 seed companies offer more than 1,000 Roundup-Ready varieties.

7. Weed Competition In Soybeans

Weeds compete with soybeans for soil moisture, nutrients, sunlight and space in the field. One cocklebur may occupy four to eight square feet of area, thereby reducing the space available for soybean growth. When weeds shade the soybean plant, less sunlight is available for soybean production. Most of the soybean yield reduction from velvetleaf and pigweeds is ascribed to shading by the weed leaves above the soybean canopy. As a result of competing with soybeans, uncontrolled weeds decrease the quantity of soybean seeds produced [26].

The efficiency of operation of harvesting equipment is also reduced by the presence of significant numbers of weeds [8]. The quality of the harvested crop is directly impacted by weeds. Increases in moisture content, foreign matter, and splits have been documented when high levels of weeds are present at harvest [8].

Two of the factors that contribute to the strong competitive nature of weeds include high seed production leading to high plant density and a long duration in the soil [28]. Weed seeds remain viable (capable of germination) for varying periods of time. Seed longevity represents a major survival mechanism for weed species; it constitutes a continuing source of emerging weeds in croplands [29].

Table 3 lists the length of survival in soil of several common weed species in U.S. soybean fields. As can be seen, the seeds of these species can survive in the soil for eight to forty years. Weed species re-infest the soil primarily due to the large amounts of seeds produced by a single plant. Table 3 lists the number of seeds produced per plant for several weed species of importance to U.S. soybean growers. A single cocklebur plant can produce 900 seeds while a single pigweed plant produces 117,000 seeds.

In Minnesota, weed seed counts at four different locations in twenty-four different plots showed from 98 to 3,068 viable weed seeds per square foot of soil six inches deep – that converts to between 4 million and 133 million seeds per acre [28]. In western Nebraska average cropland soil contained 200 million seeds per acre [32]. The number of weed seeds that germinate and emerge in any given year is quite low in relation to the total number of seeds present – perhaps only 5 – 10% of the total seed population [33]. A very high percentage of the total weed seed population in the soil survives from one year to the next.

Numerous reports in the literature quantify the effects of full- or partial-season weed interference on soybean seed yields. The data show considerable differences among species in interfering ability. Figure 4 charts the relationship between increasing density of six weed species on soybean yield. As can be seen, common cocklebur is the most interfering weed: 1 plant/m^2 depresses soybean yield by 30% while nine plants/m^2 depresses soybean yield by 80%. A less competitive weed species for soybeans is giant foxtail: 16 plants/m^2 depresses soybean yield by only 10%.

There are three categories of weeds: dicots, monocots and sedges. Dicotyledons (dicots) contain two cotyledons (seed leaves) while monocotyledons (monocots) contain only one. These two large groups of plants typically have different types, arrangements and locations of organs. Dicots have broad leaves with veins radiating from a midvein, a taproot and/or fibrous root system and flower parts in multiples of four or five. In contrast, monocots generally have long, narrow leaves with parallel veins, a fibrous root system and flower parts in multiples of three. Because of pronounced structural differences between dicots (broadleaves) and monocots (grasses), weed control methods can often be targeted specifically at one of these groups.

More than thirty plant species infest soybean fields in the major soybean producing areas of the U.S. [30]. Annual broadleaf and grass weeds are major problems. In some areas, perennial grass, broadleaf weeds and sedges are troublesome. Table 4 lists important weed species in soybean fields in two states, representative of the Midwest (Illinois) and the Delta (Arkansas). As can be seen, a combination of broadleaf and grass weed species infest a sizable portion of the soybean acreages in both states. Ragweed, foxtail, nightshade, lambsquarters, smartweeds, and velvetleaf infest more acreage in the Midwest than in the Delta. Morningglory, barnyardgrass, signalgrass, prickly sida, hemp sesbania and sicklepod infest more acreage in the Delta. Cocklebur, johnsongrass, crabgrass and pigweeds are estimated to infest sizable acreages of soybeans in both soybean producing regions.

Two to four species typically dominate the soil seed population [32]. In a typical field in the Midwest, weed control strategies are generally planned based on two grass weed species and three to five broadleaf species.

Natural weed populations in most fields are high enough to cause devastating soybean yield losses if left uncontrolled [10]. Loss figures of 50 – 90% are common for soybeans grown in natural weed infestations [9] [11] [12] [13].

Research has shown that a period of 4 to 6 weeks without weed competition at the beginning of the growing season will allow production of maximum yields under most environmental conditions [10]. Any weed emerging in the crop after this initial weed free period will not compete effectively with soybeans and will not affect yield potential due to the soybean canopy which shades emerging weeds. Similarly, a period of 4 to 6 weeks of weed interference at the beginning of the season usually can be tolerated by soybeans with no significant yield loss provided that the crop is maintained weed free for the remainder of the season [10].

TABLE 3: Weed Seed Production and Length of Seed Survival in Soil

Weed Species	# of Seeds Per Plant	Length of Survival in Undisturbed Soil (Years)
Common Cocklebur	900	8
Common Lambsquarters	72,450	39
Common Ragweed	3,380	39
Green Foxtail	34,000	39
Pennsylvania Smartweed	19,300	30
Redroot Pigweed	117,400	10
Velvetleaf	2,000	10

Source: [32]

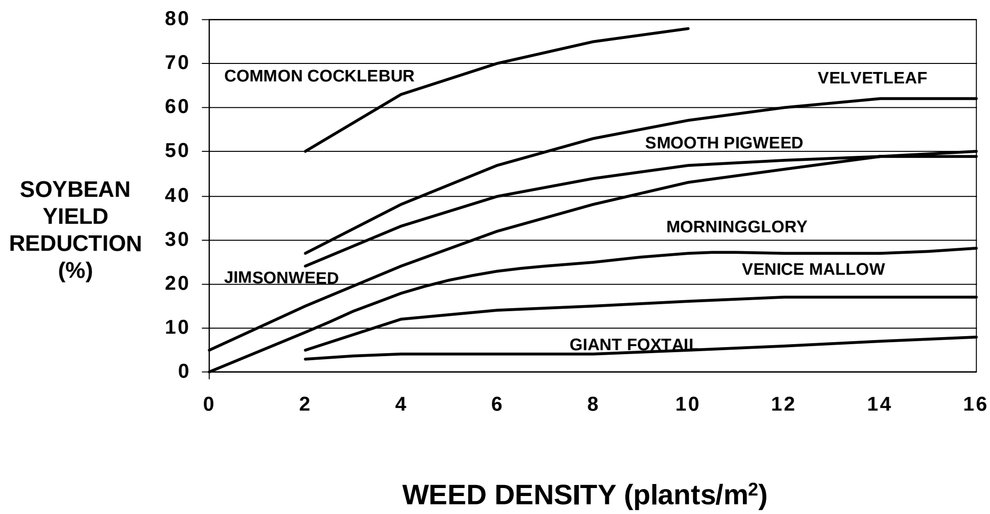
TABLE 4: Infestations in Soybeans (% Acres Infested)

Species	Type[1]	Illinois	Arkansas
Morningglory	BL	20	60
Barnyardgrass	GR	-	50
Black Nightshade	BL	10	-
Signalgrass	GR	-	50
Common Cocklebur	BL	20	67
Common Ragweed	BL	30	1
Giant Foxtail	GR	95	-
Giant Ragweed	BL	10	-
Johnsongrass	GR	30	35
Lambsquarters	BL	30	-
Large Crabgrass	GR	20	50
Pennsylvania Smartweed	BL	60	6
Pigweed	BL	85	50
Prickly Sida	BL	-	19
Hemp Sesbania	BL	-	11
Sicklepod	BL	-	20
Velvetleaf	BL	70	-

Source: [17]

[1] BL = Broadleaf
GR = Grass

FIGURE 4
INFLUENCE OF WEED DENSITY ON SOYBEAN YIELD



SOURCE: [26]

8. Weed Control in Soybeans: 1940's - 1950's

In the 1940's and 1950's, tillage was the primary method used to control weeds in U.S. soybeans. The use of several shallow cultivations prior to planting soybeans to destroy as many annual weeds as possible was regularly recommended [30]. Various implements, such as the rotary hoe and spike-toothed harrow, were used after planting to perform shallow tillage to uproot very young annual weeds between the soybean rows.

Weed control by the tillage method is achieved primarily by (1) the burial of small annual weeds in soil thrown over them through the action of tillage tools and (2) the disruption of the intimate relationship between the weed plant and the soil, whereby (a) the soil is loosened about the roots, resulting in disruption of water absorption and death by desiccation, or (b) the plant is "cut off" below ground. Care must be taken in the tillage operation so as not to injure the roots or aboveground parts of the crop plants.

Research demonstrated that rotary hoeing provided 70-80% weed control three to five days after soybean emergence with two repeat treatments at about five-day intervals [107]. However, untimely rotary hoeing applied when weed seedlings were bigger (in the 1-3 leaf stage) decreased weed control effectiveness to 50% [107].

The rotary hoe should travel at a relatively high speed (8-12 mph). The rotary hoe is most effective on small weeds. For bigger weeds, growers relied on the use of slower-moving shovel cultivators.

Researchers in the 1950's reported effective control of weeds between the rows with sweep cultivation and some control of weeds in the row by throwing soil into the row during cultivation [39]. Throwing soil into the row is risky since it may cause excessive ridging of soils in the row [30].

Timely weed removal treatments with the rotary hoe were sometimes difficult to apply because of wet conditions – mud would ball up excessively on the rotary hoe. Consequently, rotary hoeing was not always practiced when weeds were at an optimum size. To reach a maximum effectiveness, the hoeing must be done before the weeds reach a height of more than a quarter inch [34]. Prolonged rainy periods often delayed the use of the rotary hoe in farmers' fields beyond the time when it is effective. If rotary hoeing is delayed, the weeds develop extensive root systems preventing their removal with the implement [34].

The best weed removal system for soybeans in the 1950's was determined to be two timely rotary hoeings plus two shovel cultivations [35]. Even with timely use of cultivation, soybean yields were reduced because the tillage operations did not control effectively the weeds growing in the row with the soybeans.

An eleven year experiment (1952 – 1962) in Iowa estimated soybean yield reductions resulting from weed infestations which survived good cultural and mechanical weed control methods [41]. Average soybean yield reduction was 10% despite best mechanical weed control practices. Uncontrolled weeds were confined to a four to six inch band centered on the soybean row.

In the 1950's soybean farmers in the Midwest generally cultivated two to three times [34]. In 1964, soybean acreage in Illinois was cultivated two times with a shovel or sweep cultivator and an additional one time with a rotary hoe or harrow cultivator [15].

USDA estimated that the average annual national loss in the potential production of soybeans due to weeds was 17% for the period between 1951 and 1960. The USDA loss estimate includes a yield loss of 14% and a loss of three percent in quality due to weed seed dockage, damage in cleaning to remove weed seeds, split beans due to presence of weed seeds and off flavors [18].

The need to cultivate soybeans for weed control was seen as the limiting factor as far as the number of acres that one person could manage since planting had to be stopped so that cultivating could begin [36].

Each tillage operation reduces the amount of crop residue and clods on the soil surface and this, in turn, increases soil erosion susceptibility .

9. <u>Herbicides – An Overview</u>

An herbicide may be defined as any chemical agent that kills or greatly inhibits plant growth. Herbicide mode of action is the chemical interaction that interrupts a biological process necessary for plant growth and development. Herbicides injure and kill plants by interfering with the normal function of one or more of the metabolic processes vital to the life of the plant. It is generally accepted that the phytotoxicity caused by many herbicides is due to their adverse effects on normal enzyme activity [29]. Some plants possess the means to detoxify certain herbicides and are not killed by these chemicals [29].

Susceptible weeds either cannot metabolize the herbicide or metabolize it too slowly for detoxification. For example, the half-life of imazaquin in soybeans is three days, whereas in cocklebur it is 30 days [28].

Herbicides traditionally have been discovered by screening chemical compounds in a series of increasingly specific tests. Compounds are first tested for activity against a spectrum of weeds and lack of activity against targeted crops. To be commercially successful, herbicides must have potent biological activity against a broad spectrum of weeds and, at the same time, be non-toxic to crop plants. Selective toxicity of herbicides to weeds but not to crops is one of the most difficult properties to achieve, as might be expected, from the biological relatedness of weeds and crops [83].

Both crops and weeds are naturally resistant to many selective herbicides. Herbicide resistance can be due to three basic mechanisms: resistance at the site of action, metabolic detoxification, and prevention of the herbicide from reaching the site of action [30].

Resistance at the site of action generally is due to an alteration in the herbicide target site (generally a protein) that prevents the herbicide from binding to the site and inhibiting the vital process mediated by the target site. Metabolic detoxification generally means that the plant can degrade the herbicide faster than the herbicide can cause irreversible damage to the plant. For an herbicide to be effective, it must reach its molecular target site. Thus, blockage of movement to the site of action can occur at the plant surface, just inside the plant or in vascular tissue where the herbicide can be rendered metabolically inactive [30]. For a number of important classes of herbicides selectivity results from a unique or enhanced metabolic detoxification of the herbicide by the crop plant, but not by the weed [83].

Following their entry into plants, herbicide ions and molecules are subjected immediately to the influence of interactions with plant constituents and processes (chemical, biochemical and physical) that may affect their translocation or phytotoxicity [29]. Once absorbed by a plant cell, an herbicide may react with chemicals present in the cell to form complexes, or conjugates, that are insoluble or non-translocatable. Such complexes immobilize the herbicide in the cell [29].

Some herbicides are used directly on weed foliage while others are applied to the soil and depend on uptake by the weed. Certain herbicides are active in the soil and available for plant uptake for a considerable period of time while others exhibit little or no soil residual activity. The primary mechanisms that regulate soil activity are (1) the feeding of soil bacteria on herbicides and (2) the binding of herbicides to soil. Breakdown is generally fastest in warm, moist, light textured, low pH soils and slowest in cold, dry, heavy, high pH soils.

Bacteria are the most numerous of the free living micro-organisms in the soil. Their populations in soils are unevenly distributed, commonly clustered in colonies of a few to many thousands of individuals. The colonies are scattered along the walls of pores and channels in the soil, as well as over the surface of soil particles. So enormous is the total amount of live and dead micro-organisms in soil, that soil protein is composed largely of their remains [29]. Bacteria are the smallest living organisms, being about one micron in length.

Soil bacteria must have food for energy and growth. Organic compounds of the soil provide this food supply. If an organic substance is applied to the soil, micro-organisms immediately attack it. Those that can utilize the new food supply flourish and increase in number. In effect, this hastens decomposition of that organic substance [28].

Organic compounds are those chemicals that contain one or more atoms of the element carbon. Carbon atoms, alone or in a chain, are able to bond with as many as four other atoms at the same time. These atoms may be carbon or other elements. Organic herbicides, in general, are composed of molecular combinations of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus and the halogens (fluorine, chlorine, iodine and bromine) [29].

Bacteria are capable of breaking apart the molecules of organic herbicides, resulting in their deactivation. The organisms rapidly multiply as they utilize the herbicide as an energy source. Herbicidal decomposition continues indefinitely until it is reduced completely to carbon dioxide, water and basic elements. As the food source is depleted, the large microbial population dies back [29].

Adsorption in soils is the process whereby ions and molecules are bonded to the surface of soil colloids due to the electrical attraction between themselves and the colloidal particles, a process similar to the attraction of iron filings to a magnet [29]. Adsorbed herbicides are in a passive state – unavailable to biological and chemical processes.

10. Herbicide Use in Soybeans: 1960's - 1995

A. Introduction

U.S. soybean growers began to use herbicides for weed control in the late 1950's. By 1966, only about 30% of the nation's soybean acreage was treated with herbicides. However, herbicide usage in soybeans grew rapidly in the late 1960's and early 1970's and reached more than 90% acreage treated by 1982. Since 1982, soybean growers have consistently treated more than 95% of the nation's soybean acreage with herbicides to control weeds. Figure 5 charts the percentage of soybean acreage treated with herbicides 1959-1998.

Significant shifts have occurred in the specific herbicides used in soybeans. Table 5 shows estimates of the percent of the nation's soybean acreage treated with specific herbicides for selected years 1966-1995. Because national surveys were not conducted between 1982 and 1990, soybean herbicide use data collected in Illinois surveys 1982-1992 are presented in Table 6. Tables 5 and 6 show the rise and fall in usage for the major herbicides used in soybeans 1966-1995. Because the individual herbicides are used at different rates per acre, a shift in usage among herbicides can lead to overall changes in the amounts of herbicide active ingredients being applied. Figure 6 charts the national usage of soybean herbicides in terms of pounds applied. As can be seen, a large decrease in pounds applied occurred in the late 1980's as growers switched to lower use rate herbicides.

The usage trends among the major herbicides used in soybeans are described in the following parts of this section. As can be seen, soybean growers have changed their use of specific herbicides. These shifts are due to differences in effectiveness in controlling weeds, amount of crop injury to soybeans and cost of alternative herbicides.

B. Historical Overview

1. The Early 1960's

The main herbicides recommended for weed control in soybeans in the late 1950's were 2,4-D, dinoseb, naptalam, chlorpropham, and CDAA. Applications of these chemicals controlled many weed species, but occasionally severely injured soybeans and provided erratic weed control [30]. The limited acceptance of naptalam and chlorpropham was due in part to the narrow spectrum of weeds controlled relative to product cost [24].

Following its introduction in the 1950's, chloramben (Amiben) dominated the soybean herbicide market during the 1960's [24]. Chloramben applied to the soil surface before emergence of weeds in soybeans controlled annual grass weeds effectively when rainfall after application was adequate. In addition, chloramben controlled most small-seeded broadleaf weeds (ragweed, smartweed, velvetleaf) satisfactorily [40]. Chloramben provided residual control of germinating weeds for 6 to 8 weeks. Chloramben inhibited root development of seedling weeds. Soybeans tolerated chloramben due to reduced translocation of the herbicide within the soybean plant in comparison to susceptible weeds [42]. Chloramben lost effectiveness if prolonged dry weather followed its application [41]. Chloramben was used as an over the row band. As a result, weeds between the rows were not controlled by chloramben, making cultivation still necessary. Chloramben's use as a broadcast application over an entire soybean acre was considered prohibitively expensive by most producers. Unfortunately, the cost of manufacturing chloramben was relatively expensive and there was little opportunity for a price reduction in the marketed product [24]. As broadcast applications of other herbicides became popular, use of chloramben declined.

2. Soil Applied Herbicides

Trifluralin (Treflan) became available in 1963 for use as a preplant incorporated treatment for control of weeds in soybeans, applied to bare soil before planting and incorporated into the top few inches of soil with a disk. The acceptance of this treatment marked the beginning of an era in which farmer opinion shifted to allow not only a more expensive broadcast application, but also an extra tillage application for the purpose of incorporating the herbicide [30]. Trifluralin readily volatilizes from soil surfaces and performs best when incorporated into the soil soon after application because of the loss of herbicidal activity when left on the soil surface [30]. The universally recognized plant damage caused by trifluralin is a swelling of the root tip where cell division is disrupted [43]. Trifluralin is root absorbed and absorbed by the shoots of seedlings as the shoots grow and push through the soil toward the soil surface [29]. Affected weeds are prevented from emerging through the soil surface [43] Plant death primarily results from the lack of a functional root system, leading to a lack of water flowing to the aerial parts of the plant. Trifluralin inhibits cell division by binding to tubulin and blocking the formation of spindles or microtubules necessary for cell wall formation [28]. Microorganisms are believed to contribute to the degradation and disappearance of trifluralin from soil.

Trifluralin is transformed by soybeans into a number of non-toxic metabolites. Soybean tolerance to trifluralin is also due to the selective placement of the chemical above the root zone of soybeans. Trifluralin is strongly absorbed on soil and shows negligible leaching. Trifluralin has been shown on occasion to have adverse effects on soybeans. Trifluralin has been shown to inhibit soybean root growth. There have also been reports of trifluralin causing decreases in nodulation and yield of soybeans [59]. Microbial degradation of trifluralin depends on environmental conditions – it occurs faster when the weather is hotter and wetter. Under normal conditions trifluralin provides residual control of germinating weeds throughout the soybean growing season. Residues of trifluralin may persist into the following season enough to injure certain rotational crops, such as corn and sugarbeets [65].

Trifluralin controls annual grasses (foxtail, crabgrass, johnsongrass) and certain broadleaf weeds (pigweeds, lambsquarters). A factor limiting the intitial acceptance of trifluralin was the lack of an economical broadleaf herbicide that could be applied with trifluralin [24]. Since trifluralin only controls grasses and certain small-seeded broadleaf weeds, the result was often a nearly pure stand of uncontrolled broadleaf species such as jimsonweed, cocklebur, or smartweed [44]. Soybeans often required a timely cultivation to control weeds that escaped early season control measures [45].

The introduction of metribuzin (Sencor, Lexone) became the catalyst for the increased acceptance of pre-plant incorporated treatments in soybeans. Research demonstrated that metribuzin provided greater than 95 percent control of the broadleaf weeds velvetleaf and smartweed, plus 60–70% control of cocklebur [46]. A tank mix combination of trifluralin and metribuzin was evaluated extensively during 1972 – 1973 and demonstrated that the combination of metribuzin and trifluralin provided greater than 88% control of foxtail, pigweeds, velvetleaf, smartweed and morningglory, and 70% control of cocklebur [46].

Metribuzin inhibits photosynthesis by binding to a protein which results in the blocking of electron transport. This stops carbon dioxide assimilation. The most important detoxification route for metribuzin in soybeans is glucose conjugation to non-toxic metabolites [47]. Soybeans degrade metribuzin to these nontoxic metabolites far more rapidly than susceptible weed species.

Differential responses of soybean cultivars to metribuzin soil applications were reported in numerous studies in the 1970's. At the rates required for effective pre-emergence weed control, soybean tolerance to metribuzin is narrow [48]. Some soybean cultivars were determined to be more tolerant of metribuzin as a result of more rapid metabolism to nontoxic metabolites [49]. Some soybean cultivars are so sensitive that metribuzin cannot be used safely [31]. This very narrow margin of soybean selectivity is based on soil pH (more damage at higher pH), soil organic matter (more damage at lower organic content), rainfall (more damage with more rainfall), and rate (more damage at higher rates) [50] [51]. Unacceptable injury from metribuzin occurred in low areas where water stood for extended periods [46].

In the Mississippi Delta region, successful production of soybeans requires cultivars with at least a moderate level of resistance to the plant disease phytophthora rot and to the foliar diseases bacterial pustule and target spot. The cultivar Tracy has these characteristics and ranked as the top yielding cultivar on clay soils in Mississippi [61]. However, tests showed that Tracy was extremely sensitive to metribuzin injury.

A greenhouse technique was utilized for screening Tracy seedlings for metribuzin tolerance, after field observations had indicated that a small percentage of Tracy plants might be tolerant [62]. Plants were selected that survived a metribuzin rate twice as high as that required to kill normal Tracy plants [105]. Surviving seedlings were transplanted and grown to maturity. A new cultivar called Tracy-M was registered. Tracy-M is tolerant to metribuzin and is similar to Tracy in yield and disease resistance [62]. Seed was distributed in 1979 for increase in Mississippi, Alabama, Arkansas and Louisiana. These plants appear to be tolerant owing to a detoxification mechanism resulting in inactivation of metribuzin.

The herbicide alachlor (Lasso) was registered for use in soybeans in 1969. Alachlor controls many annual grasses and some small-seeded broadleaf weeds. As metolachlor (Dual) was introduced, it was in direct competition with alachlor and obtained some of that market. The mechanisms of action of alachlor and metolachlor are not well understood. Alachlor generally provides six to ten weeks of weed control while metolachlor generally provides ten to fourteen weeks of weed control [65]. The effectiveness of alachlor and metolachlor is improved when they are incorporated into the soil mechanically.

The dinitroanaline herbicide pendimethalin (Prowl) was registered for soybeans in 1976. Pendimethalin is soil-applied before planting and is absorbed by plant roots.

Pendimethalin is immobile, being strongly bound to organic matter and clay. Typical half-life in the field is 44 days. Highly susceptible annual grasses and broadleaves usually fail to emerge. Pendimethalin does not require mechanical incorporation to be effective. Pendimethalin is less volatile than trifluralin and need not be incorporated for seven days following its application. Thus, early rains can often be relied upon for its incorporation.

3. Postemergence Herbicides

Postemergence herbicides are applied to emerged weeds. Postemergence herbicide programs allow growers to adjust the herbicide rate and application date to weed growth depending on the severity of the weed problem and to target specific weed species.

Certain broadleaf weeds (such as cocklebur) which were not controlled adequately with the available preplant or preemergence herbicide treatments became greater problems in the early 1980's. Soybeans often required a timely cultivation to control weeds that escaped the early season control measures [45]. Several herbicides were introduced in the 1970's and 1980's that provided selective control of several of these troublesome weeds when applied postemergence.

Research demonstrated that postemergence application of bentazon (Basagran) over the top of weeds and soybeans selectively controlled many broadleaf weed species without reducing soybean yields [66]. Bentazon controls ragweed, smartweed, velvetleaf and common cocklebur. Bentazon proved erratic in controlling pigweeds and lambsquarters. Weed tolerance to bentazon increases rapidly as they grow larger. Research demonstrated that a preemergence treatment of metribuzin and a postemergence application of bentazon provided 92–99% control of common cocklebur [67].

Bentazon inhibits photosynthesis by binding to a protein which blocks electron transport. In soybeans bentazon is rapidly metabolized following glucose conjugation. Bentazon has little to no soil residual activity. However, bentazon applications may result in foliar bronzing of soybeans.

Soybean plants usually will outgrow the leaf burn provided they are not subjected to additional stress and have ample time to recover [129]. The use of trifluralin for grass control followed by postemergence applications of bentazon for broadleaf weed control proved popular in the 1980's.

Research demonstrated that acifluorfen (Blazer) controls a number of weeds in soybeans when applied postemergence: cocklebur, pigweed and morningglory [68]. Soybean injury from postemergence application of acifluorfen is exhibited as leaf crinkling and red speckling or bronzing. Soybeans normally recover 10 to 14 days after application and continue growing at a normal rate [68].

The primary target site for acifluorfen appears to be Protox, an enzyme of chlorophyll. Soybean tolerance appears to be due to rapid cleavage of the ether bond followed by further metabolism. Metabolism appears to be much slower in susceptible weed species. Acifluorfen is readily absorbed by leaves and is considered to be a contact herbicide since little translocation occurs and leaf injury is usually observed rather quickly. The best weed control with acifluorfen is obtained when weeds are small and actively growing.

A potential problem with postemergence mixtures deals with the timing of applications in instances where plant size between different weed species is highly variable. This is a concern in tank mixes of acifluorfen and bentazon in which the window of application of each herbicide is short and specific for the size or stage of growth of weeds [129].

Sethoxydim (Poast) and fluazifop (Fusilade) are postemergence herbicides that selectively control grass species in broadleaf crops, such as soybeans. Fluazifop and sethoxydim became available for use in soybeans in the early 1980's. Researchers have reported excellent control of may grass species with fluazifop and sethoxydim at very low rates [161]. Fluazifop and sethoxydim are absorbed rapidly into leaves and are rainfast within one to two hours. Grasses must be growing actively for these herbicides to be effective. Sethoxydim and fluazifop have short field half-lives of 5-15 days. Fluazifop occasionally controls or suppresses grass weeds germinating after application [65].

Sethoxydim and fluazifop inhibit the enzyme ACCase, that inhibits the production of membranes required for cell growth. In soybeans, transformation of sethoxydim and fluazifop to metabolites is very rapid. The slow adoption of the postemergence grass herbicides (such as sethoxydim and fluazifop) is primarily attributed to high product cost, and the risk associated with late season treatments.

One limitation on the use of postemergence herbicides in soybeans is the inability to tank mix broadleaf and grass herbicides. A tank mix of bentazon or acifluorfen with sethoxydim or fluazifop produces an antagonism which reduces the efficacy of the grass herbicides: the burning of the grasses by the broadleaf materials reduces the uptake and transformation of the grass herbicide by the weeds [70].

Additional postemergence grass control herbicides were introduced into the soybean market in the 1990's: quizalofop, clethodim and fenoxaprop. Additional broadleaf control herbicides introduced for soybeans included fomesafen, lactofen, flumiclorac and sulfentrazone. Flumetsulam was introduced in 1993 as a component in mixtures with trifluralin and metolachlor to provide broadleaf control. Clomazone was commercialized in 1985 and was targeted for the control of velvetleaf. Clomazone also controls several other broadleaf weeds and several seedling grasses [60].

Availability of effective postemergence herbicides allowed some soybean producers to switch entirely to postemergence weed control programs [69]. In some cases, postemergence applications were made to soybeans because of inadequate performance of soil applied materials during years with dry soil conditions.

4. <u>Sulfonylurea/Imidazolinone Herbicides</u>

In the mid and late 1980's, several herbicides from the sulfonylurea and imidazolinone chemical classes were introduced for use in soybeans: imazethapyr (Pursuit), imazaquin (Scepter) and chlorimuron (Classic). The sulfonylureas and imidazolinones are similar in their mode of action. They are absorbed readily by roots and foliage of plants. They are transported throughout the plant with concentration in merismatic tissues (buds), a primary site of DNA, and amino acid synthesis [112]. Herbicide activity by the sulfonylureas and imidazolinones results from inhibition of the enzyme acetohydroxyacid (AHAS, acetolactate synthase, ALS), which stops the synthesis of three essential amino acids – valine, leucine and isoleucine. This causes a disruption in protein synthesis resulting in an interference of DNA synthesis and rapid cessation of growth. Metabolic inactivation by the soybean plant serves as the basis of selectivity [113] [112].

Soybean plants rapidly metabolize imazaquin to a wide variety of compounds that either are degraded or incorporated into naturally occurring plant constituents [53]. Imazethapyr is metabolized to non-toxic forms in soybeans by hydroxylation followed by conjugation to glucose [53].

The imidazolinones and sulfonylureas are used at low rates (.004-.125 LB AI per acre) and can be applied in a variety of ways. Imazaquin and imazethapyr can be applied preplant, preemergence or postemergence. Chlorimuron is marketed for postemergence herbicide applications. Chlorimuron and imazaquin are primarily targeted at broadleaf weeds in soybeans. Research demonstrated that soil applied imazaquin provided weed control superior to metribuzin on cocklebur (93 <u>vs.</u> 74%), giant ragweed (93 <u>vs.</u> 63%) and velvetleaf (91 <u>vs.</u> 80%) [112]. Research demonstrated that imazethapyr controlled grass and broadleaf weeds effectively [114].

Applications of imazethapyr postemergence following pendimethalin applied preplant provided effective control of giant foxtail, pigweed, velvetleaf, cocklebur and lambsquarters [127]. This combination provided improved control over a broader spectrum of weeds than a combination of bentazon plus acifluorfen following pendimethalin.

Imazaquin and imazethapyr are used widely in soybeans due to their weed control advantages and the flexibility to apply them preemergence, preplant incorporated or postemergence. Imazethapyr exhibits soil residual weed control activity, yet controls a broader spectrum of weeds when applied postemergence. Imazaquin evaluations in 1985 indicated greater than 90% control of cocklebur, pigweed, lambsquarters, smartweed, ragweed and giant foxtail [128].

Research indicated that postemergence applications of imazethapyr and imazaquin discolored and restricted margins in young, expanding soybean leaves [114]. Also, imazethapyr treated soybeans were significantly shorter compared to untreated, handweeded soybeans. However, it was reported that this condition did not affect soybean yield [114].

Because of its limited spectrum on grasses, imazaquin was labeled for tank mixing with such soil-applied grass herbicides as pendimethalin and trifluralin. However, research demonstrated that mixtures of imazaquin with the postemergence herbicides sethoxydim and fluazifop had an antagonistic effect and reduced grass control [112].

The sulfonylureas and imidazalones remain active in the soil after application for season-long weed control. They are degraded in the soil via environmental routes, such as microbial activity and plant uptake [112]. The greatest contribution to breakdown by microorganisms occurs in warm, moist soils. Conversely, cool temperatures and either very wet or very dry soil conditions lead to a slower microbial degradation of the sulfonylureas and imidazalones [113]. Since sufficient concentrations of the imidazalones or sulfonylureas can remain in soil to injure certain sensitive rotational crops (such as corn, sorghum, cotton and rice) restrictions have been placed on the crops that can follow soybeans [113]. Susceptible crops that are seeded in years following imazethapyr application can be damaged severely by imazethapyr residues in the soil [138].

One limitation to the use of ALS herbicides is the development of resistant weed populations. Weeds that have evolved resistance to ALS inhibitors have evolved altered ALS that is resistant to the herbicide. Common waterhemp is a widespread problem in Midwest soybean production because of resistance to ALS-inhibiting herbicides [135]. In Kansas resistance to ALS-inhibiting herbicides has been confirmed in kochia, Russian thistle, common waterhemp, Palmer amaranth, common cocklebur, shattercane and common sunflower [136]. The occurrence of herbicide resistant weed biotypes in the Midwest increased in the mid 1990's. Biotypes of common and tall waterhemp and kochia demonstrating resistance to ALS-inhibiting herbicides have been reported [132].

The prevalence of herbicide resistant weeds has led to postemergence mixtures of active ingredients for broad range weed control.

Early field testing in the U.S. indicated that imazaquin provided excellent control of common cocklebur, that was not controlled adequately by the standard treatments available at the time. Tests in Mississippi demonstrated that imazaquin controlled sicklepod. In 1985, the EPA permitted growers in five southern states to use imazaquin under a Section 18 Emergency Exemption Clause for control of sicklepod since there were no registered herbicides that controlled this weed in soybeans. In 1986, imazaquin was registered for use in soybeans in the U.S., where it is sold under the trade name of Scepter.

In 1982, field testing of imazethapyr was begun in U.S. soybeans. Initial results demonstrated that imazethapyr controlled a wide spectrum of weeds, including velvetleaf, pigweeds, nightshades and foxtails. University researchers in Minnesota demonstrated that imazethapyr was highly effective in controlling Jerusalem artichoke. Because there were no labeled herbicides that controlled this weed in soybeans, EPA permitted

Minnesota growers to use imazethapyr under a Section 18 Emergency Exemption Clause in 1987 and 1988. In 1989, imazethapyr was registered for use in soybeans in the U.S.

Pendimethalin's (Prowl) use increased from 3 to 23% acreage treated as a result of its availability in formulated mixtures with imazaquin and imazethapyr , and its use for surface application with reduced tillage. The soybean acreage treated with trifluralin, which requires preplant incorporation, declined from 61% treated in 1982 to 50% in 1990.

In a reduced tillage system, it is not possible to incorporate herbicides into the soil as a preplant treatment.

The use of metribuzin decreased from 1982 to 1990 as soybean producers used other herbicides in an effort to reduce crop injury and broaden the spectrum of weed control. Herbicides used as metribuzin replacements included chlorimuron and imazaquin. Use of these herbicides resulted in a 50% decline in metribuzin use within one year of their introduction in 1987.

An aggressive imazaquin marketing campaign led to its widespread use in 1988, a drought year in the Midwest. The subsequent widespread carryover injury to corn in 1989 resulted in a change in the label and elimination of imazaquin use in the northern two-thirds of the Corn Belt.

The introduction of imazethapyr in 1989 resulted in a decline in both metribuzin and imazaquin use.

Because chlorimuron and thifensulfuron are broadleaf herbicides, they need to be mixed with grass herbicides for broad-spectrum postemergence weed control in STS soybeans. Because many fields contain ALS-resistant waterhemp, postemergence treatments are often tank mixed with lactofen or acifluorfen. For preemergence applications, a copack of thifensulfuron and chlorimuron plus sulfentrazone provides control of waterhemp and nightshade in addition to broadleaf weeds [27]. This copack can be combined with a grass herbicide such as pendimethalin.

5. Burndown Herbicides

Soybeans often follow corn, which has a large amount of crop residue. Leaving residues intact or mixing them into the soil surface is highly desirable for the purpose of controlling erosion. Prior to the widespread use of herbicides, surface trash was unacceptable because it clogged the cultivator that was used for weed control. Conservation tillage practices, including no-till, that greatly reduced preplant tillage for weed control increased reliance on herbicides for weed control [10]. In the 1970's the herbicide paraquat (Gramoxone) was introduced to kill existing vegetation at the time of crop planting. After spraying paraquat, all the plants were killed and completely dead, almost as if they had been burned with fire [162]. For this reason, the term "burndown" was coined. Another commonly-used burndown herbicide is glyphosate (Roundup). It also provides broad-spectrum control of both grass and broadleaf, annual and perennial vegetation. A commonly-used burndown herbicide is 2,4-D, an inexpensive herbicide that is effective on winter annuals/perennials and annual broadleaves, but is not effective on annual grasses.

Control of existing vegetation at planting is absolutely critical to successful no-till farming. If any weeds survive, they will have a head start on the soybean plants and will be extremely difficult to control.

C. Summary of Usage: 1995

By the early 1990's, there were at least 70 registrations for individual herbicides or packaged herbicide mixtures for weed management in soybeans. As a result, most weeds in soybeans could be adequately controlled with the herbicides available in the early 1990's in well-planned management systems [23].

During the 1990's soybean growers increasingly shifted towards postemergence herbicide use. Table 7 shows that by 1995, only 23% of the nation's soybean acreage received only a before or at-plant soil applied herbicide – representing a 50% reduction in such treatments between 1990 and 1995. The average number of treatments per acre rose from 1.5 in 1990 to 1.7 in 1995 (Table 7) as it was more common for soybean growers to make both an at-plant and postemergence treatment or make two postemergence treatments.

Table 8 compares 1988 with 1995 in terms of the distribution of soybean acreage by the number of herbicide applications. As can be seen, there was a decline in acreage that received a single herbicide application (51 to 41%) with a concomitant rise in the number of acres that received two treatments (34 to 44%).

As can be seen in Table 9, a steady increase in the number of herbicide active ingredients applied to treated soybean acreage occurred between 1986 and 1995: from 1.4 to 2.7.

In 1995, 23% of the nation's soybean acreage was treated with a combination of four or more active ingredients while 28% received three active ingredients, 35% received two active ingredients and 12% was treated with just a single herbicide active ingredient. (See Table 10.)

Annual soybean yield loss to weeds in the U.S. was estimated at 17% during the 1951-60 time period [18]. In 1992, U.S. soybean yield losses to weeds was estimated at 7% [14].

The increased use of herbicides led to significant reductions in the number of cultivations of U.S. soybean acreage. In 1994, only 43% of U.S. soybean acreage received any cultivation during the growing season [16]. The average cultivated acre was cultivated one time during the season in 1994.

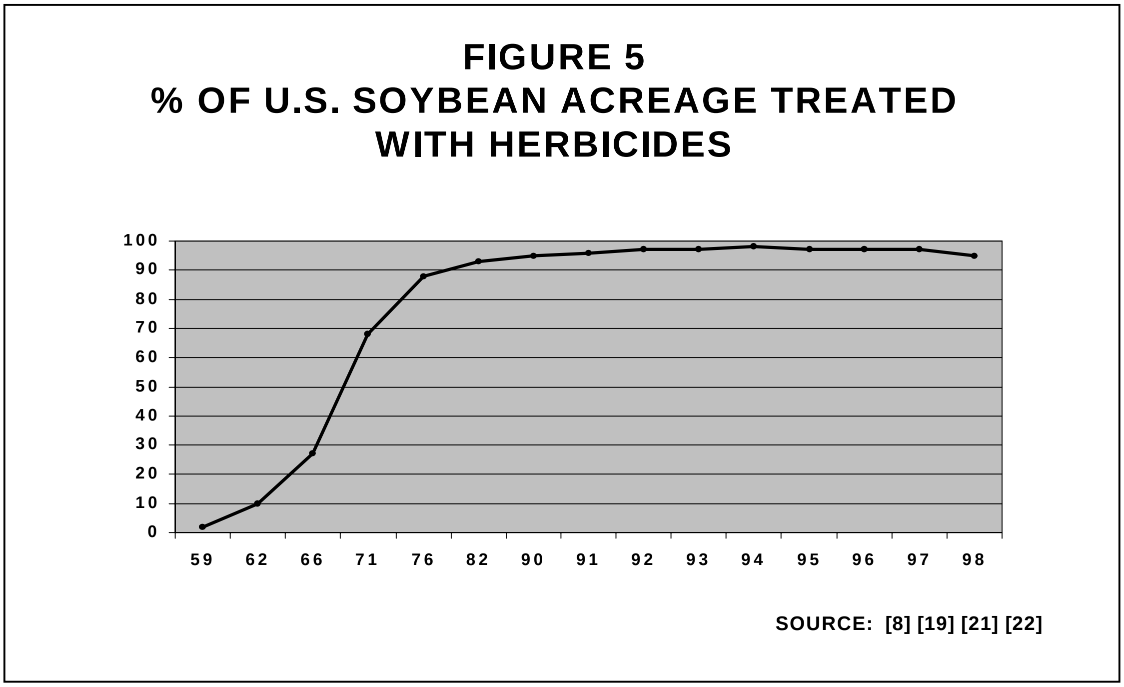


FIGURE 5
% OF U.S. SOYBEAN ACREAGE TREATED
WITH HERBICIDES
100
90
80
70
60
50
40
30
20
10
0
59
62
66
71
76
82
90
91
92
93
94
95
96
97
98
SOURCE: [8] [19] [21] [22]

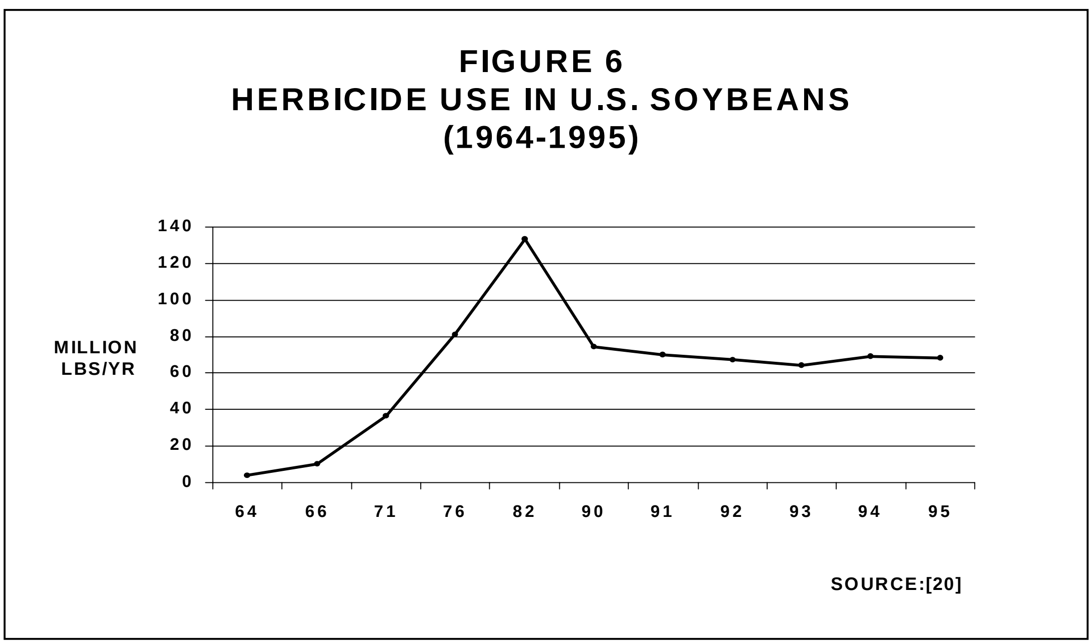


FIGURE 6
HERBICIDE USE IN U.S. SOYBEANS
(1964-1995)
140
120
100
80
60
40
20
0
MILLION
LBS/YR
64
66
71
76
82
90
91
92
93
94
95
SOURCE:[20]

TABLE 5: Herbicide Use on U.S. Soybean Acreage: (% Acreage Treated, 1966-1995)

Active Ingredient	1966	1976	1982	1990	1991	1992	1993	1994	1995
Acifluorfen				7	9	10	10	10	12
Alachlor		37	25	13	12	9	8	7	4
Bentazon		11	16	16	12	14	12	14	12
Chloramben	11	7	6	1	<1	-	-	-	-
Chlorimuron				20	17	17	17	15	16
Fluazifop				6	5	6	9	8	10
Glyphosate				5	5	7	15	15	20
Imazaquin				16	15	18	17	18	15
Imazethapyr				11	24	29	32	42	44
Metolachlor			10	10	8	6	7	8	7
Metribuzin		17	33	19	16	14	13	10	11
Pendimethalin				14	17	21	22	25	26
Sethoxydim				4	5	6	6	6	7
Trifluralin	8	48	47	37	35	35	25	24	20
Thifensulfuron				4	5	7	10	14	12

Selected major herbicides only

Source: [6], [64], [19], [22]

Table 6: Herbicide Use on Illinois Soybean Acreage (1982-1992)

	% Acres Treated				
Active Ingredient	1982	1985	1988	1990	1992
Acifluorfen	1	6	9	8	11
Alachlor	25	15	11	13	9
Bentazon	20	26	30	32	24
Chloramben	4	3	1	1	-
Chlorimuron			9	17	20
Imazaquin			27	12	15
Imazethapyr				11	28
Metolachlor	10	14	10	8	5
Metribuzin	51	46	23	16	13
Pendimethalin	3	7	15	24	32
Trifluralin	61	48	43	39	27

Source [25], [22]

TABLE 7: Herbicide Use Practices on Soybean Acreage in Major Producing States (1990-95)

	Units	1990	1991	1992	1993	1994	1995
Area Receiving Herbicides	%	96	97	98	98	98	98
Before or at plant only	%	44	39	36	28	28	23
After plant only	%	20	26	28	30	29	32
Both	%	32	32	34	35	42	42
Avg. # treatments/acre	#	1.5	1.5	1.6	1.6	1.7	1.7

Source: [20]

TABLE 8: Soybean Area by Number of Herbicide Applications (%)

	1988	1995
No Applications	4	3
One Application	51	41
Two Applications	34	44
Three or more Applications	11	12

Source: [115] [116]

TABLE 9: Number of Herbicide Active Ingredients Applied per Treated Soybean Acre

Year	Number
1986	1.4
1988	1.6
1990	2.3
1991	2.3
1992	2.4
1993	2.5
1994	2.7
1995	2.7

Source: [115] [116] [117]

TABLE 10: Soybean Area by Number of Active Ingredients Applied (%)

	1995
No Active Ingredients	3
One Active Ingredient	12
Two Active Ingredients	35
Three Active Ingredients	28
Four or more Active Ingredients	22

Source: [115]

11. <u>Transgenic Herbicide Tolerant Soybeans</u>

A. <u>Glyphosate – An Overview</u>

Glyphosate was first introduced as an herbicide under the trade name of Roundup by Monsanto in 1974. Glyphosate is a nonselective broad spectrum herbicide that normally cannot be applied over crops without severe plant injury. Some plant species are somewhat tolerant to glyphosate, probably because of uptake or translocation mechanisms, but no crop has sufficient tolerance for glyphosate to be used directly on the crop without damage. Glyphosate is a systemic herbicide that is transported rapidly from the foliar tissue to the metabolically active regions of shoots and root tips [102]. Within these tissues, glyphosate inhibits the biosynthesis of aromatic amino acids by inhibiting the enzyme EPSPS, an enzyme essential for amino acid synthesis. This pathway for aromatic amino acid biosynthesis is required in plants, not only for the formation of the aromatic amino acids, but also for a number of vitamins, phytohormones and lignins. It is estimated that 40-60% of the carbon fixed by plants is fixed through this pathway [102]. Glyphosate inhibits EPSPS, thus preventing the plant from making the aromatic amino acids essential for the synthesis of proteins and some secondary metabolites [72].

EPSPS is the only physiological target of glyphosate in plants. EPSPS is present in all plants, bacteria and fungi, but not in animals. In plants, EPSPS is localized in the chloroplasts or plastids [72]. Only glyphosate-based herbicides have this biochemical mode of action. No other class of commercial herbicides are known to target this enzyme [103].

Glyphosate is bound tightly to soil particles and is metabolized readily by soil micro-organisms into plant nutrients. As a result, glyphosate exhibits no residual soil activity [103]. The initial inactivation of glyphosate in soils is believed to result primarily from the formation of insoluble complexes with aluminum, iron and calcium ions bound to the soil [103].

Degradation by soil microflora is the predominant route by which glyphosate is metabolized in the environment. Studies on the degradation of glyphosate in the soil indicate the formation of ammonia, phosphates, and carbon [103].

Section 6 includes a description of the development of transgenic soybean plants that tolerate glyphosate applications following the insertion of a gene from a soil bacteria that provides the soybean plant with a version of the enzyme EPSPS to which glyphosate cannot bind. The transgenic soybeans were introduced to U.S. growers in 1996. As can be seen in Table 5, glyphosate had been used on 20% of the nation's soybean acreage in 1995 – the year before the introduction of the transgenic varieties.

Prior to the development to the transgenic cultivars, glyphosate could not be used directly over the top of soybean plants. Glyphosate's use in soybeans prior to 1996 was confined to use as a non-selective "burndown" of weeds prior to soybean planting, spot spraying and in a recirculating sprayer.

High clearance sprayers, known as "bean buggies," were developed, allowing operators to spot spray glyphosate directly on individual weed escapes in soybean fields, avoiding the soybean plants [111].

For tall-growing weeds in soybeans, the recirculating sprayer may be used. It relies on a weed-to-crop height differential by which tall weeds can be controlled selectively with a non-selective herbicide in a short-statured crop [109]. The basic concept of the sprayer is to direct the spray above the crop. A catch basin over the row for each spray nozzle catches any spray that is not intercepted by tall weeds and recirculates it through the sprayer [109]. Weeds must be at least 16 cm taller than the soybeans. Research demonstrated that use of glyphosate in the recirculating sprayer to control johnsongrass greatly increased soybean yields [110].

B. Performance of Roundup Ready Soybeans

The use of glyphosate over-the-top of Roundup Ready soybeans was researched extensively prior to and immediately after their introduction in 1996. Roundup Ready soybeans were evaluated under an EUP in 1995 in 14 states in the Midwest and Mid Atlantic. A single application of glyphosate at 0.63-0.84 kg/ha provided annual weed control throughout the entire growing season [137].

In a 1997 experiment, all application timings of glyphosate (at weed heights of 7.5, 15, 23 and 36 cm) provided season-long control of giant foxtail, common lambsquarters and common cocklebur. The 7.5 cm timing was the only treatment to provide better than 90% control of smartweed. (All other glyphosate treatments provided 78-89% control.) All glyphosate applications provided yields equal to the weed-free comparison. All comparison treatments yielded significantly lower than weed-free comparison [120].

Glyphosate applications provided excellent control of ALS-resistant waterhemp when applied postemergence over glyphosate tolerant soybeans [121]. In Missouri, common waterhemp resistant to the sulfonylureas and imidazolinones had become the principal weed problem in soybeans with over 900,000 acres of soybeans infested. In 1996 experiments, imazaquin, imazethapyr and chlorimuron provided 15-25% control while .75 lb. AI of glyphosate applied over glyphosate tolerant soybeans provided 98% control [122].

Experiments in 1995 and 1996 in Illinois demonstrated that the use of a tank mix partner or a preplant herbicide followed by a single glyphosate application improved weed control compared with a single application of glyphosate [123]. However, the highest levels of weed control were always obtained with two sequential applications of glyphosate [123].

Experiments compared the yield impact of glyphosate and alternative herbicide treatments. At seven locations in 1996, yield averaged across seven locations was higher in the glyphosate treated acres in comparison with those treated with imazethapyr, acifluorfen, bentazon and sethoxydim [124].

Glyphosate's lack of residual activity raises concerns of the optimal time to apply glyphosate and whether sequential applications will be needed. Experiments demonstrated that glyphosate applied at 5 or 8 weeks after soybean planting provided season-long control of almost all weed species [125].

When glyphosate is applied early (when weeds are 7.5 cm), a late flush of a weed species such as velvetleaf is not controlled, and yield can be reduced [120]. In a 1998 experiment, yield loss due to weeds germinating after glyphosate application was observed with the one-pass, two to four inch weed height application [131].

Proper timing of glyphosate application is necessary because applications too early following soybean planting (10 days) allow significant reinfestation of weeds late in season. Also, delaying treatment past 28 days allows an unacceptable level of weed competition reducing soybean yield due to early season weed pressure [133].

Experiments were established at 15 sites in 1997 and 12 sites in 1998 across the north central and northeast U.S. to evaluate the time of application of Roundup (based on weed size) on soybean yield in a Roundup Ready system. When weeds were treated at the 7.6, 15.2 or 22.8 cm stage, the Roundup plots were among the highest yielding treatments at all sites. When weeds were treated at the 30.5 cm stage, yield reductions occurred at 6 of the 15 sites [134].

In southern states, rainfall distribution throughout the growing season results in several flushes of weeds.

In Mississippi, with a long growing season and a more difficult weed complex, a minimum of two applications of Roundup is necessary [63].

Due to the extended emergence period of broadleaf signalgrass and large crabgrass, at least two applications of Roundup are generally required for season-long control of these weeds [95].

The University of Arkansas recommendation is to apply one pint of Roundup 10 to 14 days after the soybeans and weeds emerge and to repeat the treatment in 7 to 10 days. This treatment schedule consistently has produced equal control across all weed species [38].

As with any other herbicide, weed species vary in their tolerance to glyphosate. Highly susceptible weeds, such as giant foxtail, can be controlled with lower rates than required to control weeds with a high level of tolerance, such as velvetleaf. The typical use rates of Roundup (24 to 32 ounces per acre) were chosen since they are the lowest rates that will provide consistent control of most major weeds found in soybean fields [37].

C. <u>Herbicide Ratings</u>

Each year, state extension services release weed control guides for field crops which include soybeans [3] [5] [7]. The guides advise growers on the expected performance of available herbicide treatments to control specific weed species. The guides also include a rating as to the likely injury to soybeans that may result from the treatment. The efficacy rating for a particular herbicide treatment to control a particular weed species is developed by extension specialists based on experimental work and the experiences of farmers in the state. Generally, the rating tables include five broad categories of control effectiveness: none, poor, fair, good and excellent. These ratings are associated with a range of percent control of the weed species. For example, a common range for a "good" rating is 80 to 90% control. The rating tables group herbicide treatments according to timing: preplant incorporated (ppi), pre-emergence (pre), or postemergence (post).

The most extensive set of soybean herbicide treatments rated in a university weed control guide is issued annually by Michigan State University (MSU) [3]. The MSU guide rates the effectiveness of 182 herbicide treatments in controlling 24 different weed species. The MSU guide rates the effectiveness of 47 treatments that contain a single active ingredient, 103 treatments that contain two active ingredients and 33 treatments that contain 3 active ingredients. The MSU guide rates the potential for soybean crop injury from the treatments as follows: 47 treatments have a "minimal risk of crop injury," for 53 treatments "crop injury can occur under certain conditions," and for 66 treatments "severe crop injury can occur."

Roundup (glyphosate), used over Roundup Ready soybeans, is one of the 182 treatments rated by MSU. MSU rates the effectiveness of Roundup as "good" or "excellent" on 23 of the 24 weed species. Roundup receives a rating of "fair" for yellow nutsedge control. The 23 good/excellent ratings is the highest total of good/excellent ratings for the 182 herbicide treatments. In addition, the Roundup treatment is assigned a rating of minimal risk of crop injury. The MSU control rating for Roundup for each species is shown in Table 11 which also displays the weed control ratings for all the alternative treatments discussed below. The next highest total of good/excellent ratings (18) is for a preemergence application of either dimethenamid, or alachlor in combination with metribuzin plus chlorimuron. This combination is also assigned one fair rating, 3 poor ratings, and 2 ratings of no control. This treatment is assigned a crop response rating of "severe crop injury can occur." The most effective postemergence treatment in terms of the number of good/excellent ratings (17) is the prepackage mixture of bentazon and acifluorfen plus sethoxydim (this combination is rated as two separate treatments in the MSU table). This treatment also receives a rating of "severe crop injury."

Of the 47 herbicide treatments that have a rating of "minimal" crop injury, the most highly rated alternative to the Roundup treatment is a preemergence combination of either dimethenamid, alachlor or metolachlor in combination with imazaquin. This treatment is assigned 16 ratings of good or excellent. The most effective postemergence herbicide treatment with a minimal crop injury risk rating is the combination of chlorimuron and thifensulfuron for use with STS soybeans, which is assigned 11 good and excellent ratings with 12 ratings of “none” – mostly for annual grasses. A combination not rated by MSU because it would require two trips across the field is the use of fluazifop for grass control followed by a chlorimuron/thifensulfuron treatment for broadleaf weed control. These products are assigned a rating of minimal crop injury. Two passes are required for this treatment since the treatment is not labeled for tank mixture due to an antagonistic interaction which reduces effectiveness on grasses. This treatment receives 20 good/excellent ratings with three ratings of “none” due to a lack of control of certain perennials.

The MSU weed control guide for soybeans also includes a table advising growers of the maximum broadleaf weed heights for consistent control. Table 12 shows these maximum weed heights for five of the postemergence treatments for 11 broadleaf weed species. As can be seen, for all weed species glyphosate can be applied up to a maximum weed height of at least four inches. The combination of chlorimuron plus thifensulfuron can also be used on weeds up to four inches. However, imazethapyr will only control lambsquarters if they are less than one inch tall while the combination of acifluorfen plus bentazon can be used to control redroot pigweed at a maximum height of only two inches.

Because of the potential for injury to sensitive crops, restrictions on the label identify the minimum interval required before certain crops can be grown in rotation with soybeans treated with certain herbicide treatments. Table 13 shows these minimum intervals for four herbicide treatments. Because of its lack of soil residual activity, there are no rotation restrictions on the glyphosate label for any crop. As can be seen in Table 13, a very sensitive crop such as sugarbeets cannot be planted into a soybean field for 26 to 40 months following the applications of chlorimuron/metribuzin, imazaquin, imazethapyr or thifensulfuron/chlorimuron. The common soybean rotational crop is corn, that can be planted eight to ten months following the application of chlorimuron, metribuzin, imazethapyr or thifensulfuron in soybeans.

D. Adoption Impacts: 1995 – 1998

Table 14 shows estimates of U.S. soybean acreage treated with individual active ingredients for the years 1995-1998. As can be seen, glyphosate usage increased to 46% of acreage treated in 1998, from 20% in 1995, while most other active ingredients recorded declined in acreage treated.

The impacts of the adoption of Roundup Ready soybeans and the associated use of glyphosate are presented in the next sections by comparing herbicide use in 1995, the year before their introduction, with 1998, the latest year for which herbicide use data are available. The comparison is based on USDA surveys of soybean growers in the same thirteen states in both 1995 and 1998. These thirteen states represent approximately 80% of U.S. soybean production. Consequently, the results determined from the thirteen state comparison are assumed to equal 80% of the national impacts. Other states that were included in either the 1995 or 1998 surveys (but not both) were excluded from the calculations since comparisons between the two years could not be made.

1. Herbicide Costs

Roundup Ready soybean weed control programs have been priced to be competitive with conventional programs. In addition to the cost of the herbicide, soybean growers must pay the equivalent of $6 per acre as a “technology fee” when purchasing Roundup Ready seed. Table 15 displays estimates of the costs of different weed control programs for soybeans. These estimates are exclusive of any burndown preplant herbicide treatments. As can be seen, depending on the rates used, numbers of applications and combinations of products, the cost of a conventional program can range from $14 to $25 per acre, an STS program can range from $11 to $28 per acre, while a Roundup Ready program can range from $16 to $32 per acre (including the technology fee).

Potential savings depend on which program growers choose. Growers tailor weed control programs to fit particular weed pressure in each field, choosing herbicides and combinations of herbicides to control particular weed species. There are no data on the specific weed control programs growers are using.

The introduction of the competitively-priced Roundup Ready weed control systems led to reductions in the prices farmers paid for competitive herbicides. Table 16 charts the per pound cost of selected herbicide active ingredients from 1990 to 1998. As can be seen, the costs of chlorimuron and imazethapyr were reduced significantly (40-50%) in 1997 and 1998.

The price of glyphosate was also reduced in 1998 by 22%. The result of lower priced Roundup Ready treatments in comparison with competitive herbicides and the lowering of the price for key herbicides including glyphosate meant that soybean growers spent significantly less on herbicides in 1998 than in 1995. Table 17 displays estimates of aggregate expenditures on soybean herbicides in the thirteen states in the USDA surveys for 1995 and 1998. These aggregate expenditure estimates have been calculated by multiplying the USDA survey estimates of pounds of each active ingredient applied times an estimated price per pound of each active ingredient.

As can be seen, the aggregate expenditure amount for the thirteen states declined from $1.5 billion in 1995 to $1.2 billion in 1998. Assuming that the thirteen states represent 80% of national production implies a reduction in herbicide expenditures from $1.86 billion in 1995 to $1.48 billion in 1998, representing a reduction of $380 million in annual herbicide expenditures by U.S. soybean growers. In addition to the cost of the herbicide glyphosate, soybean growers who purchase Roundup Ready seed paid the equivalent of an extra $6 per acre in a technology fee. As shown in Table 1, 37% of the soybean acres, or 27 million acres, were planted to the Roundup Ready soybeans in 1998, implying a total annual technology fee of $160 million. Thus, soybean growers spent $220 million less on weed control in 1998 due to lower costs after netting out the technology fee.

2. Soybean Yields

There are different points of view regarding the impacts of the wide-spread planting of Roundup Ready soybean on yield. On one hand, some argue that university performance data show the Roundup Ready varieties lagging behind other varieties in terms of yield. On the other hand, Monsanto has presented data that show that Roundup Ready soybeans outyielded national averages in 1998.

Determining the impact that Roundup Ready soybeans have had on yields is somewhat problematic at this stage. With such a new technology, field data on yields are scarce. However, some expectations may be developed from available research results. Two areas of research are relevant to addressing the issue of the impact that Roundup Ready soybeans have had on yields. The first type of research is variety trials, where the yield potential of conventional and Roundup Ready varieties have been compared under weed free conditions. The second type of research is weed control trials, which compare weed control strategies.

Variety trials from more than 3,000 side-by-side comparisons from forty university performance tests conducted across eight states in 1998 were summarized by a University of Wisconsin agronomist [146]. When averaged across all tests, Roundup Ready varieties were 4% lower in yield than conventional varieties. On average, the top five Roundup Ready varieties yielded 5% less than the top five conventional varieties in 200 comparisons. The most often heard explanation for the yield lag is that the Roundup Ready gene often has not been put into the most elite lines that some companies have to offer. A more plausible explanation, say some soybean breeders, is that in the rush to get Roundup Ready lines on the market, many companies have not made enough backcrosses to capture all of the yield potential in the parent lines. In either case, the conventional wisdom says that will be corrected in a reasonably short time, and yield lags will be eliminated [147].

1999 University variety trial data have not been compiled as yet to compare the Roundup-Ready variety yields to those of non-Roundup-Ready varieties.

Some have argued that the university trials show a "yield drag" from Roundup Ready soybeans and that national soybean yields could decline by 2–2.5% as a result of widespread planting of the Roundup Ready cultivars [148].

This analysis ignores potential weed control benefits of the new technology, as it is based solely on the results of the variety trials.

Upon further analysis of the university trial data, Monsanto has pointed out that the notion of a yield drag is based on the overall average yields of conventional varieties in comparison with the overall average yield of the Roundup Ready varieties. Monsanto argues that such an analysis can be misleading since fewer Roundup Ready varieties were tested than the number of conventional varieties. As a result, one poor performing variety has a large impact on the overall average. Monsanto points out that many of the elite varieties of Roundup Ready soybeans have not been included in the variety trials. Monsanto's position is that the top-selling elite Roundup Ready varieties have yields comparable to top-selling elite conventional varieties [149].

In soybean fields the potential yield advantage of conventional varieties may not be realized because of poor weed control. In addition, injury from herbicides may reduce the potential yield advantage of conventional varieties.

In weed control trials, weed control programs are compared as to their efficacy in controlling weeds, and yields are often recorded. Several herbicides are usually included in these trials, alone or in combination, at variable rates and application timing. The purpose of these types of studies is to determine optimal rates and timing to achieve control of various weeds. In general, these tests are conducted using one variety in order to eliminate variety as a variable. Many of these studies are now conducted using Roundup Ready varieties, in order to include Roundup treatments in the studies.

The yield differences in weed control trials are due to differences in weed control and/or crop injury associated with the different herbicides tested but do not take into account the yield potential of the variety used in the study. It is difficult to generalize about the results of these weed control studies, except to note that at this point there seems to be no resounding yield advantage or disadvantage in Roundup Ready systems compared to conventional programs.

Increasingly, weed science researchers have focused on the relationship between crop injury and lower soybean yields. Injury from contact postemergence herbicides such as acifluorfen and lactofen has been observed for a number of years. Combinations of thifensulfuron and imazaquin that achieve acceptable lambsquarters control also result in unacceptable soybean injury and possible yield loss [142].

Soybean injury may alter the canopy such that late germinating weeds may escape control. Bentazon, acifluorfen, thifensulfuron, lactofen and clethodim treatments reduced leaf area and reduced soybean yield 209 to 215 kg/ha compared to the untreated control [130]. Soybean injury from postemergence herbicides reduces yields [139]. A perception that injury from postemergence herbicides may reduce soybean yield potential has been triggered by the minimal to no soybean injury observed with foliar applied glyphosate on glyphosate resistant soybeans.

Surveys conducted by Monsanto and USDA have compared yields in Roundup Ready soybeans and conventional soybeans. Monsanto reported that Roundup Ready soybeans out yielded the national average in 1998. A 4.5 bushel per acre difference was observed, based on a comparison of USDA estimated national soybean yields and Monsanto grower survey results [150]. However, this comparison does not take into account differences in other characteristics between adopters and non-adopters of the technology. The observed difference may be due to adoption of the technology by better managers who would obtain higher than average yields normally. The difference may also be due to adoption by growers who utilize production methods, such as narrow row spacing, that would account for higher yields.

In USDA surveys of soybean producers, yield differences were observed for growers using herbicide-tolerant varieties compared to other growers who purchased seed [151] [160]. Table 18 shows USDA survey results for 1996-1998. Statistically significant differences were observed in three production regions in 1997 and one region in 1998. All of these differences showed higher yields for growers planting herbicide-tolerant varieties. However, the USDA analysis of the survey data does not take into account any confounding factors, which may have influenced the observed differences between herbicide-tolerant varieties and conventional varieties. Similar to the Monsanto results discussed above, the results are differences in means, which may be driven by factors other than the adoption of herbicide-tolerant varieties. In fact, further analysis indicated that only a small increase in yields, less than 1% for a 10% change in adoption, was related to adoption of herbicide tolerant varieties when other factors were considered and controlled. Results of the extended analysis showed that larger operations and more educated operators were more likely to use herbicide-tolerant soybean seed. Use of conventional tillage was higher for conventional growers, which would be expected if Roundup Ready soybeans are adopted in conservation tillage systems, as is widely believed [152].

Researchers in Minnesota concluded that yields in a Roundup system compared to conventional herbicide systems were equal [54]. In a summary of weed control research published in the 1997 North Central Weed Science Society Research Report, in which Roundup-only treatments were compared to conventional programs, Roundup-only plots out-yielded conventional plots by 5.3 bushels per acre [56]. However, some conventional treatments in those comparisons may have been tested against weeds they do not control, whereas Roundup has a broad weed spectrum. This may make Roundup Ready systems appear to be more effective in these types of studies than they would be in reality, where a grower would tailor a weed control program for the particular weed species present in the field.

3. **Returns**

Researchers have compared net returns for conventional and Roundup Ready programs, taking into account both cost and yield differences between the programs. Based on data from field trials conducted in Tennessee in 1995, 1996 and 1997, comparing several conventional and Roundup treatments on Roundup Ready varieties, the Roundup only program was the most profitable system, providing a 13% higher net return than the next best alternative [154].

In Arkansas trials conducted during 1996 and 1997, conventional herbicide programs on nontransgenic and glyphosate tolerant soybean were compared to glyphosate programs on glyphosate-tolerant soybean. Two conventional programs had the highest net returns ($37.33 and $49.72 per acre), while only two of the Roundup Ready programs had positive returns ($14.21 and $15.99 per acre) [155].

A 1997 trial conducted in Louisiana, comparing conventional programs with programs combining conventional herbicides with glyphosate or glyphosate alone on glyphosate-tolerant varieties, resulted in the highest yielding treatment being a combination of metribuzin at a reduced rate applied preemergence followed by Roundup. Though this treatment was more costly than a Roundup only program, only a 0.5 bushel increase in yields would be needed most years to pay for the difference [156].

Trials were conducted in 1997 in Mississippi, using the three highest yielding Roundup Ready and conventional cultivars. Net returns were more than $60.00 per acre higher with the labeled rate glyphosate system at two of three locations compared to the labeled rate conventional system [157].

A study at the Leopold Center that analyzed 1998 crop survey data for Iowa concluded that returns to land and labor essentially were identical for the genetically modified and non-genetically modified soybeans [52]. Results from 365 soybean fields indicated that

1998 yields from the genetically modified soybeans were slightly lower than from the conventional varieties, but so were the costs [52].

4. Other Aggregate Studies

Two previous studies have been conducted analyzing the aggregate impacts of the introduction of Roundup Ready soybeans. Auburn University researchers analyzed the impact of herbicide tolerant soybeans in 1997, using yield and cost change assumptions based on USDA survey data. Overall U.S. growers gains were estimated at $800 million. Table 19 shows assumptions used in the analysis and results of U.S. farmer surplus by region. The authors note that the results are extremely sensitive to the values chosen for certain variables, the supply elasticity in particular. Farmer surplus estimates are also presented using a different assumed elasticity, which reduces the gain to $126 million. Further, it should be noted that the assumptions on yield and cost changes are differences between adopters and non-adopters of the technology from the USDA survey data, as discussed above. These observed differences may be due to factors other than the adoption of herbicide tolerant varieties [158].

Monsanto cites the results of the Auburn study, concluding that Roundup Ready soybeans have reduced herbicide costs for U.S. soybean farmers by almost $700 million [143]. However, this statement mischaracterizes the result of the analysis by attributing the impact to reductions in herbicide costs alone, while the correct interpretation of the Auburn study's result is that farm level impact estimates are based on a combination of yield and cost impacts.

Researchers at Iowa State University have also modeled the impact of adoption of Roundup Ready varieties. Several scenarios were considered, including varying assumptions about adoption patterns, market structure for the technology and yield changes. In the scenario most closely depicting what would be expected for crop year 1999-2000, an adoption rate of 55% for the U.S. is assumed, growers are assumed to realize a $20/ha cost savings and no yield advantage or disadvantage using Roundup

Ready varieties. Change in producers surplus under this scenario was estimated at $156 million [159].

5. Herbicide Treatments

The USDA surveys of herbicide use in soybeans summarizes estimates by state for individual active ingredients that show the estimated percent of acres treated and the average number of applications per treated acre. Estimates of the total number of acres treated and average number of treatments per treated acre are summarized in Table 20 as summed from the data for the thirteen states included in the USDA surveys for both 1995 and 1998.

By multiplying the number of treated acres by the average number of applications per treated acre for each active ingredient, estimates are made of the total number of "application-acres" for each active ingredient. An application-acre is the number of different active ingredients applied per acre times the number of repeat applications. It is different than number of treatments, or passes over the field, in that a single treatment containing two ingredients is counted as two acre-treatments, as is two treatments containing a single ingredient. A reduction in the number of application-acres reflects a reduction in the number of active ingredients used and/or the number of treatments.

These estimates are also shown in Table 20 for each active ingredient. As can be seen, in 1995, on average, most active ingredients were used just slightly more than once per treated acre. In 1998, most active ingredients were used just once per treated acre. As can be seen, glyphosate was used on average 1.3 times per treated acre in 1998, which may represent a combination of a pre-plant burndown treatment with a postemergence spray, or two postemergence sprays on some acreage. Overall, the number of herbicide "application acres" totaled 12.5 million fewer in 1998 in comparison with 1995 in the thirteen states. As can be seen, the overall total of herbicide application acres declined by 9% between 1995 and 1998. This reduction occurred even though the total number of soybean acres increased by 12% between 1995 and 1998.

Inflating this subtotal to represent all soybean acreage implies an annual reduction of 16 million herbicide treatments on U.S. soybean acreage between 1995 and 1998. In 1995, the average treated soybean acre received 2.7 herbicide active ingredient applications while in 1998, the average treated acre received 2.2 applications, representing a 19% reduction (Table 21).

A 1997 survey analysis by USDA's Economic Research Service (ERS) reported that there were statistically significant reductions in herbicide acre treatments in three regions by growers of herbicide tolerant soybeans in comparison with other growers [151]. For two regions, the ERS study reported a higher number of herbicide acre treatments by the herbicide tolerant soybean adopters, but these results were not statistically significant.

It is believed that the use of glyphosate as a postemergence spray was the primary cause of the decline in usage of other active ingredients. In many cases, one glyphosate application substituted for two or more active ingredients, which would account for the decline in the number of herbicide application acres in U.S. soybeans between 1995 and 1998.

6. Herbicide Use Amounts

The USDA surveys of herbicide use include estimates of the total pounds of individual active ingredients used on soybeans by state. The USDA reports include totals by active ingredient, including usage during the growing season as well as preplant "burndown" applications. Table 22 summarizes the estimates of total pounds of herbicide active ingredients applied to soybeans in the thirteen states included in the USDA surveys for 1995 and 1998. As can be seen, the aggregate pounds applied went up by 14% (from 52 to 59 million pounds). One factor that led to the increased poundage was an increase in soybean acreage of 12% between 1995 and 1998.

The average per acre use rate rose between 1995 and 1998. In 1995, there were 51.5 million soybean acres in the thirteen states, of which 97% were treated with herbicides, implying an average treatment rate of 1.04 lbs. In 1998, there were 57.8 million soybean acres in the thirteen states, of which 95% were treated with herbicides, implying an average application rate of 1.08 lbs. per acre.

Figure 7 shows the overall trend in herbicide use in soybeans in terms of millions of pounds per year from 1964 to 1998 (this figure is an extension of Figure 6). As can be seen, the overall poundage of herbicides went up between 1995 to 1998 – largely due to the increase in soybean acreage and to an increase in the rate per treated acre.

It is not possible to determine whether the increase in herbicide use amounts occurred on the Roundup-Ready acreage or the non-Roundup-Ready acreage. For example, increases in the per acre treatment amount was recorded for 10 active ingredients in addition to glyphosate. (See Table 22.)

Table 22 shows the average application rate per treated acre for each active ingredient in 1995 and 1998. As can be seen, the average rate for glyphosate increased from 0.61 to 0.92 lbs. AI per acre, as the herbicide was used increasingly to control weeds during the growing season in addition to burndown or spot treatments. Many of the herbicides that glyphosate displaced are used at lower rates. This can be seen in Table 22. The rates of alternative herbicides are lower than glyphosate's: e.g., acifluorfen (0.24 lb/acre), chlorimuron (0.02 lb/acre), imazethapyr (0.04 lb/acre), etc. However, most of the alternatives to glyphosate are used in combinations, increasing the total number of pounds per acre. For example, a common treatment is pendimethalin and imazethapyr with an overall rate of 1.09 lbs. per acre. There is an absence of information regarding the distribution of the exact programs used by soybean growers in 1995 and 1998. Undoubtedly, some growers in a Roundup Ready program made two postemergence applications while others may have made one postemergence in combination with a preemergence trifluralin or pendimethalin application.

Monsanto commissioned two studies (for 1996 and 1997) that compare the in-season use of herbicides in Roundup-Ready soybean fields with in-season herbicide use in non-Roundup-Ready fields [163]. These data are summarized in Figures 8 and 9. As can be seen, in-season herbicide use, as measured in terms of pounds of active ingredient used in-season per acre, was lower in the Roundup-Ready fields in all regions for both years.

Monsanto reports that 77% and 70% of those surveyed said that they only had to apply glyphosate once to control weeds for the entire growing season in 1996 and 1997, respectively. Twenty-two to 29% made two applications with glyphosate and 1% made three or more trips [163].

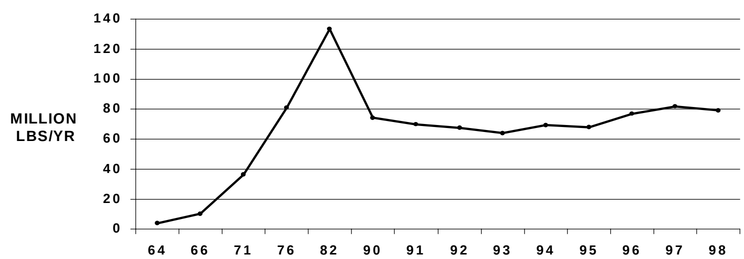
One analyst has claimed that farmers growing Roundup Ready soybeans use two to five times more herbicides measured in pounds applied per acre [148]. However, this statement is based merely on a comparison of the rate of glyphosate with the rates of other postemergence herbicides with no survey data on actual usage.

7. <u>Other Impacts</u>

As noted above, the use of glyphosate in soybean fields imposes no minimum interval before a different crop can be planted in a rotation. Since many of the alternative commonly-used herbicides do have minimum intervals, it is likely that at some point additional crops will be grown in rotation with soybeans. However, it is still too early to tell whether crop rotations have (or will) shift because of the greater flexibility in planting a different crop following soybeans.

Weed populations resistant to many commonly-used soybean herbicides had developed to a significant level in the mid 1990’s. As noted above, herbicide-resistant kochia and waterhemp infested a sizable portion of the soybean growing regions of Missouri and Kansas. Following the introduction of Roundup Ready soybeans, soybean growers had an effective alternative to control these resistant weed populations. As a result, many more acres of soybeans were planted. As Figure 10 shows, soybean acreage in Kansas grew by about 500,000 acres following the introduction of Roundup Ready soybeans, which may be due to growers' ability to control herbicide-resistant weed populations with Roundup.

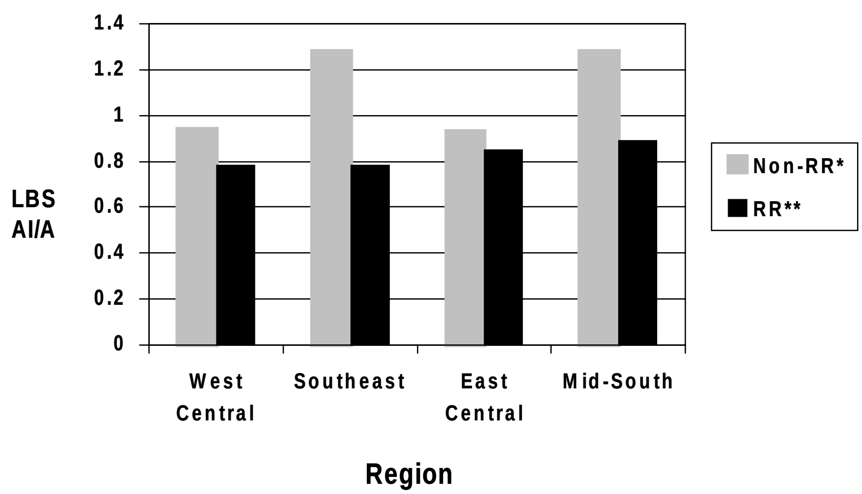
FIGURE 7
HERBICIDE USE IN U.S. SOYBEANS
(1964-1998)



SOURCE:[20]*

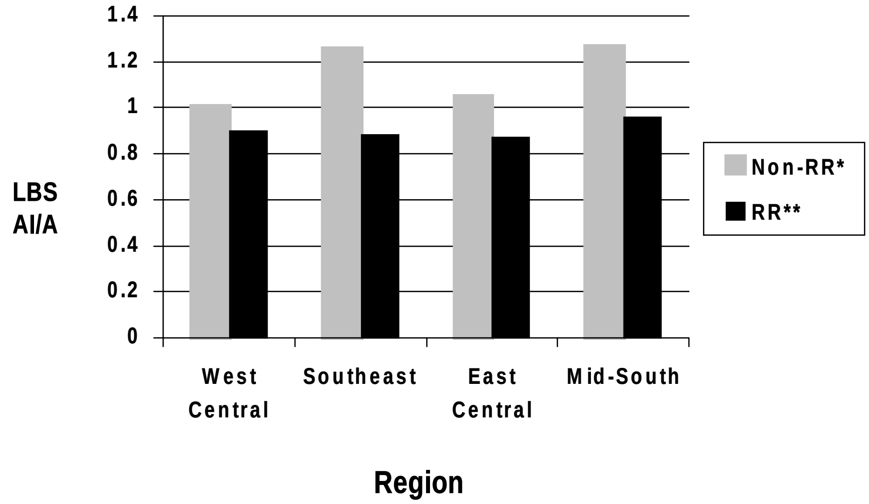
* Estimates for 1996-1998 are extrapolations from USDA survey [22] to include all states producing soybeans based on acreage.

Figure 8: 1996 Herbicide Use In-Season
Roundup Ready Soybeans vs. Non-Roundup Ready Soybeans



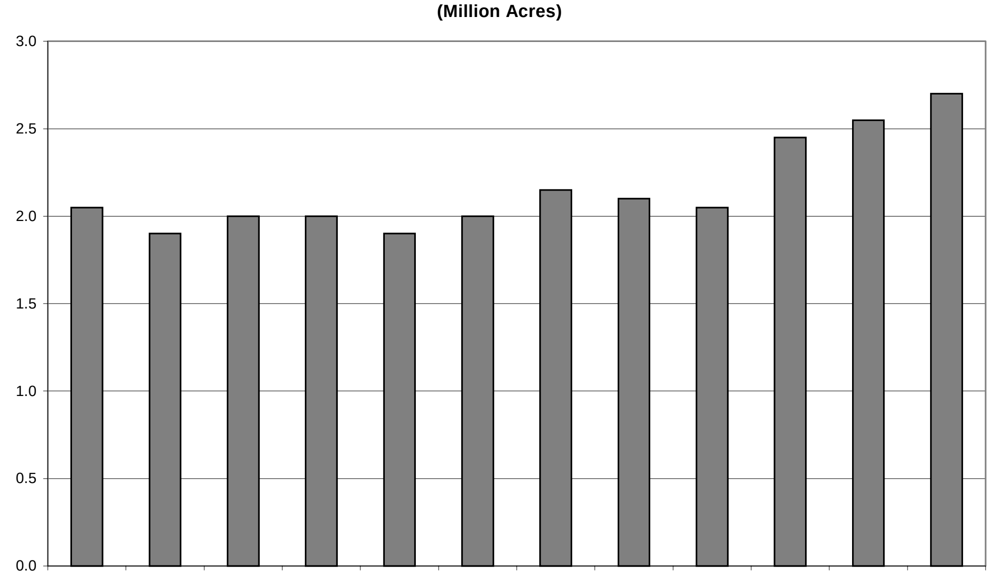
Source: [163]
* Based on a proprietary brand use survey of 7,100 growers, calculated by Sparks Companies, Inc. for Monsanto
** Based on a survey of 1,058 growers conducted by Marketing Horizons for Monsanto

Figure 9: 1997 Herbicide Use In-Season
Roundup Ready Soybeans vs. Non-Roundup Ready Soybeans



Source: [163]
* Based on a proprietary brand use survey of 6,869 growers, calculated by Sparks Companies, Inc. for Monsanto
** Based on a survey of 800 growers conducted by Marketing Horizons for Monsanto

FIGURE 10
KANSAS SOYBEAN ACREAGE
(Million Acres)



Source: [58]

TABLE 11: Weed Control Ratings for Soybean Herbicide Treatments

	Glyphosate	Dimethenamid Or Alachlor + Metribuzin + Chlorimuron	Bentazon + Acifluorfen + Sethoxydim	Chlorimuron + Thifensulfuron	Dimethenamid Or Alachlor Or Metolachlor + Imazaquin	Chlorimuron + Thifensulfuron + Fluazifop (2 passes)
Cocklebur	E	G	E	E	G	E
Jimsonweed	E	G	G	G	G	G
Lambsquarters	G	E	G	G	G	G
Nightshade (Black)	G	G	G	N	G	N
Pigweed (Redroot)	G	E	E	E	E	E
Common Ragweed	G	G	G	G	G	G
Giant Ragweed	G	G	F	G	G	G
Smartweed	G	E	E	E	G	E
Velvetleaf	G	G	G	E	F	E
Wild Mustard	G	E	E	E	G	E
Horseweed	E	E	F	G	P	G
Barnyardgrass	G	E	E	N	E	E
Crabgrass	G	E	G	N	E	G
Giant Foxtail	E	E	E	N	E	E
Green Foxtail	E	E	E	N	E	E
Yellow Foxtail	E	E	E	N	E	E
Fall Panicum	G	G	E	N	G	E
Witchgrass	G	G	E	N	G	E
Sandbur	G	P	E	N	P	E
Bindweed (Field)	G	P	P	N	N	N
Bindweed (Hedge)	G	P	P	N	N	N
Canada Thistle	G	N	F	F	N	F
Quackgrass	E	N	F	N	N	G
Yellow Nutsedge	F	F	F	E	F	E
Timing	Post	Pre	Post	Post	Pre	Post
Crop Injury	Minimal	Severe	Severe	Minimal	Minimal	Minimal

Source: [3]

N= None
P= Poor
F= Fair
G= Good
E= Excellent

TABLE 12: Maximum Broadleaf Weed Heights for Consistent Postemergence Control in Soybeans

	(Weed Height in Inches)			
	Glyphosate	Thifensulfuron + Chlorimuron	Imazethapyr	Acifluorfen + Bentazon
Cocklebur	6	8	8	6
Jimsonweed	6	5	3	6
Lambsquarters	5	4	<1	2
Nightshade (Black)	4	No	2	<2
Pigweed (Redroot)	6	8	6	2
Common Ragweed	6	4	2	3
Giant Ragweed	8	4	3	6
Smartweed	4	8	3	6
Velvetleaf	5	8	2	5
Wild Mustard	6	5	3	4
Horseweed	6	5	No	5

Source: [3]

TABLE 13: Herbicide Crop Rotation Restrictions (in months)

	Field Corn	Oats	Sugarbeets	Potatoes
Glyphosate	0	0	0	0
Chlorimuron + Metribuzin	10	30	30	30
Imazethapyr	8.5	18	40	18
Thifensulfuron + Chlorimuron	9	3	30	30

Source: [3]

TABLE 14: Herbicide Use on U.S. Soybean Acreage: (% Acreage Treated, 1995-1998)

Active Ingredient	1995	1996	1997	1998
Acifluorfen	12	11	12	7
Alachlor	4	5	3	2
Bentazon	12	11	11	7
Chlorimuron	16	14	13	12
Fluazifop	10	7	7	5
Glyphosate	20	25	28	46
Imazaquin	15	15	13	8
Imazethapyr	44	43	38	17
Metolachlor	7	5	7	4
Metribuzin	11	9	10	6
Pendimethalin	26	27	25	18
Sethoxydim	7	9	7	5
Trifluralin	20	22	21	16
Thifensulfuron	12	10	9	5

Source: [22]

Selected major herbicides only. Includes preplant and in-season use.

TABLE 15: Soybean Herbicide Program Costs

Herbicide Program	Rate per Acre	Cost ($/acre)
Conventional		
Pursuit Plus	2.5 pt	13.50
Fusion	6 oz	
+Pursuit	1.44 oz	
+Blazer	8 oz	23.56
Treflan	2 pt	
+Pursuit	1.44 oz	
+Blazer	8 oz	25.70
Roundup Ready		
Roundup	1 qt	16.45
2x Roundup	2 qt	25.90
Pursuit Plus	1.44 oz	
+Roundup	1 qt	22.95
Command	1 pt	
+Prowl	2.4 pt	
+Roundup	1 qt	32.78
STS		
Synchrony	0.5 oz	
+Fusion	6 oz	20.00
Reliance	0.5 oz	
+Assure II	8 oz	11.23
Treflan	2 pt	
+Reliance	0.5 oz	13.26

Source: [153]

TABLE 15a: Glossary

Product Name	Active Ingredient(s)
Pursuit Plus	Pendimethalin + Imazethapyr
Fusion	Fluazifop + Fenoxaprop
Pursuit	Imazethapyr
Blazer	Acifluorfen
Treflan	Trifluralin
Assure II	Quizalofop
Roundup	Glyphosate
Command	Clomazone
Prowl	Pendimethalin
Synchrony	Chlorimuron + Thifensulfuron
Reliance	Chlorimuron + Thifensulfuron

TABLE 16: Herbicide Prices, 1990-1998 ($/LB/AI)

Active Ingredient	1990	1991	1992	1993	1994	1995	1996	1997	1998
Acifluorfen	30	29	29	29	30	30	31	32	34
Alachlor	6	6	6	6	6	7	7	6	6
Bentazon	15	16	16	16	17	17	18	18	18
Chlorimuron	1126	1164	1137	1123	1123	1210	1220	620	722
Fluazifop	94	97	59	59	59	66	66	62	63
Glyphosate	21	18	15	16	16	17	18	18	14
Imazethapyr	287	284	290	299	310	318	329	340	200
Metribuzin	30	32	32	34	36	40	35	36	27
Pendimethalin	7	9	9	8	8	8	8	9	8
Sethoxydim	70	77	60	59	59	59	56	56	51

Source: [5]

TABLE 17: Aggregate Herbicide Expenditures: Soybeans (13 States)						
	$/LB/AI [1]		000 LB AI [2]		000$/YR[3]	
Active Ingredient	1995	1998	1995	1998	1995	1998
2,4-D	3	4	2,248	1,910	7,643	7,411
Acifluorfen	30	34	1,362	1,041	40,656	34,946
Alachlor	7	6	2,749	746	18,785	4,590
Bentazon	17	18	4,241	1,839	73,115	33,966
Chlorimuron	1,210	722	151	140	182,687	101,125
Clethodim	120	97	200	288	23,977	27,825
Clomazone	22	20	605	1,281	13,358	25,569
Cloransulam	-	491	0	2	0	982
Dimethenamid	13	13	286	438	3,850	5,895
Ethalfluralin	11	11	0	0	0	0
Fenoxaprop	114	102	316	315	36,011	32,250
Fluazifop	66	63	359	153	23,522	9,581
Flumetsulam	188	188	12	36	2,257	6,771
Fomesafen	33	46	552	830	18,183	38,495
Glyphosate	17	14	6,313	24,944	105,238	358,695
Imazamox	-	600	0	113	0	67,800
Imazaquin	160	158	716	360	114,718	56,772
Imazethapyr	318	200	1,332	401	423,829	80,200
Lactofen	60	66	145	43	8,651	2,851
Linuron	21	22	217	22	4,659	495
Metolachlor	9	10	6,745	3,673	57,400	37,722
Metribuzin	40	27	1,259	674	50,612	18,269
Paraquat	14	15	365	264	4,979	3,889
Pendimethalin	8	8	12,897	10,280	107,690	85,632
Quizalofop	154	156	134	59	20,592	9,178
Sethoxydim	59	51	646	559	37,849	28,685
Sulfentrazone	-	78	0	250	0	19,500
Thifensulfuron	2,249	2,464	19	4	42,740	9,855
Trifluralin	9	9	8,125	8,633	69,117	76,963
TOTAL			**51,994**	**59,298**	**1,492,115**	**1,185,913**

[1] From [5]

[2] From [22] Includes the states: AR, IL, IN, IA, KY, LA, MN, MS, MO, NE, NC, OH, TN, which account for 80 % of U.S. soybean acreage

[3] Calculated by multiplication

Includes preplant and in-season use.

TABLE 18: Yields Comparison of Herbicide-tolerant Soybeans to Conventional Varieties by Production Region (% difference)

Region	1996	1997		1998	
Heartland	-2.4	13.6	**	4.4	
Mississippi Portal	2.8	-6.2		-3.6	
Northern Crescent	id	-4.6		4.8	
Prairie Gateway	id	21.0	**	24.2	
Southern Seaboard	id	13.3	*	21.4	*
Eastern Uplands	id	id		-8.0	

Herbicide tolerant varieties include both Roundup Ready and STS varieties.
id-insufficient data to estimate
* significantly different from all other at the 10 percent level.
** significantly different from all other at the 5 percent level.
Source [151] ERS Study

TABLE 19: Soybean Area, Adoption Rate, Yield and Cost Changes, and Farmer Surplus by Region, 1997 from Auburn Study

Region	Acres Planted to Herbicide Tolerant Soybeans (1000)	Acres Planted to Soybeans (1000)	Percent Yield Change (HT-conv)	Percent Pesticide, Tillage, Cultivation and other Cost Change (HT-conv)	US Farmer Surplus ($1000)
Corn Belt	5,960	35,266	13.0	3.7	830,194
Southeast	737	4,027	18.2	4.9	109,706
Delta	2,369	7,093	-14.7	6.2	-295,113
Northern Plains	1,213	9,476	15.4	2.5	163,507
Total	10,279	55,863			808,295

Source: [118]

HT: Herbicide Tolerant

TABLE 20: Herbicide Applications to Soybeans (13 States)

Active Ingredient	Percent Acres Treated 1995	Percent Acres Treated 1998	# of Applications Per Treated Acre 1995	# of Applications Per Treated Acre 1998	Total Application-Acres (000) 1995	Total Application-Acres (000) 1998
2,4-D	10	8	1.00	1.00	5,077	4,613
Acifluorfen	11	7	1.10	1.02	6,420	4,376
Alachlor	3	1	1.01	1.04	1,501	490
Bentazon	12	5	1.03	1.00	6,462	2,672
Chlorimuron	15	13	1.08	1.02	8,600	7,908
Clethodim	4	4	1.01	1.01	2,212	2,115
Clomazone	2	3	1.00	1.00	1,081	1,809
Cloransulam	-	<1	-	1.00	-	128
Dimethenamid	1	1	1.00	1.00	284	415
Fenoxaprop	5	4	1.08	1.03	2,842	2,467
Fluazifop	10	5	1.09	1.04	5,657	2,873
Flumetsulam	<1	1	1.00	1.00	236	663
Fomesafen	4	6	1.05	1.02	1,957	3,316
Glyphosate	20	47	1.01	1.30	10,478	35,163
Imazamox	-	7	-	1.00	-	3,990
Imazaquin	15	7	1.10	1.01	8,346	4,231
Imazethapyr	44	16	1.00	1.00	22,522	9,413
Lactofen	4	1	1.00	1.00	1,840	539
Linuron	1	<1	1.00	1.00	451	44
Metolachlor	7	4	1.02	1.00	3,520	2,098
Metribuzin	9	6	1.04	1.00	5,080	3,184
Paraquat	1	1	1.12	1.00	645	531
Pendimethalin	26	17	1.10	1.00	14,810	9,791
Quizalofop	5	2	1.03	1.00	2,582	1,159
Sethoxydim	7	4	1.00	1.00	3,601	2,580
Sulfentrazone	-	4	-	1.00	-	2,087
Thifensulfuron	12	4	1.04	1.00	6,701	2,540
Trifluralin	19	16	1.01	1.00	10,069	9,256
Total					**132,974**	**120,445**

Source: Derived from [22]

Includes preplant and in-season use.

TABLE 21: Herbicide Application Treatments per Treated Soybean Acre

	1995	1998
Total Soybean Acreage (millions)	62.5	72.3
Percent Treated[1]	97.0	95.0
Acreage Treated (millions)	60.6	68.7
Number of Application Acres (millions)[2]	166.0	150.0
Number of Herbicide Application Treatments per Acre	2.7	2.2

[1] From [22]

[2] Calculated by assuming that the 13 state total in Table 15 represents 0.8 of the national total. Includes preplant and in-season use.

TABLE 22: Herbicides: Pounds Applied to Soybeans (13 States)

Active Ingredient	LBS AI (000)		Acres Treated (000)		LBS AI/A/YR	
	1995	1998	1995	1998	1995	1998
2,4-D	2,248	1,910	5,077	4,613	0.44	0.41
Acifluorfen	1,362	1,041	5,824	4,281	0.23	0.24
Alachlor	2,749	746	1487.3	471	1.85	1.58
Bentazon	4,241	1,839	6270.6	2671.5	0.68	0.69
Chlorimuron	151	140	7,974	7,791	0.02	0.02
Clethodim	200	288	2,201	2,089	0.09	0.14
Clomazone	605	1,281	1,081	1,809	0.56	0.71
Cloransulam	0	2	0	128	0	0.02
Dimethanamid	286	438	283.5	415	1.01	1.06
Fenoxaprop	316	315	2,620	2,404	0.12	0.13
Fluazifop	359	153	5,193	2,771	0.07	0.06
Flumetsulam	12	36	236	663	0.05	0.05
Fomesafen	552	830	1,858	3,266	0.30	0.25
Glyphosate	6,313	24,944	10,423	26,985	0.61	0.92
Imazamox	0	113	0	3,990	0	0.03
Imazaquin	716	360	7,613	4,181	0.09	0.09
Imazethapyr	1,332	401	22,522	9,413	0.06	0.04
Lactofen	145	43	1,840	539	0.08	0.08
Linuron	217	22	451	44	0.48	0.50
Metolachlor	6,745	3,673	3,454	2,098	1.95	1.75
Metribuzin	1,259	674	4,877	3,184	0.26	0.21
Paraquat	365	264	577	531	0.63	0.50
Pendimethalin	12,897	10,280	13,437	9,791	0.96	1.05
Quizalofop	134	59	2,514	1,159	0.05	0.05
Sethoxydim	646	559	3,601	2,580	0.18	0.22
Sulfentrazone	0	250	0	2,087	0	0.12
Thifensulfuron	19	4	6,418	2,540	0.003	0.002
Trifluralin	8,125	8,633	9,970	9,256	0.81	0.93
Total	**51,994**	**59,298**				

Source: Derived from [22]

Includes preplant and in-season use.

12. Summary and Conclusions

Soybeans are an extremely important and valuable crop in the United States. Controlling weeds is the major pest problem that soybean growers must plan for every year. Before the introduction of herbicides, soybean growers made many trips across fields with cultivators, and yet, the nation lost about 17% of national production to weeds because of limited effectiveness of the mechanical operations.

The use of herbicides in soybeans for weed control is one of the most important technological changes that has made possible consistent high yields for U.S. soybeans. Since herbicides are more effective than non-chemical controls, soybean yields increased dramatically as growers switched to them and reduced tillage operations.

Soybean cultivars have been improved genetically through crossbreeding for many decades. All currently grown U.S. soybean cultivars are the result of genetically improving cultivars imported from Asia. As important as herbicides are for soybean production, genetically improving soybean cultivars to tolerate applications of these chemicals has not been a focus of conventional crop breeding. As a result, chemicals have been selected for herbicidal use in soybeans based on screening compounds that kill weeds without causing major damage to soybeans. Although soybean plants detoxify these chemicals, several of the most widely used herbicides in soybeans can cause serious injury to the crop under certain environmental conditions.

As a result of changing one enzyme out of the approximately 10,000 enzymes in a soybean plant, scientists created a transgenic plant that tolerates the use of the broad-spectrum herbicide glyphosate. The plant is called transgenic because the gene that conferred glyphosate tolerance was taken from a soil bacterium and inserted into soybean DNA by a non-sexual method. Glyphosate tolerance in the transformed plant is based on the insertion of DNA for a different form of an enzyme already present in the soybean plant. The difference in the enzyme from the bacterium means that glyphosate does not interrupt the functioning of the enzyme.

U.S. growers have planted the transgenic herbicide tolerant soybean varieties on a large percentage of U.S. soybean acreage primarily because of the following factors:

- Less complicated weed control
- Broader spectrum weed control
- Less crop injury
- More flexibility in timing treatments
- Less concern of carryover to rotational crops

In addition to these factors, the herbicide tolerant seed and associated herbicides were priced competitively with existing herbicide programs for soybeans. Following the introduction of the herbicide tolerant transgenic soybeans, the prices of alternative herbicide treatments were lowered significantly. Overall, U.S. growers saved approximately $220 million in 1998 due to lower herbicide costs.

The broad spectrum of weeds controlled by glyphosate means that soybean growers no longer need to make as many multiple applications with combinations of herbicides. As a result of the widespread substitution of one active ingredient application for an application that might include three or four active ingredients, U.S. soybean growers made 16 million fewer herbicide active ingredient applications in 1998 in comparison with 1995. A 19% reduction occurred in the number of herbicide active ingredient applications made to the average treated soybean acre.

13. References

[1] ASA, SoyStats, 1999.

[2] Smith, Keith J., and Wipada Huyser, "World Distribution and Significance of Soybean" in Soybeans: Improvement, Production, and Uses, Second Edition, American Society of Agronomy, 1987.

[3] Kells, James J., and Karen A. Renner, 1999 Weed Control Guide for Field Crops, Michigan State University, Extension Bulletin E-434.

[4] Fehr, Walter R., "Breeding Methods For Cultivar Development" in Soybeans: Improvement, Production, and Uses, Second Edition, American Society of Agronomy, 1987.

[5] 1999 Illinois Agricultural Pest Management Handbook, University of Illinois Extension Service.

[6] USDA, Quantities of Pesticides Used by Farmers in 1966, Economic Research Service, Agricultural Economic Report Number 179, April 1970.

[7] 1999 Cultural and Chemical Weed Control in Field Crops, University of Minnesota Extension Service, BU-3157-5.

[8] USDA, A Survey of Extent and Cost of Weed Control and Specific Weed Problems, Agricultural Research Service, ARS 34-23-1, August 1965.

[9] Anderson, J.M., and C.G. McWhorter, "The Economics of Common Cocklebur Control in Soybean Production," Weed Science, July 1976.

[10] Jordan, T.N., H.D. Coble, and L.M. Wax, "Weed Control," in Soybeans: Improvement, Production and Uses, Second Edition, American Society of Agronomy, 1987.

[11] Barrentine, W.L., "Common Cocklebur Competition in Soybeans," Weed Science, November 1974.

[12] Coble, H.D., and R.L. Ritter, "Pennsylvania Smartweed Interference in Soybeans," Weed Science, November 1978.

[13] Coble, H.D., et al., "Common Ragweed Interference in Soybeans," Weed Science, May 1981.

[14] Bridges, David C., ed., Crop Losses Due To Weeds In The United States – 1992, Weed Science Society of America, 1992.

[15] Weed Control Used By Illinois Farmers, 1964, Illinois Cooperative Crop Reporting Service, December 1964.

[16] USDA, AREI Updates: 1994 Pest Management on Major Field Crops, Economic Research Service, Number 19, 1995.

[17] Pike, David R., et al., Biologic and Economic Assessment of Pesticide Use In Corn and Soybeans, USDA, NAPIAP Report Number 1-CA-95.

[18] USDA, Losses In Agriculture, ARS, Agriculture Handbook No. 291, August 1965.

[19] USDA, Inputs: Outlook and Situation, Economic Research Service, IOS-2, October 1983.

[20] USDA, Agricultural Resources and Environmental Indicators, 1996-97, Economic Research Service, Agricultural Handbook No. 712, July 1997.

[21] USDA, Farmers Use of Pesticide in 1971…, Extent of Crop Use, Economic Research Service, Agricultural Economic Report No.268, September 1975.

[22] USDA, Agricultural Chemical Usage: Field Crops Summary 1990-1998 (Separate Volumes), National Agricultural Statistics Service.

[23] Wilcut, John W., et al., "The Niche for Herbicide Resistant Crops in U.S. Agriculture" in Herbicide Resistant Crops, CRC Press, 1996.

[24] Pike , David R., et al., "A Case Study of Herbicide Use," Weed Technology, July-September 1991.

[25] Pike, David R., et al., Pesticide Use in Illinois: Results of a 1990 Survey of Major Crops, University of Illinois, Cooperative Extension Service, DP-91-1.

[26] Stoller, Edward W., et al., "Weed Interference in Soybeans," Reviews of Weed Science, 1987, 3:155-181.

[27] "STS Soybeans: They're Still a Good Choice," Soybean Digest, November 1997.

[28] Ashton, Floyd M., and Thomas J. Monaco, Weed Science: Principles and Practices, Third Edition, John Wiley and Sons, 1991.

[29] Anderson, Wood Powell, Weed Science: Principles, Second Edition, West Publishing Company, 1983.

[30] Wax, L.M., “Weed Control,” in Soybeans: Improvement, Production and Uses, American Society of Agronomy, 1973.

[31] De Weese, W.W., et al., “ Response of Soybean Cultivars to Metribuzin in the Field and Greenhouse,” Weed Technology, October-December 1989.

[32] Wilson, Robert G., and John Furrer, “Where Do Weeds Come From?” NebGuide, University of Nebraska, G86-807, July 1986.

[33] Taylorson, R.B., “The Role of Seed Dormancy and Germination in Devising Weed Control Methods,” in Proceedings World Soybean Research Conference III.

[34] Peters, Elroy J., et al., “Rotary Hoeing in Combination with Herbicides and Other Cultivations for Weed Control in Soybeans,” Weeds, 1959, 7:449-458.

[35] “Weeds – Still Your Worst Enemy,” Soybean Digest, April 1960.

[36] “Herbicides for Use in Soybeans,” Soybean Digest, April 1966.

[37] Hartzler, Bob, “Are Roundup Ready Weeds in Your Future?,” Iowa State University, November 3, 1998.

[38] Baldwin, Ford, “Make Use of Roundup Ready Program Flexibility,” Delta Farm Press, July 10, 1998.

[39] Probst, A.H., and O.W. Luetkemeier, “How Much Cultivation for Best Soybean Results?” Soybean Digest, March 1959.

[40] Wax, L.M., and C.G. McWhorter, “Prescription for Weed Control,” Soybean Digest, February 1968.

[41] Staniforth, D.W., et al., “Role of Herbicides in Soybean Production,” Weeds, 11:96-98.

[42] Colby, S.R., “The Mechanism of Selectivity of Amiben,” Weeds, 1966, 14:197-201.

[43] Parka, S.J., and O.F. Soper, “The Physiology and Mode of Action of the Dinitroaniline Herbicides,” Weed Science, January 1977.

[44] Kirkpatrick, B.L., et al., “Competition of Jimsonweed with Soybean,” Agronomy Journal, September-October 1983.

[45] Steckel, Lawrence E., et al., "Integrating Reduced Rates of Postemergence Herbicides and Cultivation for Broadleaf Weed Control in Soybeans," Weed Science, November 1990.

[46] Webster, H.L., et al., "Trifluralin and Metribuzin for Broad Spectrum Weed Control in Soybeans," Proceedings North Central Weed Control Conference, Volume 28, 1973.

[47] Hargroder, T.G., and R.L. Rogers, "Behavior and Fate of Metribuzin in Soybean and Hemp Sesbania," Weed Science, May 1974.

[48] Abusteit, Ezzaldin G., et al., "Absorption, Translocation and Metabolism of Metribuzin in Diploid and Tetraploid Soybean Plants and Cell Cultures," Weed Science, September 1985.

[49] Mangeot, B.L., et al., "Differential Metabolism of Metribuzin by Two Soybean Cultivars," Weed Science, May 1979.

[50] Coble, H.D., and J.W. Schrader, "Soybean Tolerance to Metribuzin," Weed Science, July 1973.

[51] Moshier, L.J., and O.G. Russ, "Metribuzin Injury in Soybeans as Influenced by Application Timing and Cultivation," Agronomy Journal, July-August 1981.

[52] Duffy, Mike, "Does Planting GMO Seed Boost Farmers' Profits?" Leopold Letter, Fall 1999.

[53] Shaner, Dale L., and N. Moorthy Mallipudi, "Mechanisms of Selectivity of the Imidazolinone Herbicides," in The Imidazolinone Herbicides, CRC Press, 1991.

[54] Breitenbach, F., and T. Hoverstad, "Roundup Ready Soybeans," Crop News, December 18, 1998, 4(29), 162-163.

[55] Sebastian, S.A., et al., "Semidominant Soybean Mutation for Resistance to Sulfonylurea Herbicides," Crop Science, November-December 1989.

[56] Fawcett, R.S., North Central Weed Science Society Research Report Studies Investigating Yields of Roundup Ready Soybeans with and Without Roundup Herbicide Treatments in 1997, Huxley, IA: Fawcett Consulting, 1997.

[57] Hahn, Kevin L., and Michael R. Hughes, "Weed Control Options for 'STS' Soybeans," in Proceedings North Central Weed Science Society, 1993.

[58] USDA, Agricultural Statistics, National Agricultural Statistics Service

[59] Koskinen, William C., et al., "Effects of Trifluralin Soil Metabolites on Soybean Growth and Yield," Weed Science, 1986, 34:471-473.

[60] Ross, Merrill A., and Carole A. Lembi, Applied Weed Science, Prentice-Hall, Second Edition, 1999.

[61] Barrentine, William L., et al., " Tolerance of Three Soybean Cultivars to Metribuzin," Weed Science, July 1982.

[62] Hartwig, E.E., et al., "Registration of Tracy-M Soybeans," Crop Science, 1980, 20: 825.

[63] "Research Compares Roundup Ready, Conventional Beans," Delta Farm Press, May 1, 1998.

[64] USDA, Farmers' Use of Pesticides in 1976, Economics, Statistics and Cooperative Services, Agricultural Economic Report Number 418, December 1978.

[65] WSSA, Herbicide Handbook, Seventh Edition, 1994.

[66] Anderson, Robert N., et al., "Controlling Broadleaf Weeds in Soybeans with Bentazon in Minnesota," Weed Science, March 1974.

[67] McWhorter, C.G., and J.M. Anderson, " Bentazon Applied Postemergence for Economical Control of Common Cocklebur in Soybeans," Weed Science, July 1976.

[68] Lee, Steve D., and Lawrence R. Oliver, "Efficacy of Acifluorfen on Broadleaf Weeds: Times and Methods for Application," Weed Science, September 1982.

[69] "Why Postemerge is Looking Good," Successful Farming, June 1989.

[70] "Growers Utilizing Postemergence Options," Agrochemical Age, April 1991.

[71] Delanney, X., et al., "Yield Evaluation of a Glyphosate-Tolerant Soybean Line After Treatment with Glyphosate," Crop Science, September-October 1995.

[72] Padgette, S.R., et al., "Development, Identification and Characterization of a Glyphosate Tolerant Soybean Line," Crop Science, September-October 1995.

[73] Schroder, David, et al., The Contribution of Herbicides and Other Technologies to Soybean Production in the Corn Belt Region, 1965 – 1979, University of Missouri, Ag Econ Paper, 1981-33, 1981.

[74] Liu, KeShun, Soybeans: Chemistry, Technology and Utilization, Chapman and Hall, 1997.

[75] Fehr, Walter R., “Breeding,” in Soybean Physiology, Agronomy and Utilization, Academic Press, 1978.

[76] Bernard, Richard L., “Soybean Germplasm, Breeding and Genetic Activities in the United States,” in Soybean Research in China and the United States, INTSOY Series Number 25, College of Agriculture, University of Illinois, July 1983.

[77] Caviness, C.E., “Estimates of Natural Cross-Pollination in Jackson Soybeans in Arkansas,” Crop Science, March-April 1966.

[78] Poehlman, John Milton, Breeding Field Crops, Holt-Dryden, 1959.

[79] Collins, G.B., and D.F. Hildebrand, “Biotechnology in Soybean Breeding,” Proceedings: World Soybean Research Conference V, 1994.

[80] Goodman, Robert M., and Jack Kiser, “Biotechnology and its Impact on Future Developments in Soybean Production and Use,” in Proceedings World Soybean Research Conference III.

[81] Dyer, William E., “Applications of Molecular Biology in Weed Science,” Weed Science, July-September 1991.

[82] Dyer, William E., “Techniques for Producing Herbicide-Resistant Crops,” in Herbicide Resistant Crops, CRC Press, 1996.

[83] Mazur, Barbara J., and S. Carl Falco, “The Development of Herbicide Resistant Crops,” Annu. Rev. Plant Physiol. Plant Mol. Biol., 1989.

[84] Hart, Stephen E., et al., “Herbicide-Resistant Crops from Cell Selection,” Reviews of Weed Science, 1994, 6:251-263.

[85] Specht, James E., and James H. Williams, “Contribution of Genetic Technology to Soybean Productivity – Retrospect and Prospect,” in Genetic Contributions to Yield Gains of Five Major Crop Plants, CSSA Special Publication Number 7, 1984.

[86] Christou, Paul, et al., “Soybean Genetic Engineering – Commercial Production of Transgenic Plants,” Bio/Technology, 8:145-151, 1990.

[87] Christianson, Michael L., “Fun with Mutants: Applying Genetic Methods to Problems of Weed Physiology,” Weed Science, July-September 1991.

[88] Carlson, John B., and Nels R. Lersten, "Reproductive Morphology," in Soybeans: Improvement, Production and Uses, Second Edition, American Society of Agronomy, 1987.

[89] Christou, Paul, et al., "Stable Transformation of Soybean Callus by DNA-Coated Gold Particles," Plant Physiology, Volume 87, 671-674, 1988.

[90] Heady, Earl O., and Ludwig Auer, "Imputation of Production to Technologies," Journal of Farm Economics, Volume 48, Number 2, May 1966.

[91] Wood, D.R., ed., Crop Breeding, American Society of Agronomy, 1983.

[92] Harten, A.M. van, Mutation Breeding: Theory and Practical Applications, Cambridge University Press, 1998.

[93] Sebastian, Scott A., and Roy S. Chaleff, "Soybean Mutants with Increased Tolerance for Sulfonylurea Herbicides," Crop Science, September-October 1987.

[94] Salisbury, Frank B. and Cleon Ross, Plant Physiology, Wadsworth Publishing Company.

[95] Scott, Robert, et al., "Glyphosate Tank Mixtures with SAN582 for Burndown or Postemergence Applications in Glyphosate Tolerant Soybean," Weed Technology, January-March 1998.

[96] Saari, Leonard L., and C. Jeffrey Mauvais, "Sulfonylurea Herbicide-Resistant Crops," in Herbicide Resistant Crops, CRC Press, 1996.

[97] Sipos, Endre, F., et al., "Processing and Utilization Activities of Soybeans in the United States," in Soybean Research in China and the United States, INTSOY Series Number 25, College of Agriculture, University of Illinois, July 1983.

[98] Orthoefer, F.T., "Processing and Utilization," in Soybean Physiology, Agronomy and Utilization, Academic Press, 1978.

[99] USDA, Oil Crops: Situation and Outlook Yearbook, Economic Research Service, October 1998.

[100] Smith, C. Wayne, Crop Production: Evolution, History and Technology, John Wiley and Sons, Inc., 1995.

[101] Padgette, Stephen R., et al., "New Weed Control Opportunities: Development of Soybeans with a Roundup Ready Gene," in Herbicide Resistant Crops, CRC Press, 1996.

[102] Kishore, Ganesh M., et al., "History of Herbicide-Tolerant Crops, Methods of Development and Current Status of the Art – Emphasis on Glyphosate Tolerance," Weed Technology, July-September 1992.

[103] Franz, J.E., et al., Glyphosate, a Unique Global Herbicide, ACS Monograph 189, 1997.

[104] Sigurbjornsson, Bjorn, "Induced Mutations in Plants," Scientific American, January 1971.

[105] Barrentine, W.L., et al., "Development of Tracy Soybeans Tolerant to Metribuzin," in Proceedings Southern Weed Science Society, 1979.

[106] Palmer, Reid G., and Thomas C. Kilen, "Qualitative Genetics and Cytogenetics," in Soybeans: Improvement, Production, and Uses, Second Edition, American Society of Agronomy, 1987.

[107] Lovely, W.G., et al., "Effectiveness of the Rotary Hoe for Weed Control in Soybeans," Agronomy Journal, 50:621-625.

[108] Hartnett, Mary E., et al., "Herbicide-Resistant Plants Carrying Mutated Acetolactase Synthase Gene," in Managing Resistance to Agrochemicals, ACS Symposium Series 421, 1990.

[109] Carlson, D.R., and O.C. Burnside, "Use of the Recirculating Sprayer to Control Tall Weed Escapes in Crops," Weed Science, March 1981.

[110] McWhorter, C.G., "Weed Control in Soybeans with Glyphosate Applied in the Recirculating Sprayer," Weed Science, March 1977.

[111] "Homemade Bean Buggies Put Weeds on the Spot," Farm Journal, May 1985.

[112] Congleton, W. Franklin, et al., "Imazaquin (Scepter): A New Soybean Herbicide," Weed Technology, April 1987.

[113] Claus, Jon S., "Chlorimuron-Ethyl (Classic): A New Broadleaf Postemergence Herbicide in Soybean," Weed Technology, January 1987.

[114] Cantwell, John R., et al., "Imazethapyr for Weed Control in Soybean," Weed Technology, October-December 1989.

[115] USDA, Pest Management on Major Field Crops, AREI Updates 1997, Economic Research Service, Number 1, February 1997.

[116] USDA, Agricultural Resources: Situation and Outlook Report, Economic Research Service, AR-13, February 1989.

[117] USDA, Agricultural Resources: Inputs Situation and Outlook Report, Economic Research Service, AR-5, January 1987.

[118] Schaub, James, et al., The U.S. Soybean Industry, Economic Research Service, USDA, Agricultural Economic Report, Number 588, May 1988.

[119] Lux, James F., et al., "Influence of Glyphosate Application Timing on Weed Control and Yield in Glyphosate Tolerant Soybeans," in Proceedings North Central Weed Science Society, 1997.

[120] Dobbels, Anthony F., and Mark M. Loux, "Weed Competition in Glyphosate Tolerant Soybeans," in Proceedings North Central Weed Science Society, 1997.

[121] Wax, Loyd M., et al., "Control of an ALS-Resistant Waterhemp Biotype in Soybeans with Glyphosate," in Proceedings North Central Weed Science Society, 1997.

[122] Null, Donald E, "Herbicide Evaluation for Common Waterhemp Control in Northwest Missouri Soybeans," in Proceedings North Central Weed Science Society, 1996.

[123] Gonzini, Lisa C., et al., "Herbicide Combinations for Weed Control in Glyphosate Tolerant Soybeans," in Proceedings North Central Weed Science Society, 1996.

[124] Aldridge, Grant, et al., "Performance of Glyphosate Tolerant Soybean as Influenced by Weed Control Systems," in Proceedings North Central Weed Science Society, 1996.

[125] Mulugeta, Dawit and Chris M. Boerboom, "Critical Period of Weed Management in Glyphosate-Resistant Soybean Systems," in Proceedings North Central Weed Science Society, 1996.

[126] Ateh, Comfort M., and Robert G. Harvey, "Models Which Predict Soybean Yield Reduction from Postemergence Herbicide Injury," in Proceedings North Central Weed Science Society, 1996.

[127] Allen, S.M., and T.J. Hartberg, "Weed Control in Soybeans with Applications of Imazethapyr Postemergence Following Pendimethalin Preplant Incorporated," in Proceedings North Central Weed Science Society, 1989.

[128] Nau, H.H., et al., "Imazaquin: Summary of 1985 EUP Results," in Proceedings North Central Weed Science Society, 1985.

[129] Martin, James R., and James W. Herron, "Pro's and Con's of Soybean Postemergence Herbicide Combinations," in Proceedings North Central Weed Science Society, 1985.

[130] Nelson, Kelly A., and Karen A. Renner, "Soybean Variety Response to Glyphosate and Postemergence Herbicide Tank Mixtures," in Proceedings North Central Weed Science Society, 1998.

[131] Potter, Bruce D., et al., "Competitive Effects of Annual Weeds in Glyphosate Tolerant Soybeans," in Proceedings North Central Weed Science Society, 1998.

[132] Hager, Aaron G., et al., "Identification of a Smooth Pigweed Biotype in Illinois Resistant to Various ALS-Inhibiting Herbicides," in Proceedings North Central Weed Science Society, 1998.

[133] Evers, Mandy L., and Reid J. Smeda, "Impact of Weed Competition on Rowed versus Drilled Glyphosate Tolerant Soybeans," in Proceedings North Central Weed Science Society, 1998.

[134] Horak, Michael J., et al., "Early Season Weed Control in Roundup Ready Soybean: Effect on Yield," in Proceedings North Central Weed Science Society, 1998.

[135] Smeda Reid J., "Suppression of Common Waterhemp Emergence and Growth with Preemergence Herbicides in Soybean," in Proceedings North Central Weed Science Society, 1998.

[136] Peterson, Dallas E., "The Impact of Herbicide Resistant Weeds on Kansas Agriculture," in Proceedings North Central Weed Science Society, 1998.

[137] Sprankle, Paul L., and William B. Parker, "Glyphosate Tolerant Soybean EUP Results," in Proceedings North Central Weed Science Society, 1995.

[138] Bresnahan, Gail A., and Alan G. Dexter, "Imazethapyr Carryover in Wheat, Susceptible Corn and IR Corn," in Proceedings North Central Weed Science Society, 1994.

[139] Ateh, Comfort M., and Robert G. Harvey, "Effects of Injury from Postemergence Herbicides on Soybean Development and Grain Yield," in Proceedings North Central Weed Science Society, 1994.

[140] Moshier, Loren J, et al., "Development of STS Soybean Varieties and Weed Control Systems," in Proceedings North Central Weed Science Society, 1994.

[141] Wells, Barbara H., "Glyphosate Tolerant Crop Update," in Proceedings North Central Weed Science Society, 1994.

[142] Vollmer, Jennifer L., et al., "Affects of Imazethapyr and Thifensulfuron Methyl Tank Mixes on Weed Control and Soybean Injury," in Proceedings North Central Weed Science Society, 1991.

[143] Monsanto, "Monsanto Responds to GAO Report on Soybean Pricing," February 22, 2000.

[144] USDA, Cropping Patterns of Major Field Crops and Associated Chemical Use, AREI Updates, Economic Research Service, Number 18, December 1996.

[145] 1998 Summary of Illinois Farm Business Records, Department of Agriculture and Consumer Economics, University of Illinois, October 1999.

[146] Oplinger, Ed, "Performance of GMO's in Northern U.S.," University of Wisconsin, Department of Agronomy.

[147] "Roundup Ready Yields Lag," Soybean Digest, vol. 59, no. 5, 1999.

[148] Benbrook, Charles, Evidence of the Magnitude and Consequences of the Roundup Ready Soybean Yield Drag from University-Based Varietal Tests in 1998, Ag BioTech Info Net Technical Paper Number 1, July 13, 1999.

[149] "Monsanto's Response to Ag Bio Tech Info Net Technical Paper Number 1 (re: Roundup Ready Soybeans)," October 1999.

[150] Monsanto, "Roundup Ready Soybeans Outyield National Average," January 22, 1999.

[151] USDA, "Genetically Engineered Crops for Pest Management," Economic Research Service, June 25, 1999.

[152] USDA, "Impacts of Adopting Genetically Engineered Crops in the U.S. – Preliminary Results," Economic Research Service, July 20, 1999.

[153] Rawlinson, Jeff, and Alex Martin, "Weed Management Strategies in Soybeans," University of Nebraska, November 10, 1998.

[154] Roberts, Roland K., et al., "Economic Analysis of Alternative Herbicide Regimes on Roundup Ready Soybeans," Journal of Production Agriculture, vol. 12, no. 3, 1999.

[155] Webster, Eric P., et al., "Weed Control and Economics in Nontransgenic and Glyphosate-Resistant Soybean (*Glycine max*)," Weed Technology, vol. 13, 1999.

[156] Miller, D.K., et al., "Evaluation of Reduced Rate Preemergence Herbicides in Roundup Ready Soybean Weed Control Programs," 1998 Proceedings, Southern Weed Science Society.

[157] Arnold, J.C., et al., "Roundup Ready Programs Versus Conventional Programs: Efficacy, Varietal Performance, and Economics," 1998 Proceedings, Southern Weed Science Society.

[158] Falck-Zepeda, Jose B., et al., "Rent Creation and Distribution From Biotechnology Innovations: The Case of Bt Cotton and Herbicide-Tolerant Soybeans in 1997," Agribusiness, vol. 16, no. 1, 2000.

[159] Moschini, Giancarlo, et al., "Roundup Ready Soybeans and Welfare Effects in the Soybean Complex," Agribusiness, vol. 16, no. 1, 2000.

[160] McBride, William D. and Nora Brooks, "Survey Evidence on Producer Use and Costs of Genetically Modified Seed," Agribusiness, vol. 16, no. 1, 2000.

[161] Parker, William B., et al., "Integrating Sethoxydim into Soybean Weed Management Systems," Weed Science, January 1985.

[162] Mueller, Thomas C., and Robert M. Hayes, "No-Till Farming and Weed Control," Tennessee Agri Science, Summer 1996.

[163] Monsanto, The Roundup Ready Soyabean System: Sustainability and Herbicide Use, Backgrounder, April 1998.

APPENDIX 1

Soybean Processing – A Description

When soybeans enter the processing plant, they are screened to remove broken or damaged beans and foreign materials. The beans are then crushed into four to six small pieces between rollers. The hulls are removed by air. Steam heating raises the moisture content of the crushed soybean pieces to 11%. The soy meats are flaked by being passed between horizontal smooth rollers, that produce flakes approximately 0.01 inch thick. The passage between the rollers ruptures the oil cells [74]. Oil is removed from the flakes by an organic solvent, hexane, that selectively dissolves the oils. The solvent mixture is extracted from the flakes, and the oil-hexane solution obtained after flake extraction consists of 75% oil and 25% hexane. To remove hexane from the oil, a solvent recovery system is used. It consists of a stripping evaporator where the hexane vaporizes and subsequently condenses [74]. The defatted flakes from the extractor contain about 30% hexane. They are processed through a desolventizer-toaster to remove remaining solvent. Steam contacts the flakes and the heat of vaporization released from the condensing steam vaporizes the hexane [74].

Degumming is the removal of gums or lecithin. Direct addition of water to the oil is the most commonly used method of degumming. The water attracts the polar phospholipids. The water and oil phases then are separated by centrifugation [74]. Neutralization is the removal of free fatty acids from the oil. They must be removed as they reduce the smoking point of the oil and increase foaming [74]. Alkali refining is the most common method for removing acids from oil. Soy oil usually is pretreated with phosphoric acid before alkali refining. Free fatty acids are separated by the addition of alkali in the form of sodium hydroxide to the oil [74].

Bleaching is important in enhancing oil appearance. Bleaching is an adsorption process whereby minor oil components are bound by a fine, powdered adsorbent. Natural bleaching earths, such as Fuller's earth or bentonite and activated carbon, are mixed with the oil followed by removal in filter presses [98]. Deodorization is a steam stripping process in which good quality steam is injected into the soybean oil at a sufficiently high temperature to vaporize undesirable volatile compounds that impart a bitter beany flavor [74]. During the latter stage of deodorization, citric or phosphoric acid is added to the oil. These chelate the traces of heavy metals present [98].

After deodorization of the oil, it is filtered prior to storage. Nitrogen gas is used to blanket the oil to prevent oxidation [98].

Once a high purity oil has been obtained it may be processed further in order to change its physiochemical properties for use in certain food products. Hydrogenation increases the oxidative stability and melting point of the oil. Hydrogenation consists of the addition of gaseous nitrogen with the aid of a nickel catalyst to increase the solidification part of the oil to convert liquid vegetable oil into margarine or shortening. The catalyst is filtered from the oil.

Winterization involves the removal of solids that settle out from the oil at about 4-10º C [74]. Winterization is used to remove waxes that may precipitate during storage in domestic refrigeration [74].

The crude soy lecithin is quite brown and viscous. The crude lecithin is dried, de-oiled by acetone (the phospholipids are insoluble in acetone) and subsequently may be chemically modified to enhance specific properties. The dark colored lecithin may be bleached by addition of hydrogen peroxide prior to drying [98].

Soy meal is produced by grinding defatted and desolventized flakes, containing a little over 50% protein. Grits are obtained by coarsely grinding the defatted flakes; soy flour is produced by grinding the flakes to very fine particles.

Soy protein concentrates are prepared by removing soluble carbohydrates from defatted meal. Three basic processes have been used for carbohydrate removal: acid leaching, aqueos ethanol extraction and moist heat-water leaching. Proteins become insolubilized while a portion of the carbohydrates remains soluble so that their separation becomes possible by centrifugation [74]. The soybean protein concentrates consist of 70% protein on a dry weight basis.

Extrusion texturization involves mixing soy flour with water, feeding to a continuous cooker-extruder, heating under pressure and extruding. The heated, compressed mass expands resulting in a sponge-like mass. After hydration, the textured product has a chewy resilient texture similar to meat [98].

Soybean meal must be heated sufficiently in order to inactivate anti-nutritional factors, but not enough to damage the protein [118].