Chapter 3

Weed Management in Soybean — Issues and Practices

Rafael Vivian, André Reis, Pablo A. Kálnay,
Leandro Vargas, Ana Carolina Camara Ferreira and
Franciele Mariani

Additional information is available at the end of the chapter

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1. Introduction

Weed management is essential for any current system of agricultural production, especially for large monoculture areas, which exert high pressure on the environment. Soybean is among the largest monoculture registered worldwide, with 102 million hectares harvested only in 2010. The leading countries of production are Argentina, Brazil and the United States, with more than 70% of the total cultivated area. Along with China and India, these five countries represent 90% of all produced soybean. The production incentive is related to growing global demand for oil and protein for food and feed, as well as the feasibility of crops for biodiesel production, extremely important for the global economy.

Meanwhile, weeds are considered the number one problem in all major soybean producing countries. Even with advanced technologies, producers note high losses due to interference by weeds. According to estimates, weeds, alone, cause an average reduction of 37% on soybean yield, while other fungal diseases and agricultural pests account for 22% of losses [1]. In the United States, it is considered that weeds cause losses of several millions of US dollars annually. In Brazil, with an average production of 75 million tons, it is estimated that expenses on weed control represent between 3% and 5% of total production cost, which means more than US$ 1.2 billion used in that country, only for weed chemical control in soybeans.

Disregarding the high cost, weed might be controlled in soybean crop using good management practices of all available methods, combining them in an integrated weed management (IWM). Crop rotation is a rather efficient method, since it allows an easy control of the most troublesome weeds. In order to achieve success on crop rotation, weeds must be managed throughout the growing soybean season. Using full capacity of crop competition is another alternative, yet this tool is often overlooked.

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Despite differences between soybean cultivars used worldwide and the main weed species which attack these cultivars, there are many resemblances in management practices and control. The species hairy fleabane, *Conyza bonariensis* (L.) Cronq., horseweed, *Conyza canadensis* (L.) Cronq., goosegrass, *Eleusine indica (L.)* Gaertn., barnyardgrass, *Echinochloa crus-galli (L.)* Beauv., johnsongrass, *Sorghum halepense* (L.) Pers., beggarticks, *Bidens pilosa* L. and common ragweed, *Ambrosia artemisiifolia* L., are common weeds in Argentine, Brazilian and American soybean crops. The burndown and subsequent post-emergence (POST) spraying of crop with glyphosate usually occur from south to north in the American continent, with some distinctions among products used in mixture with glyphosate for managing resistant weeds. All these factors increase the selection pressure even more.

The introduction of GR (glyphosate-resistant) soybean, genetically modified (GM), contributed to standardization of weed management. With a large adoption of this technology, there are many concerns regarding the control and the high selection pressure on common weed species in soybean. In the US, more than 93% of soybean has the GR technology. In Brazil and Argentina, these values represent 80% and 99%, respectively.

The use of very similar technologies as well as the facility of proliferation of weeds has intensified reported herbicide resistance. Since the first report of *E. indica* resistance, in Malasia (1997), 22 species (biotypes) are already not controlled by glyphosate and 10 show multiple resistance. The number of reports increases every year and, in 2011, 7 weed resistance cases were recorded. The evolution of weed resistance to glyphosate also worries members of the Weed Science Society of America, mainly by the spread rate and by the impact on ecosystems.

New technologies derived from genetic alteration of cultivars resistant to herbicides are part of management alternatives to glyphosate. Many of them still under test should be available on short notice. In Brazil, both soybean resistant to ALS (acetolactate synthase) inhibitors and those resistant to 2,4-D should take up areas with a history of weed glyphosate resistance. In the US, besides soybean resistant to dicamba and that resistant to glyphosate + ALS, mixtures are used on crop pre-emergence (PRE), for example, dimethenamid and saflufenacil (new active ingredient). Spraying of encapsulated ingredients (acetochlor) at soybean POST and at weed PRE also come up as management alternatives.

Despite efforts on weed control in soybeans, the benefits of IWM based on preventive and cultural controls will always be fundamental to the maintenance of monocultures. However, it appears that much of what is discussed about IWM is slightly practical, with corrective measures mostly. This chapter aims to present some focal issues related to weed management in soybean growing areas, which include weed potential to cause severe damages and yield losses by weeds, the evolution of resistant weeds in GR soybean monoculture, the soybean management characterization in the main producing countries and discussions about the benefits of IWM use as an accurate control measure. It presents a set of information for researchers and experts on weed management service area, reporting clear and objectively the major impacts of the current management used and the outlook for soybean farming.

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2. Implication of weed management in soybean

Weed control is a practice of great importance for obtaining high soybean yields. Weed species is a serious problem for the soybean crops and its control is needed especially in infested sides. Therefore, weed management is an integral part of soybean production. Recently, research has reported that the density and distribution of weed species in the soybean plantations are significant parameters on yield losses. This happens because the weed species competes with the sunlight, water and nutrients, and may, depending on the level of infestation and species, hamper harvesting operations and compromise the quality of soybean grains [2]. Current studies on weed biology are changing, largely due to the effects of agricultural practices on weeds, cropping systems, and the environment. Research emphasis has been altered based on the need to understand basic weed biology [3]. It is our job to predict how weed species, populations, and biotypes evolve in response to selection pressure primarily due to agricultural practices. This knowledge helps developing weed management practices in the soybean crops. Other important biological factors in weed management decisions include weed and crop density, seedbank processes, demographic variation, weed-crop competition, and reproductive biology [4]. Development of economic thresholds for weed species made significant progress in the last decade. Integrated weed management has focused on the effects of crop planting dates, row spacing, cultivators, use of cover crops and reduced herbicide rates.

Selection and adaptation of weed populations occur at the level of the individual. Weeds interfere with crop production, and the yield losses incurred are the aggregate consequence of competition between heterogeneous weed phenotypes and homogeneous crop phenotype [5]. Because weed selection results in diversity, a population of weeds on a field consists of a heterogeneous collection of genotypes and phenotypes that allows exploitation of many niches left available by crops. Weed species respond to these opportunities with an impressive array of adaptions: phenotypes plasticity in response to microsite resource availability, somatic polymorphism of plant and seed form and function, density-dependent mortality (population size adjustment), density-independent mortality (disease, predator, stress resistances), and chemical inhibition of neighbors by allelopathic interference [6]. When all else fails, many weed seeds can remain dormant and extend their life for several years in the soil seedbank, waiting for the right opportunity to grow [7].

Weed populations possess considerable heterogeneity at many levels, consequence of adaptation for colonization and survival. In order to select the most appropriate herbicides or devise the optimum weed control system, one must be able to properly identify the weeds present within a field. Weed identification immediately following emergence is essential since the effectiveness of most herbicides depends on weed size. Maps of weeds by species in fields prior to harvest will aid in the choice of herbicide program for the following year.

2.1. Issues on weed management

All the characteristics cited are essential for soybean weed management. However, starting from the identification of species, three leading questions must be answered in order to suitably

handle weeds: i) What are the available tools for weed management? ii) How should one use them for reducing weed interference? and iii) When should one use them?

The available tools are those that enable the reduction of weed-crop competition. It integrates all traditional control — cultural, physical, chemical, among others — and it should be evaluated in accordance with locally grown system. Currently, due to countless resistance cases, preferences are for those that integrate cultural and physical controls together with chemical ones, and the following ones can be cited: no tillage system, crop rotation, using of cover crops, autumnal herbicide management directed to key-weeds, and new GM soybean resistant to herbicide from different modes of action.

All tools should be adapted to use availability, particularly considering the ratio income/investment. Many of these tools are easy to be used and have high impact. The no tillage system, for example, changes weed management completely, so that the mulch formed reduces weed survival [8] and also encourages the germination of negative photoblastic species [9], in addition to all other benefits found in the tropical regions of soybean production [10]. The advantages of no tillage over conventional tillage systems in improving soil quality are generally accepted, resulting in benefits for physical, chemical and biological properties of the soil [11]. Nowadays, no tillage is practiced on over 100 million ha worldwide, mostly in North and South America, but also in Australia and in Europe, Asia and Africa [12,13]. Among the advantages, one can cite the control of soil erosion, moisture conservation, favorable soil temperatures, increased efficiency in nutrient cycling, improvement on soil structure, machinery conservation and time saving in terms of human and animal labor [12,14]. The system also ensured changing among the population of arthropods, which are usually favored by the system because they find greater protection to natural enemies or use many of weed seeds as a feed source.

The crop rotation system constitutes another important management tool, often overlooked by producers. It allows the variation primarily at chemical control. Corn rotating, despite inconvenient profitability decreases, compared with soybean, allows an important POST emergent apply against glyphosate-tolerant weeds, in areas where GR corn is not used. Several studies carried out from 1970 to 1990, associated with cultivation of soybeans in crop rotation systems with diverse grasses (rice, maize, sorghum, wheat, sugar cane) and cotton, have shown that nitrogen residual effect, fixed by soybean crop and its residues, replaces partial the nitrogen on following crop, resulting in field optimization and alleviating part of the production costs [15]. In China, for example, soybean is commonly grown continuously in monoculture rather than rotated with other crops, like maize or wheat. The soybean monoculture results in yield decline, as well as its quality. The yield reduction on soybean in 2, 3 and 4-year monoculture was 15%, 20%, and 30%, respectively [16,17], highlighting the significance of rotational system in the preservation of crop production. Furthermore, several experiments suggest that carbon and nitrogen from microbial biomass (particularly nitrogen) are sensitively affected by soil- and crop-management regimens, being directly influenced by crop rotation [18].

Using cover crops between the main crops (fallow period) is also part of conservation practices and it represents a breakthrough in weed management. Besides, competing against weeds,

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many cover crops allow using selective herbicide in the fallow period, reducing hard-to-control species. Despite the high costs, it saves on using herbicides along cultivation years for the primary crop, as the infestation plant is reduced by ongoing practice of this system. Nutrient cycling is also favored by means of cover crops, especially for those who exhibit high mobility on the ground, such as nitrogen [19]. For other nutrients, arbuscural mycorhizal development is favored in areas in which cover plants are used. This arbuscural mycorhizal promotes phosphorus absorption [20]. Nitrate loss in annual row crops could also be significantly mitigated by the adoption of no tillage and cover crops or greater reliance on biologically based inputs, according to [21]. In general, cover crops increase the primary productivity of the system and diversify basal resources for higher trophic levels.

However, the selection of proper cover crop is essential for the success of the system. Plant-feeding nematodes, for example, were less abundant in plots with Poaceae cover crops, while bacterivorous, omnivorous and root-hair-feeding nematodes were more abundant with Fabaceae cover crops than with bare soil, indicating that cover crop identity or quality greatly affects soil food web structure [22]. Other species, such as those from genus Desmodium, may be used suppressing *Striga hermonthica* (Del.) Benth. by means of an allelopathic mechanism. Their root exudates contain novel flavonoid compounds, which stimulate suicidal germination of *S. hermonthica* seeds and dramatically inhibit its attachment to host roots [23].

Herbicides, in the broad action spectrum, are and will be essential tools in weed management, even for those with a great number of resistant weeds. But the trend is that using different herbicide is increasingly related to GM crops which show resistance to more than one active ingredient. For new GM soybean, 2,4-D and dicamba resistance traits will always be used in stacks with at least one other herbicide-resistant trait. Glyphosate and ALS trait stack, recently deregulated in the US, possibly will allow the use of ALS-inhibiting herbicides with soil residual that are too phytotoxic to use on conventional crop cultivars [24]. In reference [25], diversification may make weed management more complex, but growers must not use new GM crop resistant to herbicides in the same way that some used initial GM crops, in order to rely only on one herbicide until it is no longer effective and then switch herbicides. Research alerts that "if growers use the new GM crops and the herbicides that they enable properly, GM crops will expand the utility of currently available herbicides and provide long-term solutions to manage resistant weeds".

Answering the question related to the period when control tools should be used, different opinions arise. Many specialists recommend to use tools, especially chemical control, only when economic loss level is reached, ie, when population density finds a minimum threshold at which costs of controlling are lower than economic damage coming from losses by weed interference. Nevertheless, by following the concept of integrated management, it is recommended the use of many available tools, even at fallow periods or at low weed densities. In reference [26], as opposed to pest and pathogens which attack crops in epidemic cycles, weeds are endemic, regenerating from the seed and/or vegetative propagules that are introduced into the soil; thus, the continuous management allows the best result. Besides, confining weed management to a narrow temporal window increases the risk of unsatisfying weed management outcomes due to unfavorable weather [27]. Coupled with this agreement, good man-

agement models for weed control may join forces to the definition of weed control periods according to their competitive ability and the local crop conditions set out during the growing (climate, cultivar, sowing density, etc).

So far, absence of management or misuse of control tools may undermine the productivity, the sustainability of system production and the agricultural activity, also interfering in the preservation and balance between species. Thus, interactions among weeds and further organisms (fungi, viruses, bacteria, mites, insects, nematodes, etc.) as well as their handling may have a direct or indirect impact into the production system.

2.2. Impact of weed management on nontarget organisms

Many studies have attempted to relate the intensification of certain pathogenic diseases of shoot plants in areas annually treated with herbicides, being placed on proof the intensive use of those mainly in no tillage system. Glyphosate, for example, is a highly effective broad-spectrum herbicide that is phytotoxically active on a large number of weeds and crop species across a wide range of taxa [28]. Glyphosate inhibits the biosynthesis of aromatic aminoacids, thereby reducing biosynthesis of proteins, auxins, pathogen defense compounds, phytoalexins, folic acid, precursors of lignins, flavonoids, plastoquinone, and hundreds of other phenolic and alkaloid compounds [29]. These effects could increase the susceptibility of glyphosate-sensitive plants to pathogens or other stress agents [30]. Engineered to express enzymes that are insensitive to or are able to metabolize glyphosate, GR crops have enabled farmers to easily apply this herbicide in soybean, corn, cotton, canola, sugar beet and alfalfa, besides controlling problematic weeds without harming the crop [28].

For glyphosate and its interspecific transfer from weeds to nontarget organisms, in [31] it was related the increasing remark number of plant diseases growing in long term [32]. But the herbicide influence on disease incidence at glyphosate-resistant crops has varied. While in [33,34] it was observed an increase of *Fusarium solani* (Mart.) Sacc. in soybean, others showed a reduction of *Phakopsora pachyrhizi* Sidow at this crop [35]. For nitrogen-fixing microorganisms in soybean, negative interference of glyphosate has been proven by different authors [36-39], usually in laboratory experiments, with clear differences among rhizobial strains, as well as among glyphosate formulations, having roughly deleterious effects according to combinations of these.

Disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, for example, occurs in numerous weeds considered plant hosts. Crop rotation is essential in this case, specially when it uses non host crops and some herbicides with effects over the weed hosts and, consequently, the disease. In [40], the use of chemical weed management with sethoxydim, an important herbicide on soybean system, had the biggest toxicity rate together with cycloxydim. Other herbicides tested, such as cycloxidim and haloxyfop-ethoxy-ethyl, had less impact on *S. sclerotiorum*, but negative action on *Trichoderma* sp..

In other cases, not only herbicides, but also weeds, can supply the decrease of several crop diseases, so that their management is extremely important. In [41] it was investigate the efficacy of three common weeds, i.e., *Amaranthus viridis* (L.), *Lantana camara* (L.) and *Malvastrum coroman-*

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delianum (L.) Garcke against four bacterial species, *Xanthomonas axonopodis*, *Pseudomonas syringae*, *Corynebacterium minutissium*, *Clostridium difficile* and major seed-born fungi *Aspergillus niger*, *Alternaria alternata*, *Drechslera biseptata*, *Fusarium solani* in vitro. Leaf extracts of these weeds exhibit antimicrobial effects and all were moderately active against seed-born fungi.

Some experiments found preliminary details, which suggest that the presence of weeds that serve as hosts of both tobacco rattle virus (Corky ringspot disease) and *Paratrichodorus allius* (root nematode) may nullify the positive effects of growing alfalfa or Scotch spearmint for Corky ringspot control conducted [43]. For all species researched, *Solanum sarrachoides* Sendtn presented positive correlation with Corky ringspot disease.

Weed management is also associated with most pests on crop cultivation; ecological relationships set out among organisms (weeds, insects, mites, etc.) allow their maintenance and proliferation. Examples of pest and weed interactions established in soybean has been reported by [44], who found anticipation of 14 days at critical period of weed control when crop was 60% defoliated by insects. Increasing of *Anticarsia gemmatalis* Hübner oviposition was also logged in [45] when soybean presented a high infestation of *Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill. Thus, S. exaltata management reduces *A. Gemmatalis* population. Overall, monoculture areas tend to present higher mites and pest infestation and reduced biological diversity when maintained free of weeds. At the same time, weeds help insect diversity and natural biological control [46]. Mites, important arthropods in agricultural systems, currently constitute themselves key pests for soybeans in regions of hot and dry weather. Some predatory mites can be used against them into the biological management scope. Therefore, a fundamental aspect is the alternative feed sources for predatory mites during periods in which mite pests are at low populations. Among feed sources, there are many weeds, especially *Ageratum conyzoides* L., commonly encountered in citrus orchards and further agricultural areas. Overall, dicotyledonous weeds that produce a lot of pollen are preferred by predatory mites, in particular the genus Euseius [47]. Phytophagous mites, especially the web mite family Tetranychidae, were traditionally considered secondary pests in soybean. However, in recent years, it has been recorded severe and frequent attacks of these in different producing regions in Brazil [48]. Into surveys about GR soybean carried out in the state of Rio Grande do Sul, six phytophagous mite species were identified, five tetranychid — *Mononychellus planki* (McGregor), *Tetranychus desertorum* (Banks), *T. gigas* (Pritchard & Baker), *T. ludeni* (Zacher) and *T. urticae* (Koch) — and white mite tarsonemid *Polyphagotarsonemus latus* (Banks) [49,50]. In most of the sampled sites, more than one tetranychid were reported, being directly influenced by weed management.

The integrated management of weeds and pests, despite essential, is not easy to be performed on extensive production systems, especially because there are interactions of many species having various relations, either symbiosis, predation or parasitism. Knowing the interactions and the organisms that comprise production system is the great challenge and it can bring good results. Examples can be viewed in [51], with the reporting of lepidopterous in corn, in cotton [52], in *Heliothis zea* [53] and *H. virescens* [54] with *Bemisia tabaci* (Genn), among others. Maintaining biodiversity and sustainable production are some of the main advantages of using these systems [55].

3. Evolution of weed resistance in soybean

Herbicide-resistant weeds represent the evolution of plants as a consequence of environmental changes, which are usually caused by human action. This process is aligned with the theory of evolution. The process of natural selection, according to Darwin's theory of evolution, may be summarized by three guiding principles: i) principle of variation – there are variations in physiology, morphology and between behavior of individuals of any population, ii) principle of heredity – descendents are more similar to their parents than unrelated individuals, and iii) principle of selection – some individuals are more successful at survival and reproduction than others in a particular environment [56].

Therefore, a whole species keeps changing its composition because the individuals evolve in the same direction. The next generation will have a higher frequency of individuals that have been most successful in surviving and multiplying on environmental conditions. Frequencies of individuals within a population will change over time and those better adapted to the environment become predominant [56]. The biotype selection in a population by the same repeated herbicide application and its multiplying are shown bellow (Figure 1).

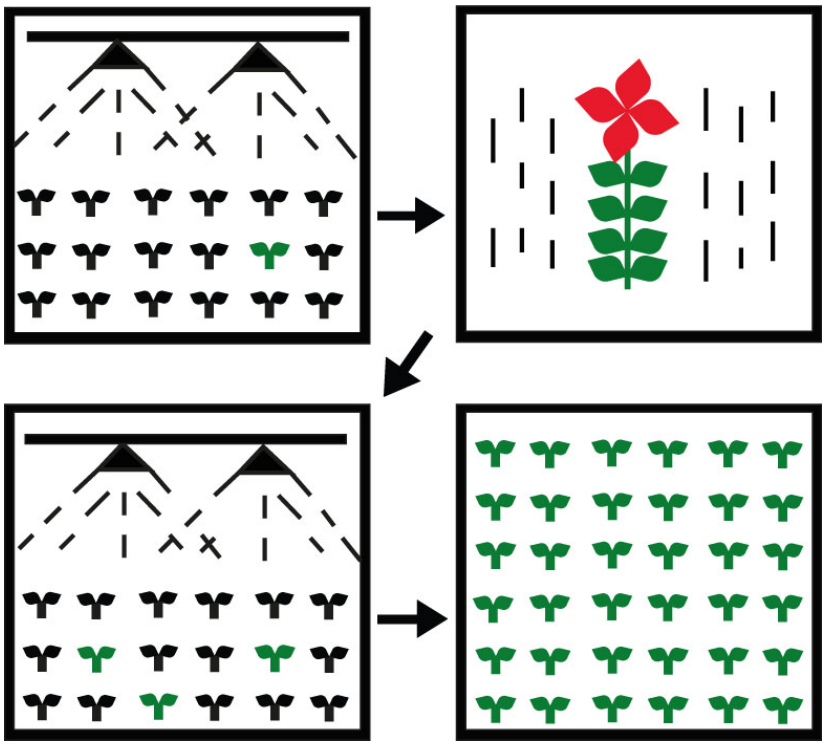

Figure 1. Illustration of a resistant biotype selection of a sensitive species [57].

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Considerable evidence suggests that the appearance of herbicide resistance in a plant population comes with the selection of a resistant biotype, which is pre-existing. According to the selection pressure, this individual finds favorable conditions to reproduce [58]. The perception of resistance is only possible when the number of resistant plants or failure in control are clearly identified (Table 1). Unfortunately, for most cases, the seedbank already has seedlings of the resistant biotype in this time and eradication becomes arduous and expensive. The resistant biotypes may exhibit less ecological adaptation in these environments and become predominant due to elimination of sensitive plants. In terms of natural selection, biotypes with greater ecological adaptation reveal greater production than less adapted biotypes [59].

Years	Nº resistant plants	Nº sensitive plants	Control (%)	Progress
0	1	1,000,000	99.9999	unnoticeable
1	1	100,000	99.999	unnoticeable
2	1	10,000	99.99	unnoticeable
3	1	1,000	99.9	unnoticeable
4	1	100	99.0	unnoticeable
5	1	10	90.0	barely noticeable
6	1	5	80.0	noticeable
7	1	2	50.0	apparent

Table 1. Evolution of resistance in a population of resistant weed biotypes [60].

Most of the ecological issues associated with evolution of herbicide resistance involve the understanding of relationship between adaptation, gene frequency, inheritance and gene flow [61] because the interactions among these factors shall determine the time required for resistant biotypes to become predominant.

The time for resistant plants' appearance and resistant and non-resistant weed proportion change frequently with herbicide use and its biological effects, which may be fairly short (two years from commercial use — ALS inhibitors) or take more than 20 years, as happened with glyphosate (EPSP – 5-enolpyruvylshikimate-3 phosphate synthase inhibitors) [62] (Table 2). Weeds resistant to sulfonylureas were identified after four or five years of a continuous use of this herbicide group [63]. In Australia, *Lolium rigidum* Gaudin biotypes resistant to diclofop-p-methyl have been selected into three generations, starting from a sensitive population and by using a normal herbicide dose.

Herbicides with a high level of safety, i.e., high efficiency and specificity play a huge selective pressure. Examples include inhibitors of the enzymes ALS and ACCase (acetyl coA carboxylase), which have great chances to select resistant weed biotypes, since any change in its action point (enzyme) may result on activity losses and resistant weed increase.

Herbicide	Introduction year	Confirmation year	Place
2,4-D	1948	1957	EUA and Canada
Triazines	1959	1970	EUA
Propanil	1962	1991	EUA
Paraquat	1966	1980	Japan
EPSP syntase inhibitor	1974	1996	Australia
ACCase inhibitor	1977	1982	Australia
ALS inhibitor	1982	1984	Australia

Table 2. Year of introduction and its first confirmation of weed resistance to different herbicide action mode [64].

There are six factors related to plant population, which interact and determine the probability as well as the time of resistance evolution. They are the following: *number of alleles* involved in strength expression, *resistant allele frequency* in an initially sensitive population, mode of *resistance inheritance* (cytoplasmatic or nuclear), *reproductive traits* of species, *rate crosses between resistant and sensitive biotypes* and *selection pressure* [65].

The **number of genes** that confer resistance is important because, when inheritance is polygenic, the likelihood of the resistance to appear is low. However, when a single gene is responsible for resistance (monogenic), there is a high probability of occurrence. Most cases of resistance are conferred on a single gene. It is due to two factors. First of all, modern herbicides are specific, acting upon specific enzymes in metabolic pathways. Incidence of gene mutations responsible for coding that enzyme may change the plant sensitivity to the product, resulting in resistance. The second factor refers to the high selection pressure exerted by high efficiency of these herbicides. In order to occur polygenic resistance, the recombination between individuals for several generations would be necessary to obtain adequate number of alleles and to confer high plant resistance level [66].

Frequency of resistant allele(s) in sensitive population is usually between 10^{-16} and 10^{-6} [65]. So, the higher is the frequency of these alleles, the greater is the probability of selecting a resistant biotype. The frequency of resistant allele in the population becomes more significant in the evolutionary process when herbicide requires low selection pressure. However, if allele frequency is high, evolution of resistance may be faster, regardless of selection pressure.

Inheritance resistance type is fundamental for the establishment of resistance in a plant population. There are two basic types of inheritance: cytoplasmatic (maternal) and nuclear. Cytoplasmatic inheritance happens when hereditary traits are transmitted by cytoplasm, so only the mother plant can pass the trait to the descendants, as an example, resistance to triazines. On the other hand, if the inheritance is nuclear, transmission is by chromosomes, and both father and mother might forward its resistance, such as resistant plants to ALS inhibitors. In case of maternally inherited resistance, allelic migration between adjacent populations does not occur [66], so the development of this type of resistance is slower than nuclear, where migration of alleles occurs via pollen.

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The **reproductive traits**, such as pollen scattering and number of propagules generated, influence directly the spread of resistant plants. Dispersal of resistance by pollen is affected by scattering efficiency and pollen longevity [67].

The **cross rate between resistant and sensitive biotypes** determines the spread of resistant alleles in a population. Pollen exchange between resistant and sensitive plants allows dispersion of the resistance, mainly in plants with high cross-fertilization rate, since the contribution of seed displacement is relatively small [59]. Gene flow is correlated with pollen flow distribution and varies on the species, with pollination mechanism and climatic conditions during flowering [68]. Species which presents more resistant biotypes and effective propagule dispersion may spread itself quickly, even though the inheritance of this resistance is maternal.

Repeated use of herbicides to plant control exerts high **selection pressure**, causing changes on flora of some regions, especially those with predominance of monoculture, such as soybean in major producing countries. Usually, better biotypes of a species adapted to a particular practice are selected and then they multiply rapidly [69]. Species exhibit different features and several responses to herbicide treatment. Therefore, an association of species characteristics with those of herbicides creates different periods needed for selection of resistant biotypes (Table 3).

Weed	Herbicide sprayed	Years
Alopecurus myosuroides Huds.	Chlortoluron	10
Avena fatua L.	Diclofop methyl	4-6
Avena fatua L.	Triallate	18-20
Carduus nutans L.	2,4-D or MCPA	20
Hordeum leporinum Link	Paraquat or Diquat	25
Kochia scoparia (L.) Schrad	Sulfonylurea	3-5
Lolium multiflorum Lam.	Diclofop methyl	7
Lolium rigidum Gaudin	Diclofop methyl	4
Lolium rigidum Gaudin	Amitrole + Atrazine	10
Lolium rigidum Gaudin	Sethoxydim	3
Senecio vulgaris L.	Simazine	10
Setaria viridis (L.) Beauv.	Trifluralin	15

Table 3. Number of years required for natural selection of resistant biotypes of a weed population according to the herbicide used [61].

In summary, the evolution process of herbicide resistance goes through three stages: removal of biotypes highly sensitive, remaining only the most tolerant and resistant; elimination of all biotypes except those resistant and selecting them in a population with high tolerance; intercrossing among survivors biotypes, generating new individuals with higher level of

resistance, which may be selected later [65]. This process resulted in 383 resistant biotypes, 208 species (122 dicotyledonous and 86 monocotyledonous) and over 570,000 fields [62].

There are no doubts that selection pressure by use of herbicides at cultivated soybean areas contributed to the increasing of resistant weeds. Among the main representative countries, Argentina, Brazil and the USA, there is a positive correlation between soybean expansion areas and intensive use of herbicides, as well as between the increasing of resistance incidence and massive adoption by the same technology in these countries, i.e., one or few herbicide action modes.

In the USA, country with the largest number of resistance cases, 139 occurrences have been recorded, approximately 119 resistant species to different states and mechanism actions. From the 139 cases, around 25.9% are resistant species to two or more herbicide mechanism actions [62]. The first resistance case in the US, to auxin herbicides, was *Commelina diffusa* Burm. f., in 1957. Then, in 1964, it was reported a *Convolvus arvensis* L. case, resistant to 2,4-D. In the years 1970 and 1972, resistance cases of *Senecio vulgaris* L. and *Amaranthus hybridus* L. to PSII inhibitors were reported. In 1973, it was recorded *Eleusine indica* (L.) Gaertn resistant to dinitroanilines and, in 1979, *Chenopodium album* L. resistant to PSII inhibitors. With soybean advance in the 80s, the resistance cases increased to 28 reports with PSII inhibitors and 10 cases with ALS inhibitors. In the 90s, the intensive use of ALS and ACCase inhibitors in soybean contributed to 68 ALS resistance events and 26 to ACCase. From 2000, resistance cases to glyphosate became more common. Between 2000 and 2011, it was registered more than 70 resistance events to glycine group as the result of larger glyphosate use at GR soybean, genetically modified to glyphosate resistance (RR1). Among reports so far, the largest number of species is related to ALS inhibitors (44), triazines (25), ACCase inhibitors (15) and glycines (13).

In Brazil, selection of tolerant or resistant species started in the 70s, with repeated metribuzin use. This herbicide was introduced to control *Bidens pilosa* L., but it had low efficiency against *Euphorbia heterophylla* L.. *E. heterophylla* showed tolerance to metribuzin and so was selected and became a major weed to be fought in crops. Concerns with *E. heterophylla* control were solved by imazaquin herbicide (ALS inhibitor) in the 80s, which had been used widely, becoming the main herbicide used in soybean fields. But at the end of the 90s, *E. heterophylla* and *B. pilosa* became resistant to imazaquin, including the selection of *Cardiospermum halicacabum* L..

The control of resistant species to ALS inhibitors was solved with GR soybean. History repeated itself with glyphosate and this has become practically the only herbicide hold on soybeans, imposing great selection pressure of tolerant and resistant species. Thus, the continuous glyphosate spraying has selected tolerant weeds such as *Ipomoea* sp., *E. heterophylla*, *Richardia brasiliensis* (Moq.) Gomez and *Commelina* sp., as those resistant species, such as *Lolium multiflorum* Lam., *Conyza bonariensis (L.)* Cronq., *C. canadensis*, *C. sumatrensis* (Figure 2) and *Digitaria insularis* (L.) Mez ex Ekman.

Resistance of *L. multiflorum* to glyphosate was identified in Brazil in 2003, and this forced ALS inhibitors and ACCase to become the main control options for this species. The continuous

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use of ALS inhibitors (iodosulfuron-methyl) and ACCase to *L. multiflorum* control resulted in biotypes resistant to ALS and ACCase in 2010 and in 2011, respectively. These biotypes have multiple resistance to glyphosate, glyphosate + ALS and glyphosate + ACCase. Certainly, the resistance to the three mechanisms in the same biotype will not take long to happen.

For soybeans and wheat, ACCase inhibitors are the main alternative to *L. multiflorum* control. Thus, impact selection of resistant and tolerant species in Brazil is mainly focused on cost production, since the farmer will have to use alternative herbicides in the area, usually more expensive than glyphosate and less efficient.

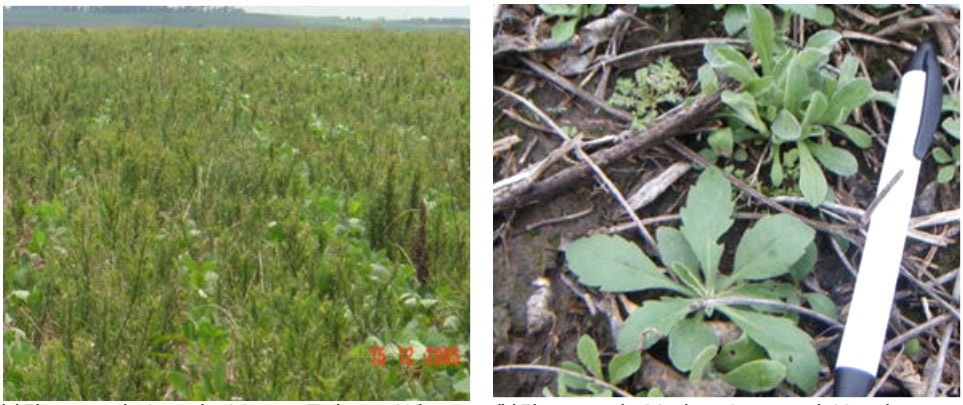
(a)Photograph: Leandro Vargas, Embrapa Wheat, (b)Photograph: Marlene Lazzaretti, Unnoba,

Figure 2. (a) Illustration of *Conyza* sp. resistant to glyphosate in Brazilian soybean field; b) rossettes of *Conyza bonariensis* (L.) Cronq. (smaller, smooth lobes) and *C. sumatrensis* (wider, serrated lobes), germinating in the fall, in Argentina.

In general, weed resistance to herbicides in Argentina became important after 2005 and is also related to the intensive use of glyphosate in GR soybean crop. The introduction of the RR technology in 1996 quickly masked the incipient problem of herbicide resistance in the country, marked by the appearance, in the northern part of Argentina, of an *Amaranthus* sp. resistant to ALS inhibitors herbicide (sulfonylureas and imidazolinones), officially confirmed as resistant in 1996. For many years, the problem faded into obscurity and farmers enjoyed the efficacy of an herbicide that seemed to elude the perils of resistance selection, again ignoring the advice of the few experts that protested against the practice of monoculture and lack of herbicide rotation. Reports of *Sorghum halepense* (L.) Pers. escapes in the province of Salta (NW Argentina), even after repeated applications of glyphosate, started in 2003, and the resistance was confirmed in 2006. This was the first case of resistance to glyphosate in Argentina, followed by *Lolium rigidum* Gaudin (and *L. multiflorum*) in 2007. In 2010, it was reported the case of *Avena fatua* L. resistant to ACCase inhibitors and two cases of multiple resistance *L. multiflorum*, resistant to ALS inhibitors plus glycine and ACCase inhibitors plus glycine as well [62], followed by *Echinochloa colonum* (L.) Moench (2011) and *Cynodon hirsutum* (2012).

The outlook is that the main crops (soybean, corn, cotton) from Brazil, the USA and Argentina will be resistant to glyphosate. In this context, succession and crop rotation with conventional seeds is a strong chance in the field. There is the necessity to convince farmers that repeated and continuous use of glyphosate-resistant crops in few years could cripple the weed control with the use of glyphosate-based products.

Evolution of glyphosate-resistant populations is an imminent threat in areas where there is dominance of glyphosate-resistant crops, intense selection pressure and no diversity [70]. Certainly other glyphosate-resistant weeds will be identified in the coming years. But when and how it is related to use of glyphosate-resistant crops? The use of practices to reduce selection pressure and switch mechanisms is important to protect and prolong the use of important molecules such as triazines, ALS inhibitors, ACCase, and glycines.

4. Management of weeds in soybean areas: Argentina, Brazil and the USA

4.1. Weed management in Argentina

The first recorded experience with soybeans in Argentina was in 1862, just a few years after their introduction to the US, but back then the country was a stronghold of cattle production, and there was little interest in agriculture. The first variety trials and commercial harvests occurred during the 60s. At the turn of the century, soybeans in Argentina were reaching the 10,000,000 hectares mark, coinciding with the adoption of transgenic GR soybeans. Soybean production increased over 1000-fold to a record of 52 million metric tons in 2010. The most productive area for soybeans is comprised by the northern portion of the province of Buenos Aires, the central and southern part of the province of Santa Fe, and the southeastern part of Córdoba (humid pampas), but in recent years the expansion has been more noticeable in other provinces, like Entre Ríos, Santiago del Estero, Tucumán, Salta and Chaco, in the northern part of Argentina. Another factor influenced by the adoption of GR soybeans was the oversimplification of the weed control programs, which eventually led to the selection of resistant biotypes and hard-to-control weeds.

The development and early expansion of the crop in Argentina was accompanied by the constant introduction of new herbicide molecules. During the 70s, as farmers in Argentina were learning how to grow this crop, the most common weed control methods in soybeans were a combination of tillage and pre-emergent (PRE) herbicides such as trifluralin, dinitramine (dinitroanilines), cloramben (benzoic acid), naptalam (amide), flucloralin (chloroanilin), vernolate (thiocarbamate), metribuzin, prometrin (triazines), alaclor (chloracetamide), and linuron (phenylurea). Bentazon, one of the first post-emergent options, did not become available until the end of that decade. The dinitroanlinies, flucloralin, and vernolate were used on pre-planting incorporated (PPI) for annual grasses and broadleaves control, clormaben was one of the few burndown options for broadleaves, naptalam was applied PRE for annual grasses and broadleaves, the triazines also PRE, for small seeded broadleaves, often in combination with alachlor to improve annual grass control, and linuron offered broad spectrum control also applied PRE.

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As a result of the limited choices in herbicides in soybean, there were several weed problems, such as the perennial grasses *Sorghum halepense* (L.) Pers. and *Cynodon dactylon* (L.) Pers., several annual grasses, such as *Digitaria sanguinalis* (L.) Scop., *Echinochloa crus-galli* (L.) Beauv., *E. colonum* (L.) Moench, *Eleusine indica* (L.) Gaertn., and the typical broadleaf weeds of summer crops — *Amaranthus* sp., *Chenopodium album* L., *C. cordobense* Aellen, *C. pumilio* R. Br., *Datura ferox* auct. non L., *Tagetes minuta* L., *Ipomoea* spp.. It was mention at least 6 species of Ipomoea, *Xanthium strumarium* L., *X. cavanillesii* Shouw, *Anoda cristata* (L.) Schlecht. and *Portulaca oleracea* L. [71] — among the broadleaf weeds in the humid pampas (Table 4). These plants represented a challenge and slowed the initial expansion of the crop. Most of the weeds described here are the same or very similar to the weeds commonly found in conventional-tillage systems around the world. A very interesting point is that none of the broadleaf weeds that are posing a challenge today to glyphosate in the temperate region is in this list, and most of the emerging weeds are local weeds, not common in other regions.

Economically important weeds	Secondary weeds	Emerging weeds
Echinochloa crus-galli (L.) Beauv; *E. colonum* (L.) Moench	*Xanthium* spp.	*Physallis angulata* L.
Cyperus rotundus L.	*Sida rhombifolia* L., *S. spinosa* L.	*Solanum sisymbriifolium* Lam.
Datura ferox auct. *non* L.	*Galinsoga parviflora* Cav.	*Aeschynomene virginica (L.) B.S.P.*
Tagetes minuta L.	*Bidens* spp.	*Nicandra physalodes (L.) Gaertn.*
Cynodon dactylon (L.) Pers	*Ipomoea nil* (L.) Roth., *I. purpurea* (L.) Roth.	*Abutilion theophrasti* Medik
Anoda cristata (L.) Schlecht	*Setaria viridis* (L.) Beauv., *S. verticillata* (L.) Beauv.	*Solanum chacoense* Bitter, *S. nigrum* L.
Digitaria sanguinalis (L.) Scop.	*Helianthus annuus* L. (volunteer)*	*Acanthospermum hispidum* DC.
Chenopodium album L.	*Eleusine indica* (L.) Gaertn.	*Flaveria bidentis* (L.) Kuntze
Portulaca oleracea L.	*Alternanthera philoxeroides (Mart.) Griseb.*	
Amaranthus quitensis Kunth	*Euphorbia heterophylla* L.	
Sorghum halepense (L.) Pers.	*Wedelia glauca* (Ort.) Hoffm. ex Hicken	

*Sunflower was a common component of the rotation systems.

Table 4. Most important weeds in the humid pampas in 1997, before the adoption of GR soybeans [72].

Usually a moldboard plow was used in the fall to incorporate the previous crop residue and destroy existing vegetation. Herbicides were part of the control methods from the beginning, given the timing of the introduction of soybeans in Argentina, so a mechanical-only technology was never developed for the region, except for specific purposes, like organic soybeans. In the spring, residual herbicides were applied after the preparation of the seedbed, incorporating them if needed. There were several escape problems given the limitation of POST options, especially with large seeded broadleaf weeds like *D. ferox*, *A. cristata*, and *Ipomoea* spp. The problem was so common that in many areas a special device called "Chamiquera" (Figure 3)

was used to separate the harvested soybeans from "Chamico" (*D. ferox*) and sometimes "Bejucos" (*Ipomoea* spp.) seeds before the beans could be delivered at the grain elevators.



Figure 3. Special device "Chamiquera", Rojas, Buenos Aires, circa 1980.

During the 80s and 90s, until the introduction of GR soybeans, the development of several new molecules improved the control of many weeds, but still in combination with mechanical methods, leading to a steady expansion of both the area planted with soybeans and the average yields (Figure 4). Gradually, new herbicides allowed technology developments that replaced, at least in part, mechanical control methods with chemical ones. The need of field cultivators was reduced or replaced by the application of pre-emergent combinations of alachlor and metribuzin that offered a wide spectrum of control and proven residuality, replacing other herbicides — like trifluralin — that required mechanical incorporation.

Acifluorfen became a common tool for rescuing treatment, even though it caused severe crop injury. This herbicide allowed the control of large seeded broadleaf weeds — *Xanthium* spp., *D. ferox*, late flushes of *Ipomoea* spp. —, all common problems in most of the soybean area, but the injury it caused to the crop was something the farmer was not used to dealing with. It was replaced in part by another diphenylether, fomesafen, although it did not have the same efficacy or control spectrum. The registration of ALS inhibiting herbicides (sulfonylureas, imidazolinones and triazolopyrimidines) ushered a new era of weed control in soybeans in Argentina, allowing for PRE/POST combinations that offered effective and lasting control of the most important weeds with less crop injury than the previous options. Imazaquin, imazethapyr, chlorimuron, diclosulam and flumetsulam were launched in Argentina during the second half of the 80s (the first registration of imazaquin was actually in Argentina, in 1984) and allowed the

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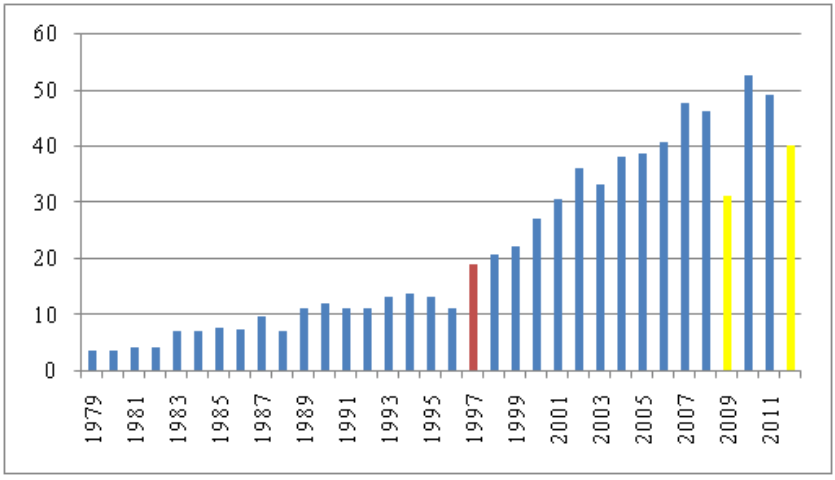
first approach to no tillage in soybeans. The resistance problems associated with this group were not noticeable in Argentina, although the first resistant weed in the country is resistant to this herbicide group (ALS inhibitors), because it coincided with the introduction of the GR technology, and the quick adoption of the new varieties masked the problem.



Sources: 1979-2004, Secretaría de Agricultura, Ganadería, Pesca y Alimentos de la República Argentina. 2005-2012, Diario La Nación, May 24, 2012.

Figure 4. Soybean production, in million metric tons, from 1979 to 2012. In red: first year with commercial GR soybeans. In yellow: droughts of the 08-09 and 11-12 seasons.

New inhibitors of the protoporphyrinogen oxidase enzime herbicides were introduced during the late 90s. Carfentrazone, sulfentrazone (aryl triazinones) and flumioxazin (N-phenylphtalimides derivative) offered new options for burndown (carfentrazone) and residual control (sulfentrazone, flumioxazin), but the introduction of the GR soybean varieties prevented its adoption, thus the most dramatic expansion of soybean production in Argentina was the introduction of the glyphosate resistant varieties in 1996.

Nearly all the soybeans in Argentina are transgenic (GR1). Argentina had the fastest adoption of glyphosate-resistant soybeans in the world. This fast adoption coincided with the expansion of no tillage technology in the region, fueling a synergism between GR soybeans and no tillage. AAPRESID, the national association of no tillage farmers, had held its first national symposium a few years prior to the launching of this technology, and its members welcomed and quickly embraced a new biotech development that allowed them to fully implement their preferred technology.

Until the adoption of GR soybeans, tillage was an important weed control method, complementing chemical control options, but it had a negative impact on erosion, soil structure and organic matter mineralization. The introduction of herbicide-resistant varieties increased not only the use of glyphosate, but also the practice of no tillage as well, replacing mechanical con-

trol almost completely in soybean production. The high efficacy of this herbicide combined with the simplicity of the system resulted in a quick replacement of other herbicides used in soybeans, both over the top applications and during the chemical fallow period. In 2005, over 92% of the herbicide volume used in chemical fallow was glyphosate, while some hormonal herbicides were commonly tank-mixed with glyphosate to improve the control of thistles and other "new" weeds. Overall costs of weed control in soybeans decreased dramatically as new generic glyphosate brands entered the Argentine market. Another aspect that contributed to the simplification of the system, including soybean monoculture, was the general economic situation of the country. Corn required a higher investment, while soybeans, especially GR soybeans, as described above, allowed farmers to plan their soybean season with less financial requirements (in Argentina, the law allows farmers to save seeds for their own use) in times when the prices of commodities were uncertain and financial means were limited, or expensive.

Glyphosate effectively controlled not only the most problematic weeds in soybean fields; it replaced herbicide combinations that required a deep knowledge of the weed spectrum, careful planning to avoid escapes, tank mix problems, timing concerns and crop injury, and still did not offer the satisfaction of a field completely clean of weeds. RR technology simplified the business of growing soybeans like no other technology ever developed. Today, soybean system is characterized by over-reliance on glyphosate, low crop rotation, absence of mechanical control methods and limited monitoring (of both weeds present at the time of application and results). The lack of monitoring practices is a direct result of the high efficacy of glyphosate control in the early years of the biotech age. As a result, the weed spectrum has shifted and there are several glyphosate-resistant weeds, combined with hard-to-control ones, while the presence of weeds with resistance to other modes of action is still limited.

Glyphosate is still a very valuable weed control tool, in spite of the weed shift that Argentina has experienced due to its over-use. In [73] it was studied the effectiveness of glyphosate applications at two stages (vegetative and reproductive) on 31 weeds that represented the typical weed spectrum of the region. The herbicide had complete control on 58% of the species at both stages, complete control at the vegetative stage but deficient control at the reproductive stage on 32% of the species and poor control on only 10% of the species at either stage. Disregarding the poor control at the reproductive stage-only, which is not recommended, it is clear that glyphosate satisfactorily controlled 90% of the weeds. The remaining 10% can be managed easily combining glyphosate with the proper herbicides, providing a cost-effective complement. The control of some of these difficult weeds is improved when glyphosate is combined with atrazine or metsulfuron applied during fall [74]. For example, *Bowlesia incana* Ruiz & Pavón and *Parietaria debilis* G. Forst., increased when glyphosate was applied as a tank mix to these herbicides, compared to glyphosate by itself. These herbicides are readily available and are cost-effective alternatives to combine with glyphosate.

The selection of herbicide-resistant biotypes was a consequence of lack of crop and herbicide rotations. Daniel Tuesca, a weed scientist in the University of Rosario, states that, in the years preceding the introduction of the GR soybean, farmers mentioned, on surveys, the use of 16 different herbicides in the fallow process and 13 on the crop (either PRE or POST), but a few years after the introduction of the technology, there were only 3 herbicides applied in fallow,

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and only glyphosate over the crop. From the three herbicides mentioned in the surveys, there were no specific graminicides (Table 5), so it is not a surprise that all the weeds that have been confirmed as resistant to glyphosate are grasses.

Before 1997 (1995-1997)		**After 1997**	
Fallow applications	Picloram	Fallow applications	Atrazine
	Flumetsulam		2,4-D
	Metribuzin		Metsulfuron
	MCPA		
	Dicamba		
	Atrazine		
	2,4-D		
	Metsulfuron		
	Other herbicides		
	No applications		
PRE/Over the top	Flumioxazim	PRE/Over the top	
	Clorimuron		
	2,4-DB		
	Imazaquin		
	Acetochlor		
	Graminicides (FOP's)		
	Flumetsulam		
	Diclosulam		
	Imazetapyr		
	Other herbicides		
	No applications		

Based on individual responses to surveys, % for each answer is omitted. FOPs (aryloxyphenoxypropionate herbicide group)

Source: courtesy of Professor Daniel Tuesca, Universidad Nacional de Rosario, AR.

Table 5. Herbicides, other than glyphosate, used in soybean production before and after the introduction of GR soybeans in Argentina, according to surveys with farmers.

Apart from the confirmed cases of glyphosate-resistant weeds, there are several problems caused by the excessive use of glyphosate. To better understand the problem, it is important to state that in Argentina about 70% of the farming is done in rented land, and during the last decade the rental price has increased constantly. In many cases, this situation prevented the traditional early fallow procedures and resorted to burndown practices with weeds that had grown beyond their optimal control stage. One particular case is *C. bonariensis*, which has been confirmed to be resistant to glyphosate in Brazil, although the resistant biotype is not present in Argentina yet. When treated at the rosette stage, the plant is susceptible to be controlled with glyphosate, but when it has elongated (early in the spring), it becomes resilient, even

when using 2 and 3 times the dose of glyphosate. The situation changes when residual herbicides are applied in the fall (flumioxazin, metsulfuron, atrazine, and diclosulam have proved to be effective). There was a lot of confusion when this weed began to emerge as a problem because it co-exists with another species, *C. sumatrensis*, more susceptible to be controlled by glyphosate applications at later stages, leading to a general belief that there are resistant biotypes that escape control. Again, the lack of monitoring practices is evident here. These weeds are strongly associated with no-tillage practices, since they do not progress at all in tilled soil.

Today, there are many efforts to revert the reliance on glyphosate and the selection of resistant biotypes and hard-to-control weeds. Universities, professional associations and the industry are advocating the rational use of herbicides with different sites of action, in a crop rotation program, to prevent the selection of new resistant biotypes, not only to glyphosate but to others as well, especially biotypes with multiple resistance. It is only fair to mention that academics from different institutions such as INTA, Universidad Nacional de Buenos Aires, Universidad Nacional de Rosario, Universidad Católica de Córdoba, Universidad Nacional de Tucumán, Estación Obispo Colombres, just to mention a few, have been working hard on this matter in the previous years, when glyphosate was still the undisputed weed control method of choice. Argentina is shifting from a simple and effective system to a more complex one that requires a stronger commitment from farmers, advisors, the academic sector and the industry. The soybean sector is facing a turning point, and this new reality will have to include more crop rotations, more herbicides and also mechanical and cultural weed control methods.

4.2. Weed management in Brazil

According to professor Gustavo Dutra, from Cruz das Almas, Bahia, it may be inferred that, since its introduction in the country in 1882, soybean crop has transformed the Brazilian agriculture. Initially planted in the state of Rio Grande do Sul, first recorded in 1914, in Santa Rosa, the soybean "tropicalization" has found space coming out from southern pampas to the midwestern region of the country. While only 2% of national soybean production had been recorded in this region in the 70s, more than 47% of national production was reported in midwestern region in 2010/2011 harvest. Hence, Brazil represents one of the most important regions with a growing potential in soybean production. Probable areas to produce soybean ponder between 20° S and 20° N. However, the largest portion of this production belt is concentrated in the Brazilian lands, with estimated increases of 2.3% up to the year 2020.

Weed control has bothered growers from the beginning of soybean cultivation, especially since 1950, with the expansion of southern region. Adaptation of production system allowed the satisfactory management, even when using only mechanical tools to control. Cost constraints and limitations set by this control led to its quick replacement by the chemical control, which became a primary tool of weed management. Due to its importance, Brazilian pesticide market has expanded from 1977 to 2006, on average, 10% per year. Even after many decades, the use of soybean herbicide has been restricted to spraying in incorporated pre-plant (eg triflurallin) and pre-emergence (eg metribuzin, alachlor and linuron) along with plowing and harrowing, to prepare conventional soybean field.

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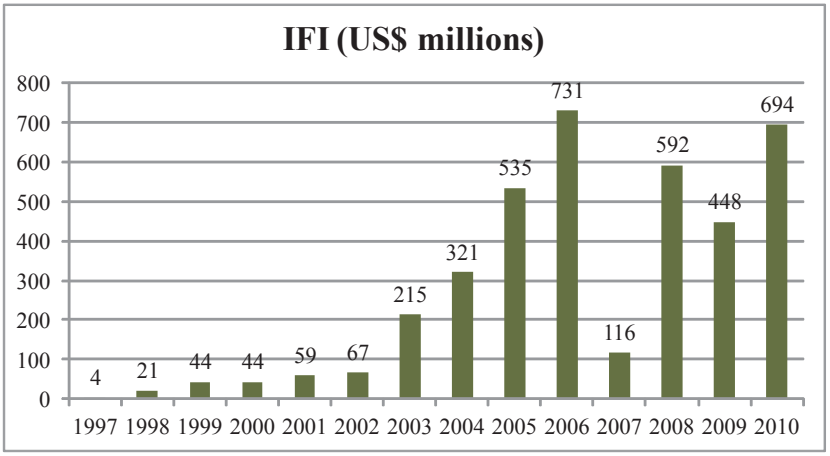
Few herbicides used previously restricted the implementation period, affecting more specific actions for managing the weeds emerged in advanced stages of the crop. The launch of bentazon POST herbicide revolutionized the market, allowing the control of major dicotyledonous weeds on soybean. Introduction of new molecules from the 80s and 90s afforded efficiency on the control of several species, in particular those belonging to genders Amaranthus, Digitaria, Brachiaria, Euphorbia and Bidens. The main herbicides applied belonged to the chemical groups ALS and ACCase inhibitors, with monocotyledonous and dicotyledonous actions.

Since the introduction of no tillage system, weed management has changed and, as a consequence, moved to consider factors other than chemical control on the production system. The main benefit of no tillage system is the reduction of weed germination over time [75] and greater use of crop control. Furthermore, species not commonly observed in the conventional system demand better preparation and expertise of producers. Such modifications are related to the absence of soil disturbance, favoring perennial cycle weeds, as well as changes in patterns of temperature and light incidence, influencing seeds' mechanisms of dormancy. Cover crops result in greater amount of organic residue, with higher C/N ratios, and are more efficient in weed management, by composing a thicker layer of mulch on surface soil [76]. The weed density decreases linearly with organic residues increasing on surface soil, mainly by reduction on weed germination.

Originally, no tillage system in Brazil used 2,4-D and paraquat herbicides as burndown to prepare cultivation areas. At the time there was no product like glyphosate, with non-selective and desiccant action. Despite the effective action, there were limited control with paraquat and some residual effects of 2,4-D on soybeans, hindering the sowing immediately after spraying. With glyphosate releasing in Brazil in 1982, the technology suited local and producers' needs, gaining the market by its control efficiency. But the POST application was still limited to the same herbicides (bentazon, imazethapyr, setoxydin, tepraloxydym, etyl-chlorimuron, diclosulam, clorasulan-methyl, etc.). Doses were necessarily higher and the number of resistance cases to ALS inhibitors started to increase, since the first record of *Bidens pilosa* L., which is resistant to imazaquin and chlorimuron-ethyl, appeared in 1993.

With the introduction of GR soybean, most of the herbicides were replaced in 2003/2004 harvest in Brazil. The system that provides a single application of glyphosate at early stages of the crop gained market for its easy adoption, undeniable efficiency in weed control and guarantee of profitability. According to data, nearly 81% of all soybeans cultivated area in Brazil is GR and its contribution to farmers is unquestionable (Figure 5). The impact of using GR soybeans has been similar to that identified in the US and Argentina, although the net savings on herbicide costs are larger in Brazil, due to higher average costs of weed control [77]. The average cost savings originated from a combination of reduced herbicide use, fewer spray runs, labor and machinery savings, were between US$30/ha and US$81/ha in the period 2003-2010, which means that the net cost saving after deduction of the technology fee (assumed to be about US $19/ha in 2010) has been between US$9/ha and US$61/ha in recent years, with increased farm income levels of US$694 million in 2010 by the GR soybean adoption.



Source: adapted [77].

Figure 5. Impact of using GR soybean on farm income (IFI), at a national level. Brazil, 1997-2010.

Unfortunately, the overuse of the technology (GR soybean + glyphosate) in tillage and no tillage system led to strong selection pressure. Apart from the variation of biotypes selectivity, the level of herbicide application also contributes to the tolerance of species. It was checked the Brazilian herbicide usage data for the periods 2001-2003 and 2007-2009, as well as information from industry and extension advisers and was concluded that the annual average use of herbicide active ingredient per ha in the early years of GR soybean was lesser than 2007-2009, an estimated difference of 0.22 kg/ha [77]. From 2007-2009 data, it was observed an average active ingredient use of 2.37 kg/ha for GR soybean compared to 1.96 kg/ha for conventional soybeans.

These data clearly illustrate the current weed management in soybean in the country. Nowadays, Brazilian producers are using sequential spraying of glyphosate in order to control species which are difficult to manage in crops, such as *Bidens* spp., *Chamaesyce hirta* (L.), *Spermacoce latifolia* Aubl., *Chloris polydactyla* (L.) Sw., *Ipomoea grandifolia* (Dammer) O'Donell, *Commelina benghalensis* L., etc. along with glyphosate herbicides. They also associate herbicides of other chemical control groups and, especially on southern and southeastern regions, producers are using the autumn management, in areas where these species are present [2]. Other herbicides — such as imazethapyr and imazapic — are frequently applied to reduce the emergence of weeds during the fallow period and/or associated with the herbicide 2,4-D on burndown, about 15-20 days before the sowing, for dicotyledonous management of complex control by glyphosate. A relevant number of not highlighted weed species are worrying Brazilian soybean producers. *Borreria* spp., *Tridax procumbens* L. and *Alternathera tenella* Colla, among others (Table 6), are species with high adaptability to different ecological niches throughout the national territory and they are on the list of species likely to be capable of developing resistance to herbicides used in cultivation, being it a GMO or not.

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Scientific name	Common name	Scientific name	Common name
Acanthospermum hispidum DC.	Starbur	*Eleusine indica* (L.) Gaertn	Goosegrass
Amaranthus retroflexus L.	Pigweed	*Euphorbia heterophylla* (L.)	Wild poinsettia
Bidens pilosa L.	Hairy beggarticks	*Galinsoga parviflora* Cav.	Smallflower
Brachiaria plantaginea L.	Alexandergrass	*Ipomoea purpurea* (L.) Roth	Morningglory
Cenchrus echinatus L.	Sandbur	*Panicum maximum* Jacq	Urochloa maxima
Commelina benghalensis L.	Dayflower	*Pennisetum setosum* Rich	Bufflegrass
Cynodon dactylon (L.) Pers	Bermudagrass	*Setaria geniculata* auct. non (Willd.) Beauv.	Foxtail
Conyza bonariensis (L.) Cronq.	Hairy fleabane	*Sida rhombifolia* L.	Sida
Conyza canadensis (L.) Cronq.	Horseweed	*Sorghum halepense* (L.) Pers.	Johsongrass
Digitaria insularis (L.) Mez ex Ekman	Sourgrass	*Spermacoce latifolia* Aubl.	Buttonweed
Digitaria horizontalis Willd.	Jamaica crabgrass		

Table 6. Some weed species on soybean Brazilian crop [2].

For the management of weeds resistant to glyphosate, the alternative control, besides herbicide mixtures, includes crop rotation, autumnal management or even return of non transgenic soybeans, as well as herbicides spray recommended in 80s and 90s. To reduce *Conyza* spp. competition, which can cause yield losses above 70% for soybean [78] it is recommended winter management by mixing residual herbicides and glyphosate + 2,4-D [79] ever sprayed on initial growth stage and on plant less than 10 cm-height. For *L.* multiflorum control in the south region, clethodin or haloxyfop-p-methyl herbicides in a glyphosate mix can be used. *S. halepense* is another glyphosate-resistant species which has a reasonable control with haloxyfop-p-methyl application. Nevertheless, this last management must be made with young plants.

In the US, saflufenacil is being used as a major product mixed to glyphosate for controlling resistant weeds. This PPO inhibitor empowers the action of glyphosate as desiccant and it is applied on off-season management or before crop sowing. Though, its release in Brazil has not occurred yet and it should be soon on the market to assist the producers. One of its advantages is the low residual rate in the soil at recommended doses, which allows its implementation and subsequent planting without requiring longer intervals before sowing.

The steady cost increase in weed control by intensive herbicides use and their mixtures emphasizes the need of changing. Since introduction of GR soybean technology in 2003, until 2006, there has been a reduction in herbicide application in soybeans in the country, deriving mainly from efficiency control and range of action of the glyphosate (Table 7). However, amount of active ingredients utilized on crop has risen since 2006, as a result of the intense use of glyphosate and other herbicides. New generic glyphosate brands entered the Brazilian market and it contributed to glyphosate use indefinitely.

Year	ai saving (kg; negative sign denotes increase in ai use)*	% decrease in ai (- = increase)
1997	22,333	0.1
1998	111,667	0.3
1999	263,533	0.7
2000	290,333	0.7
2001	292,790	0.7
2002	389,145	0.8
2003	670,000	1.2
2004	1,116,667	1.7
2005	2,010,000	2.9
2006	2,546,000	4.0
2007	-5,808,563	-8.8
2008	-5,704,705	-17.6
2009	-6,642,000	-18.7
2010	-7,529,650	-20.0

Sources: Kleffmann & AMIS Global;

* Including herbicides (mostly glyphosate) used in no/low tillage production systems for burndown.

Table 7. National level changes in herbicide use (active ingredient – ai) by GR soybean. Brazil, 1997-2010 [77].

In spite of weed shift in Brazil, glyphosate is still a helpful weed control tool. To extend its use as a major tool in chemical control strategies on tillage and no tillage sowing, GR and no-GR soybean, current management in soybean aims to integrate methods that minimize the effects to the environment and offer adequate security control. Therefore, in addition to new technologies afforded by the chemical industry, producers should also cooperate in the process, even though this implies the return of already used tools, as the conventional soybean (no GM). Among the alternatives, there is the rotation area with conventional soybeans, the use of offseason management (autumn), the spraying of non-selective herbicides that reduce shifts on further glyphosate applications, the advanced management in spraying installment — being the first 30 days before sowing and the second between five and seven days prior of planting —, the sowing of cover crops in fallow period and the spraying of recommended herbicide doses in order to avoid progressive biotypes selection [30,80].

4.3. Weed management in the USA

Soybean production in the US is undoubtedly part of the greatest productions worldwide and it has an expressive occupation of agriculture area in the country. According to the USDA projections, last average yield was around 3.7 tons/ha crop; in 2012, there will be about 29.9 million hectares crop in the country. Most of soybean cultivated area in the US (93%) uses GR soybeans. The first scientific record of soybean cultivation in the US took place in 1879 at the Rutgers Agricultural College, in New Jersey [81]. Initially, the crop was mainly used as animal fodder. However, the growing interest in culture, sponsored by the demand for oil and meat,

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forced soybean to expand rapidly and occupy many areas previously cultivated with corn, in the extensive Corn Belt.

Despite high yields, the country also passed through difficulties at the beginning of crop establishment. Even with great advances in farmland during the 50s, farming tools were limited, especially the ones related to weed management. There was no PRE or POST herbicides. Usual control practices were restricted to the use of mechanical weeding, fundamental on conventional crop system. Wide-row spacings were used in order to provide effective mechanical weeding and post-sowing. The 2,4-D was used over-the-top at the end of crop growing, prior to the harvest. This allowed reduction on dicotyledonous weeds and on subsequent crops, but did not control the monocotyledonous ones. These have become the main weeds and *Sorghum halepense* (L.) Pers was a major problem weed in many fields.

Until glyphosate and, mainly, GR soybean advents, weed management in the US was restricted to mechanical control and some PRE and POST herbicides to monocotyledonous and dicotyledonous control. Trifularin was a major narrowleaf herbicide used for years, which was applied in autumn or in spring before sowing. Its use requires tillage system but did not aid weed management in early-season, especially *S. halepense* and *Amaranthus* sp. control. Between the 70s and 80s, glyphosate and paraquat came into use as preplant burndown, being helpful on no tillage system. These herbicides replaced preplant tillage and fostered the currently used stale seedbed planting system. Not so far, PRE and POST selective herbicides became available to most monocotyledonous and dicotyledonous weed control. Narrow-row and no tillage system challenged soybean farmers to introduce a new management concept. In the US, the first POST herbicide formulations were available years before their release in Argentina and Brazil and some chemical alternatives on weed management in the country had always been more accessible. Nevertheless, the order of release was followed, initially by bentazon, with a broad spectrum of action, and after, ACCase inhibitors, diphenylethers, imidazolinones and sulfonylureas (ALS inhibitors).

Traditionally, soybean is the rotational crop with rice in most farming areas, particularly in midsouth region. Prior to rapid rice expansion area in the 70s, the common rotation involved 2-year soybean and 1-year rice. Today, rice is often grown for 2 or 3 years before another crop, especially where the land is unsuited for other crops, and soybean is predominant. Major conventional herbicides that have been used in soybean include trifluralin, pendimethalin, metolachlor, alachlor, dimethenamid, clomazone, imazethapyr, sethoxydim, fluazifop, quizalofop, and clethodim [82]; many of them are useful against *S. halepense*.

The main herbicides such as trifluralin, pendimethalin, imazethapyr and imazaquin were widespread until the mid 90s, but with glyphosate effectiveness, mainly linked to GR soybean, there was a massive replacement of the "out-of-fashion" herbicides. During the period considered, 1995-2006, the treated areas with pendimethalin decreased from 26% to 3%; areas treated with imazethapyr suffered a reduction from 44% to 3% [83]. Especially for imazethapyr, whose decrease was greater than pendimethalin, many resistant weeds had been selected, even in the first using years, encouraging technology exchangings.

Many advantages provided by glyphosate on GR soybean weed control overlapped other management tools, leading to a replacement of herbicides and conventional soybean for the new

technology. No tillage systems became widely used and weed control costs were lowered. Total applied herbicides and labor inputs declined initially and narrow-row on soybean became the standard. In 1995 the GR soybean areas treated with glyphosate were only 20%, but they took over 96% in 2006 [84]. Currently, the GR soybean represents over 94% of the soybeans grown in the US, and more than 90% of soybeans produced worldwide are considered GR.

The initial advice for GR soybean system was only one spray and its late application would not undermine crop yield. In extremely wet sites with late sowing — Iowa, for example —, weeds emerged early and single POST glyphosate spray was enough for effective control till the end of the cycle [85]. But for the midwest region, the sowing scheduled occurred earlier, thus only one application was unsuitable for weed control, usually requiring additional sprays.

Concerns about the definition of better periods of spraying, along with the appearance of the first glyphosate resistance case, registered for *Lolium rigidum* in 1998, have collaborated with gradual increase in herbicide use. For the period 2003-2009, herbicides applied to GR soybean increased 30%, whereas consumption remained stable for conventional soybeans [83]. Among changes observed in the global agricultural production, there is the search for socioeconomic and environmental efficiency. Farmers want new tools for weed management. New GM crops have allowed simple and effective solutions, but if producers keep outdated manners when using new tools with GR soybean and glyphosate, these tools will soon become obsolete [25].

As a result, a second generation of GR soybean was launched recently in the US in 2009. Although this technology offers the same soybean resistance to glyphosate as the first generation (RR1), it has a higher yield potential, between 7% and 11%. Some farmers reported no increasing yield in relation to first GR soybean generation; perhaps others found positive yield effect. In 2010, soybean farmers pointed that second GR soybean generation has, on average, about 5% of yield improvement.

Many soybean farmers currently use glyphosate mixed with residual herbicides employed previously. The increase of these mixtures permits earlier glyphosate sprays promoting weed management for a larger period. Using conventional herbicides into new GM soybeans are also essential to ensure its resilience, since new traits will be released to use with former herbicides. New technologies include GM soybeans resistant to glufosinate "Liberty Link", to 2,4-D "Optimum GAT", to dicamba and also to glyphosate plus ALS inhibitors. Despite the creation of technologies for landing efficiency and easy management on weed control, good practices at all soybean crop system are rather necessary. Also, weaknesses and difficulties on weed management in many regions of the US have attracted the interest for non-GM soybeans. Differentiated prices in the international market have also stimulated this substitution, yet it is constrained to small and middle producers.

5. Benefits of integrated weed management

Effective weed management is very important to maintain agricultural productivity. By competing for light, water and nutrients, weeds can reduce crop yield and quality and can lead

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to billions of dollars in global crop losses annually. Because of their ability to persist and spread through the production and dispersal of dormant seeds or vegetative propagules, weeds are virtually impossible to eliminate from any given field. The importance of weed management to successful farming systems is demonstrated by the fact that herbicides account for the large majority of pesticides used in agriculture, eclipsing inputs for all other major pest groups. To no small extent, the success and sustainability of our weed management systems shapes the success and sustainability of agriculture as a whole [86].

Integrated pest management (IPM) concept was introduced in the 60s comprising many definitions from then. The primary goals of IPM programs are to reduce pesticide use and the subsequent environmental impact and to rely more on alternative strategies to control pests [87]. Integrated weed management (IWM) comes as a secondary effect of IPM, but it has similar proposal of using multiple management tactics and incorporating the knowledge of weed biology and crop physiology into the weed management system. The goals of IWM range from maximizing profit margins to safeguarding natural resources and minimizing the negative impact of weed control practices on the environment [88].

Integrated Weed Management combines multiple management tools (biological, chemical, mechanical and others) to reduce a pest population to an acceptable level while preserving the quality of existing habitat, water, and other natural resources. The integrated management provides connection of all the involved organisms, whether weeds, pests or diseases, and should focus on decision-making with case studies. There are many practices set out in the integrated management systems, whose benefits have been extensively studied by several authors (Table 8). These studies demonstrate many benefits and the efficiency of integrated tools in crop management systems.

Practices evaluated in IWM	Study
Monitoring weeds in crop fields	[90,91]
Use economic thresholds to determine when to apply herbicides	[91-93]
Crop rotation	[80,91]
Using the biological and chemical control	[94,95]
Using cultural and chemical control	[96]
Using mechanical and chemical control	[97]
Using rotation of herbicides	[90,91]
Plant cover crops	[90,98]
Using the tillage, no-tillage or reduced tillage system	[90,92]

Table 8. Practices evaluated in previous studies as part of an Integrated Weed Management (IWM).

However, there are no more ready-made and generalized solutions without risk of errors. IWM is characterized by reliance on multiple weed management approaches that are firmly

underpinned by ecological principles [89]. As its name implies, IWM integrates tactics, such as crop rotation, cover crops, competitive crop cultivars, the judicious use of tillage, and targeted herbicide application, to reduce weed populations and selection pressures that drive the evolution of resistant weeds. Under an IWM approach, a grain farmer, instead of relying exclusively on glyphosate year after year, might use mechanical practices such as rotary hoeing and interrow cultivation, along with banded PRE and POST herbicide applications in a soybean crop one year, which would then be rotated to a different crop, integrating different weed management approaches.

Earlier studies have also demonstrated that IWM strategies are effective in managing herbicide-resistant weeds. For example, glyphosate-resistant horseweed in no tillage soybean can be controlled by integrating cover crops and soil-applied residual herbicides [100]. In a recent experiment in which the integration of tillage and cover crops was evaluated for controlling glyphosate-resistant *Amaranthus palmeri* in Georgia, the combination of tillage and rye cover crops reduced *A. palmeri* emergence by 75% [101]. In addition to cultivation and cover crops, other practices can be used to manage resistant-weed populations.

In another experiment, it was experienced biological and chemical control to *Sesbania exaltata* [Raf.] Rydb. ex A.W. Hill in soybean field. Different concentrations of *Colletotrichum truncatum* (Schwein.) Andrus & Moore were tested alone and in combination with glyphosate. Positive results suggest that it might be possible to utilize additive or synergistic herbicide and pathogen interactions to enhance *S. exaltata* control [94]

Despite many results, researchers suggest that implementation has been slow, and that farmers rarely move beyond incorporating cost-effective, targeted pesticides application [102]. Many growers are not adopting integrated management because current assessment methods are inadequate [99]. In their study, evaluating data from eastern North Carolina, US, they considered four components of the integrated management: weed, pest, environmental and general management of the properties. The component weed had the highest percentage (79%), indicating that growers were undertaking its management.

In [97] it was evaluated a cropping system, including various combinations of seeding rate and date, herbicide timing and rate, and tillage operations, by measuring weed response to six IWM systems, in a wheat–oilseed rape–barley–pea rotation. Changes in weed communities assessed over 4 years indicated a gradual increase of *Thlaspi arvense*, *Chenopodium album*, *Amaranthus retroflexus* and *Fallopia convolvulus* in the no herbicide/high tillage system. Winter and early spring annuals and perennials increased in most systems, but particularly in the low herbicide /zero tillage and medium herbicide/zero tillage systems. This study confirms the potential of contrasting IWM systems under the challenging environmental conditions.

Some mathematical models are also used into IWM. It allows to model scenarios and to compare long-term economic and weed population outcomes of various integrated management tools. In southern Australia, species like *Lolium rigidum* and *Raphanus raphanistrum* were managed for many years with selective herbicides. But these species became resistant and are widespread now. In [93] it was tested an integrated model to compare the management over

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a 20-year period and found that differences between scenarios are not due to weed densities but differences in total cost on weed control.

In fact, despite all the benefits, the implementation of IWM is extremely challenging for researchers and especially for farmers. In a recent paper — *True integrated weed management* — was highlighted in glowing way the need for a single platform development, including sensors and decision-support software, that has multiple application technologies for weed management [103]. According to the actor, "*Ideally, a self-guided machine is needed that could comb the field in a systematic way to identify weeds and then apply the necessary control tool (eg spray, mow, cultivate) at the individual plant or patch scale*". The illustration of a machine model (Figure 6), which allows the required operations case by case is utopian, although it is believed that efforts to achieve this goal are unlimited.



Figure 6. Illustration of a robotic weed control using multiple tools designed [103].

6. Conclusions

Weed management has always been inserted into the soybean crop system, contributing decisively to the success of this crop in major producing countries nowadays. The evolution of weed management practices in Argentina, Brazil and the US has been developed similarly, by means of mechanical growers and massive use of GM soybean. However, weeds also have evolved and as new tools were used, new species or new biotypes appeared.

Despite the persistent search for weed control in the soybean areas, it is observed that management of those has increased considerably in the last 10 years. There are numerous cases of

weed resistance to various chemical herbicide groups used in the crop and some weed species are resistant to more than two chemical groups.

Even with the biotechnology advances and other GM soybean introduction, history must repeat itself, since the tendency to standardize production systems favors the weeds, allowing better adaptation response as it increases the selection pressure. The application of glyphosate to GM crops like soybeans, corn, cotton, canola, wheat, among others — all resistant to this herbicide — is not the best alternative to properly manage weeds. In regions where RR technology is predominant, shifts on weed control are increasing, as well as new weed problems, including weeds resistant to glyphosate which are infesting other crops. In this case, soybean producers must use all available technologies, considering both socioeconomic and environmental efficiency.

The use of IWM is the most suitable alternative to maintain weed populations below damage threshold on the soybean crop. Besides difficulties on IWM implementation, there are concerns about farmers' awareness and variations into each farm. The use of IWM without considering the integration of control methods of other organisms (pests and diseases) does not allow the sustainability of used practices.

Even with prediction models to IWM implementation, weed control is not indefinitely assured if it is not continuously adapted to new changes in soybean production system. In this context, there is no single solution, ready and with indeterminate validity on weed management. Choosing intelligent systems, which integrate the basic concepts of ecology and biology of species to the available tools (GM crops, herbicides, biological control, etc.), should assist weed management.

Author details

Rafael Vivian[1*], André Reis[2], Pablo A. Kálnay[3], Leandro Vargas[1],
Ana Carolina Camara Ferreira[4] and Franciele Mariani[5]

*Address all correspondence to: rafael.vivian@cpamn.embrapa.br

1 Brazilian Department of Agriculture, Agriculture Research Service – Embrapa Mid-North, Teresina, PI, Brazil

2 Department of Civil and Environmental Engineering, Waseda University, Shinjuku-ku, Okubo, Tokyo, Japan

3 National University of Buenos Aires/Northwest, Pergamino, Buenos Aires, Argentina

4 Federal University of Piauí, M.Sc. student – Soil conservation – Teresina – PI, Brazil

5 Federal University of Pelotas, Ph.D. student – Weed management – Passo Fundo – RS, Brazil

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