TRANSGENIC CROPS IN SPAIN

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9.1 Introduction

The rate of introduction of transgenic crops in Spanish agriculture has been limited by a number of adverse factors, some of which are linked to specific local circumstances, while others are common to most European Union (EU) member countries. Because of its dry climate, Spain is the main European importer of feed grains, mainly soybeans and corn. The public was introduced to transgenic crops through the appearance of press headlines reporting demonstrations by nongovernmental organizations protesting transgenic grains at ports of entry.

The main purpose of this chapter is to examine the present status and future prospects of some key transgenic crops, such as corn and cotton. However, in order to understand the current impact and future potential of such crops in Spanish agriculture, some background facts and a brief description of the general scenario are pertinent. We will begin by briefly considering some relevant characteristics of Spanish agriculture, the R&D potential of the country, and the legal backdrop.

9.2 Some Facts About Spanish Agriculture

Spain has over 19 million ha under cultivation. Approximately 3.6 million ha are irrigated, which represents a sizable fraction of the 11.6 million irrigated ha in the 15 EU countries. Average rainfall over the last 15 years has been about 630 mm, but there is considerable variation, both from year to year (553 mm to 893 mm) and among the different river basins (from 1250 mm in the North to less than 300 mm in the South East). Maximum water storage capacity is over 51,500 million m^3.

The size of the Spanish population is stabilized around 40 million people, of which 3.3 million are engaged with agricultural production activities. The active agrarian population (about 1.25 million) represents a rather constant percentage of the total active population (8.7%), although the number of individuals fully employed in agriculture has been reduced by 50% since 1980. Farm owners over 55 years of age comprise 60% of the total—less than 6% are under 35 years old. On average, Spanish farm workers are employed only one third of their time, and Spain has the highest percentage of temporary workers in the European Union.

Gross national product (GNP) has multiplied by a factor of three from 1983 to 1997, and is currently 78 billion pesetas. During this period, the contribution of agriculture to GNP has decreased from 6.1% to 3.1%.

Cultivated area and production figures for some of the main crops are presented in Table 9.1 and Table 9.2. The figures suggest that most of the crops for which transgenic cultivars have been produced are potentially relevant to Spanish agriculture (with the exception of soybeans, which are not commonly cultivated in this country). Annual imports of corn and soybeans are in the order of two million and one million metric tons, respectively. Approximately 70% of imported grain is used for animal feed and 30% for human consumption, such as starches and sugars in the case of corn, and as oil in the case of soybean.

Seed companies are licensed by the Ministerio de Agricultura Pesca y Alimentación (Department of Agriculture, Fisheries and Food), under the advice of the Instituto Nacional de Semillas y Plantas de Vivero (National Institute of Seeds and Nursery Stocks), which has been recently transferred to the Ministerio de Ciencia y Tecnología (Dept. of Science and Technology).

Table 9.1 *Main crops in Spain, 1996.*

Crop	Hectares (thousands)	Metric Tons (thousands)	Pesetas (millions)
Cereals	6.767	22.378	356.758
Olive	2,214	1.772	335.000
Industrial Crops	1.526	—	180.164
Grapes	1.198	3.350	260.000
Fodder Crops	1.189	—	—
Fruits (except citrus)	967	3.081	316.000
Grain Legumes	705	488	32.802
Vegetables	356	11.406	652.202
Citrus	272	4.767	248.500
Tubers	167	3.416	78.201
Woody Crops & Others	76	150	101.800

Table 9.2 *Cultivated area (thousands ha) of different plant species.*

Species	Nonirrigated	Irrigated	Total
Wheat	1,898	229	2,126
Barley	3,303	252	3,555
Rice	—	54	54
Corn	97	260	357
Potato	98	108	206
Sugarbeet	40	132	172
Cotton	—	31	31
Tomato	2	42	
		11 (greenhouse)	55
Tobacco	—	18	18
Vineyards	1,125	73	1,198
Olive	2,045	178	2,223
Canola	13	74	87
Soybean	—	3	3

Approximately 350 seed companies are currently operating in Spain and share a market of about 60 billion pesetas, which means the annual sales of the average company does not exceed US$1 million. In fact, the average size of seed companies has not changed significantly since 1985, although the number of companies and total seed sales has nearly doubled. Of course, the sizes of the companies vary significantly and the larger ones, with few exceptions, are transnationally owned.

9.3 R&D in Plant Biotechnology

With a substantial agricultural sector at hand, Spain has shown an early interest in biotechnology. The application of plant transformation techniques in basic research was first introduced into Spanish labs in 1982, just before scientists in Belgium and the US achieved foreign gene expression in plants. At present there are approximately 1,000 scientists applying molecular techniques to plant research, integrated into some 170 research groups, which are distributed over 70 different institutional locations. The main research centers dealing with plant biotechnology are: Centro de Biología Molecular y Celular de Plantas, which has been established in Valencia under the joint sponsorship of the Universidad Politécnica de Valencia and the Consejo Nacional de Investigaciones Científicas (CSIC); Centro de Investigación y Desarrollo de

Barcelona, which is a CSIC lab; Departamento de Biotecnología de la Universidad Politécnica de Madrid; and the plant division of the Centro Nacional de Biotecnología, which is a CSIC lab located in the campus of the Universidad Autónoma de Madrid.

The most widely used technology are molecular markers (e.g., RFLPs, RAPIDS, etc.) as applied to cultivar identification and breeding programs. There have also been significant contributions in research areas of biotic (e.g., plant-pathogen interactions) and abiotic (e.g., drought, salinity) stress. The first patent application including plant biotechnology was submitted in 1991. Since then, a limited number of patents that originated in Spain have reached the international market, including registration in the USA.

Public support to plant biotechnology research has been contributed through the Planes Nacionales de Investigación (National Research Programs) and implemented during the periods 1988-1991, 1992-1995, and 1996-1999, under the legal framework of the Ley de la Ciencia (Science Law) of 1986. During these years, scientific research, including plant biotechnology, increased in both quantity and quality.

Research in plant biotechnology is largely carried out in public institutions and supported through government funds. The contribution of the private sector is very limited. Spanish universities contribute a majority of the scientists (54%), followed by the CSIC (39%) and other public labs (7%).

Field trials of transgenic crops were first initiated in 1993 with just three experiments, and increased to over 150 experiments by 2000 (Figure 9.1). The number of trials has declined due to the reluctance of regional governments to give the necessary clearance. Each of these trials includes a variable number of plots in a particular location or region, with Andalucía (a region in the south of Spain) being the site of over half of the trials. The transgenic species tested are indicated in Table 9.3, and the agronomic traits involved are summarized in Table 9.4.

9.4 The Legal Framework

European directives 219/1990/CEE and 220/1990/CEE, concerning transgenic crops, have been incorporated into Spanish law 15/1994 (June 3, 1994) and the corresponding implementation norms into the Real Decreto 1951/1997 (June 20, 1997). These legal instruments—Law and Royal Decree—apply to the confined use, voluntary release, and commerce of genetically modified organisms, in order to prevent possible risks to human health and the environment (*Boletín Oficial del Estado* no. 133, 4/6/1994). European Regulations 257/1997/CEE and 1139/1998/CEE, concerning the labeling of foods

Figure 9.1 *Field trials (deliberate releases) approved in Spain.*

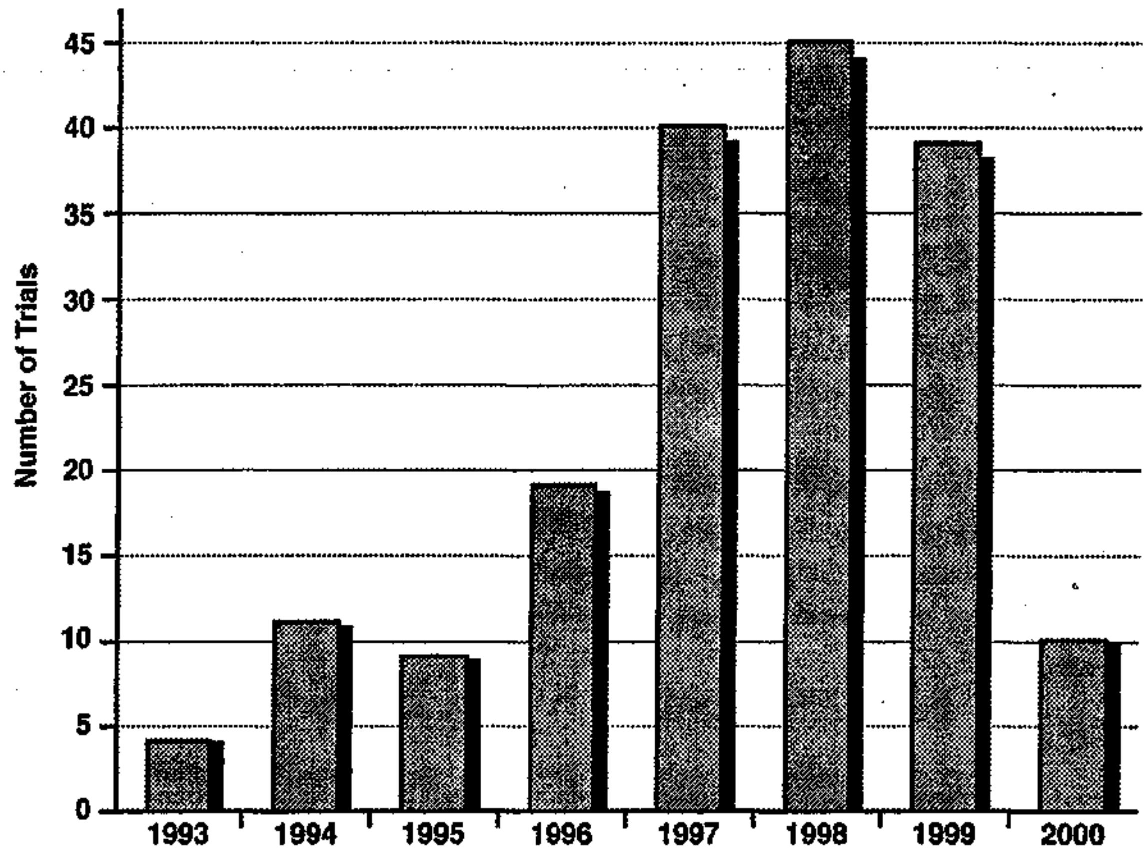

Table 9.3 *Number of trials per species.*

Species	Number	Species	Number
Tomato	14	Canola	2
Zucchini	2	Sugarbeet	20
Tobacco	5	Alfalfa	1
Melon	6	Potato	9
Rice	2	Soybean	2
Eucalyptus	1	Orange Trees	1
Sunflower	1	Wheat	4
Prune Trees	1	Cotton	10
Strawberry	2	Corn	39
Rhizobium (symbiotic bacteria)	3		

and ingredients produced through genetic engineering, apply in Spain and do not require national legislative actions.

The concept of confined use affects both academic labs and industrial pilot or production plants. One regulatory difference is that prior authorization is required of industrial research and development on pathogenic organisms. In

Table 9.4 *Types of traits in tested transgenic plants.*

Type of Modification	Number of Trials
Tolerance to Water Stress	1
Gene Expression	16
Modification of Biological Traits	24
Androsterility/Herbicide Tolerance	3
Insect Resistance/Herbicide Tolerance	12
Virus Resistance	14
Insect Resistance	29
Herbicide Tolerance	50
Androsterility	4

all other cases, the confined activity has to be notified to the authorities. Field trials with transgenic plants or microbes have to be approved by the corresponding regional government.

To support and advise both central and regional authorities, the Real Decreto 1951/1997 created the Comisión Nacional de Bioseguridad (National Bio-safety Commission), which is coordinated by the Ministerio de Medio Ambiente (Department of the Environment). The National Bio-safety Commission is an amalgamation of government departments and scientific institutions such as: Health & Consumers; Agriculture, Fisheries & Food; Industry & Energy; and Education & Culture. Additionally, a number of experts are coopted to this commission. The authorization is finalized by the Órgano Colegiado (Collegiate Committee), which includes representatives from the departments of the central government.

Registration and agronomic evaluation of transgenic cultivars are completed by the Registro de Variedades Comerciales de Plantas (Register of Commercial Plant Varieties). To this end, a ministerial order (Min. Agr. Pesca y Alimentación; order 6951; March 23, 1998) has been issued to modify the internal norms of the registration of transgenic varieties. This order immediately preceded a second order that approved the first transgenic corn varieties (Order 6952). The order stipulates that all transgenic cultivars must be submitted to the registry with a prevention plan, as well as a description of specific actions to be implemented in case of failure of the prevention plan.

At present, only two corn varieties have been authorized for cultivation in Spain. These are both resistant to the European corn borer, through the expression of the Bt (*Bacillus thuringiensis)* toxin gene from 950242 Compa CB and 950243 Jordi CB, progeny of the genetic modification event CG17, that have been commercialized by Novartis Seeds S.A. (now Syngenta). In total, there are over a dozen varieties from different companies containing genetic modifications approved by the European Union (CG176; Mon 810; T25), but not yet authorized for sale in Spain.

Three genetic modifications submitted to the European Union through Spain are pending approval. These modifications include a tomato with delayed ripening from Zeneca (now Syngenta), which has been recently recommended for approval by the EEC Scientific Committee on Plants, and two cotton modifications that affect resistance to *Lepidoptera* and glyphosate tolerance, respectively.

A monitoring plan should be followed for each approved variety according to the Official Bulletin. BOE March 23, 1998 requires:

- Monitoring occurs for a five-year period from the date of approval.
- Yearly sales and client information should be supplied.
- A prevention plan considering all risks should be approved.
- Notification of insect resistance within 30 days of detection.

9.5 Transgenic Corn

The European corn borer (ECB; *Ostrinia nubialis,* Hübner) and the Mediterranean corn borer (MCB; *Sesamia nonagroides*, Lefèbvre) are damaging to corn in the Mediterranean region, including Spain. The first records of damage by these pests date back to 1902, when they caused severe losses in the northern province of Asturias (Spain). Because of their endophytic larval behavior, chemical treatments are relatively ineffective; resistant plants offer a rational strategy to avoid yield losses, as well as an opportunity to reduce the environmental impact associated with the use of conventional insecticides (Alcalde, 1998; Farinós et al., 2000; González-Nuñez et al., 2000; Eizaguirre et al., 2000).

Although two corn varieties, "Jordi" and "Compa CB" (Novartis, now Syngenta), have been approved for cultivation in Spain, only the latter has been cultivated, because it is better adapted for the Spanish corn-growing regions. Out of a total of 455,000 ha devoted to corn in 1998, an estimated 22,000 ha of the "Compa CB" variety was grown, and in 1999, the total increased to 25,000 ha out of 397,000 ha. Because transgenic corn represents only 5% of the area dedicated to corn, it would seem that there is considerable room for expansion. However, expansion is limited by the official reluctance to approve additional resistant cultivars, which are required for some of the growing areas with insect pest problems.

As a requisite for the approval of a commercial introduction of Bt maize, a monitoring program has been established and funded by the Spanish Ministry of the Environment. In the following sections, we will first consider the agronomic performance of Bt corn in Spain and summarize preliminary results of the monitoring program.

9.5.1 Corn Borer Infestation in the Field

The EEC authorities approved corn hybrids and products originating from Novartis event 176 (January 23, 1997), after evaluation from three different scientific committees. The evaluation included three years of trials (1995-1997) focusing on the agronomic performance of the Compa CB variety under the predominating long-cycle conditions in major Spanish corn-growing areas, ascertaining potential advantages. Following commercial release in 1997, more extensive field tests have been conducted.

The 1995-1997 trials followed the protocol approved by the Spanish National Bio-safety Commission (rules 90/220 applying to intentional release). Five locations (three replicas per location) were selected in the main long-cycle growing areas: Andalucía, Aragón, Cataluña, and Castilla-La Mancha. These locations have a history of corn borer attacks; this allowed for experimentation under natural infestation conditions (Alcalde, 1998).

After authorization in 1997, the observations were made in farmer's fields, using routine growing conditions in plots of 3,000 m^2 and under natural infestation conditions. A total of 96 fields were involved in the trials to determine infestation levels and economic losses due to the insects. The following parameters were measured:

- number of plants affected (by at least one larva);
- length of galleries in different parts of the plants;
- number of ECB and MCB larvae;
- grain yield at 14% moisture.

"Dracma," which is isogenic for Bt resistance with respect to Compa CB corn and is also the most widely grown cycle-700 corn in Spain, was used as the control for corn borer damage (Alcalde, 1998). Results of the 1995-1997 trials in small scale plots are summarized in Table 9.5.

Although a year-to-year variation was observed, mainly due to different weather conditions, pest attacks were consistently above 10%. Table 9.6 shows more precise, location-by-location data obtained from larger plots in 1997. These results indicate considerable variation among plots (minimum-maximum), along with a high average and mode (>20%). In particular, provinces with a high minimum (such as Girona, Huesca, Lleida, Navarra, and Zaragoza) have a high probability of infestation in a given field.

Table 9.5 *Corn borer infestation trials, 1995-1997.*

Year	Average % of Infested Plants	Number of Locations
1995	13	5
1996	10.6	2
1997	38	4

Table 9.6 *Corn borer infestation in large-plot trials, 1997.*

Province	Average % Infested Plants	Maximum %	Minimum %	Mode
Albacete	38	60	5	30-40
Girona	62	70	50	50-60
Huesca	47	80	15	30-40
Lleida	36	60	15	20-30
Madrid	30	60	0	30-40
Navarra	35	40	25	30-40
Zaragoza	61	80	35	40-50
Overall	**46**			**30-40**

Table 9.7 *Yield and economic losses due to corn borer infestation.*

Year	Yield Loss (%)	Yield Loss (kg/ha)	Economic Loss (pesetas/ha)
1995	9	941	21,643
1996	6.1	735	16,905
1997	26.4	2,415	55,545

9.5.2 Losses from Corn Borer Infestation

Yield and economic losses calculated from the small plot trials are presented in Table 9.7, assuming a price for grain of 23 pesetas/kg (around $3.40 per bushel). The data suggests heavy losses in infested fields, but does not represent average losses for the whole growing area. A more precise evaluation can be obtained from the large plot trials in farmer's fields (Table 9.8).

The overall yield-loss average, including affected and nonaffected fields of long cycle corn, is 6.1%. Losses above 20%, such as those recorded in Girona and Huesca, make the crop economically nonviable. Even losses of 8-10%, which are quite frequent, are a severe handicap for corn cultivation. The economic consequences of the decreased yields are presented in Table 9.9, assuming a price of 23 pesetas/kg.

Table 9.8 *Yield loss produced by corn borer infestation in large-plot trials, 1997.*

Province	Average Loss %	Mode %	Maximum %
Albacete	6.4	8-9	9.6
Girona	12.9	14-16	24.6
Huesca	6.5	8-9	21.2
Lleida	4.4	8-9	9.1
Madrid	2.9	2-4	5.9
Zaragoza	6.1	8-9	22.0
Overall	**6.1**	**8-9**	

Table 9.9 *Economic losses due to corn borer infestation, based on data from the 1997 large-plot trials (14% moisture).*

Province	Yield of Compa CB (kg/ha)	Yield of Isogenic Line (kg/ha)	Average Loss (pesetas/ha)	Maximum Loss (pesetas/ha)
Albacete	14,200	13,340	19,775	31,353
Girona	13,629	12,070	35,848	77,110
Huesca	13,350	12,535	18,740	65,096
Lleida	13,719	13,134	13,440	28,714
Madrid	14,700	14,276	9,752	19,949
Zaragoza	12,013	11,316	16,023	60,784
Overall	13,278	12,511	17,632	47,168

9.5.3 Farmer Perceptions

From an agronomic point of view, transgenic corn has been accepted by farmers, as their willingness to grow it is practically unanimous. This high acceptance level has supported the relatively low price of transgenic seed, which was initially set only slightly above that of the isogenic line—18,000 versus 17,000 pesetas per 50,000 seeds. Comparatively, the additional benefits in a year of heavy infestation have been up to 40,000 pesetas per ha. A yield increase of over 10% is mostly responsible for the benefit, as insecticides have not been traditionally used in the area of cultivation because they are not effective.

In view of the successful cultivation of Compa CB corn, the Asociación General de Productores de Maíz de España (AGPME; General Association of Spanish Maize Producers) had submitted, in February 2001, an official request to the Minister of Agriculture for the commercial authorization of other Bt corns that have already been approved by the European Union authorities.

Against this favorable influence is the perception by the farmer of explicit or implicit reluctance to acquire transgenic corn by their main clients, whom in turn reflect the hostile social climate created by ecological organizations and, to a lesser extent, by consumer groups. In spite of the positive attitude of the farmers directly involved in corn production, the hostile social reaction also pervades some of the national farmer associations. However, demand of transgenic seed is increasing, and it seems that in the current campaign, seed suppliers have run out of stock at a much earlier date than in previous years.

9.5.4 Effects of Bt Maize on the Spanish Corn Borer

As part of the transgenic corn-monitoring program, susceptibility to Bt-maize of corn borer populations has been investigated (Gonzalez-Nuñez et al., 2000; Farinós et al., 2000; Eizaguirre et al., 2000). Baseline susceptibility to the Cry1Ab delta-endotoxin from *Bacillus thuringiensis* (Berliner) has been established for field-collected Spanish populations of the Mediterranean corn borer (MCB), *Sesamia nonagrioides* (Lefèbvre), and the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), collected at the locations indicated in Figure 9.2.

The first species was found to be at least as susceptible to the toxin as the second (Table 9.10). In addition, no significant differences in susceptibility among ECB populations were observed, whereas small differences in susceptibility observed among the Spanish MCB populations have been attributed to natural variation, because there are no records of Bt insecticide formulations being used on maize crops in Spain (González-Nuñez et al., 2000).

Annual monitoring of field populations of both species collected from Bt maize in the same geographical areas did not reveal changes in susceptibility after two years of Bt maize cultivation in Spain, whereas laboratory selection for four generations has yielded MCB and ECB strains that are 2.5- and 3.3-fold less susceptible to Cry1Ab than the unselected control strains, respectively (Farinós et al., 2000). The relevance of laboratory selection to forecast the development of insect resistance in the field has been questioned, because selection pressures could be lower than those in the field, where larvae could be exposed to a high Bt dose. However, in the case of Compa CB, the expression of the toxin in the tissues is not maintained all season; therefore, laboratory selection might be more relevant than in other situations where a high expression of Bt toxin is maintained throughout the maize cycle (Farinós et al., 2000).

Studies are underway to determine genetic flux in the Mediterranean corn borer, *S. nonagrioides*, by following dispersal of adult males and females

Figure 9.2 *Locations where S. nonagriodes and O. nubilalis larvae were collected in Spain.*

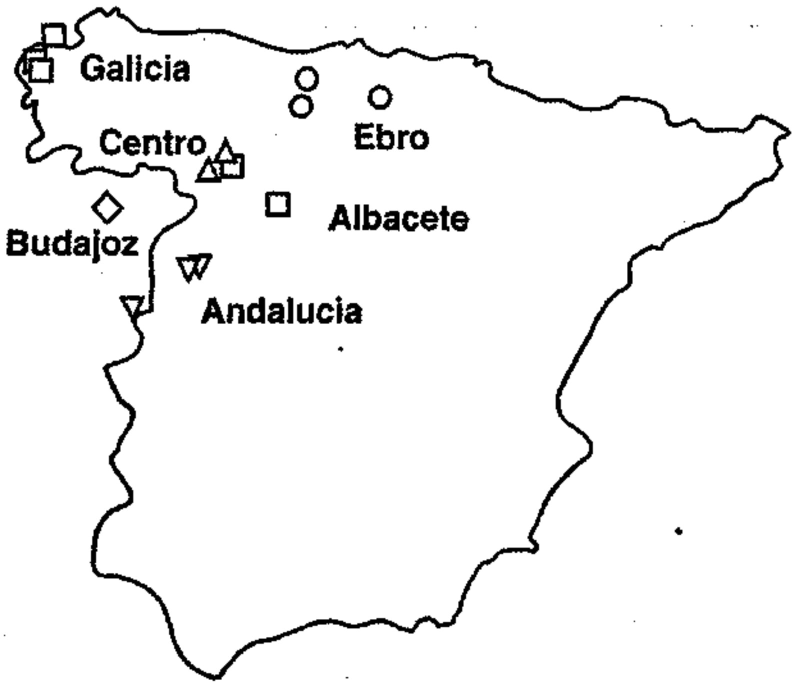

Table 9.10 *Results of probit analysis indicating susceptibility of field-collected larvae of S. nonagrioides and O. nubilalis from different Spanish maize growing areas to native Cry1Ab thuringiensis protein.*

		Lethal Dose LD_{50} (95% CL)		
Species	Region	1998 Toxin-1	1999 Toxin-1	Toxin-2
S. nonagrioides	Central Spain	23(16-30) NT	32(19-45) T	5(1-9) T
	Andalucia	27(16-39) NT	36(19-66) T	3(2-4) T
	Galacia	55(19-115) NT	—	—
	Ebro	70(56-87) NT		23(14-31) T
	Albacete	—		14(10-20) T
O. nubilalis	Ebro	109(77-162) NT	81(51-122) T	
	Central Spain	104(82-140) NT	—	—
	Badajoz	—		5(2-9) NT

Dose expressed as nanograms of native Cry1Ab protein per square centimeter of diet surface area. NT = nontransgenic maize. T = transgenic maize. Data from Farinós et al., 2000; González-Nuñez et al., 2000

through the fields, as well as the maximum migration distances of larvae. The efficiency of refuges for this species can be evaluated (Eizaguirre et al., 2000).

9.5.5 Impact of Bt Corn on the Maize Ecosystem

Two aspects of the possible impact of Bt corn on the environment are under investigation: the effects on other insects feeding on maize, and the consequences to parasitoids and predators (Farinós et al., 2000; Eizaguirre et al., 2000). Additionally, laboratory studies focusing on the abundant aerial predator, *Orius majusculus,* and its susceptibility to the Bt toxin have been initiated (Eizaguirre et al., 2000). This predator is used because it is polyphagous and also feeds on pollen and plant juices.

The greater specificity of Bt toxin compared to conventional insecticides, and the likely reduction of pesticide use under a transgenic crop system, should result in a more favorable environment for the natural enemies of the target pests. This creates greater possibilities for their use in integrated pest management programs. However, it is suspected from some laboratory experiments that Bt corn may have a negative impact on natural enemies, due to a combined effect of Bt exposure and nutritional deficiency caused by a reduction in the quantity and quality of their food supply (Farinós et al., 2000). This matter is now being pursued in field studies in commercial plots, in which Bt maize and cv. Compa CB is compared with the isogenic cv. "Dracma" under conventional farm practices, with or without Imidacloprid treatment (Farinós et al., 2000). Species monitored in different studies include *Orius* spp. (Anthocoridae), the most abundant species throughout the sampling period, followed by spiders, as well as representative Chrysopidae, Coccinellidae, and Syrphidae, which show little or even negligible abundance (Eizaguirre et al., 2000; Farinós et al., 2000). Carabids and spiders were the most abundant predators found. No negative effects associated with the transgenic cultivar have been detected thus far, although no firm conclusions can be drawn until these studies are completed (Farinós et al., 2000; Eizaguirre et al., 2000).

9.6 Transgenic Cotton

Insect pests cause important crop losses in cotton worldwide; thus, considerable efforts and expenditures are dedicated to their control. In southern Spain (the primary cotton region), the control of three lepidopteran species (namely *Helicoverpa armigera, Pectinophora gossypiella,* and *Earias insulana* [common names are heliothis, pink bollworm, and earias, respectively]) requires more than half of the total insecticide treatments during the cotton season (Novillo et al., 1999; Soto et al., 2000).

H. armigera and *E. insulana* attack squares and bolls, whereas *P. gossypiella* larvae feed only on bolls. *H. armigera* and *P. gossypiella* are widely distributed, although the intensity of pink bollworm attacks can be different in each area. In the Jaén province, *E. insulana* causes important damage to cotton fields. The control of the three lepidoptera involves an average of 3-4 treatments every year—1-2 against heliothis and 1-2 against pink bollworm and/or earias—but eventually this number can reach higher values (Novillo et al., 1999).

The predominant treatments against these insects are as follows: heliothis is controlled with endosulfan and a mixture of this product with methomyl; pink bollworm is controlled with pyrethroid sprays; and earias is controlled with mixtures of all these products. In the first case, treatment is directed against small larvae, and requires a very detailed scouting program on eggs and larvae for a precise timing. In the case of the pink bollworm, adults must be controlled, so it is necessary to follow their flight by pheromone traps. Finally, control of earias is generally less effective, as scouting on eggs is not feasible and pheromone traps do not work well under field conditions (Novillo et al., 1999).

Since 1979, a program has been promoted in Andalucía to scout cotton fields using treatments only after the economic thresholds have been reached, thus favoring the effects of beneficial insects. This program has been coordinated by the Asociación para Tratamientos Integrados en Algodón (ATRIA; Association for the Integrated Treatment of Cotton)

9.6.1 Field Trials of Transgenic Cotton

The approval for Bt cotton (Bollgard) varieties in the European Union was submitted in 1996. Since that year, field trials have been carried out in Andalucía, with excellent recorded performance (Novillo et al., 1999; Soto et al., 2000). During 1999, the submission for field trials was not answered, so these evaluations had to be discontinued. In 2000 and 2001, field performance had been evaluated under the permit issued in the B/ES/00/01 and B/ES/01/01 notification from the Andalusian government, after a positive evaluation by the Spanish Biosafety Commission. Authorization was subjected to the following conditions:

- A minimum buffer of 40 meters surrounding each trial without any cotton plants.
- Destruction of each trial not exceeding 45 days after the appearance of the first flowers.
- Available field book, where all operations in each trial plot had to be recorded.
- Trial seeding and destruction had to be supervised by regional government staff.

These field trials, which were established in fields from cooperating farmers, were to evaluate the protection and benefits of Bt cotton varieties while refining their management under local conditions. The following seeds, developed by the seed company Delta & Pineland, were planted in different locations:

- DP20B (Bollgard version);
- DP20 (conventional, registered in Spain under the label CORONA);
- NUCOTN35B (Bollgard version);
- DP5690 (conventional, registered in Spain under the label LINDA).

Bollgard varieties were not treated against bollworms, but received standard treatments against mites, aphids or other lepidopteran pests, whereas conventional varieties were either treated against bollworms or not treated (Novillo et al., 1999; Soto et al., 2000).

The possible effects of Bt cotton on the following beneficial insects were also monitored: *Orius* spp., *Chrysoperla carnea, Nabis* spp., *Geocoris* spp., *Deraeocoris* spp., and Coccinellidae. Phytophagous bugs (*Creontiades pallidus, Nezara viridula, Lygus gemellatus*) and larvae from other pests (*Spodoptera littoralis* or *Spodoptera exigua*) were also counted concomitantly with the evaluation of beneficial insects. Mites and aphids were monitored while scouting for heliothis and earias (Novillo et al., 1999; Soto et al., 2000).

Results from these trials confirmed that Bt transgenic cotton offered improved control of bollworms, compared to conventional programs based on pesticide applications (Table 9.11). Economic thresholds were not reached in Bt plots for any trial; thus, apart from occasional insecticidal treatments against lepidopteran leaf feeders such as *Spodoptera* spp., it was not necessary to complement the control offered by transgenic varieties against *H. armigera* (Novillo et al., 1999; Soto et al., 2000).

Concerning *E. insulana,* the observed transgenic protection against this species in some trials suggested an even greater efficiency than that against *H. armigera* and clearer advantages versus control with insecticides, which only offered partial protection. Commercial experience in other countries, as well as susceptibility analysis of Spanish pink bollworm populations under lab conditions, suggest a higher susceptibility for this species than for *H. armigera.* Field trials during 1998 in Greece and 2001 in Spain have also confirmed an excellent protection against this species (C. Novillo, personal communication).

Bt varieties saved an average of 2.3 applications and 10.7 liters of a broad spectrum of insecticides per ha. This would have saved approximately 533,500 liters of insecticides, if 50% of this region had been planted with this kind of bollworm-protected varieties. Yields of Bt varieties in the 1998 trials were 10-20% higher than those of the conventional yields under standard con-

Table 9.11 *Buds and capsules damaged in transgenic (IPC 531) and conventional treated (T) or nontreated (NT) cotton*.*

Treatment	Locations 1	2	3	4
IPC 531	14.7 a	14.3 a	8.3 a	8.5 a
Nontransgenic T	74.8 b	127.0 b	63.0 b	79.2 b
Nontransgenic NT	214.0 c	256.0 c	153.5 c	178.0 c

**Average damaged buds and capsules per 20 plants between the 2nd week of June and the first week of September.*

ditions (Novillo et al., 1999). No adverse effects on fiber quality or induction of resistance were observed.

Some species of phytophagous bugs (*Creontiades* spp., *Oxycarenus* spp., and *Lygus* spp.) could potentially reach high levels at the end of the crop season, affecting either seed or fiber quality. In conventional cotton, these species are considered secondary pests, as current broad-spectrum insecticides, applied against worms, also control these pests. It has been speculated that a reduction of insecticide use in Bt cotton varieties could favor incidence of these secondary pests, and additional control might be required. However, factors such as increased beneficial arthropod populations also need to be considered. Monitoring of these populations in the 2000 trials suggest a slight increase of these species in untreated plots (either Bt or non treated conventional cotton). Large-scale trials conducted before harvest time or commercial launch will probably offer the best way to evaluate their impact and improve the appropriate management (Soto et al., 2000).

9.7 Other Crops

As indicated previously in Table 9.3, field trials have been conducted in Spain for a variety of crops. Under present circumstances, commercial approval of those targeted for direct human consumption (such as tomatoes, potatoes, and others) is progressing slowly. Among these, sugar beets should be the least controversial, as the product is a purified chemical compound. For this reason, data concerning this crop will be briefly summarized.

The main agricultural problem of sugar beet cultivation is weed control, due to the limited effectiveness and selectivity of currently used herbicidal treatments, which involve four to five applications of four or five different herbicides, often complemented with manual weeding. Herbicide tolerant varieties offer the possibility of better control with a single herbicide. Field trials, with glyphosate tolerant varieties (Roundup Ready), have been carried out since 1995 (Costa et al., 1999; González & Costa, 2000).

Excellent weed control has been achieved with two to three applications of a herbicide formulation at two to three L/ha. Predominant weeds were species of *Chenopodium*, *Salsola*, *Orobanche*, *Cirsium*, *Abutilon*, *Cyperus*, and *Beta*. This approach allowed a 50% reduction in the amount of herbicide applied and the use of a product that is more environmentally friendly than those previously used. Additionally, a greater flexibility for conservation agriculture, including cultivation under cover, is provided. Sugar yield increases of approximately 4% were observed in demonstration trials (Costa et al., 1999; González & Costa, 2000).

9.8 Future Trends

It is difficult to forecast future developments in Spanish cultivation of transgenic crops because of the confusing public scenario. Over two thirds of the population is opposed to transgenic food, although this opinion is not supported by advocate knowledge of the new technology. This attitude has been formulated, in part, by recent outbreaks of "mad cow disease" in Spain, as well as in other European countries. However, the prohibition of animal residues in feeds is likely to increase imports of feed grains, including corn and soybeans, and this circumstance will in the medium term facilitate the diffusion of transgenic versions of these crops among Spanish farmers. On the other hand, it appears that the acceptance of transgenic crops for direct human consumption will be slowed by the reluctant official decisions taken by the European Parliament and the European Commission, which have been slow in their development.

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