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# Pollen Mediated Gene Flow in GM Crops: The Use of Herbicides as Markers for Detection. The Case of Wheat

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## 1. Introduction

ISAAA has estimated that genetically modified (GM) crops, mainly soybean, maize, cotton and canola, are cultivated worldwide in an area that has increased from 1.7 million hectares in 1996 to 134 million hectares in 2009, of which more than 80% have an herbicide-tolerant trait (ISAAA 2010). This work reviews the agricultural and environmental concerns about the likelihood for gene flow from GM wheat (*Triticum aestivum* L.). Wheat is the world's most important crop species, grown on over 210 million hectares. There are no GM wheat varieties commercially available but transgenic wheat varieties are being successfully developed and field-tested. That makes wheat in the pipeline of genetically engineered crops to be cultivated. Although wheat is predominantly a self-pollinating crop, pollen from one plant can travel via wind to other receptive plant, being outcrossing between wheat cultivars possible at variable rates. Coexistence problems in wheat could thus arise if no measures are taken before releasing and marketing any transgenic cultivar, as has occurred with other GM crops such as oilseed rape or maize, where measures were implemented after commercial transgenic introduction. Besides this, wild *Aegilops* species like *Ae. geniculata* Roth., *Ae. cylindrica* Host., *Ae. biuncialis* Vis. or *Ae. triuncialis* L. can form natural interspecific hybrids with wheat where they grow in sympatry. These natural hybrids are highly sterile, although seeds may occasionally be found. Data presented aim to contribute to the determination of the extent of this phenomena. These data are necessary to manage the possible impact of transgenic wheat hybrids before the transgenic crop can be grown under field conditions. Herbicide-tolerant wheat parental varieties can be used to obtain resistant progeny detectable by herbicide selection, providing a high approach to the potential occurrence of intra and interspecific pollen mediated gene flow.

## 2. Herbicide resistance as a marker for gene flow

In spite of the knowledge of GM herbicide tolerant wheat cultivars, whose use is limited by availability and regulatory constraints, in the experiments presented in this book chapter we have used non GM wheat cultivars possessing homozygous dominant genes for herbicide response. Chlorotoluron and difenzoquat tolerant wheat cultivars were used to obtain hybrid-resistant progenies detectable by herbicide selection.

The herbicide chlorotoluron is a commercially available selective phenylurea that is widely used for broad-leaf and annual grass weed control in winter cereals. The genetic control of tolerance to chlorotoluron in bread wheat is determined by a major single dominant gene, *Su1*, located on the short arm of chromosome 6B (Krugman et al., 1997). This herbicide is selective in winter wheat crops although there are wheat cultivars susceptible to chlorotoluron (Sixto et al., 1995; Bozorgipour & Snape, 1997). Wheat wild relatives as *Aegilops* spp. are also susceptible to it. In the presence of herbicide selection pressure, herbicide resistance allows for the detection of hybrids between resistant wheat cultivars and susceptible ones and between *Aegilops* spp. and resistant wheats. In our studies, we have used chlorotoluron tolerant wheat cultivars as Castan or Deganit.

The herbicide difenzoquat is a mitosis inhibitor used for the post-emergence control of wild *Avena* spp. in winter cereals. *Aegilops* species are susceptible to this herbicide. Chinese Spring (CS) is a wheat cultivar possessing herbicide resistance alleles endowing resistance that can be used to obtain hybrid resistant progeny. The genetic control of tolerance to difenzoquat in bread wheat is determined by a major single gene (Busch et al., 1989).

During our work we have conducted two types of assays which have enabled us to identify resistant hybrids: growing plants with herbicides in hydroponic assays and herbicide spraying assays.

### 3. Pollen dispersal in wheat

Wheat pollen dispersal is not a new issue in agriculture. The varietal purity of the seed has always played a fundamental role in the development, yield and final quality of crops. It has long been known that pollen contamination not only takes place in cross-pollinated crops, it is also possible in self-pollinating crops when different varieties of the same crop are cultivated and sufficient separation distance is not maintained (Sanchez-Monge, 1955).

One of the most effective methods for preventing pollen contamination between crossable genotypes is the use of isolation distances. The isolation distance required will depend on flower characteristics, compatibility with neighboring crops, pollen quantity and viability, mode of pollen dissemination and environmental conditions, which are of the utmost importance. Not all genotypes show the same ability in crosses. Wheat cultivars could show differences in the factors included in their reproductive biology; the flowering period of a wheat plant takes around 8 days. During these days each flower is open from 8 to 60 minutes. Wheat produces a low number of pollen grains (10,000 per anther) only the 5 to 7% of the pollen drops on the stigma, the great majority is dispersed by wind (de Vries, 1971). The period of pollen viability is low, never above three hours (D'Souza, 1970). Pollen viability declines, with time and exposure to environmental stresses. From a hybridization rate of 86% obtained with fresh pollen maintained at 15° C (at RH 65 ± 5%), hybridization was only 12% after one hour at 25°C, while no seeds were found at 30°C. At 15°C seed set declined 14% and 23% at 20°C (Loureiro et al., 2007). Receptivity of stigma and flower opening were also environmental and genetically dependent (de Vries, 1971). Under our circumstances, in a year with favourable conditions (77% RH and 20 ± 2°C), a maximum seed set of 78% was obtained for Pavon x CS wheat cultivars hand crosses. These values were of 39% in a less favorable year.

### 4. Outcrossing in wheat. The problem of coexistence

Wheat is a self-pollinating crop but outcrossing is possible between cultivars at variable rates that are related with populations, genotypes and environmental conditions (Jain, 1975).

The main studies on pollen dispersal in wheat appear in two stages. In the 1960s and beginning of the 1970s managing pollen drift was a major concern within the context of commercial production of hybrid wheat, where achieving high levels of genetic purity and satisfactory seed set on male sterile plants were essential (Pickett 1993). In recent years, pollen dispersal in wheat has again received considerable attention, within the context of the legislation applied to cultivars issued from the advances in biotechnology. Transgenic wheat varieties are being successfully developed and field-tested, primarily as glyphosate-tolerant wheat (Blackshaw & Harker, 2002; Zhou et al., 2003), and there is extensive research on a wide range of GM wheat traits (e.g. Fusarium resistance, drought resistance); probably in the next few years certified cultivars of transgenic wheat shall be commercially available.

There is concern that once transgenic wheat is released for commercial production, there will be a potential pollen flow from GM wheat to non GM-wheat (van Acker et al., 2003). As a consequence the product could not fulfil all the requirements of some international markets and farmers could lose the ability of choose between conventional, organic or GM-based crop productions, in compliance with the relevant EU legislation on labelling and/or purity standards. EU regulations framework establishes a 0.9% labelling threshold for the adventitious presence of GM material in non-GM products. Thus, problems could appear in wheat if no measures are taken prior to the release and commercialisation of any transgenic cultivars to establish the basis that allows the coexistence of all type of wheat with the GM wheat.

Outcrossing studies between *T. aestivum* cultivars have been conducted by different authors in the absence of any pollen competition on male sterile receptor plants. In this sense emasculate plants provide information on the upper levels of outcrossing under specific conditions and help in evaluating safety distances that avoid outcrossing and potential pollen-mediated gene-flow. Outcrossing rates in these studies are very different among experiments in terms of frequency of hybrid seed set and maximum seed set distance (from 12 to 73% at distances near to the pollen source, from 0.3 to 9 % at around 10 m distance) (Khan et al., 1973; de Vries 1974). In a three-year study we assessed the maximum potential outcrossing under field conditions between the wheat cultivars Pavon (receptor) and Chinese Spring (3 x 3 m source donor). Bread wheat can also coexist in the field with the second major cultivated wheat species, the durum wheat tetraploid *Triticum turgidum* L. (tetraploid, AABB) that is closely related to bread wheat which bulk of production is concentrated in the Middle East, North America and the Mediterranean region. For this reason durum wheat *T. turgidum* L. var. *durum* cultivar Nita was also included in the study. Outcrossing was measured by seed set on emasculated recipient plants. Frequencies of seed set at 0 m distance were 45% (37-56%) for *T. aestivum* cultivars and 18% (5-30%) with *T. turgidum* (Loureiro et al., 2007). Under semiarid conditions of this assay, viable pollen was found at 14 m from the source, the maximum distance analyzed, with a distance of 8 m at which cross-pollination decreases below 1%. There is a strong positive correlation between outcrossing and the amount of pollen in air, for this reason hybridisation at distances close from the pollen source are similar to maximum hybridisation when emasculated plants were used as receptors. However as the distance from the pollen source increases the pollen concentration rapidly decline, 90% of the pollen in wheat remains within 6 meters from its source (Jensen, 1968; Loureiro, 2005). A mean seed set of 45% at 0 m decrease to 10% at 2 m (Figure 1). At 10 m seed set was of 1% in agreement with data of Stopkopf & Rai (1972) ; de Vries (1974) and Zhao et al. (2000) and slightly higher than data of Lu et al. (2002). Other authors have found a slower decrease on seed set in relation to distance from the pollen

source (Johnson et al., 1967; Bitzer & Patterson 1967; Khan et al., 1973). An exponential predictive curve (Figure 1) provides the upper level of the magnitude of this event (Loureiro et al., 2007). In these circumstances, 5 m would be required to avoid adventitious GM presence above the 0.9% marked by the European legislation. This isolation could be higher downwind with 7 m required to meet the threshold.

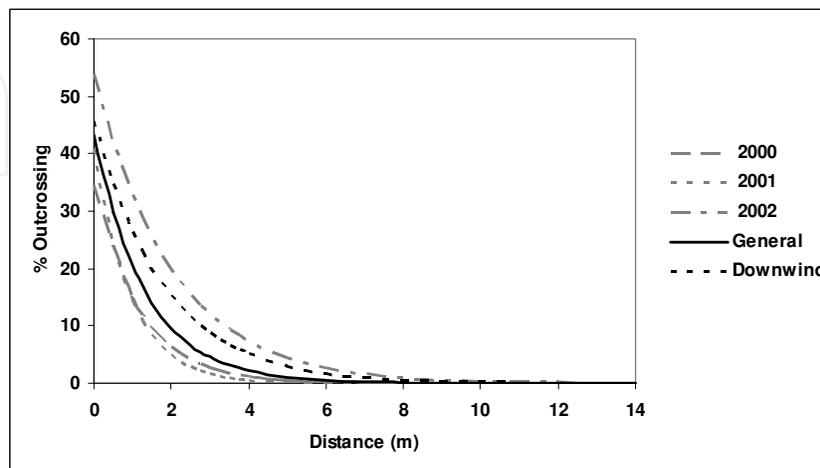


Fig. 1. Mean seed set related to distance under no pollen competition in field assay.

The outcrossing between wheat cultivars have been also assessed natural conditions of pollen competition. Experiments were carried out in the year 2005 at “La Canaleja (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA) and at “El Encín” (Instituto Madrileño de Investigación y Desarrollo Rural y Agrario, IMIDRA) experimental stations, Madrid, Spain. The layout of the experiment was such that it permitted observations on the extent of natural crossing of a wheat pollen donor with different recipient cultivars and in different directions and distances. The experimental field design consisted in a 50 x 50 m central square plot sown with a *T. aestivum* chlorotoluron tolerant cultivar pollen donor (Castan in “El Encin” and Deganit in “La Canaleja”) at field density and chlorotoluron susceptible receptors (Altria and Recital) placed in the four sides of the pollen source at distances of 0, 1, 3, 5, 10, 20, 40, 80 and 100 m. In any case the mean of outcrossing reached 2% at 0 m distance. This value was always below 5% downwind even in close proximity (Loureiro et al., 2005). Outcrossing was detected at the very low level of 0.07% at 100 m from the source.

These outcrossing rates are in the range of published frequencies averaging 1%, but that can vary between 0 to 6.7% at distances below 1 m (Griffin, 1987; Hucl, 1996; Zhao et al., 2000; Hucl & Matus-Cadiz, 2001; Loureiro et al., 2005), although hybrid seed set is also possible at greater distances.

## 5. Hybridization with wild relatives

Genes could also be transferred from GM crops to wild relatives through interspecific hybridization. Prior to the commercialization of GM crops the research on the natural hybridization between crops and related wild species was very limited. Most of the research was done with the purpose of breeding and with the aim of transferring desirable traits between species, with crops always used as female parent in intergeneric and interspecific crosses. But the picture is quite different and numerous crops are known to have wild

relatives that can hybridize with them somewhere in the world. Gene flow between cultivated species and their weedy and wild relatives has been documented in species such as oilseed rape (*Brassica napus* L.) (Jørgensen & Andersen, 1994), maize (*Zea mays* L.) (Doebley, 1990), sorghum (*Sorghum halepense* (L.) Pers) (Arriola & Ellstrand, 1996), sunflower (*Helianthus annuus* L.) (Arias & Rieseberg, 1994) and sugarbeet (*Beta vulgaris* L.) (Bartsch & Pohl-Orf, 1996). Hybridization with wild relatives has been a real issue implicated in the evolution of some of the most aggressive weeds. In order to prevent the diffusion of a character that could provide adaptative advantages, thus making weed and wild species more invasive (Darmency, 1994), it is important to understand the potential for gene flow and transgene introgression from cultivated wheat into other species, mainly their wild relatives.

Any future market launch and use of genetically modified wheat must be undertaken with extreme care, since a number of closely related species, primarily of the genus *Aegilops*, share their habitat with wheat and some natural hybrids between *Aegilops* spp. and wheat have been documented in field borders (van Slageren, 1994). Hybridization of herbicide-resistant genetically modified wheat with populations of free living relatives could make these plants increasingly difficult to control, especially if they are already recognized as agricultural weeds and if they acquire resistance to widely used herbicides (Darmency, 1994). The transfer of herbicide resistance genes from wheat to *Aegilops cylindrica* Host., a noxious weed in the wheat producing areas of the western United States, has been detected in the field and created problems for its control (Seefeldt et al., 1998; Wang et al., 2001; Gandhi et al., 2006). Other wild *Aegilops* species like *Ae. geniculata* Roth., *Ae. biuncialis* Vis. and *Ae. triuncialis* L. also form natural intergeneric hybrids with bread wheat where they grow in sympatry and with overlapping flowering times (van Slageren, 1994; Loureiro et al., 2006; Zaharieva & Monneveux, 2006), a phenomenon underlining the close genetic links of the two genera. Hybrids between *Ae. geniculata* and *Ae. triuncialis* and wheat have been found in several countries of Europe, mainly in Spain and France, while *Ae. biuncialis*-wheat natural hybrids have been described in Lebanon (van Slageren, 1994). These natural hybrids are highly sterile, although seeds may occasionally be found in *Ae. geniculata* hybrids (van Slageren, 1994; Loureiro et al., 2008).

In order to study the extent of natural hybridization, we collected spikes from one *Ae. geniculata* population that was spread extensively along a wheat field (in close proximity, Fig. 2A) where one natural hybrid has been previously detected (Fig. 2 B). A total of 3200 seeds were collected and grown in the greenhouse. Six hybrid individuals were identified from 3158 germinated seedlings, so the spontaneous hybridization rate was of 0.19% (Loureiro et al., 2006). This natural hybridization rate was similar to the 0.24% and 0.39% obtained in the assays carried under simulated field conditions explained below (Loureiro et al., 2007). Our semiarid field conditions, with frequent high temperatures and low relative humidity during the flowering periods, negatively affect to the viability and dispersal of the wheat pollen (Waines and Hegde, 2003; Loureiro, 2005). Therefore, rates of crop-wild hybridization may be higher under environmental conditions that are more favorable to hybridization.

An useful herbicide resistance screening test has been conducted to detect the potential occurrence of gene flow from *T. aestivum* to *Aegilops* using herbicide tolerant wheat cultivars as pollen donors. *Aegilops* spp. seeds are sown at appropriate depths in 1 L plastic pots (10 cm diameter, 10 seeds per pot) containing soil and sand in a 1:1 (V/V) mixture. Plants were treated at the three leaf stage with a commercially formulated herbicide at the amount of



Fig. 2. A) An extensive stand of *Ae. geniculata* with some *Ae. triuncialis* in a roadside near Zamora, Castilla-León, Spain. B) Spikes of a natural hybrid plant between *Ae. geniculata* and *T. aestivum* on the edge of wheat field. Hybrids were identified in the field by their intermediate spike morphology.

herbicide recommended in the field. In the case of Chinese Spring used as parental in crosses, the spraying was done with difenzoquat (Superaven, 330 g a.i. kg<sup>-1</sup>, Cyanamid Ibérica, S.A.) at 3 kg a.i. ha<sup>-1</sup>. For Castan and Deganit, plants were sprayed 1 day after planting with a commercial formulation of chlorotoluron (Oracle, 500 g a.i. L<sup>-1</sup>, DuPont Ibérica, S.A.) at 2 kg a.i. ha<sup>-1</sup>.

The damage produced by the herbicide to the growth of the susceptible plants was apparent 21 days after treatment. The response to the herbicides was evaluated visually 30 days after treatment. Herbicide applications were made using a Research Track Spray Cabinet (Devries Manufacturing, Hollandale, MN, USA) equipped with a Teejet 8002-E flat fan nozzle calibrated to spray 176 L ha<sup>-1</sup> at 130 kPa. After spraying, the pots can be placed in the glasshouse or in a growing chamber and watered as required. Temperature was maintained at 24/16 ± 2°C (day/night temperature).

We can see in the Figure 3A that the herbicide killed the *Ae. geniculata* plants 30 days after treatment, while the Deganit tolerant wheat cultivar and the F<sub>1</sub> hybrid plants survived the treatments. Figure 3B shows the response to difenzoquat, with the CS tolerant wheat cultivar and the hybrids between this cultivar and *Ae. biuncialis* surviving the herbicide treatment while the *Ae. biuncialis* plants are dead. The results indicated that the bioassay was adequate for detecting hybrids. This kind of bioassay will be useful for the identification of hybrids in *Aegilops* wild populations growing near fields sown with wheat carrying a dominant trait for resistance to herbicides and in the quantification of the rate of hybridization.

These bioassays using herbicides as markers for hybrid detection were used to evaluate the hybridization between cultivated wheat and two *Aegilops* wild relatives during two seasons in simulated field conditions under Central Spain conditions (Loureiro et al., 2007). Ten 1 m x 1 m pollinator experimental plots sowed with *T. aestivum* cv Deganit at field density (400 seeds m<sup>-2</sup>) were established per *Aegilops* spp. for each of two consecutive years of experimentation. Two to 3 days before anthesis one pot of *Aegilops* spp. was placed inside each pollinator plot. The wheat flowering period was monitored each year. Spikes from *Aegilops* plants were collected at maturity separately from each individual. Progeny from



Fig. 3. A) Response to the herbicide chlorotoluron ( $2 \text{ kg a.i. ha}^{-1}$ ) 30 days after treatment of *Triticum aestivum* cv Deganit (left), *Ae. geniculata* (right) and their  $F_1$  hybrids. B) Response to difenzoquat ( $3 \text{ kg a.i. ha}^{-1}$ ) 21 days after treatment of *T. aestivum* cv Chinese Spring (left), *Ae. biuncialis* (right) and their  $F_1$  hybrids. The herbicide application allows the identification of the hybrids.

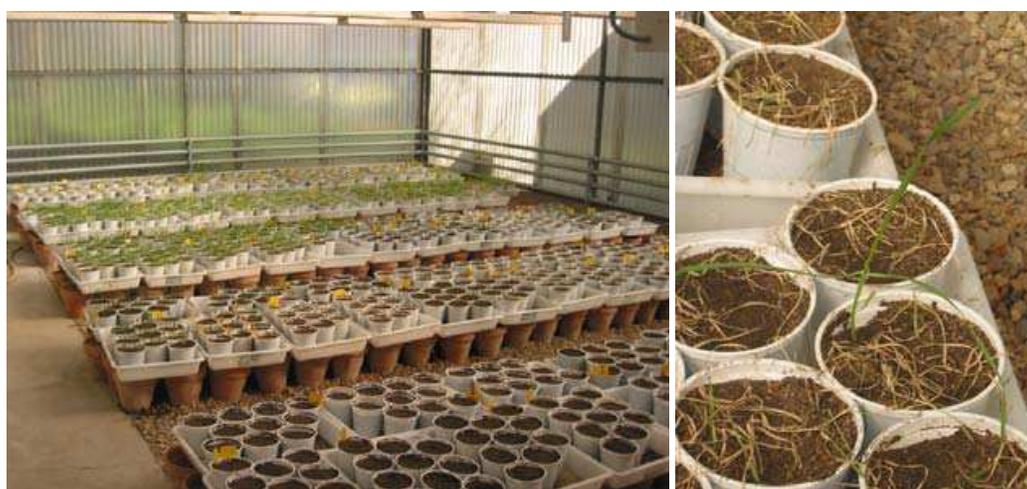


Fig. 4. A) *Aegilops-Triticum* hybrid detection by herbicide screening in the greenhouse. B) Herbicide resistant hybrid between *Ae. geniculata* and *T. aestivum* cv Castan wheat identified by screening with the chlorotoluron applied at  $2 \text{ kg a.i. ha}^{-1}$ .

each *Ae. geniculata* and *Ae. biuncialis* plant was screened separately to check for resistance to chlorotoluron in the greenhouse (Fig. 4A). Percentage of hybridization was estimated as a ratio of survivor chlorotoluron-resistant hybrids to the total number of *Aegilops* seeds sprayed. Figure 4B shows a chlorotoluron resistant hybrid between *Ae. geniculata* and Castan. The spike morphology of interspecific hybrids, intermediate between wheat and *Aegilops*, was similar to that of those obtained previously by hand-crossing under greenhouse conditions and allowed for their identification. The different ploidy levels of *T. aestivum* ( $2n = 42$ ) and the two *Aegilops* spp. ( $2n = 28$ ) also enabled us to confirm the hybrid status of all surviving individuals on the basis of their chromosome number in root meristems ( $2n = 35$ ). The estimated hybridization rates using the data from both years were similar in both species and averaged 0.34% for *Ae. biuncialis* and 0.31% for *Ae. geniculata*. Assuming these hybridization rates and that the average seed production per plant is of 58.8 and 80.2

seeds/plant for *Ae. biuncialis* and *Ae. geniculata*, respectively, in a hypothetical field population of 100 plants growing in wheat close proximity in 1 year, the next year we would find around 17 *Ae. biuncialis* x wheat and 24 *Ae. geniculata* x wheat hybrids that could germinate or remain viable in the soil for more than 1 year. This study was carried out under experimental conditions where the factors that influenced cross-pollination as experimental plot layout or flowering synchrony, were optimized to promote hybridization. Thus, the results provided are a better indication of the maximum potential for hybridization under field conditions than of actual hybridization in agronomic settings, although it can vary within and probably among wild *Aegilops* populations and wheat varieties (Farooq et al., 1989; Hedge & Waines, 2004).

Hybridization frequency is only a component of the rate of interspecific gene flow; the ability of the hybrids to reproduce and survive in nature for the first generations is another limiting factor in terms of introgression. Fertile progenies of an *Ae. geniculata* x wheat hybrid were described as early as early as 1838 in the South of France (van Slageren, 1994). After a few years of cultivation, seed producing fertile plants that increasingly looked like wheat were obtained. The fact that hybrids between wheat and *Aegilops* spp. can be partially fertile, with low male fertilities and some female fertility that allows for backcrosses with the parents to occur (Mujeeb-Kazi, 1995), raises the question of whether a wheat gene could be transferred when other wheat fields are grown near the hybrid zone. *Aegilops* x wheat hybrids showed some female fertility by backcrossing when placed inside a wheat plot. Seeds were found in *Ae. biuncialis* and *Ae. geniculata* x Deganit hybrid plants when they were placed inside 1 x 1 m wheat plots for backcrossing. Mean fertility rates were of 3.17% for *Ae. biuncialis* hybrids (0-9.26%) and 2.87%(0-8.33%) for *Ae. geniculata* hybrids, with great variability among plants (Loureiro et al., 2007). These backcrossing rates are in the range of that obtained by Snyder et al. (2000) for *Ae. cylindrica* in an experiment with one *Ae. cylindrica* x *T. aestivum* cv Madsen hybrid plant inside a 1 m<sup>2</sup> plot of wheat: they obtained average seed sets of 1.8% (1-2.5%) and 6% (3-9.2%) in each year. Morrison et al. (2002) found that a 44% of the 754 *Ae. cylindrica* x wheat hybrids produced BC<sub>1</sub> seeds at an average rate of 1%, but up to 8% can be achieved for some hybrid plants. Higher BC<sub>1</sub> seed set rates of near to 30% in some hybrid plants have been found for other wheat cultivars (Loureiro et al., 2009). Besides, BC<sub>1</sub> partial self-fertility can be restored to 37% in the second backcross generation using jointed goatgrass as the recurrent parent, indicating that only two backcrosses are needed to restore fertility (Wang et al., 2001).

Dose-response analysis was conducted on F<sub>1</sub> and BC<sub>1</sub> hybrids between *Ae. geniculata* (Loureiro et al., 2008) and *Ae. biuncialis* (Loureiro et al., 2009) and wheat. Herbicides (chlorotoluron and/or difenzoquat) were applied at 0, 0.5, 0.75, 1, 1.5 and 2X (X = recommended dose). The hybrids were extracted with their roots 15 days after treatment, washed with water and roots dried with paper to obtain the fresh weight. Three replicates and 3 seeds per replicate were used in each treatment. A log-logistic model (Seefeldt et al., 1995) was used to analyze the data to predict the trend of herbicide resistance. In this model, the equation

$$y = f(x) = C + (D - C) / (1 + (x/LD_{50})^b)$$

was used to fit the data (LD<sub>50</sub> = 50% inhibitory dose, *b* = slope of the curve at LD<sub>50</sub>, *C* = lower limit and *D* = upper limit). Figure 5 shows the herbicide dose-response curves based on fresh weight 15 days after treatment of *Ae. geniculata*, F<sub>1</sub>, BC<sub>1</sub> and wheat cultivars with the herbicides chlorotoluron and difenzoquat.

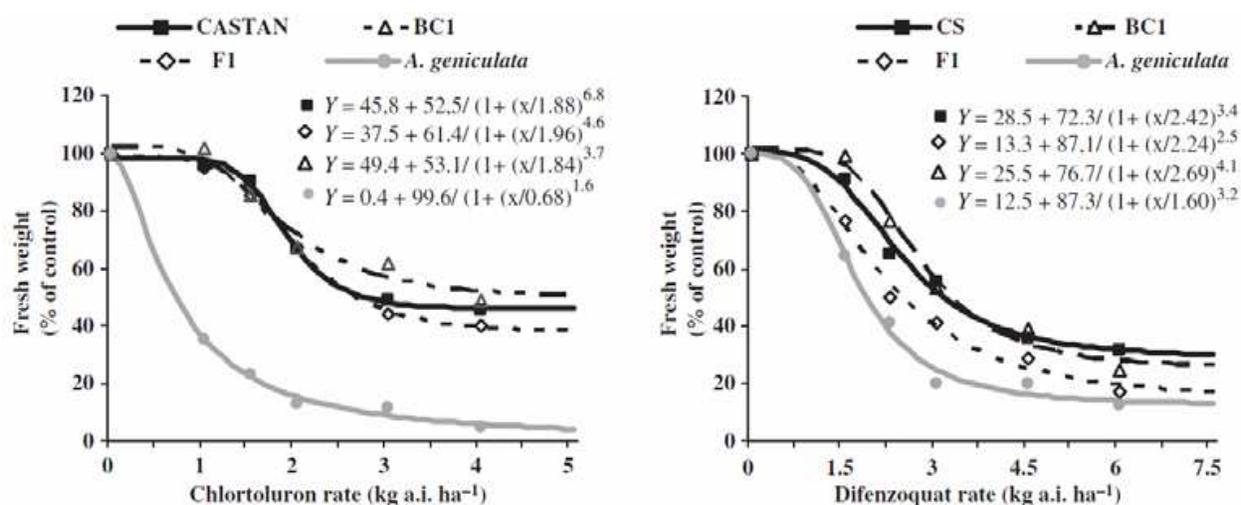


Fig. 5. Herbicide dose-response curves. *Ae. geniculata*, F<sub>1</sub>, BC<sub>1</sub> and wheat cultivars with the herbicides (A) chlorotoluron and (B) difenzoquat.

As hybrids could maintain the herbicide resistance from wheat, as is shown by the LD<sub>50</sub> values of the F<sub>1</sub>s and BC<sub>1</sub>s, the spread of these plants will be favoured by the use of the herbicide. At this point, herbicide resistance could be used as a good marker gene for hybrid detection and for the study of the herbicide resistance transference in the subsequent generations.

The hybridization ability, the partial fertility of *Aegilops*-wheat hybrids, the expression of herbicide tolerance from wheat in the cytoplasmic background of *Aegilops* and the successful backcross seed production indicate that hybrids could facilitate the transfer of herbicide resistance from cultivated wheat to *Aegilops* in the hypothesized case of backcrossing with *Aegilops* as male parent. Until now, no case of herbicide-resistance in *Ae. geniculata* or *Ae. biuncialis* harmful to farmers have been reported, which could be an indication of the real low level impact of hybridization. However, there is evidence of past gene-flow and natural, sporadic introgression from wheat into related *Aegilops* species (Weissman et al., 2005). This fact could give to the introgressed hybrids and successive generations a selective advantage and could increase the weediness of these species under an agronomic scenario of herbicide-resistant wheat, as is pointed out by Schoenenberger et al. (2006) for *Ae. cylindrica*. Broader research is needed on the fertility and fitness of the hybrids and their progenies when *Ae. geniculata* is the male parent in the backcrosses. This information could let us predict the relative advantage of hybridization on the adaptive ability of *Aegilops* spp. and hybrid derivatives and its impact on the environment and agricultural system.

## 6. Conclusions

Gene flow dynamics need to be considered in planning future field experiments with transgenic wheat. Agricultural reality shows that the degree of autogamy is high in wheat and that, generally, gene flow can be managed, provided that some precautionary measures are taken, such as keeping enough spatial isolation from other non GM wheat fields or from *Aegilops* wild relatives which wheat can hybridize. More research in this field is needed in order to establish coexistence measures to avoid unintended presence of GM in non-GM wheat, with cross-pollination being studied case by case and region by region. The fertility and fitness of the hybrids and their progenies must be also further evaluated in order to

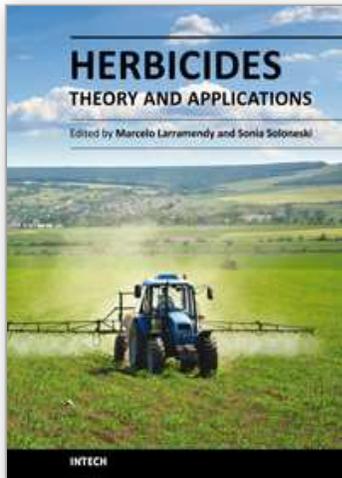
determine the potential introgression of the herbicide resistance genes into the wild species, a phenomenon that must be adequately assessed to avoid any potential risk derived of gene transfer.

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## **Herbicides, Theory and Applications**

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The content selected in Herbicides, Theory and Applications is intended to provide researchers, producers and consumers of herbicides an overview of the latest scientific achievements. Although we are dealing with many diverse and different topics, we have tried to compile this "raw material" into three major sections in search of clarity and order - Weed Control and Crop Management, Analytical Techniques of Herbicide Detection and Herbicide Toxicity and Further Applications. The editors hope that this book will continue to meet the expectations and needs of all interested in the methodology of use of herbicides, weed control as well as problems related to its use, abuse and misuse.

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