DuPont™ ExpressSun™ Herbicide Technology in Sunflower

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ABSTRACT

- The DuPont™ ExpressSun™ technology has been a revolution in aiding sunflower growers internationally with a genetic herbicide trait system designed to maximize weed control in sunflower crops, thereby enhancing production and yield. The DuPont™ ExpressSun™ trait combines herbicide tolerant sunflower seed with the Express® herbicide to provide improved over-the-top weed control compared to conventional systems.
- Traditional herbicide options for sunflower offer very limited broadleaf (BL) control and have significant crop injury risk. Express® or tribenuron-methyl has a broader spectrum of annual and perennial broadleaf weed control and is post-applied for greater flexibility, efficacy, economy and crop safety. Express® research trials have demonstrated in-season control of Canada thistle and control of many other annual broadleaf weeds in conventional, minimum-till or no-till production systems. Combined with a suitable graminicide the tank mix can control most key broadleaf weeds and grass species.
- Pioneer initially launched sunflower hybrids with the DuPont™ ExpressSun™ trait in Europe and North America for the 2007 growing season. We continue to introgress this trait into elite germplasm to develop new sunflower hybrids with the DuPont™ ExpressSun™ trait for the future. In Europe and North America, hybrids with the DuPont™ ExpressSun™ trait make up a significant percentage of our overall sales.

Key words: herbicide tolerance – sulfonylurea – sunflower – tribenuron methyl – weed control

INTRODUCTION

In the past, sunflower growers lacked effective options for post-emergence control of broadleaf weeds. Additional weed control options are needed in sunflower to provide better weed spectrum control options to growers. Sulfonylurea (SU) herbicides applied post-emergence provide both contact and residual control of annual and perennial broadleaf weeds. Conventional sunflower hybrids are susceptible to SU herbicides. Sulfonylurea herbicides control weeds by inhibiting acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS) (Kolkman et al., 2004). Due to the lack of effective conventional herbicide options in sunflower development of novel herbicide traits was initiated.

Scientists at DuPont created a sunflower line, designated M7, containing a highly heritable and dominant trait conferring tolerance to sulfonylurea herbicides wherein the tolerance trait was obtained using induced mutagenesis, ethyl methanesulfonate (EMS), and artificial selection initiated by applying a sulfonylurea herbicide solution to the imbibing mutated seeds of "HA89B" (Gabard and Huby, 2004).

MATERIALS AND METHODS

Trait discovery for ALS herbicide tolerant sunflower began in 1990 by producing mutants derived through EMS mutagenesis of the inbred line 'HA89B' (Gabard and Huby, 2004). Five plants demonstrating resistance to sulfonylurea herbicides were found during various selection experiments conducted as large-scale hydroponic screening in the greenhouse (Table 1). M11 and E24 were selected from the same M_1 mutagenized seed batch, but with different sulfonylurea herbicides. As M11 was later found to be not tolerant to rimsulfuron, M11 and E24 were likely due to different mutagenesis events. E24 and M14 were sterile and not developed further. Of the five rescued plants demonstrating resistance to sulfonylurea herbicides, only M7, M11 and M12 were fertile and could be developed further through breeding. M7 and M12 may have come from the same mutation event as they came from the same seed batch.

Table 1. Name, origin, selection herbicide and frequency of confirmed sulfonylurea tolerant plants.

Name	Seed Batch	Selection Herbicide	EMS Treatment Concentration	Selection Frequency*
M7	M_2-2	thifensulfuron-methyl	30 mM	4.2×10^{-6}
M11	M_2-1	thifensulfuron-methyl	35 mM	5.5×10^{-6}
M12	M_2-2	thifensulfuron-methyl	30 mM	4.2×10^{-6}
M14	M_2-2	thifensulfuron-methyl	30 mM	2.1 to 4.2×10^{-6}
E24	M_2-1	rimsulfuron	35 mM	5.5×10^{-6}

* Selection frequency is calculated as the ratio of surviving mutant plants compared to the total number of seeds sown and treated with the sulfonylurea selection agent.

For the conduction of small field trials, the following sunflower varieties, mutant lines and hybrids were created (Table 2).

Table 2. Description of sunflower varieties, including mutant inbred lines and hybrids.

Code	Name	Description
A	Hybrid Control: H89A x RHA274	USDA CMS H89A ("USDA" means United States Department of Agriculture; "CMS" means cytoplasmic male sterile) crossed restorer line USDA RHA274
B	Inbred Line Control: H89A	USDA CMS H89A (cytoplasmic male sterile inbred line)

C	GA-M_4M12 x RHA274	Hybrid mutant with M_4 generation of mutant line M12 on female side
D	GA-M_4M11 x RHA274	Hybrid mutant with M_4 generation of mutant line M11 on female side
E	GA-M_4M7 x RHA274	Hybrid mutant with M_4 generation of mutant line M7 on female side
F	H89A x M_4M12	USDA CMS H89A crossed with M_4 generation of mutant line M12 on male side
G	H89A x M_4M11	USDA CMS H89A crossed with M_4 generation of mutant line M11 on male side
H	H89A x M_4M7	USDA CMS H89A crossed with M_4 generation of mutant line M7 on male side
I	BC3-M_4M12	Third generation backcross of inbred line mutant M12
J	BC3-M_4M11	Third generation backcross of inbred line mutant M11
K	BC3-M_4M7	Third generation backcross of inbred line mutant M7

RESULTS

The evaluation of the maximum injury to sunflower varieties by sulfonylurea herbicides was measured by utilizing visual ratings; mean values are listed in Table 3. A rating of 0% phytotoxicity means no crop injury. A rating of 15 to 20% injury, indicating the plants were not significantly or adversely affected and rapidly and completely recovered. A rating of 100% means the complete death of all plants. The control sensitive hybrid and inbred (A and B) were destroyed by all sulfonylurea herbicides tested, except nicosulfuron. Homozygous inbred lines (I, J and K) were resistant to metsulfuron-methyl, tribenuron-methyl and to a lesser extent triflusulfuron-methyl at 20 g a.i./ha, marginally tolerant to nicosulfuron and less susceptible than the standards to thifensulfuron-methyl and primisulfuron-methyl. All three lines were as susceptible as the standards to rimsulfuron and triasulfuron at the application rates tested. The heterozygous resistant hybrids (C to H) were as tolerant as the three homozygous resistant lines (I, J and K) to metsulfuron-methyl and tribenuron-methyl, but the homozygous resistant lines were more tolerant to primisulfuron-methyl, nicosulfuron and thifensulfuron-methyl. These results are indicative of highly heritable (i.e. dominant or semi-dominant type) mutations.

Table 3. Maximum phytotoxicity following sulfonylurea treatments (4 replications/treatment).

		Sunflower Varieties/Hybrids										
Active Ingredient	**Rate (g a.i./ha)***	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Primisulfuron-methyl	30	100	100	71	75	74	66	73	69	35	55	51
Nicosulfuron	30	33	54	12	13	13	15	20	16	8	23	14
Rimsulfuron	15	100	100	99	99	98	98	98	96	90	95	95
Rimsulfuron	30	100	100	100	100	100	100	100	100	97	98	97
Triflusulfuron-methyl	20	100	100	19	19	20	16	15	10	4	6	8
Triflusulfuron-methyl	40	100	100	38	40	38	33	36	33	13	14	10
Thifensulfuron-methyl	35	100	100	75	81	83	78	81	80	34	52	36
Thifensulfuron-methyl	70	99	100	88	92	93	89	90	90	53	79	79
Metsulfuron-methyl	6	100	100	8	7	0	4	6	4	7	11	8
Tribenuron-methyl	22.5	100	100	0	6	2	0	3	0	4	0	4
Triasulfuron	20	100	100	94	99	97	98	98	98	93	95	95

* Application rates in treatments refer to amount of active ingredient in formulated herbicides in units of g/ha.

An additional greenhouse experiment was conducted to evaluate the spectrum of tolerance of three homozygous resistant mutants to various herbicides inhibiting acetolactate synthase (ALS) as their mode of action (Table 4). The inbred lines tested were the 5th generation self-pollinated maintainers, M_5M7, M_5M11 and M_5M12, as well as the 4th generation male sterile backcrosses BC_4-M_5M7, BC_4-M_5M11 and BC_4-M_5M12. Treatments of formulated herbicides were applied at the 4-leaf stage. Application rates of the test herbicides were chosen to coincide with the rates commonly used in the crops for which they are registered. The results indicate very good tolerance of the homozygous progeny of the three mutant sources to tribenuron-methyl. All mutants were also found extremely resistant to ethametsulfuron-methyl. Marginal tolerance to a low use rate of imazethapyr was also observed.

The DuPont™ Express® herbicide is a member of the sulfonylurea class of herbicides. The active ingredient is tribenuron-methyl. Express® herbicide has been used extensively in other crops for more than 15 years to control broadleaf weeds, and is effective at controlling Canada thistle (Table 5).

Table 4. Maximum phytotoxicity following herbicide treatments (2 replications/treatment).

		Sunflower Varieties					
Active Ingredient	**Rate (g a.i./ha)***	**M_5M7**	**M_5M11**	**M_5M12**	**BC_4-M_5M7**	**BC_4-M_5M11**	**BC_4-M_5M12**
Halosulfuron-methyl	45	50	60	60	60	65	65
Flupyssulfuron-methyl-sodium	10	62.5	50	57.5	42.5	60	50
Amidosulfuron	30	52.5	60	45	47.5	57.5	55
Tribenuron-methyl	22.5	12.5	0	7.5	10	17.5	20
Rimsulfuron	15	52.5	65	55	45	47.5	65
Sulfosulfuron	22.5	50	60	65	47.5	57.5	50
Ethametsulfuron-methyl	15	10	0	0	5	10	0
Chlorsulfuron	20	47.5	50	45	45	50	45
Imazethapyr (ammonium salt)	25	22.5	30	22.5	20	25	30
Pyrithiobac-sodium	70	37.5	50	40	35	42.5	40

* Application rates in treatments refer to amount of active ingredient in formulated herbicides in units of g/ha.

Table 5. Condensed list of broadleaf weeds controlled or suppressed

- Buckwheat, wild*
- Chamomile, mayweed
- Chamomile, wild
- Chickweed, common
- Falseflax, smallseed
- Flaxweed
- Kochia**
- Lambsquarter, common
- Lambsquarter, slimleaf
- Lettuce, miner's
- Lettuce, prickly**
- Nightshade, hairy*
- Pennycress, field
- Pineapple-weed
- Pigweed; redroot, tumble
- Puncturevine
- Purslane, common
- Sherpard's-purse
- Smartweed, Pennsylvania*
- Sowthistle, annual*
- Tansymustard
- Thistle: Canada, Russian**

• Marshelder • Mustard: black, blue (purple mustard), wild	• Vetch*: common, hairy • Wallflower, bushy (treacle mustard)
* Partially controlled weeds exhibit a visual reduction in numbers as well as a significant loss of vigor. ** Resistant biotypes were not always adequately controlled.	

From 2000-2004, trait introgression (TI) into elite inbreds was initiated for the development of tribenuron-methyl tolerant sunflower hybrids. During this same period, DuPont Crop Protection began registration activities for Express® herbicide and the DuPont™ ExpressSun™ trait trademark in the United States and countries in Europe.

The first registered commercial sunflower hybrids from Pioneer were launched in 2007 and were heterozygous for the M7 SU trait. It was later decided to accelerate TI and forward breeding activities and to focus only on the development of homozygous hybrids to offer greater flexibility, increased herbicide efficacy, trait durability and crop safety. The first homozygous hybrid with the M7 SU trait was commercialized in 2008.

Tribenuron has been registered in more than 60 countries globally for use in sunflower (Table 6). Additionally, the DuPont™ ExpressSun™ trademark has been officially registered in more than 30 countries in Europe and the United States (Table 7).

Table 6. Countries where tribenuron herbicide is registered.

Albania	Ethiopia	Luxembourg	Slovenia
Algeria	Finland	Macedonia	South Africa
Australia	France	Mexico	Spain
Austria	Germany	Moldavia	Sweden
Azerbaijan	Greece	Morocco	Switzerland
Belarus	Hungary	Netherlands	Syria Arab Republic
Belgium	Iraq	New Zealand	Tazikistan
Bulgaria	Ireland	Norway	Tunisia
Canada	Israel	Pakistan	Turkey
China	Italy	Poland	Turkmenistan
Croatia	Kazakhstan	Portugal	Ukraine
Cyprus	Kenya	Romania	United Kingdom
Czech Republic	Kirgizistan	Russia	United States
Denmark	Latvia	Saudi Arabia	Uzbekistan
Egypt	Lebanon	Serbia & Montenegro	Zambia
Estonia	Lithuania	Slovakia	Zimbabwe

Table 7. Countries where DuPont™ ExpressSun™ Trademark is registered.

Austria	Greece	Romania
Belgium	Hungary	Slovak Republic
Bulgaria	Ireland	Slovenia
Croatia	Italy	Serbia
Cyprus	Latvia	Spain

Czech Republic	Lithuania	Sweden
Denmark	Luxembourg	Ukraine
Estonia	Malta	United Kingdom
Finland	Netherlands	United States
France	Poland	
Germany	Portugal	

Pioneer has commercialized sunflower hybrids resistant to tribenuron-methyl herbicide in Europe and the United States. We have also out-licensed this technology to several competitor companies in the United States. We are currently working with competitor companies to consider out-licensing this technology in Europe.

DISCUSSION

The use of herbicide resistance crops has been the dominant weed management technology for the past 15 years (Green, 2011). Glyphosate resistant crops became available when growers desperately needed the technology to effectively control problem weeds (Green & Owen, 2011). Most herbicide tolerance genes are transgenic and therefore must be approved by the appropriate government agencies prior to cultivation. The DuPont™ ExpressSun™ trait is a non-transgenic trait and therefore did not require the same approval process as transgenic traits. However, we did require "Plants with Novel Traits" (PNT) approval in Canada and global regulatory approvals for use of the Express® herbicide in combination with the DuPont™ ExpressSun™ trait.

The evolution of herbicide resistance weed development is a rare event, but continuous use of the same herbicide family or same mode of action can have unintended consequences. Stewardship plans are drafted to manage the development of resistant weeds. Crop rotations and rotating modes of herbicide action can be effective resistance management tools.

As we look to the future, identification of the next-generation sunflower herbicide tolerance trait should be a priority to create new weed control options for commercial sunflower growers globally. The next-generation herbicide trait may be a combination or stack of multiple herbicide tolerance genes that would provide growers with more weed management options and help reduce reliance on a single mode-of-action or could be the creation of an entirely new resistance trait to a new herbicide. The development of glyphosate resistant maize and soybean also led to reduced emphasis on the creation of new herbicide compounds; therefore, no commercial herbicide with a new MOA has been discovered for nearly three decades (Stuebler et al., 2008).

Since seventy (70) percent of the global sunflower production is in Europe, Russia and Ukraine, where adoption of transgenic crops has been met with resistance, the next-generation trait is likely to be more quickly adopted if its development involves non-transgenic technology.

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