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Die CLEARFIELD® Technologie – Ein neues Nachauflauf-Unkrautbekämpfungssystem für den europäischen Sonnenblumenanbau

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Summary

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in Europe with a total planted area of about 9.2 million hectares in 2006. Weeds are a major production problem in sunflower cultivation. Sunflower is a poor competitor during the early growth stages until canopy closure. Therefore, weeds compete successfully during these growth stages for light, water and nutrients. Limitation of available herbicides, especially herbicides to control broadleaf weeds, causes considerable yield losses to sunflower producers. The CLEARFIELD technology has been developed in sunflower to allow the use of imidazolinone herbicides as a post-emergence weed control option. The mode of action of imidazolinone herbicides is the inhibition of the enzyme acetohydroxyacid synthase (AHAS). While conventional sunflower is sensitive to imidazolinone herbicides, CLEARFIELD sunflower hybrids have been modified to survive an otherwise lethal application of these herbicides. The trait for tolerance to imidazolinone herbicides in CLEARFIELD sunflower goes back to a naturally occurring mutation in the AHAS gene detected in a wild population of *Helianthus annuus*. This technology does not involve the introduction of foreign genetic material from other sources and thus is characterized as a non-GMO (genetically modified organism) process. CLEARFIELD herbicides provide exceptional foliar and soil activity to control a broad spectrum of weeds occurring across regions and cropping systems where sunflowers are produced.

Key words: acetohydroxyacid synthase (AHAS), CLEARFIELD, herbicide, imazamox, imidazolinone tolerance, mutant/mutation, weed control

Zusammenfassung

Unter den Ölkulturen in Europa nimmt die Sonnenblume (*Helianthus annuus* L.) mit einer Anbaufläche von ca. 9,2 Millionen Hektar (2006) eine bedeutende Stellung ein. Unkräuter stellen ein wichtiges Problem beim Anbau dar. Die frühe Entwicklungsphase bis zum Reihenschluss der Sonnenblume ist durch eine geringe Konkurrenzkraft gegenüber Unkräutern gekennzeichnet. Die Anzahl von kulturselektiver Herbizide ist sehr begrenzt und besonders die Bekämpfung zweikeimblättriger Unkräuter stellt in vielen Anbauregionen ein Problem dar. Mit der Entwicklung und Einführung der CLEARFIELD-Technologie für die Sonnenblume wurde der Einsatz von Herbiziden aus der Wirkstoffklasse der Imidazolinone als Nachauflauf Anwendung ermöglicht. Der Wirkmechanismus dieser Herbizidklasse beruht auf der Hemmung der ALS-Synthase. Herkömmliche Sonnenblumen reagieren sensitiv auf die Anwendung von Imidazolinon-Herbiziden, während CLEARFIELD-Sonnenblumen diese Anwendung tolerieren. Die Toleranzeigenschaft ist zurückzuführen auf eine natürliche Mutation des AHAS-Gens in wilden Sonnenblumen. Diese Technologie beruht nicht auf der Nutzung genetischen Materials artfremder Organismen und ist deshalb als „Nicht GVO“ klassifiziert. Das CLEARFIELD-Herbizid-System in Sonnenblumen ermöglicht die Bekämpfung eines breiten Spektrums ein- und zweikeimblättriger Unkräuter im Nachauflauf.

Stichwörter: Acetohydroxyacid-Synthase (AHAS), CLEARFIELD, Herbizid, Imazamox, Imidazolinon-Toleranz, Mutante/Mutation, Unkrautbekämpfung

1 Introduction

As oilseed crop, sunflowers play a major role in Europe. Sunflower oil is regarded as high quality oil for food use, and lately there is also an increasing demand for sunflower oil for industrial uses, mainly for the bio-diesel industry. Despite of these facts, sunflower remains in many areas an extensive low input crop. Weed management is an important component of successful sunflower production. Sunflowers are usually planted in low densities and grow slowly during the first weeks till row closure. Weeds that emerge and establish during this time are competitive and ultimately reduce sunflower yield. Generally, weed control options, especially for broadleaf weeds, in sunflower are limited. Pre-plant or pre-emergence applications of Dinitroanilines as well as Chloroacetamides are common herbicides in use, often accompanied with a tillage operation. Most of these herbicides are primarily controlling grass weeds with narrow broadleaf weed control spectrum, especially on difficult-to-control weeds like *Xanthium strumarium*, *Datura stramonium*, *Ambrosia artemisiifolia*, and *Sonchus* spp. Weeds insufficiently controlled in sunflower also include parasitic weeds like *Orobanche* spp. Weeds which germinate and emerge before activation of the herbicide will most likely not be controlled by a pre-emergence treatment. Weed control options are also limited in minimum and no-tillage systems. Available post-emergence sunflower herbicides are mainly graminicides for grass control. From an industry perspective, sunflower unlike corn or cereals has never been in the focus for new herbicide developments. This is influenced also by the fact that it is increasingly difficult to discover new herbicides with either known or a novel mode of action (Gressel 2002). Hence there is a strong demand to enlarge the utility of existing molecules to crops which do not possess initial tolerance to specific herbicides.

2 Materials and methods

2.1 Herbicide tolerant crops

One effective approach to allow the herbicide to be used in a sensitive crop is to change the crop genetically with either traditional or transgenic techniques (TAN et al 2006). After the genetic change, the enzyme targeted by the herbicide is no longer sensitive to the herbicide, or the modified plant can degrade the herbicide before the herbicide reaches the target enzyme. These crops are commonly identified as Herbicide Tolerant Crops or HTCs. HTCs in combination with their corresponding herbicides have been widely and increasingly adopted around the world because they have several advantages over other weed management methods (DUKE 2005; TAN et al. 2005). With the HTC technology one is able to control some weeds that cannot be controlled by other means, or to control weeds more effectively than other methods in the crop for which the HTC is designed. The imidazolinone-tolerant sunflower in combination with an imazamox-based herbicide enables very effectively the post-emergence control of a wide range of weeds in sunflower including parasitic weeds such as broomape (*Orobanche* spp.) (TAN et al. 2005).

2.2 The imidazolinone herbicides

Imidazolinone herbicides, which include imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin, were discovered and developed in the 1980s and 1990s (SHANER and SINGH 1997). Imidazolinone herbicides control a wide spectrum of grass and broadleaf weeds. They are effective at low application rates, have low mammalian toxicity, and possess a favourable environmental profile. Thus imidazolinone herbicides have many ideal characteristics for utilization in a herbicide-tolerant crop. Imazamox is the active ingredient in PULSAR® 40 herbicide and is the newest member of the imidazolinone herbicide family. Imazamox is registered throughout the world for use in leguminous crops, including soybeans, alfalfa and edible beans as well as in imidazolinone-resistant crops (Tab. 1). Imazamox became the cornerstone imidazolinone herbicide for the CLEARFIELD production system in Europe due to its favourable and versatile characteristics. Although imazamox is much more effective applied post-emergence, it possesses certain residual activity. In comparison to other imidazolinone herbicides, imazamox however, dissipates more rapidly in the soil.

2.3 Imidazolinone tolerance

Imidazolinone herbicides are active against the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS). AHAS is an enzyme found in bacteria, certain other micro-organisms and plants. This enzyme catalyses the first step in the biosynthesis of the essential branched chain amino acids isoleucine, leucine and valine. Herbicide-induced AHAS inhibition results in a lethal decrease of protein synthesis. Unmodified sunflowers are not tolerant to imidazolinone herbicides. A single amino acid substitution in the AHAS gene alters the binding site such that imidazolinone herbicides no longer bind to the AHAS enzyme, resulting in the herbicide- tolerant phenotype. There are five commonly known AHAS mutations which confer tolerance to AHAS inhibiting herbicides. The AHAS mutation found in imidazolinone-tolerant wild sunflower (BAUMGARTNER et al. 2004) has no or negligible cross tolerance to sulfonylureas (Tab. 2).

2.4 Variety development

Since the commercial launch of the CLEARFIELD herbicide tolerant maize (*Zea mays*) in 1992, five other imidazolinone-tolerant crops have been developed and commercialized from mutations, including oilseed rape (*Brassica napus*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), sunflower (*Helianthus annuus*) and lentil (*Lens culinaris*). All are originated by using conventional breeding methods such as cell culture selection with or without chemical mutagenesis. CLEARFIELD sunflower is derived from imidazolinone-tolerant wild sunflowers that were discovered in

Tab. 1: Regional overview of the different imidazolinone-tolerant crops with the respective imidazolinone herbicides.
Tab. 1: Regionale Übersicht der verschiedenen Imidazolinon toleranten Kulturen mit den jeweiligen Imidazolinon Herbiziden.

Commercialized imidazolinone -tolerant crops	Region			
	North America	South America	Europe* (incl. Turkey)	Australia
Maize	Imazapyr Imazethapyr	Imazapyr Imazethapyr Imazapic	Imazamox	
Oilseed rape	Imazamox Imazethapyr			Imazapyr Imazapic
Rice	Imazethapyr	Imazethapyr Imazapic	Imazamox	
Wheat	Imazamox			Imazapyr Imazapic
Sunflower	Imazamox	Imazapyr	Imazamox Imazapyr	

*Geographical Europe

Tab. 2: Relative cross-resistance of imidazolinone-tolerant wild sunflower to other ALS inhibitors.
Tab. 2: Relative Kreuzresistenz von Imidazolinon-toleranten wilden Sonnenblumen zu anderen ALS-Inhibitoren.

	GR25* values (in g ai/ha)	
	Susceptible	Resistant
Imazamox	4.10	1235.00
Thifensulfuron	0.48	1.30
Chlorimuron	1.54	1.70

* GR25, herbicide rate required to reduce growth by 25 %

Kansas, USA in 1996 (Al-Khatib 1998). The tolerance trait occurred spontaneously, so the mutation is the result of the mutagenic effects of the natural environment. The herbicide tolerance trait is conferred by a single point mutation in the acetohydroxyacid synthase (AHAS) gene (R gene), with an alanine to valine substitution at position 205 (*Arabidopsis* alignment) such that herbicides have reduced binding and inhibiting efficiency to the modified AHAS enzyme. Seeds of the tolerant wild sunflower population were collected and bred as imazamox-tolerant gene donors (maintainer HA425 and fertility restorer RHA426 and RHA427) to introduce the tolerance trait into cultivated sunflowers (Miller 2002). Besides the R-gene as basis for the tolerance, the background genotype (E-gene) has also a significant effect on the tolerance of the phenotype. The mutated imidazolinone-tolerant AHAS gene is semi-dominant; as a result, crop tolerance increases with gene dosage. Therefore, CLEARFIELD sunflower hybrid production requires the conversion of both, male and female parent lines. The CLEARFIELD sunflower phenotype is characterized as Phenotype = Genotype ($G_{AHAS\text{-}R}$ + $G_{background}$) + Environment. CLEARFIELD sunflower hybrids are not commercially cross-tolerant to the sulfonylurea herbicides, which are also ALS inhibitors (Fabie and Miller, 2002). Therefore, herbicides of this chemical class will seriously damage or kill CLEARFIELD sunflower. After the trait was introduced into a domestic sunflower variety, the seeds were made available by the United States Department of Agriculture to sunflower breeders for the development of imazamox-tolerant CLEARFIELD sunflowers. Since then, several commercial seed companies have introduced the tolerance trait into their own sunflower lines and the first commercial tolerant hybrids have been brought to the market as CLEARFIELD sunflower hybrids in the United States, Argentina and Turkey in 2003.

3 Results

3.1 Weed control

One of the major advantages of the CLEARFIELD sunflower system is the post-emergence broad-spectrum weed control by imazamox (Tab. 3). It also controls weed species related to sunflowers like *Xanthium strumarium* or *Ambrosia artemisiifolia* (Asteraceae), which are common weeds infesting sunflower fields in the Mediterranean area. No effective herbicide is currently registered in sunflower for their control. Depending on local needs caused by multiple flushes of weeds, a soil residual herbicide like pendimethalin complements the residual activity of imazamox very well. *Orobanche cernua* (broomrape) poses a serious threat to sunflower cultivation in many countries as there is no effective method of controlling this parasitic weed. About 80 % of the sunflower production area in Turkey is infested by broomrape (Kaya et al. 2004). PULSAR® 40 (imazamox) or in the case of Turkey INTERVIX® (imazamox + imazapyr) controls these three weeds effectively in problem areas. Thus, the CLEARFIELD system also complements the genetic broomrape resistance of sunflower cultivars in an effective way when used in an alternating manner.

3.2 Crop response to imazamox

The selectivity of CLEARFIELD sunflower results from a combination of the characteristics of the altered AHAS gene

Tab. 3: Weeds controlled by post-emergence application of 1.25 l/ha PULSAR®40.
Tab. 3: Wirkungsspektrum von PULSAR®40 mit 1,25 l/ha im Nachauflauf.

95 to 100% Control	84 to 94% Control	70 to 84% Control
Amaranthus hybridus (5)	*Abuthilon theophrasti (11)*	*Cirsium arvensis (8)*
Amaranthus retroflexus (38)	*Ambrosia eliator (15)*	*Digitaria sanguinalis (14)*
Anagallis arvensis (11)	*Ammi majus (6)*	*Lolium spp.(3)*
Brassica napus (volunteer (5)	*Bromus diandrus (3)*	*Matricharia chamomilla (9)*
Brassica nigra (4)	*Capsella bursa-pastoris (3)*	*Polygonum convolvulus (43)*
Chenopodium hybridum (13)	*Chenopodium album (82)*	*Portulaca oleracea (9)*
Datura stramonium (9)	*Chrysanthemum coronarium (4)*	*Sonchus asper (11)*
Fumaria officinalis (6)	*Echinochloa crus-galli (66)*	*Veronica persica (10)*
Helianthus annuus (volunteer / wild) (9)	*Euphorbia helioscopia (3)*	
Kickxia spuria (3)	*Galium aparine (14)*	
Lamium amplexicaule (3)	*Hibiscus trinonium (5)*	
Matricharia inodora (3)	*Lamium purpureum (3)*	
Orobanche spp.(15)	*Lolium multiflorum (7)*	
Polygonum lapathifolium (6)	*Mercurialis annua (31)*	
Setaria viridis (7)	*Panicum miliaceum (7)*	
Sinapis arvensis (15)	*Pennisetum glaucum (3)*	
Solanum nigrum (45)	*Polygonum aviculare (15)*	
Stachys annua (5)	*Polygonum persicaria (26)*	
Stellaria media (10)	*Raphanus raphanistrum (3)*	
Thlaspi arvense (3)	*Setaria verticillata (10)*	
Xanthium strumarium (9)	*Sonchus arvensis (10)*	

Trial results from Europe (2001–2006); ( ) = number of trials per weed.

Tab. 4: CLEARFIELD sunflower tolerance qualification trials – Hungary 2006.
Tab. 4: CLEARFIELD Sonnenblumen Toleranz-Qualifikationsversuche – Ungarn 2006.

	Imazamox - 1X use rate								
	7 DAT			14 DAT			21 DAT		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Loc1	4.00	2.00	2.45	3.50	1.50	2.55	1.00	1.00	1.00
Loc2	3.25	1.25	2.23	2.00	1.00	1.16	2.50	1.00	1.56
Loc3	3.50	2.00	2.55	4.00	2.00	2.45	1.50	1.00	1.04
Loc4	2.13	1.13	1.84	2.13	1.13	1.84	2.00	1.00	1.15
Average	3.00	1.72	2.29	2.50	1.47	1.97	1.63	1.00	1.17

	Imazamox - 2X use rate								
	7 DAT			14 DAT			21 DAT		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Loc1	5.00	3.50	4.29	4.00	1.50	2.64	1.25	1.00	1.01
Loc2	4.00	1.75	2.79	2.50	1.00	1.77	3.00	1.00	2.26
Loc3	4.00	2.25	2.96	5.00	3.50	4.29	1.50	1.00	1.03
Loc4	3.00	1.75	2.68	3.00	1.75	2.68	3.50	1.00	1.98
Average	3.69	2.50	3.13	3.41	1.50	2.74	2.06	1.00	1.49

Data compiled from 24 sunflower hybrids; DAT = days after treatment; tolerance expressed as % visual injury compared to untreated control; scale 0 % = no Injury, 100 % plants completely killed.

and the effect of background genotype (E-gene) in each individual inbred line. Factors like crop stage at application, use rate, adsorption and translocation influences herbicidal metabolism and therefore the crop response. Occasionally, reduction of plant height or temporary yellowing of crop plants may occur, following imazamox application. These effects can be more pronounced if crops are growing under stressful environmental conditions (heat, drought, water-logged soils etc.). Symptoms are transient, without any recorded indication of yield depressions. Normal growth and appearance should resume within 1 to 2 weeks. To avoid as much as possible these effects, all commercial hybrids are undergoing a regional qualification procedure. Candidate hybrids will only qualify as CLEARFIELD sunflower, if they show no greater crop injury than the designated regional tolerant hybrid standard (Tab. 4) when treated with imazamox (2 x use rate).

3.3 Stewardship guidelines

Management of herbicide-resistant weeds and gene flow from crops to weeds are issues that must be considered with the development of any herbicide-resistant crop. One concern with a HTC system is the possibility for selection of herbicide resistant weeds. There are two likely paths for potential weed resistance to occur. The selection of resistant biotypes by an overuse of herbicides with the same mode of action and the tolerance gene out-crossing to closely related species of the HTC. The gene flow from herbicide tolerant sunflower to wild sunflower (Rafael et al. 2003) may have certain relevance, but only in areas where wild sunflower is a known weed problem (Vischi et al. 2006), whereas selection pressure exerted by herbicide use is a key issue in all agricultural areas. To preserve the long-term sustainability of the CLEARFIELD system, a stewardship program has been developed to address these issues (Tan et al. 2005). It is aimed at preventing weed resistance resulting from trait out-crossing as well as from selecting spontaneous mutations in the field. The main measurements are the recommendations on crop rotation, rotating herbicides with different mode of action, volunteer plant control and controlling key related weeds in areas adjacent to CLEARFIELD sunflower. Education of other stakeholders in sunflower production including seed breeders, dealers and distributors complements the stewardship recommendations.

4 Discussion

The CLEARFIELD® production system, by combining imidazolinone-tolerant sunflower with imidazolinone herbicides, is able to control a wide range of weeds, including certain weeds that no other herbicide can control in sunflower. Imazamox or a combination of imazamox + imazapyr has been registered for the use in CLEARFIELD sunflowers in countries like Bulgaria, Hungary Romania, Spain, Turkey and Ukraine. Additional registrations are expected in South Eastern Europe as well as in Russia. A stewardship program for this HTC has been developed and implemented to reduce gene flow and weed resistance and to preserve this very effective weed management tool. Because CLEARFIELD sunflower cultivars were developed using traditional breeding methods, there is no additional regulatory restriction regarding their commercialization in comparison to other conventionally developed sunflower cultivars. This makes this innovative cropping system readily accessible today for farmers in Europe, allowing in most cases a one pass control of grass and broadleaf weeds in sunflower.

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