

## **Amaranthus develops resistance to HPPD inhibitors in seed corn production fields - another example of lack of diversity in the weed control program**

*Amarant entwickelt Resistenz gegen HPPD-Hemmer in Saatmais Produktionsfeldern - ein weiteres Beispiel für fehlende Vielfalt in den Unkrautbekämpfungsverfahren*

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### **Summary**

In 2010, a hybrid seed corn production field was confirmed to be infested with a population of *Amaranthus tuberculatus* that was not controlled by postemergence applications of all tested commercially available HPPD-inhibitor herbicides. In glasshouse and field studies, resistance to post-emergence applications of mesotrione, tembotrione and topramezone was confirmed. In addition, this population was identified to be resistant to ALS-inhibitors and to triazines.

*A. tuberculatus* is a very competitive weed, which can appear in high population density and can emerge even very late during the season. *A. tuberculatus* is dioecious and therefore an obligate outcrosser, which is leading to high genetic variability within a population. All these biological features increase the risk of developing resistance to herbicides.

Therefore, the field history explains clearly that this resistance developed under specific conditions. In this field, inbred corn was grown for at least seven consecutive years. Inbred corn is much less competitive with weeds compared to hybrid field corn and herbicide options are reduced vs. hybrid field corn production. In addition due to the resistance to triazines, only one effective mode of action (HPPD) has been used post-emergence to control *A. tuberculatus* for over seven consecutive years. The resistance development in this *A. tuberculatus* population is clearly a result of the lack of diversity in the weed management plan which should include crop and herbicide rotation.

Field testing showed pre-emergence applications of mesotrione combinations (Lumax<sup>®</sup>, Lexar<sup>®</sup>) provided good control of this *A. tuberculatus* population. In addition alternative herbicides to control this population have been identified: Glyphosate, glufosinate, paraquat, PPGO-inhibitors, auxins, and triazinones were very effective.

**Key words:** *Amaranthus tuberculatus*, inbred corn, mesotrione, waterhemp

### **Zusammenfassung**

In 2010 wurde in McLean County, Illinois, in einem Maissaatgutproduktionsfeld eine *Amaranthus tuberculatus* Population bestätigt, die nicht mit Nachauflaufbehandlungen von HPPD-Hemmern bekämpft werden konnte. In Gewächshaus- und Feldstudien wurde die Resistenz gegen Nachauflaufbehandlungen der HPPD-Hemmer Mesotrione, Tembotrione and Topramezone gezeigt. Zusätzlich wurde eine Kreuzresistenz gegen ALS-Hemmer und gegen Triazine festgestellt.

*A. tuberculatus* ist ein sehr konkurrenzstarkes Unkraut, das in hoher Populationsdichte auftreten und sogar noch sehr spät in der Saison auflaufen kann. Es ist ein zweihäusiges Unkraut und daher ein obligater Fremdbestäuber, welches zu hoher genetischer Variabilität in der Population führt. Diese biologischen Charakteristika erhöhen das Risiko einer Resistenzentwicklung gegen Herbizide.

In dem Feld in dem die resistente Population gefunden wurde, wurde für mindestens sieben aufeinanderfolgende Jahre Hybridsaatmais aus Inzuchtlinien produziert. Inzuchtlinien sind im Vergleich zu Hybridmais sehr konkurrenzschwach gegenüber Unkräutern und zusätzlich existieren nur begrenzte Möglichkeiten der chemischen Unkrautbekämpfung. Da diese *A. tuberculatus* Population eine Resistenz gegen Triazine aufwies, war der HPPD-Hemmer für mehr als sieben aufeinander folgende Jahre der einzige effektive Wirkmechanismus in der Nachauflaufbehandlung. Die Entwicklung der Resistenz gegenüber HPPD-Hemmern ist daher eine klare Folge von einer nicht vorhandenen Diversität im Unkrautmanagement, welches ackerbauliche Massnahmen und Herbizidwechsel beinhalten sollte.

Feldversuche haben gezeigt, dass alternative Bekämpfungsmöglichkeiten dieser Population bestehen. So waren im Mais Voraufaufbehandlungen von mesotrionehaltigen Kombinationsmitteln (Lumax®, Lexar®) erfolgreich in der Bekämpfung dieser Population. Zusätzlich wurden weitere Herbizide mit guter Wirkung gegen diese Population identifiziert: Glyphosat, Glufosinat, Paraquat, PPO-Hemmer, Auxine und Triazinone waren sehr wirkungsvoll.

**Stichwörter:** *Amaranthus tuberculatus*, Herbiziresistenz, Inzuchtlinien, Mesotrione

## 1. Introduction

Herbicide resistant weeds are increasing worldwide. Among them there are three species from the *Amaranthus* family in the top ten (HEAP, 2011). In the midwestern USA Common *A. tuberculatus* (*Amaranthus tuberculatus*) has become a widespread weed.

Waterhemp (*A. tuberculatus*) is an obligate outcrosser with huge genetic variability (TRANSEL et al., 2011). Single female plants can produce up to 1 million seeds under full light conditions (STECKEL, 2007). The plant has a huge plasticity comprising small to large plants and an extended emergence pattern from spring throughout the summer month with germination even late in July resulting in plants able to produce viable seeds (HARTZLER et al., 1999). *A. tuberculatus* is a very competitive  $C_4$  plant that can grow up to 2-3 m tall (HORAK and LOUGHIN, 2000). Different populations of this weed have developed resistance to ALS, PSII, PPGO inhibitors or to glyphosate.

Resistance development is often linked to and caused by an overuse of a particular herbicide or herbicide mode of action. Good examples are the development of glyphosate resistance in the US after overreliance of glyphosate applications (VAN GESSEL, 2001) and ALS resistant *Echinochloa crus-galli* in Italy after overreliance on ALS inhibitors to control grasses in corn and rice in combination with lack of crop rotation (PIGNATA et al., 2008).

Inbred corn lines are grown in seed corn production fields to produce seed for commercial hybrid corn. Inbred lines are much slower in growth and the plants do not reach the height of hybrid corn. Therefore, row closure is much slower and in some varieties never achieved throughout the season. In addition, the male flower of the mother plants is mechanically removed before flowering reducing its height and soil coverage even more and male pollinators are removed after pollination leaving an empty row without any crop.

HPPD-inhibitor herbicides are the newest available class of herbicides in corn. They act by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) leading to bleaching of susceptible plants and its subsequent death. They are widely used for pre- and post-emergence herbicide applications due to their good corn selectivity and weed control efficacy. In hybrid corn, they are mainly used in premixes or tankmixes with other modes of action, mainly from the acetanilide and triazine group that inhibit the formation of very long chain fatty acids (VLCFA) and the photosystem II (PS II) at the D1 protein, respectively. An example is Lumax, a herbicide premix consisting of a combination of the HPPD-inhibitor mesotrione, the VLCFA-inhibitor S-metolachlor (SMOC) and the PSII-inhibitor atrazine. The combinations of HPPD- and PSII-inhibitors such as atrazine have been found to have synergistic activity and can overcome PSII-inhibitor target site resistance (HUGIE et al., 2008).

In 2009, plants from a *A. tuberculatus* population were identified in a seed corn production field in McLean County, Illinois which had survived post-emergence application of HPPD-inhibitor herbicides. The site had been under a continued monoculture (min. 7 years) of seed corn production with heavy reliance on post-emergence applications of HPPD-inhibitor herbicides to control the *A. tuberculatus* population (Tab. 1). This paper describes the resistance and cross-resistance pattern of this population, discusses factors leading to its occurrence, and identifies alternative control options.

**Tab. 1** Field history at the McLean County, Illinois, location.

**Tab. 1** *Feldhistorie des Feldes in McLean County, Illinois.*

Year	Crop	Post-emergence herbicide application	Post-emergence herbicide application
2003	Seed corn production	SMOC + simazine	Mesotrione + atrazine
2004	Seed corn production	SMOC + simazine	Mesotrione + atrazine
2005	Seed corn production	SMOC + simazine	Mesotrione + atrazine
2006	Seed corn production	SMOC + simazine	Topramezone + atrazine
2007	Seed corn production	SMOC + simazine	Topramezone + atrazine
2008	Seed corn production	SMOC + simazine	Tembotrione fb mesotrione
2009	Seed corn production	SMOC + simazine	Tembotrione fb mesotrione

## 2. Materials and methods

### 2.1 Glasshouse trials

Seed heads from female plants surviving previous HPPD-herbicide applications were collected at the end of the 2009 season and dried. Seeds were manually harvested and stratified in 0.1 % agarose solution at 4 °C for 30 days as described in HAUSMAN et al. (2011). Seeds from several plants from this site were combined and referred to as: McLean County. As sensitive comparison a commercial available *A. tuberculatus* population from Azlin seeds was used and referred to as Azlin 1999.

Plants were grown from seeds in individual pots (10x10 cm) and grown under glasshouse conditions to the required height. For post-emergence treatments, plants were 7-10 cm tall and four replicates with 1-4 plants per pot were used.

Herbicide application was made using a herbicide sprayer delivering 150 l/ha fitted with 8002E TeeJet Nozzles. For all treatments, commercially available herbicides were used. Herbicidal activity was assessed at indicated times after treatment by visual assessment.

### 2.2 Field evaluations

In 2010 field trials herbicides were treated at the appropriate growth stage of *A. tuberculatus* at the McLean County field, where surviving *A. tuberculatus* plants had been collected in 2009. Crops planted were hybrid field corn or soybeans. Herbicides were applied by backpack sprayer with a TeeJet 8002 flat fan nozzle delivering 200 l/ha. All treatments were replicated three times in a complete randomized trial design. Herbicides were used as commercially available formulated products and were tested at their recommended 1x field rate, if not indicated otherwise. Herbicide activity was assessed visually at indicated timings after herbicide treatments. For post-emergence applications plants were 5-8 cm tall.

## 3. Results

### 3.1 Glasshouse trials

The glasshouse trial (Tab. 2) showed that post-emergence application of the three most widely used HPPD-inhibitors failed to control the McLean County *A. tuberculatus* population at the 1x and even 4x field rate, whereas the susceptible standard Azlin 1999 was controlled completely at the 1x rate. This clearly demonstrates the post-emergence resistance to HPPD-inhibitors in the McLean population. In fact a separate rate response experiment (data not shown) revealed a resistance factor of ~15x to post-emergence applied mesotrione at the ED<sub>75</sub> level.

**Tab. 2** Response of *A. tuberculatus* populations to post-emergence applications of different HPPD-inhibitors.**Tab. 2** Wirkung der Nachauflaufapplikation verschiedener HPPD-Hemmer auf *A. tuberculatus*-Populationen.

Herbicide	Application rate	Control Azlin 1999	Control McLean County
	(g ai/ha)	23 DAA **	23 DAA **
Mesotrione (Callisto + MSO + AMS)	105*	99 %	72 %
	210	100 %	85 %
	420	100 %	86 %
Tembotrione + isoxadifen (Laudis + MSO + AMS)	92*	100 %	75 %
	184	100 %	78 %
	368	100 %	92 %
Topramezone (Impact + MSO + AMS)	18.5*	100 %	87 %
	37	100 %	85 %
	74	100 %	96 %

\* = recommended 1x field rate; \*\* DAA = days after application; MSO = methylated seed oil; AMS = ammonium sulfate

When alternative herbicides with different modes of action were tested (Tab. 3) it became clear that the McLean County *A. tuberculatus* has a cross-resistance to the triazine (atrazine) and to the ALS-inhibitor (nicosulfuron). Also the Azlin 1999 population was found to be resistant to atrazine (confirmed in separate experiment; data not shown). The alternative herbicides fomesafen, glyphosate, glufosinate, dicamba, as well as the combination of mesotrione + atrazine provided full control of the McLean County population in post-emergence applications.

**Tab. 3** Response of *A. tuberculatus* populations to post-emergence applications of different herbicides.**Tab. 3** Wirkung der Nachauflaufapplikation verschiedener Herbizide auf *A. tuberculatus*-Populationen.

Herbicide	Application rate	Adjuvants	Control Azlin	Control McLean
	(g/ha)		1999	County
			23 DAA *	23 DAA *
Atrazine (Aatrex <sup>®</sup> )	560 (ai)	1 % v/v COC 2.5 % w/v AMS	44 %	39 %
Mesotrione + atrazine (Callisto <sup>®</sup> + Aatrex <sup>®</sup> )	105 + 560 (ai)	1 % v/v COC 2.5 % w/v AMS	100 %	100 %
Glyphosate (Touchdown Total <sup>®</sup> )	880 (ae)	2.5 % w/v AMS	98 %	100 %
Dicamba (Clarity <sup>®</sup> )	560 (ae)	0.25 % v/v NIS 2.5 % w/v AMS	100 %	100 %
Nicosulfuron (Accent <sup>®</sup> )	70 (ai)	0.25 % v/v NIS 2.5 % w/v AMS	83 %	42 %
Glufosinate (Ignite <sup>®</sup> )	450 (ai)	2.5 % w/v AMS	100 %	100 %
Fomesafen (Flexstar <sup>®</sup> )	197 (ai)	1 % v/v COC 2.5 % w/v AMS	100 %	100 %

\* DAA = days after application; ai = active ingredient; ae = acid equivalent

### 3.2 Field trial results

Out of the pre-emergence herbicide applications at the McLean County field trial the best solution for corn was a full rate of Lumax (93 % control) and for soybeans the application of Boundary<sup>®</sup> with 99 % control (Tab. 4). Half rate of Lumax<sup>®</sup> and the ALS-inhibitor containing products failed to provide good control of this population. The standard pre-emergence application by the farmer (SMOC + simazine) provided only 40 % control and was the weakest option for controlling the McLean County *A. tuberculatus* population in corn.

After post-emergence and pre-emergence followed by (fb) post-emergence herbicide applications very good control levels were achieved (tab. 5). In corn 95 % to 98 % *A. tuberculatus* control were

achieved by pre-emergence application of Bicep II Magnum<sup>®</sup> followed by Callisto<sup>®</sup> + Aatrex<sup>®</sup>, Callisto<sup>®</sup> + Status<sup>®</sup>, Touchdown Total<sup>®</sup> or Halex GT<sup>®</sup>.

In soybeans the ALS-resistance of this population was visible by the failure of Pursuit<sup>®</sup> (38 % control). The sequential applications Prefix<sup>®</sup> followed by Touchdown Total<sup>®</sup> and Boundary<sup>®</sup> followed by Flexstar GT<sup>®</sup> provided a total control (100 %) of the population and were the best treatments.

**Tab. 4** Response of *A. tuberculatus* at McLean County to pre-emergence applications of different herbicides.

**Tab. 4** Wirkung der Voraufauf Applikation verschiedener Herbizide auf *A. tuberculatus* in McLean County.

Crop	Herbicide	Application rate GPR or LPR / ha *	Application timing	Control 54 DAA **
Corn	Dual II Magnum <sup>®</sup> + Princep 4L <sup>®</sup> (SMOC + simazine)	2.5 + 2.34 LPR/ha	PRE	40 %
	Corvus <sup>®</sup> (isoxaflutole/thiencazabazone/cyprosulfamide)	0.4 LPR/ha	PRE	62 %
	Verdict <sup>®</sup> (saflufenacil/dimethenamid-p)	1.17 LPR/ha	PRE	81 %
	Lumax <sup>®</sup> half rate (mesotrione/SMOC/atrazine)	3.5 LPR/ha	PRE	62 %
	Lumax <sup>®</sup> full rate (mesotrione/SMOC/atrazine)	7 LPR/ha	PRE	93 %
Soybeans	Sharpen <sup>®</sup> (saflufenacil)	70 GPR/ha	PRE	57 %
	Pursuit DG <sup>®</sup> (imazethapyr)	98 GPR/ha	PRE	20 %
	Prefix <sup>®</sup> (fomesafen/SMOC)	2.34 LPR/ha	PRE	85 %
	Boundary <sup>®</sup> (metribuzin/SMOC)	2.34 LPR/ha	PRE	99 %

\* GPR = gram product; LPR = liter product; \*\* DAA = days after application; SMOC = s-metolachlor

**Tab. 5** Response of *A. tuberculatus* at McLean County to post-emergence applications and sequences of different herbicides.

**Tab. 5** Wirkung der Nachauf- und sequenzieller Applikation verschiedener Herbizide auf *A. tuberculatus* in McLean County.

Crop	Herbicide	Application rate GPR or LPR / ha **	Adjuvant	Application timing	Control 28 DAA *** of post-em application
Corn *	Callisto <sup>®</sup> + Aatrex <sup>®</sup> (mesotrione + atrazine)	0.22 + 1.2 LPR/ha	MSO + AMS	PRE* fb POST	95 %
	Callisto <sup>®</sup> + Status <sup>®</sup> (mesotrione + dicamba/diflufenzopyr/isoxadifen)	0.22 LPR/ha + 350 GPR/ha	MSO + AMS	PRE* fb POST	98 %
	Touchdown Total <sup>®</sup> (glyphosate)	2.34 LPR/ha	MSO + AMS	PRE* fb POST	98 %
	Halex GT <sup>®</sup> (glyphosate/mesotrione/SMOC)	4.2 LPR/ha	MSO + AMS	PRE* fb POST	98 %
Soybeans	Pursuit DG <sup>®</sup> (imazethapyr)	98 GPR/ha	MSO + AMS	POST	38 %
	Prefix <sup>®</sup> fb Touchdown Total <sup>®</sup> (fomesafen/SMOC fb glyphosate)	2.34 LPR/ha fb 1.75 LPR/ha	MSO + AMS	PRE fb POST	100 %
	Boundary <sup>®</sup> fb Flexstar GT <sup>®</sup> (metribuzin/SMOC fb glyphosate/fomesafen)	2.34 LPR/ha fb 1.17 LPR/ha	MSO + AMS	PRE fb POST	100 %

\* All treatments following a 5.6 LPR/ ha Bicep II Magnum (atrazine/SMOC/benoxacor) treatment PRE; \*\* GPR = gram product; LPR = liter product; \*\*\* DAA = days after application

#### 4. Discussion

The experiments in the glasshouse and the field confirmed a resistance to post-emergent applied HPPD-inhibitors in the McLean County population of *A. tuberculatus*. The population is also cross-resistant to triazines and ALS-inhibitors. While the farmer had used a total of three herbicidal modes of action with *A. tuberculatus* activity (VLCFA, PSII and HPPD) the field studies revealed that the pre-emergence applications only provided very limited control (40 %) and that in post-emergence the HPPD-inhibitor was the main active ingredient with activity on this *A. tuberculatus* population. Therefore the only post-emergence mode of action to control the *A. tuberculatus* population was inhibition of HPPD for the last seven years. Besides being resistant to HPPD and PSII inhibitors the combination of mesotrione + atrazine still provided good control where single rates of each failed. This clearly confirms a synergistic activity of both herbicides working together.

In addition to this *A. tuberculatus* population in McLean County, IL, one more *A. tuberculatus* population has been confirmed to have developed resistance to post-emergence applied HPPD-inhibitors. This was also in a seed corn field in Iowa (MCMULLAN and GREEN, 2011). While the field history at this site is somewhat different than that at the McLean County site, the Iowa population was found to be cross-resistant to ALS- and PSII-inhibitors as well, and HPPD-inhibitors were the only active mode of action used in inbred corn. Due to the poor control *A. tuberculatus* has built up a huge seed bank in the soil and is the only weed species visible in untreated checks at very high densities.

The biggest problem seems to be the combination of *A. tuberculatus* and inbred corn lines in the seed corn production fields. While *A. tuberculatus* is highly competitive and germinates in several cohorts up to end of July the inbred corn lines provide little competition and never completely shade the ground between the rows to outcompete *A. tuberculatus*. In addition inbred corn lines are much more susceptible to herbicide damage, which could damage the plants directly, but also could affect the male and female lines flowering synchronization. Also herbicide tolerant traits may not be present in both female and male lines limiting the use of herbicides like glyphosate and glufosinate.

The resistance development to post-emergence applications of HPPD-inhibitors of this *A. tuberculatus* population is yet another example of overuse of a chemical weed control agent for one predominant weed over consecutive years of monoculture. This is similar to glyphosate overuse in cotton and soybeans, ALS-inhibitors in corn and rice to control *Echinochloa crus-galli* and ACCase- and ALS-inhibitors in cereals to control *Alopecurus myosuroides* and *Lolium* spp. (POWLES and YU, 2010).

Our studies identified alternative herbicide regimes to control the McLean County *A. tuberculatus* population in inbred corn, including full rates of pre-emergence herbicides like Lumax®. Also glyphosate, glufosinate and dicamba were very effective in controlling this population. Only they have limited tolerance in inbred corn lines and should be only used after discussion with the breeder. The best chemical solutions contained mixtures of several modes of action with activity on *A. tuberculatus*. Our studies demonstrated the importance of using the herbicides at full rates and at the recommended timing. Alternative measures to provide selectivity like shielded sprays with paraquat and post-directed applications to increase the selectivity of herbicides are part of the potential solutions.

The best option is to integrate chemical solutions with plant production efforts (NEVE et al., 2010; 2011). Rotating to soybeans and using a pre-emergence premix herbicide like Boundary® or Prefix® has shown to be very effective. If this is followed up with effective post-emergence products such as glufosinate or glyphosate containing mixtures (e.g. Flexstar GT®) these premixes along with competition of the soybean crop provided complete *A. tuberculatus* control in our field trials. Also rotating to hybrid corn would increase the options and enable the use of more herbicidal modes of action. Using robust rates without fear of crop damage and the higher competition of hybrid corn will help control late germinating *A. tuberculatus* more effectively.

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