Influence of the integration of ALS-tolerant varieties in crop rotations on the development of herbicide-resistant weeds

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Die Natur hat sich so viel Freiheit vorbehalten, dass wir mit Wissen und Wissenschaft ihr nicht durchgängig beikommen oder sie in die Enge treiben können.

Johann Wolfgang von Goethe (1749-1832)

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*	Significant, $p \leq 0.05$
**	Highly significant, $p \leq 0.01$
***	Highest significant, $p \leq 0.001$
®	Registered trademark
2,4-D	2,4-Dichlorophenoxyacetic acid
A. myosuroides	*Alopecurus myosuroides*
ACCase	Acetyl-coenzyme A carboxylase
AHAS	Acetohydroxyacid synthase
a.i.	Active ingredient
Ala	Alanine
ALS	Acetolactate synthase
AMARE	*Amaranthus retroflexus*
APESV	*Apera spica-venti*
Arg	Arginine
Asp	Asparagine
CAPS	Cleaved amplified polymorphic sequences
CHEAL	*Chenopodium album*
dCAPS	Derived cleaved amplified polymorphic sequences
DNA	Deoxyribonucleic acid
E. crus-galli	*Echinochloa crus-galli*
ECHCG	*Echinochloa crus-galli*
Eff.	Efficacy
e.g.	Example given
EU	European Union
F	Foramsulfuron
GALAP	*Galium aparine*
Gln	Glutamine
Gly	Glycine
GM	Genetically modified
ha	Hectare
HT	Herbicide-tolerant
HR	Herbicide resistance

I	Iodosulfuron
M	Mesotrione
MATIN	*Tripleurospermum perforatum*
MoA	Mode(s) of action
N	Nicosulfuron
n.a.	Not available
NTSR	Non-target-site resistance
OSR	Oilseed rape
p	Probability of error
PAPRH	*Papaver rhoeas*
PCR	Polymerase chain reaction
Pro	Proline
PS II	Photosystem two
R	Rimsulfuron
RFLP	Restriction fragment length polymorphism
RR	Homozygous resistant
RS	Heterozygous resistant
RY	Root yield
SB	Sugar beet
Ser	Serine
SETVI	*Setaria viridis*
SOLNI	*Solanum nigrum*
STEME	*Stellaria media*
t	ton
T	Thiencarbazone-methyl
T. perforatum	*Tripleurospermum perforatum*
Thr	Threonine
Trp	Tryptophane
TSR	Target-site resistance
UK	United Kingdom
U.S.	United States
WCS	Weed control strategy
WW	Winter wheat

1 General Introduction

Arable weeds are a major cause of yield losses in crop production and result in 34 % potential loss of crop yield, on average, worldwide (Oerke 2006). Weed species are well adapted to agricultural ecosystems (Baker 1974; Thompson 1998; Neuhauser et al. 2003) and compete with the crop for environmental resources such as nutrients, water, light and space. The effect on crop yield depends on the season of weed emergence. If crop germination is accompanied or closely followed by weed germination, higher yield losses are possible than with weeds that germinate at a later stage of crop development (Kropff 1988; Swanton et al. 1999). According to Cousens et al. (1987) and Kropff (1988) the relative time of weed emergence has a major effect on the yields of cereals and sugar beet. Bräutigam (1998) demonstrated that weed competition in sugar beet did not start before the 6 to 8 leaf stage and ended with the closure of the crop canopy. Simulation analyses by Lotz et al. (1990) showed that weeds emerging in spring barely affect winter wheat yield, contrary to weeds that emerge in autumn. Besides yield losses, weeds might be toxic for animals and humans, decrease food quality, hinder harvesting and could serve as hosts for pathogens and insects (Börner 1995; Wisler & Norris 2005). For these reasons, weed infestation must be kept at a low, acceptable level. In general, weed control strategies incorporate chemical and mechanical practices, cultivation, crop rotations, fertilization and hand weeding. Another tool in weed management is the development of herbicide-tolerant (HT) crops. The most important property of HT crops is the possibility of using active ingredients to which the crop was previously susceptible. The implementation of this technology may lead to alternatives for weed control. HT crops that are widely grown as approved varieties include soybean (*Glycine max* MERR.), maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), oilseed rape (*Brassica napus* L.), sugar beet (*Beta vulgaris* L.), and alfalfa (*Medicago sativa* L.) (Reddy & Nandul 2012). However, the additional use of an agent with a common mode of action (MoA) in a crop rotation must be weighed in terms of resistance management. Herbicides are the dominant weed control technology in most cases for economic reasons. Their intensive use has been the driving force for the development of herbicide resistance. The selection of resistant biotypes is accelerated by the repeated use of a herbicide over large areas, little or no use of herbicides with an alternative MoA and soil residual activity of the herbicide (Tranel & Wright 2002). Furthermore, the number of resistant weed species has increased significantly in recent years. In total, 152 dicot and 110 monocot species with herbicide resistance to one or more MoA are known globally (Heap 2021). The highest numbers of resistant weeds have been reported in the U.S., Europe, Australia and Canada. These resistant weeds were mostly found in major crops such as wheat, corn, soybean and rice (Heap 2021). The Poaceae family has the highest number of herbicide-resistant weed species (n=82), followed by the Asteraceae (n=42) (Heap 2021).

1.1 Weed resistance to ALS-inhibiting herbicides

Acetolactate synthase (ALS) herbicides are the most prone to resistance and the most affected herbicide MoA in Germany (Heap 2021). ALS-inhibiting herbicides have been widely used in agriculture since the introduction of the first sulfonylurea herbicide in the 1980s because they combine the advantages of broad-spectrum weed control at low rates, soil residual activity, wide application windows, and low mammalian toxicities (Mazur & Falco 1989). ALS inhibitors, also referred to as acetohydroxyacid synthase (AHAS), catalyze the first step in the branched-chain amino acid biosynthesis pathway. Two pyruvate molecules are condensed to form 2-acetolactate for valine and leucine biosynthesis and for isoleucine, 2-acetohydroxybutyrate is synthesized from pyruvate and 2-ketobutyrate (Umbarger 1978; Ray 1984). ALS herbicides act by inhibiting the ALS enzyme that is the common target site for the six herbicide chemical families sulfonylurea, imidazolinone, triazolopyrimidine, triazolinones, pyrimidinyl benzoates and sulfonanilides (Heap 2021) and leads to rapid growth cessation in susceptible species. Unfortunately, the first case of resistance was found only 5 years after the introduction of sulfonylurea herbicides on the market. Since then the number of weed species affected by ALS resistance has increased steadily. Today, ALS inhibitor resistance has been reported for 64 monocot and 101 dicot weed species worldwide (Heap 2021).

Herbicide resistance mechanisms can be categorized into two broad types of mechanism: non-target-site-based resistance (NTSR) and target-site resistance (TSR) (Délye 2005; Powles & Yu 2010; Délye et al. 2013). Both mechanisms may coexist in a population and even in the same plant (Letouzé & Gasquez 2001; Rosenhauer et al. 2013; Keshtkar et al. 2015). NTSR mechanisms include reduction in herbicide penetration due to alterations in cuticle properties and/ or plant habit, altered translocation of the herbicide away from the target protein, enhanced degradation of the herbicide or enhanced neutralization of cytotoxic molecules generated by herbicide action (Délye et al. 2013). NTSR is considered the major cause of resistance to ALS-inhibiting herbicides in grass weeds (Beckie & Tardif 2012; Délye et al. 2013) while these mechanisms have rarely been reported in broadleaf weeds. Dicot weed species affected by NTSR to ALS inhibitors are *Amaranthus hybridus* (Manley et al. 1999), *Amaranthus tuberculatus* (Guo et al. 2015), *Amaranthus palmeri* (Nakka et al. 2017), *Descurainia sophia* L. (Yang et al. 2016), *Papaver rhoeas* (Scarabel et al. 2015; Rey-Caballero et al. 2017), *Sagittaria trifolia* L. (Zhao et al. 2017) and *Sinapis arvensis* (Veldhuis et al. 2000). However, the mode of inheritance of genes responsible for NTSR is relatively poorly understood (Preston 2003; Petit et al. 2010). Evolved TSR is attributed to gene point mutation(s) causing amino acid changes in a target enzyme that are associated with reduced ALS enzyme sensitivity (Délye et al. 2013; Tranel & Wright 2002). This structural change at one out of several possible positions on the herbicide target protein prevents or reduces herbicide binding. Today, 28 resistance-endowing amino acid substitutions at eight positions of the *ALS* gene (Ala-122, Ala-205, Arg-377, Asp-376, Gly-654, Pro-197, Ser-653, Trp-574) have been identified (Heap 2021). The substitution of Trp-574 endows high levels of resistance to all ALS inhibitors (Bernasconi et al. 1995; Patzold & Tranel 2002), whereas Ser-670 and Ala-122 substitutions result in high levels of resistance to imidazolinones but not sulfonylurea resistance (Sathasivan et al. 1990; McNaughton et al. 2005). In contrast, substitution of Pro-197 usually confers resistance to sulfonylureas, but not imidazolinones (Scarabel et al. 2004; Délye et al.

2015). For example, a Pro-197 mutation conferring resistance to sulfonylurea herbicides has been reported for *Lolium rigidum*, whereas the same species with a Trp-574-Leu mutation resulted in resistance to both sulfonylurea and an imidazolinone herbicide (Yu et al. 2008).

1.2 Weed dynamics

Although there are several weed species that have evolved herbicide resistance to ALS inhibitors, this study focused on a few that have gained prominence in Europe and could become problematic in the context of HT crop production.

Alopecurus myosuroides (Huds.) is a widespread annual grass weed in winter crops in North-Western and Central Europe, particularly in the UK, France and Germany. It is a highly competitive weed, with the potential to produce many seeds (Lutman et al. 2013), and if not adequately controlled, infestation can cause substantial yield losses. Moss (2013) estimated that *A. myosuroides* populations of 12 to 25 plants per square metre can reduce winter wheat yield by 0.4 to 0.8 tons per hectare. In general, the occurrence of weeds and weed patches depends on numerous cultural techniques, e.g. the cultivated crop, crop rotation, crop residues, drilling date, fertilization, herbicide use and tillage practice. The occurrence of *A. myosuroides* is favoured by an increased proportion of winter cereals, non-ploughing cultivation systems and the regular use of herbicides for weed control (Moss & Clarke 1994). Moreover, the intensive use of selective herbicides without changing the MoA has resulted in the resistance of *A. myosuroides* to several groups of herbicides, via both TSR and NTSR (Moss & Clarke 1994; Beckie 2006). Resistance in *A. myosuroides* is mainly associated with ACCase (acetyl-coenzyme A carboxylase) and ALS inhibitors that are the most resistance-prone herbicides. These herbicides are frequently used to control grass weed species due to the low number of selective herbicide active ingredients available (Moss et al. 2007). As a result, populations exhibiting multiple resistance to these MoAs appear frequently (Marshall et al. 2012; Petersen and Raffel 2020). Previously detected mutations conferring ALS resistance are Pro-197-Thr and Trp-574-Leu (Délye & Boucansaud 2008).

Echinochloa crus-galli (L.) P. BEAUV. is one of the most problematic weeds in many crops, including maize (*Zea mays* L.), rice (*Oryza sativa* L.), cotton (*Gossypium hirsutum* L.) and other annual spring crops. This polyploid, predominantly self-pollinating summer annual weed species is ranked second globally as a weed that has evolved resistance to numerous MoAs (Heap 2021). It produces a large number of seeds, which results in significant soil seed banks (Maun & Barrett 1986; Norris 1992). In general, *Echinochloa* species are highly competitive and should be controlled soon after emergence to prevent yield losses (Maun & Barrett 1986; Bosnic & Swanton 1997). Yield losses depend on the degree of *E. crus-galli* infestation, which can reach up to 5 tons per hectare of maize grain (Rusu et al. 2010).

Tripleurospermum perforatum (MÉRAT) LAINZ or *Tripleurospermum inodorum* (L.) SCH. BIP. is a widely distributed weed species that is most commonly found as a weed of cereals and other annual field crops (Kay 1941). The number of capitula on a plant normally ranges from about 15 to 200, indicating a high potential reproductive capacity of approximately 1,800 to 36,000 achenes per plant (Kay 1994). The number of seeds per plant is likely to be highly variable, as Lutman (2002) estimated that a plant produces over 300,000 seeds in the autumn and 34,600 in the spring population, whereas Woo et al. (1991) reported up to 1,700,000 seeds per plant. In addition, achenes can survive for at least 10 years in soil with an optimum depth for germination at 0 to 0.5 cm (Kay 1994). Intensive herbicide use to control this weed has resulted in resistance to several active ingredients of ALS-inhibiting herbicides. In Europe, the first case of ALS resistance in *T. perforatum* was found in the UK (2002) (Moss et al. 2011). Leaf samples from plants surviving treatment with metsulfuron-methyl were subjected to molecular analysis and revealed a Pro-197-Gln mutation (Hull et al. 2014). In the UK, *T. perforatum* and *A. myosuroides* are among the most important herbicide-resistant weed species of arable crops (Hull et al. 2014). Further cases of ALS-resistant *T. perforatum* have arisen in Norway (2006), Germany (2009), Denmark and France (2010) as well as in Poland (2014) and Sweden (2015) (Heap 2021). In Germany, *T. perforatum* populations resistant to tribenuron have been reported owing to a Pro-to-Thr or a Pro-to-Ser substitution at the Pro-197 codon (Ulber et al. 2012).

1.3 Development of HT crops

Genetically modified (GM) or transgenic crops are plants in which the genetic material (DNA) has been altered using genetic engineering methods. The aim is to introduce a new trait to the plant that does not occur naturally by mating and/or natural recombination. Most of the commercially grown GM crops have a resistance to insects, resistance to virus infections, or tolerance towards certain herbicides. Since the introduction of GM crops in the U.S. in 1994, the global area of GM crops has increased to 191.7 million hectares in 2018. On average, the most planted biotech crops are soybeans (*Glycine max* L. MERR.), cotton (*Gossypium hirsutum* L.), sugar beet (*Beta vulgaris* L.) maize (*Zea mays* L.) and canola (*Brassica napus* L.) (ISAAA 2018). In Germany, the cultivation of GM crops in commercial agriculture has raised concerns about potential adverse effects on the environment and agronomic practice and has not yet been approved. According to Märländer & von Tiedemann (2006), GM crops and the complementary non-selective herbicides can achieve considerable ecological and economic advantages. However, another way to create HT varieties is by using conventional breeding approaches like mutagenesis or selecting for naturally occurring herbicide tolerance. These methods have been used to develop varieties that tolerate exposure to ACCase inhibitors (Somer 1996), ALS inhibitors (Newhouse et al. 1991) and photosystem-II inhibitors (Beversdorf & Kott 1987). Most of the non-GM HT crops such as rice, wheat, canola and sunflower (Devine 2005) include a tolerance to ALS inhibitors. One example of the development of HT varieties can be found in oilseed rape (OSR). These imidazolinone-tolerant hybrids are known under the Clearfield®-label (patent held by BASF SE) and have been approved for commercial cultivation in Germany since 2012. HT OSR offers the opportunity to selectively control cruciferous weed species with imidazolinone herbicides. Sugar beet genotypes that are tolerant to ALS inhibitors (foramsulfuron + thiencarbazone-methyl) have also recently been approved for commercial use in Germany.

1.4 Objective of the thesis

The cultivation of HT crops provides an alternative tool for weed control management. In the context of sustainable cropping systems, important agronomic questions concerning integrated herbicide management systems and the potentially increasing risk of the development of herbicide resistance in weeds need to be answered.

The general purpose of the first and second papers presented as part of this thesis is to evaluate the impact of ALS inhibitor intensity on susceptible and ALS-resistant weed species in a crop rotation including HT crop varieties. To date, no studies have taken into account their occurrence when using ALS-tolerant sugar beet hybrids (Paper No. 1) and imidazolinone-tolerant oilseed rape varieties (Clearfield® production system, Paper No. 2) and the consequences for crop management practice. The subjects of the investigations were *A. myosuroides* and *T. perforatum.* The results will help expand knowledge about the resistance dynamics of weed species in the cultivation of HT varieties.

As *E. crus-galli* is a hexaploid species, the third paper is concerned with the question of the number of *ALS* genes conferring resistance. Dose response curves and molecular analysis provide information about resistance patterns. The results confirm the importance of differentiation of the resistance mechanisms in polyploid weed species.

The fourth paper focuses on herbicide strategies that ensure the efficacy of the complementary herbicide (foramsulfuron + thiencarbazone-methyl) in the presence of susceptible as well as herbicide-resistant weed species in sugar beet cultivation. In addition, the selectivity of the herbicide was examined. This herbicide has been approved for commercial cultivation in Germany and therefore these results demonstrate potential strategies for herbicide management.

2 Development of herbicide resistance in weeds in a crop rotation with acetolactate synthase-tolerant sugar beets under varying selection pressure

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2.1 Summary

The development of acetolactate synthase- (ALS) tolerant sugar beet provides new opportunities for weed control in sugar beet cultivation. The system consists of an ALS-inhibiting herbicide (foramsulfuron + thiencarbazone-methyl) and a herbicide-tolerant sugar beet variety. Previously, the use of ALS inhibitors in sugar beet was limited due to the susceptibility of the crop to active ingredients from this mode of action. The postulated benefits of cultivation of the ALS-tolerant sugar beet are associated with potential risks. Up to now, with no relevant proportion of herbicide-tolerant crops in Germany, ALS-inhibitors are used in many different crops. An additional use in sugar beet cultivation could increase the selection pressure for ALS-resistant weeds. To evaluate the impact of varying intensity of ALS-inhibitor use on two weed species (*Alopecurus myosuroides* and *Tripleurospermum perforatum*) in a crop rotation, field trials were conducted in Germany in two locations from 2014 to 2017. Weed densities, genetic resistance background and crop yields were annually assessed. The results indicate that it is possible to control ALS-resistant weeds with an adapted herbicide strategy in a crop rotation including herbicide-tolerant sugar beet. According to the weed density and species, the herbicide strategy must be extended to graminicide treatment in sugar beet, and a residual herbicide must be used in winter wheat. The spread of resistant biotypes in our experiments could not be attributed to the integration of herbicide-tolerant cultivars, although the application of ALS-inhibitors promoted the development of resistant weed populations. Annual use of ALS inhibitors resulted in significant high weed densities and caused seriously yield losses. Genetic analysis of surviving weed plants confirmed the selection of ALS-resistant biotypes.

Keywords: *Alopecurus myosuroides*, ALS inhibitors, genetic analysis, sugar beet, *Tripleurospermum perforatum*, yield

2.2 Introduction

Sugar beet is highly susceptible to weed competition at early stages of development. To prevent possible yield losses of up to 95% (Petersen 2003), the crop must be kept free of weeds during juvenile development until the 8-leaf-stage (Kobusch 2003; Scott et al. 1979). Therefore, mixtures of different active ingredients are usually applied three to five times in a classical herbicide strategy (Märländer & Tiedemann 2006, Vasel et al. 2012). A novel option for weed control in sugar beets are acetolactate synthase- (ALS) tolerant sugar beets developed by Bayer CropScience AG and KWS SAAT SE. The developed system (Conviso® SMART) includes sugar beet hybrids that are tolerant to a complementary ALS-inhibiting herbicide. The herbicide contains 50 g a.i. L^{-1} foramsulfuron of the chemical group sulfonylureas and 30 g a.i. L^{-1} thiencarbazone-methyl belonging to sulfonyl-amino-carbonyl-triazolinones (Wegener et al. 2015). Sugar beet hybrids and the complementary herbicide are actually under approval in the EU member states (Wendt et al. 2017; Götze et al. 2018). The postulated advantages of the system are best crop safety, an overall lower number of required herbicide applications (Wendt et al. 2017) and high efficacy against almost all economically important weed species in sugar beet (Balgheim et al. 2016). However, these benefits are associated with potential risks. Up to now, with no relevant portion of herbicide-tolerant crop cultivars in Germany, ALS inhibitors are used in various crops, especially in cereals and maize. The increasing use of ALS inhibitors in recent years has led to 62 monocotyledonous and 99 dicotyledonous weed species showing resistance to ALS-inhibiting active ingredients worldwide (Heap 2019). The occurrence of herbicide-resistant weeds additionally limits the choice of herbicides. *A. myosuroides, Matricaria chamomilla, T. perforatum, Avena fatua, Stellaria media, Echinochloa crus-galli, Amaranthus retroflexus* and (less) *Apera spica-venti* belong to the group of the most important weeds in sugar beet cultivation (Börner 1995). In Germany, ALS resistance has already been demonstrated for all of these species (Heap 2019). Regarding *A. myosuroides* Huds., a diploid, annual grass weed, resistance to various modes of action has been reported in Germany. *T. perforatum* (MÉRAT) LAINZ (or *T. inodorum* SCH. BIP.) is also becoming increasingly problematic, as new resistance findings confirm (Ulber 2014; Hull et al. 2014). *T. perforatum* is a mass-growing species producing a high number of seeds. Inadequate control leads to a direct decline in crop yields and can result in a high degree of weed distribution in the following crop.

The use of ALS inhibitors in sugar beet was limited as the crop is comparably susceptible to this mode of action. The additional use of ALS inhibitors in sugar beet cultivation could lead to crop rotations in which one (or even multiple) use of ALS-inhibitors takes place in each year of the crop rotation. This would increase the risk of selecting ALS-resistant weeds. If ALS-tolerant sugar beet varieties are approved at the national level, the benefits of the system should be maintained for sugar beet cultivation and risks ought to be known.

The aim of this study was to investigate the effect of four herbicide strategies with varying intensity of ALS inhibitors used in a three year sugar beet – winter wheat crop rotation. The following hypotheses were tested: (i) In the presence of a low initial frequency of ALS-resistant weed species, an adapted herbicide strategy can ensure successful weed control. (ii) It is possible to integrate ALS-tolerant sugar beets in a crop rotation without increasing the risk of ALS-resistant weed selection.

2.3 Materials and methods

From 2014/15 to 2016/17, field trials were conducted in Bingen (loamy sand) and in Sickte near Braunschweig (clay loam), Germany. Four herbicide strategies were tested in a sugar beet (SB) – winter wheat (WW 1) – winter wheat (WW 2) crop rotation with four replicates. Each crop was represented with 16 plots in each trial year, thus, the study included 48 permanent plots per site. The crop rotation was SB (2015) – WW 1 (2016) – WW 2 (2017), WW 1 (2015) – WW 2 (2016) – SB (2017) and WW 2 (2015) – SB (2016) – WW 1 (2017). Before starting the experiments, oilseed rape was cultivated in Braunschweig and winter wheat in Bingen. After harvest, glyphosate treatment followed and cultivation was done by cultivator at both sites. In all years and on both trial sites soil tillage was done without using the plough.

Table 1 Characterization of herbicides used in the two field trials.

Product	**Active ingredients**	**Mode of action**	**HRAC-Group**
Atlantis® WG + FHS **(ATL)**	30 g a.i. kg^{-1} mesosulfuron-methyl 6 g a.i. kg^{-1} iodosulfuron-methyl-natrium	ALS-inhibition ALS-inhibition	B B
Axial® 50 **(AX)**	50 g a.i. L^{-1} pinoxaden	ACCase inhibition	A
Bacara® forte **(BF)**	120 g a.i. L^{-1} flufenacet 120 g a.i. L^{-1} flurtamone 120 g a.i. L^{-1} diflufenican	Inhibition of cell division Inhibition of PDS Inhibition of PDS	K3 F1 F1
Betanal® maxxPro **(BET)**	60 g a.i. L^{-1} phenmedipham 27 g a.i. L^{-1} lenacil 47 g a.i. L^{-1} desmedipham 75 g a.i. L^{-1} ethofumesat	Inhibition of photosynthesis at PS II Lipid synthesis inhibitor	C1 C1 C1 N
Cadou® SC **(CAD)**	508.8 g a.i. L^{-1} flufenacet	Inhibition of cell division	K3
Conviso® One OD 80 **(CON)** + Mero® **(ME)**	50 g a.i. L^{-1} foramsulfuron 30 g a.i. L^{-1} thiencarbazone-methyl 81 % rapeseed methyl ester	ALS-inhibition ALS-inhibition Adjuvant	B B
Duanti® **(DU)**	40 g a.i. L^{-1} fluroxypyr 200 g a.i. L^{-1} MCPA 20 g a.i. L^{-1} clopyralid	Synthetic auxin Synthetic auxin Synthetic auxin	O O O
Goltix® Titan® **(GT)**	525 g a.i. L^{-1} metamitron 40 g a.i. L^{-1} quinmerac	Inhibition of photosynthesis at PS II Synthetic auxin	C1 O
Gropper® SX **(GRO)**	192,65 g a.i. kg^{-1} metsulfuron	ALS-inhibition	B
Lontrel® 720 SG **(LON)**	720 g a.i. kg^{-1} clopyralid	Synthetic auxin	O
Pointer® SX **(POI)**	500 g a.i. kg^{-1} tribenuron-methyl	ALS-inhibition	B
Select® 240 EC **(SEL)** + Para Sommer	241,9 g a.i. L^{-1} clethodim 654 g a.i. L^{-1} paraffin oil	Inhibition of ACCase Adjuvant	A

The herbicides used are characterized in Table 1 and weed control strategies are given in Table 2. For this study, a sugar beet genotype, tolerant to ALS-inhibiting herbicides (experimental hybrid, not registered yet in Germany), was provided by KWS SAAT SE, Einbeck (Germany). Field trials were conducted in a randomized block design. Each of the four blocks represented all three crops and all four herbicide strategies (12 plots per block).

In Bingen, plot size was 7.5 x 12.0 m and the distance between the plots was 9.0 m and 12.0 m in sowing direction. Plot size in Braunschweig was 6.0 x 30.0 m. The distance between the plots was 3.0 m and 18.0 m in sowing direction.

Table 2 Herbicide treatments in field trials from 2014/15 to 2016/17.

Weed Control Strategy (WCS)	**Treatment No.**	**Crop**	**Herbicide (Dose [g, l ha^{-1}])**[a]	**Treatment Timing**
Use of ALS-inhibitors in all crops and years **(WCS I)**	1	SB	CON (1.0)	post-emergent 2
	5	WW 1	POI + ATL (60 + 500)	spring, post-emergent 1
	9	WW 2	POI + ATL (60 + 500)	spring, post-emergent 1
Use of ALS-inhibitors in two of three years **(WCS II)** Site **Bingen**	2	SB	CON fb. LON fb. SEL 1.0/ 0.167/ 0.5	post-emergent and 14, 21 days later than first treatment, respectively
	6	WW 1	POI + ATL (60 + 500)	spring, post-emergent 1
	10	WW 2	BF + CAD fb. AX fb. DU (0.75 + 0.3 fb. 1.2 fb. 3.0)	autumn, pre-emergent fb. spring, post-emergent 1 fb. post-emergent 2
Use of ALS-inhibitors in two of three years **(WCS II)** Site **Braunschweig**	2	SB	GT + BET fb. SEL 3 x 1.5 + 3 x 1.25 fb. 0.5	early-post, post-emergent and 14, 21 days later than first treatment, respectively
	6	WW 1	ATL fb. GRO (500 fb. 40)	spring, post-emergent 1 fb. post-emergent 2
	10	WW 2	ATL fb. GRO (500 fb. 40)	spring, post-emergent 1 fb. post-emergent 2
Use of ALS-inhibitors only in sugar beet **(WCS III)**	3	SB	CON 2 x 0.5	post-emergent 1 fb. post-emergent 3
	7	WW 1	BF + CAD fb. AX fb. DU (0.75 + 0.3 fb. 1.2 fb. 3.0)	autumn, pre-emergent fb. spring, post-emergent 1 fb. post-emergent 2
	11	WW 2	BF + CAD fb. AX fb. DU (0.75 + 0.3 fb. 1.2 fb. 3.0)	autumn, pre-emergent fb. spring, post-emergent 1 fb. post-emergent 2
No use of ALS-inhibitors **(WCS IV)**	4	SB	GT + BET fb. SEL 3 x 1.5 + 3 x 1.25 fb. 0.5	early-post, post-emergent 2 and 14, 21 days later than first treatment, respectively
	8	WW 1	BF + CAD fb. AX fb. DU (0.75 + 0.3 fb. 1.2 fb. 3.0)	autumn, pre-emergent fb. spring, post-emergent 1 fb. post-emergent 2
	12	WW 2	BF + CAD fb. AX fb. DU (0.75 + 0.3 fb. 1.2 fb. 3.0)	autumn, pre-emergent fb. spring, post-emergent 1 fb. post-emergent 2

fb., followed by; SB, sugar beet; WW 1, winter wheat after sugar beet; WW 2, winter wheat after winter wheat; [a] see Table 1 for abbreviation of herbicides

To establish sufficient populations of susceptible and ALS-resistant weeds, a defined amount of seeds (in the ratio 1:10, resistant to susceptible) of two weed species were sown by hand once at the beginning of the field trials (Tab. 3). For the study, *A. myosuroides* (monocotyledonous) and *T. perforatum* (dicotyledonous) were selected as important weeds with ALS-resistance. Both species showed target-site resistance to ALS-inhibiting herbicides (Tab. 3). For the ALS-resistant *A. myosuroides* population, a pronounced metabolic resistance to Axial® 50 (pinoxaden) was additionally found in previous studies (data not shown). In Bingen, seeds were sown before drilling of winter wheat in October, 2014 and in Braunschweig before drilling of sugar beet in March, 2015. *A. myosuroides* had not previously been found at both trial sites. However, *T. perforatum* populations were present at both sites before the experiment started.

Table 3 Characterization of weed species used in the field trials.

Weed species	**EPPO-Code**	**Seed amount (no. per m $^{-2}$)**	**Origin**	**Biotype**
A. myosuroides	ALOMY	86	Appels Wilde Samen	Susceptible
		8	Pellworm	ALS-resistant, Trp 574 Leu Metabolic resistance to pinoxaden
T. perforatum	MATIN	385	Appels Wilde Samen	Susceptible
		42	Freiburg/ Elbe	ALS-resistant, Pro 197 Gln

The herbicide application was conducted with a one-wheel plot sprayer (Air mix 110-025 flat fan, pressure 210 kPa, water amount 200 L ha $^{-1}$, speed 4.5 km h $^{-1}$) with an effective spraying width of 2.5 m in Bingen and a pneumatic sprayer (Rau Spridomat, nozzle type TeeJet Turbo TwinJet® TTJ60-11004, pressure 320 kPa, water amount 300 L ha $^{-1}$, speed 6.1 km h $^{-1}$) with an effective spraying width of 12.0 m in Braunschweig.

Weed density assessments

Emerged *A. myosuroides* and *T. perforatum* plants were repeatedly counted during the cropping season. In winter wheat, the first assessment was done before the beginning of winter after autumn herbicide treatment and the second one was carried out after herbicide treatments in spring. In sugar beet, the weed densities were assessed two to three weeks after herbicide application of each weed control strategy. The assessments were made with a quadratic counting frame of 0.25 m^{-2}. A total of twelve squares were assessed in each plot. The number of *A. myosuroides* heads was additionally counted at the main flowering time. Other weeds occurring were also recorded at both sites.

Molecular analysis

To evaluate the genetic resistance background, surviving plants of *A. myosuroides* and *T. perforatum* were examined by means of molecular genetic analysis. Leaf samples of *A. myosuroides* and *T. perforatum* were collected from the trial plots, dried at room temperature and sent to IDENTXX GmbH (Stuttgart, Germany) for analysis of potential target-site mechanisms. For each treatment and crop, a maximum of 15 individual plants per plot and species were included in the study. Lower sample numbers were due to reduced occurrences of the species in the plots.

The molecular genetic analysis was performed for the originally established mutations, which modify the amino acid sequence of the ALS enzyme. These are the positions encoding for Trp-574 (wild type: TGG in *A. myosuroides*) and Pro-197 (wild type: CCA in *T. perforatum*) at the *ALS* gene, respectively. The DNA was extracted from the dried leaf material using a commercially available kit (chemagic Plant DNA Kit, Chemagen). PCR amplification was done using a MangoTaq DNA polymerase (Bioline, Germany) in combination with primers developed by IDENTXX GmbH. The PCR products were analyzed by pyrosequencing on a PyroMark Q24 (Quiagen, Hilden, Germany) pyrosequencer (Ronaghi & Elahi 2002).

The detected genotypes (homozygous susceptible, heterozygous resistant (RS) and homozygous resistant (RR)) are given in Table 4. Within three trial years, 1593 / 1006 (Bingen/ Braunschweig) leaf samples of *A. myosuroides* and 893 / 1453 samples of *T. perforatum* were analyzed, respectively.

Table 4 DNA-sequences and deduced amino acids at the positions Trp-574 (*A. myosuroides*) and Pro-197 (*T. perforatum*) of the *ALS* gene.

	wild type, susceptible	**heterozygous resistant (RS)**	**homozygous resistant (RR)**
***A. myosuroides* (574)**			
DNA sequence	TGG	TG/TG	TTG
amino acid	Trp	Trp/ Leu	Leu
***T. perforatum* (197)**			
DNA sequence	CCA	CC/ AA	CAA
amino acid	Pro	Pro/ Gln	Gln

Gln, glutamine; Leu, leucine; Pro, proline; Trp, tryptophan

Determination of yield

In Bingen, crop yield was assessed for three core rows (18.0 m^{-2}) of sugar beet and three core plots (16.5 m^{-2}) of winter wheat per plot and trial year. In Braunschweig, the size of the harvested core plot was 27 m^{-2} for sugar beet and 45 m^{-2} for winter wheat. A representative winter wheat sample was taken from each plot, each year and site and grain yield was determined at 14 % moisture content. Sugar beet quality parameters were determined according to standardized procedures and white sugar yield was calculated to standard formulas (Märländer *et al.*, 2003). To compare the effect of the four weed control strategies, sugar beet root yield and grain yield are therefore shown as relative data.

Data analysis

Statistical analysis was carried out using the statistic program R, version 3.2.2 (R Development Core Team, 2015). The evaluation of weed density was based on absolute values and was done separately for each weed species and weed control strategy. For the analysis of *A. myosuroides* head numbers and *T. perforatum* plant numbers a non-parametric ranking method according to Kruskal-Wallis and following Nemenyi-Test was used because of missing normal distribution. Yield data were converted to relative values in relation to the mean values of the herbicide strategy IV ("no ALS inhibitors") before the analysis. After testing for variance homogeneity and normal distribution, yield data were investigated using ANOVA, following Tukey HSD post hoc tests ($P<0.05$). No block effects were detected.

2.4 Results

Development of weed density

To assess the impact of intensity of ALS-inhibitor use in the crop rotations on weed development, the number of *A. myosuroides* heads and *T. perforatum* plants were counted and compared to the weed control strategy (WCS) using no ALS inhibitors (WCS IV). After the second trial year, a strong increase in *A. myosuroides* head number was observed in all strategies including an application of an ALS inhibitor (Fig. 1). The weed control strategy had a significant impact on the number of *A. myosuroides* heads in Bingen in 2016 and 2017. WCS I (ALS inhibitors in all crops and years) achieved the significant highest level of *A. myosuroides* infestation in 2016 and 2017.

In Braunschweig, only low numbers of *A. myosuroide*s plants occurred in all trial years. The maximum number of surviving plants varied between 5 (2016) and 23 (2017) plants m^{-2}. However, WCS I showed a trend for the highest density of *A. myosuroide*s (data not shown).

Similar to the *A. myosuroides* density, a significantly higher number of surviving *T. perforatum* plants was observed under WCS I (ALS inhibitors in all years and crops) in Bingen in 2016 and 2017 (Table 5). In Braunschweig, all WCS including an application of ALS-inhibitors (WCS I-III) showed predominantly significant higher numbers of surviving *T. perforatum* plants in 2016. In 2017, *T. perforatum* plant densities were highest under WCS I (120.8 plants m^{-2}).

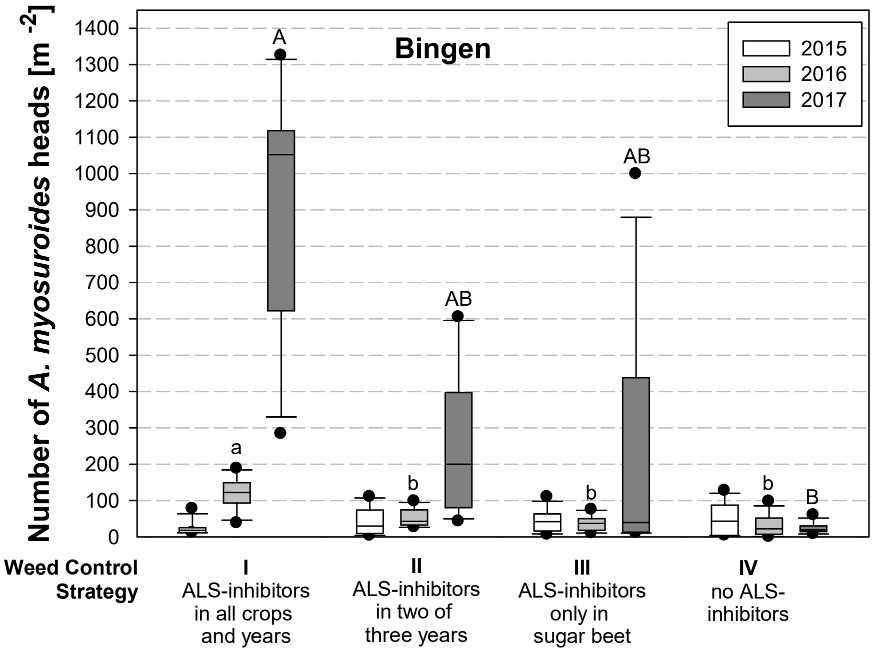

Figure 1 Development of *A. myosuroides* heads depending on weed control strategies in Bingen from 2015 to 2017. Each trial year shows the summarized results of all plots (=crops) belonging to the same WCS. The box represents the 25th and 75th quartiles, the median corresponds to the bold line and the whiskers show the minimal and the maximal values, not including outliers (black circles). Comparisons were carried out using a Kruskal-Wallis test, significant at $\alpha<0.05$.

Table 5 Number of surviving *T. perforatum* plants per m^2 from 2015 to 2017. Different capital letters and small letters indicate significant differences between weed control strategies (WCS) within each trial year and site (Kruskal-Wallis, Post-hoc test Nemenyi, $p \leq 0.05$), SE – standard error.

	2015		**2016**		**2017**	
WCS	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
Bingen						
I	2.6^{a}	2.12	5.6^{A}	5.00	9.7^{a}	19.46
II	2.6^{a}	1.71	2.0^{B}	2.16	2.8^{b}	3.20
III	4.0^{a}	2.32	1.2^{B}	1.93	1.0^{b}	1.59
IV	3.8^{a}	1.80	0.1^{B}	0.26	1.0^{b}	2.06
Braunschweig						
I	5.7^{A}	9.43	24.5^{a}	28.60	120.8^{A}	83.44
II	10.3^{A}	13.58	31.7^{a}	37.23	6.8^{B}	12.76
III	2.2^{A}	2.88	18.3^{ab}	40.28	4.1^{B}	7.32
IV	1.8^{A}	2.41	1.8^{b}	3.71	0.4^{B}	1.03

In the sugar beet plots residual weed plants of *Chenopodium album* and *Amaranthus retroflexus* were left in Bingen. WCS IV showed highest number of surviving plants (data not shown). Due to low densities no effects on yield occurred in the winter wheat plots by uncontrolled weeds (*Myosotis arvensis, Stellaria media, Veronica sp.*, *Viola arvensis*, e.g.) at both trial sites.

Molecular analysis

The results of the molecular analysis showed the distribution of susceptible and resistant fractions of *A. myosuroides* and *T. perforatum* from surviving individuals (Table 6 and 7).

Fluctuating sample numbers were due to low survival rates of the weed plants after herbicide application. For the leaf samples taken from *A. myosuroides*, target-site mutation for the codon encoding Trp-574 was mostly heterozygous at both sites. Overall, the portion of resistant plants in WCS I to III (strategies including ALS-inhibitors) was very high at 78-100 % after the third trial year. The number of homozygous resistant individuals increased from the first to the second year and decreased again in the following year.

But also in the ALS-free strategy (WCS IV), high fractions of resistant plants of *A. myosuroides* were observed with more than 70 %.

Table 6 Molecular analysis of *A. myosuroides* (Trp-574) depending on weed control strategies (WCS) at sites in the experimental years 2015-2017. WCS I- ALS-inhibitors in all crops and years, WCS II- ALS-inhibitors in two of three years, WCS III- ALS-inhibitors only in sugar beet, WCS IV- no ALS-inhibitors. Each trial year shows the summarized results of all plots (=crops) belonging to the same WCS.

		Bingen				Braunschweig			
WCS	**Year**	**Total no. of analyzed plants**	**RR (no. of plants)**	**RS (no. of plants)**	**Plants with HR (%)**	**Total no. of analyzed plants**	**RR (no. of plants)**	**RS (no. of plants)**	**Plants with HR (%)**
I	2015	135	1	111	**83**	60	12	48	**100**
	2016	135	31	68	**73**	136	72	60	**94**
	2017	135	0	135	**100**	178	51	120	**96**
II	2015	135	0	129	**96**	60	9	51	**100**
	2016	135	38	43	**60**	103	49	53	**98**
	2017	135	1	134	**100**	102	17	64	**79**
III	2015	135	1	101	**76**	23	3	20	**100**
	2016	135	8	67	**56**	56	8	43	**94**
	2017	135	0	131	**97**	120	15	79	**78**
IV	2015	132	0	49	**37**	40	3	3	**15**
	2016	111	30	37	**60**	68	11	27	**55**
	2017	135	4	92	**71**	60	5	37	**70**

RR - homozygous resistant; RS - heterozygous resistant; HR - herbicide resistance.

In the first two trial years, target-site resistance in *T. perforatum* occurred mainly heterozygous. In contrast to this, in 2017, most resistant individuals of *T. perforatum* (81 %) were homozygous for the resistant Pro-197 allele, with 19 % heterozygous individuals. In general, WCS containing an ALS application (WCS I-III) caused high rates of resistant individual plants (>94 %) after three years.

Table 7 Molecular analysis of *T. perforatum* (Pro-197) depending on weed control strategies (WCS) in the experimental years 2015-2017. WCS I- ALS-inhibitors in all crops and years, WCS II- ALS-inhibitors in two of three years, WCS III- ALS-inhibitors only in sugar beet, WCS IV- no ALS-inhibitors. Each trial year shows the summarized results of all plots (=crops) belonging to the same WCS.

		Bingen				Braunschweig			
WCS	**Year**	**Total no. of analyzed plants**	**RR (no. of plants)**	**RS (no. of plants)**	**Plants with HR (%)**	**Total no. of analyzed plants**	**RR (no. of plants)**	**RS (no. of plants)**	**Plants with HR (%)**
I	2015	83	3	78	**98**	149	3	92	**67**
	2016	130	9	69	**60**	174	72	100	**99**
	2017	135	130	4	**99**	180	108	70	**99**
II	2015	76	0	75	**99**	166	9	99	**66**
	2016	90	2	60	**69**	179	106	73	**100**
	2017	62	61	1	**100**	93	79	14	**100**
III	2015	78	1	72	**92**	66	0	44	**73**
	2016	41	0	39	**95**	134	50	68	**73**
	2017	90	84	1	**94**	54	37	16	**98**
IV	2015	55	0	15	**27**	83	0	13	**14**
	2016	10	0	1	**10**	102	5	16	**19**
	2017	43	15	4	**44**	73	34	21	**75**

RR - homozygous resistant; RS - heterozygous resistant; HR - herbicide resistance.

Determination of yield

The effect of the WCS on yield is shown relative to the ALS-free strategy (WCS IV). In Bingen, relative root yield (RY) was affected significantly by the weed control strategy in 2017 (Fig. 2). RY decreased strongly with increasing number of weeds. WCS II (ALS inhibitors in two of three years) and WCS III (ALS inhibitors only in sugar beet) even achieved higher relative RY in 2016 (not significant) and 2017 (significant) than WCS IV (no ALS inhibitors). In Braunschweig similar observations were made for WCS I (ALS inhibitors in all crops and years) with decreasing RY due to high weed densities. However, statistical analysis of RY at the trial site in Braunschweig showed no significant difference between the WCS most likely due to heterogeneous soil properties.

No significant differences in sugar content and quality parameters were found between the different treatments.

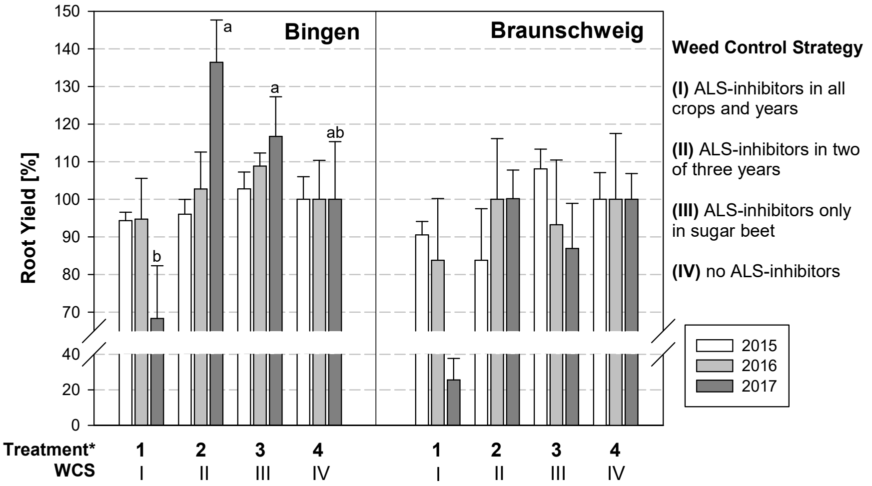

Figure 2 Root yield relative to WCS IV (no ALS-inhibitors) depending on weed control strategy from 2015 to 2017 in Bingen and Braunschweig. The whiskers show the maximal values and different small letters indicate significant differences between WCS within each trial year (Tukey, P≤0.05). *For details of weed control strategies - WCS, see Table 2.

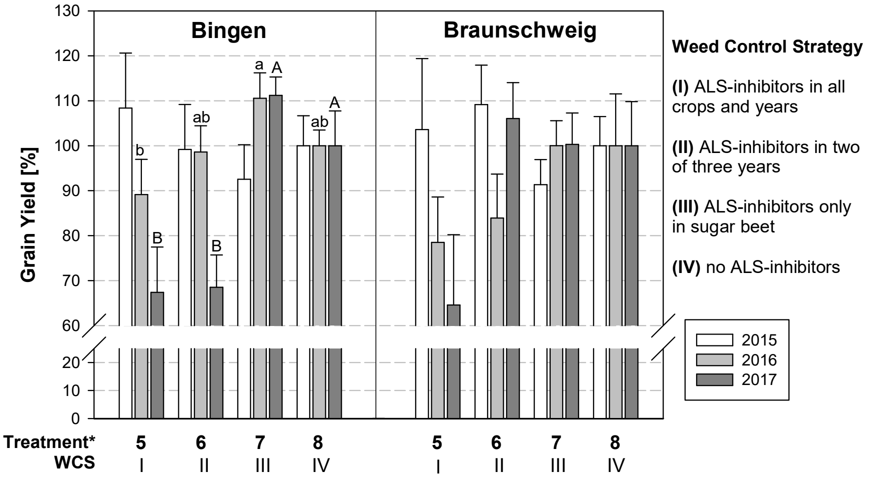

Figure 3 Grain yield (WW 1, winter wheat after sugar beet) relative to WCS IV (no ALS inhibitors) from 2015 to 2017 in Bingen and Braunschweig depending on herbicide treatment in a non-sensitive sugar beet genotype. Treatment 8 (100 %) Bingen: 7.7 t ha^{-1} (2015), 7.0 t ha^{-1} (2016), 6.0 t ha^{-1} (2017); Treatment 8 (100 %) Braunschweig: 7.6 t ha^{-1} (2015), 8.9 t ha^{-1} (2016), 8.2 t ha^{-1} (2017). The whiskers show the maximal values and different small letters and capital letters indicate significant differences between WCS within each trial year (Tukey, P≤0.05). *For treatment details, see Table 2.

Across all years and trial sites, lower grain yields were observed under WCS I and WCS II (Fig. 3 and 4) compared to WCS IV. Already in the second trial year, the relative grain yield under WCS I in Bingen was reduced for WW 1 (winter wheat after sugar beet; significant) and WW 2 (winter wheat after winter wheat; not significant). Finally, the three-time application of ALS inhibitors (WCS I) led to a significant decrease in relative yields in 2017. Although grain yields in Braunschweig were not correlated with weed density due to soil differences, WCS I showed a similar development to Bingen. Nevertheless, the grain yield decline under this herbicide strategy can be attributed to the high *T. perforatum* density.

Relative grain yield was also reduced under WCS II. While there was a clear decline in relative yields in Bingen in the third trial year, this trend could not be observed in Braunschweig.

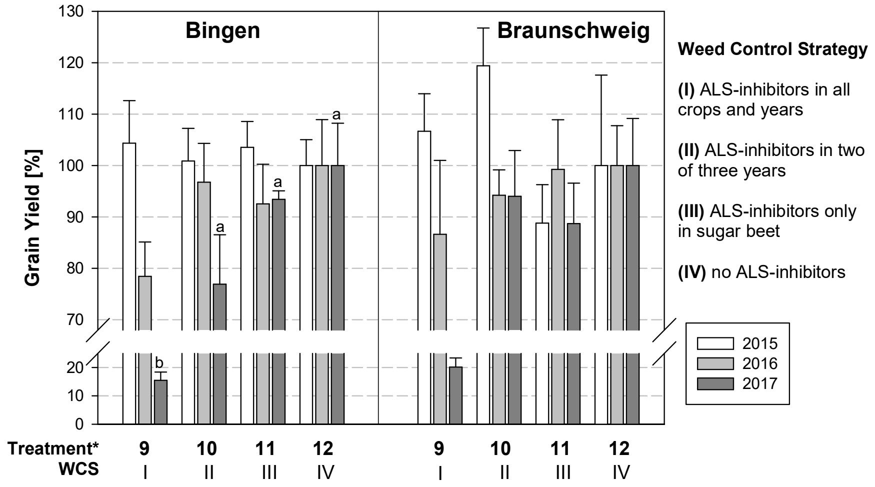

Figure 4 Grain yield (WW 2, winter wheat after winter wheat) relative to WCS IV (no ALS inhibitors) from 2015 to 2017 in Bingen and Braunschweig depending on herbicide treatment in a non-sensitive sugar beet genotype. Treatment 8 (100 %) Bingen: 7.3 t ha^{-1} (2015), 4.7 t ha^{-1} (2016), 4.7 t ha^{-1} (2017); Treatment 8 (100 %) Braunschweig: 7.6 t ha^{-1} (2015), 7.9 t ha^{-1} (2016), 7.7 t ha^{-1} (2017). The whiskers show the maximal values and different small letters and capital letters indicate significant differences between WCS within each trial year (Tukey, P≤0.05). *For treatment details, see Table 2.

2.5 Discussion

Introduction of herbicide-tolerant varieties could offer new opportunities for weed control in sugar beet. However, in the context of an increasing number of ALS-resistant weed species, this study addressed the herbicide efficacy of ALS inhibitors and the dynamics of dispersal of resistant and susceptible *A. myosuroides* and *T. perforatum* in a sugar beet, winter wheat, winter wheat crop rotation containing a herbicide-tolerant sugar beet variety. For this reason, resistant weed populations of *A. myosuroides* and *T. perforatum* were sown in the field trials before the start of the experiment.

In Bingen, a strong increase in infestation number of *A. myosuroides* (WCS I to III) was noticeable after the second trial year. A similar observation was made by Chauvel *et al.* (2001). *A. myosuroides* density increased unexpectedly after unfavourable weather conditions in two previous seasons. Decreasing root yield under WCS I (2017) was observed as well as decreasing wheat grain yields under WCS I and WCS II (2016 and 2017). These results indicate an insufficient weed control. Essentially an additional herbicide application is required.

In Braunschweig, WCS I showed a similar development to Bingen. Declining grain yields and root yields could be attributed to the high *T. perforatum* density, despite of soil heterogeneity. In principle, grain yield was not correlated to weed density as soil heterogeneity affects crop growth and weed development (Gerhards & Oebel 2006). Therefore, the use of ALS-inhibitors in two of three years is possible, but should be supplemented by alternative mode of action.

A. myosuroides head number and *T. perforatum* densities were lowest in response to herbicide applications in WCS IV (no ALS inhibitors in all three trial years). On the contrary, high numbers of residual weeds (*Chenopodium album* and *Amaranthus retroflexus*) were left in this classic herbicide treatment in Bingen, which apparently affected the yield.

Target-site resistances confirmation tests of surviving individuals of *A. myosuroides* and *T. perforatum* provided information about the resistance situation in the trials. It is known, that ALS is a nuclear gene and resistant ALS alleles are disseminated by pollen and seed (Tranel & Wright, 2002). The results of the study showed that the examined weeds had different resistance characteristics. Both susceptible and resistant biotypes of *A. myosuroides* and *T. perforatum* survived herbicidal treatments. The proportions of susceptible, heterozygous resistant and homozygous resistant genotypes varied among the two weed species as did the herbicide efficacy. Gehring & Thyssen (2014) and Moss & Hull (2009) pointed out that the expected herbicidal performance depends on the degree of resistance. Thus, increasing levels of resistance were associated with decreasing herbicidal activity. Regarding *A. myosuroides*, high rates of target-site resistance were observed in all strategies including an ALS inhibitor application (WCS I to III) throughout the trial period. Analysis of ALS-resistant *T. perforatum* fractions showed similar findings and surviving rates higher than 94 % were observed in 2017. As both resistant weeds that were initially sown to build up a resistant weed population in the field are not self pollenating species, a mixture of homozygous and heterozygous individuals can be assumed. Homozygosity and heterozygosity were determined in the leaf material of the individual plants. Therefore, we have no information about the zygosity status of the seeds. Possibly, mainly heterozygous individuals emerged in the third year. Apart from this, it is possible that some wild type individuals, that had been sown in the first year as well, emerged from the soil seed bank after herbicide application. They could have crossed with resistant individuals producing heterozygous offspring again.

This suggests that the frequency of ALS inhibitor application affects the spread of resistance for a given ALS-resistant weed population in the field. These data are consistent with previous studies which confirm the development of resistant populations by repetitive use of herbicide with similar modes of action (Christoffers 1999; Tranel & Wright 2002; Powles & Yu 2010). Consequently, the repeated use of ALS inhibitors favored the development of ALS-resistant populations. A reason for the observed high target-site resistance rate is the genetic background of ALS-inhibiting herbicide resistance, i.e., that resistant alleles are selected even when present at heterozygous condition. ALS resistance is usually conferred by a single, dominant, nuclear-encoded gene which causes the high frequency of occurrence of resistance to ALS-inhibitors (Tranel & Wright 2002).

The analysis of resistant *A. myosuroides* plants showed different proportions of the heterozygous and homozygous genotypes where heterozygousity was dominant. Wagner & Belz (2014) observed for the active ingredients cycloxydim and clethodim that plants heterozygous for ACCase target-site resistance showed a weaker phenotypic expression of resistance than homozygous plants. If similar effects were found for ALS-inhibitors, herbicidal activity could be reclassified due to the quantification of genotypes. However, in the present study, no apparent differences in herbicidal efficacy were observed under field conditions.

Surviving *A. myosuroides* plants under WCS IV (no ALS inhibitors) showed the lowest TSR levels. Furthermore, a steady increase in number of surviving plants with target-site resistance has been recorded for both sites from 2015 to 2017. This effect cannot be explained by herbicide selection during the experimental period. However, although plots were isolated from each other in the trials due to certain distances, it cannot be excluded that pollen or seed dispersal transferred the resistance trait also into plots of WCS IV. Similar observations were made by Délye et al. (2010). A massive pollen flow was thought to be responsible for the transfer of herbicide-resistant *A. myosuroides* populations from conventional to neighboring organic fields.

The proportion of surviving *T. perforatum* plants with a target-site mutation in the *ALS* gene ranged from 44 % (Bingen) to 75 % (Braunschweig) under WCS IV (no ALS inhibitors). An interesting aspect of TSR development has been observed for nearly all four WCS. While the herbicide resistance trait occurred heterozygous in the first two years of the trial, predominantly plants homozygous for the resistance trait were recorded in the third year. Homozygous biotypes may occur as a result of gene transferring and out-crossing of the resistance trait via pollen. *T. perforatum* is an insect pollinating species (Kay 1969) and thus the spread of the resistance traits depends on the means of pollen movement through insects (spatial gene transfer) (Bagavathiannan *et al.* 2013). In principle, the survival of seeds in the soil may also result in temporal gene transfer (Gruber 2004).

In this paper the management of ALS-resistant weeds in a crop rotation with herbicide tolerant sugar beet were examined for chemical options only. In any case, the principles of integrated weed management should be applied and weeds of any species should preferably or additionally be controlled through non-chemical methods. The transfer of the results into practice is not easily possible because resistant weeds were deliberately introduced and thus an artificial selection pressure was generated. However, this experimental approach shows the effects of using different ALS-inhibitors intensities in a crop rotation with herbicide-tolerant sugar beets. The benefits of the system must be carefully considered when weeds with resistance to ALS-inhibitors occur in the field.

2.6 Conclusions

The first hypothesis of the study suggested that an adapted herbicide strategy could ensure successful weed control in herbicide-tolerant varieties even in the presence of ALS-resistant weeds. The tested weed control strategies showed the feasibility of successful weed control in herbicide-tolerant sugar beet hybrids and winter wheat with the exception of WCS I (ALS inhibitors in all crops and years). However, additional herbicides with different modes of action are needed to control ALS-TSR weeds in the crop rotation and/ or in the ALS-tolerant crop. This strategy can be successful as long as effective herbicides from different modes of action are available at the scale of the crop as well as the rotation. After one crop rotation, it seems to be possible that moderate weed infestation of ALS-resistant *A. myosuroides* and *T. perforatum* can be controlled by the use of ALS-inhibitors plus alternative herbicides. But overall, it is recommended to minimize the frequency of use of ALS inhibitors when ALS-resistant weeds appear. Even the two-time use of ALS inhibitors in crop rotation could result in a high number of ALS-resistant weeds within a few years. In addition, it should be mentioned that the occurring scenario was povoked by introduction of herbicide-resistant weed seeds at the beginning of the experiment. Against this background, the speed of resistance increase has to be considered.

In the second hypothesis of the study it was assumed that it is possible to integrate ALS-tolerant sugar beets in a crop rotation without increasing the risk of selection of ALS-resistant weeds. The results of the study showed that, regardless of the weed control strategy, a progressive resistance selection process took place during the trial period. Even the alternative modes of action used in the ALS-free years could not stop the increase in resistant individuals in the weed population. However, this is due to the close neighborhood of resistant weed populations. Therefore, no correlation could be observed between herbicide-tolerant varieties and selection of ALS-resistant weeds. It must be considered that these effects could be influenced by the design of the experiments as the transport of seeds by tillage equipment and the spread of pollen by wind and insects cannot be excluded. Consequently, ALS-tolerant sugar beets were not the cause of the resistance distribution. Rather, it was the frequency of ALS-inhibitor use.

All in all, the association of the tolerant variety and its complementary ALS-inhibiting herbicide can be a useful tool for integrated weed management. The number of herbicide applications in sugar beet could be significantly reduced compared to current standards. Nevertheless, all available measures for avoiding resistance must be included throughout the crop rotation.

3 Response of *Alopecurus myosuroides* HUDS. to varying intensity of acetolactate synthase inhibiting herbicides in a crop rotation including imidazolinone-tolerant oilseed rape

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3.1 Abstract

Herbicide-tolerant winter oilseed rape (OSR) varieties offer the opportunity of using imazamox for weed control, an active ingredient belonging to the chemical group of acetolactate synthase- (ALS-) inhibitors. However, ALS inhibitors are used in many different crops and are the most resistance-prone herbicide mode of action. Their frequent application in a crop rotation increases the selection pressure for ALS herbicide resistance in weeds, which has to be considered when designing resistance management strategies. *Alopecurus myosuroides* Huds. is a frequent and economically important grass weed in Northwestern Europe, which has evolved resistance to several herbicide modes of action.

For the sustainable use of herbicide-tolerant OSR varieties, studies on the effects of the different intensities of ALS inhibitor use on *A. myosuroides* population dynamics and resistance development are required.

Two field trials were conducted including susceptible and multiple resistant *A. myosuroides* individuals and four weed control strategies varying in their intensity of ALS inhibitor use over a three-year trial period. *A. myosuroides* head numbers, the presence of target-site mutations in surviving plants, and crop yields were assessed annually, and the amount of *A. myosuroides* seeds in the soil seed bank was determined at the end of the trial period.

The results show that the intensity of ALS inhibitor use significantly influenced the density of *A. myosuroides* and the development of resistance. Under weed control strategy IV (no ALS inhibitors), an increase in *A. myosuroides* head number was observed due to multiple resistance in the *A. myosuroides* population employed in the field trial. None of the four weed control strategies was able to control *A. myosuroides* infestation to an acceptable level.

The results on *A. myosuroides* densities in the soil seed bank were highly variable and inconsistent. Molecular analysis of surviving plants showed a selection of ALS-resistant biotypes depending on the ALS inhibitor selection pressure.

This study did not reveal any specific deterioration of *A. myosuroides* infestation associated with the use of imidazolinone-tolerant OSR in a short-term crop rotation. However, this OSR production system should not be employed if ALS-resistant *A. myosuroides* plants are present in the field.

Keywords: ALS inhibitors, herbicide resistance, molecular analysis, soil seed bank, seed viability, weed density, yield

4 Occurrence, resistance factors and cross-resistance patterns to herbicides inhibiting acetolactate synthase (ALS) of *Echinochloa crus-galli* (L.) PAL. BEAUV. in Central Europe

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4.1 Abstract

Echinochloa crus-galli (L.) PAL. BEAUV. is one of the most noxious weeds in maize cultivation and has evolved target-site resistance to ALS-inhibiting herbicides. Due to the hexaploid nature of *E. crus-galli*, resistance inducing mutations can be harboured by multiple gene copies, but up to now, studies did not include an analysis of the *ALS* gene copies conferring resistance. Investigations on *E. crus-galli* populations from different countries in Central Europe revealed the presence of several point mutations conferring resistance and occurring independently at the positions Ala-122, Pro-197, and Trp-574. Moreover, an Asp-376-Glu mutation in the *ALS* gene in *E. crus-galli* and a Ser-653-Tyr amino acid change in the ALS protein of a weed were detected for the first time. Additionally, the study revealed the first case of an ALS-resistant population (Trp-574-Leu) in the Czech Republic. The detection of mutations is relevant to the evolution and management of herbicide resistance.

Dose-response experiments showed different effects depending on the population and the individual active ingredients and mixtures. Due to the genetic variability of *E. crus-galli*, results were highly variable. Overall, the populations showed a high level of resistance. For the prevailing mutated positions, i.e. Ala-122, Pro-197 and Trp-574, gene copies were examined separately using molecular genetic methods. A single mutation in at least one out of three *ALS* gene copies was sufficient to confer resistance at the positions Pro-197 and Trp-574. At Ala-122, point mutations co-occurring in the *ALS 1*, *ALS 2* and *ALS 3* gene copy were identified.

The study provides a starting point for differentiation of the resistance mechanisms in polyploid *E. crus-galli*.

Keywords: ALS mutation, gene copy, herbicide sensitivity, target-site resistance

5 Examination of efficacy and selectivity of herbicides in ALS-tolerant sugar beets

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5.1 Abstract

The development of herbicide-tolerant sugar beet varieties offers the possibility to use only herbicides from the group of the aceto-lactate synthase (ALS) inhibitors. At present, ALS inhibitors are used in many crops of the rotation to a significant extent, in particular in cereals and maize. However, more than 159 weed species with resistance to ALS inhibitors are known. Consequently, this is the mode of action with most cases of herbicide resistances. The advancing spread of ALS-resistant weeds is a major challenge for herbicide management, if herbicide ALS-tolerant crops are used.

The present study was concerned with the question of which herbicide strategies ensure the efficacy of the new herbicide Conviso® with and without presence of ALS-resistant weeds. Furthermore, the selectivity in ALS-tolerant sugar beet was investigated. Without presence of ALS-resistant weeds one treatment with Conviso® was sufficient for weed control. With a splitting application and use of an additive the Conviso® efficacy could be improved. The application of Conviso® herbicide caused no visual symptoms or yield loss in the sugar beet. ALS-resistant weeds were only partly controlled by Conviso® herbicide. By using tank mixtures with other modes of action efficacy could be improved, but complete control of ALS-resistant weeds was not possible.

Keywords: ALS inhibitors, Conviso®, herbicide tolerance

5.2 Introduction

Sugar beets have a low competitiveness against weeds during the juvenile development. Consequently, without any weed control the loss of yield can be up to 95 % (PETERSEN, 2003). Accordingly, farmers usually treat the crops 3 to 5 times with mixtures of different active ingredients (MÄRLÄNDER and TIEDEMANN, 2006). CONVISO® SMART is a new system for weed control developed by Bayer CropScience AG and KWS SAAT SE. This system consists of an ALS-tolerant sugar beet hybrid and a complementary ALS-inhibiting herbicide (50 g l^{-1} foramsulfuron plus 30 g l^{-1} thiencarbazone-methyl). It offers the chance to control major weeds with low dose rates of product and reduced number of applications (WEGENER et al., 2015). The registration of Conviso® was requested with an application rate of 1.0 l ha^{-1} or 2 x 0.5 l ha^{-1} in ALS-inhibitor tolerant sugar beets (BALGHEIM et al., 2016).

However, the intensive use of ALS-inhibitors in crop rotations favored the development of herbicide resistance in numerous weed species. In the last 30 years, resistant populations have spread widely in Western and Central Europe (HEAP, 2017). Until now, there are 97 dicot and 62 monocot species known having an ALS-resistance (HEAP, 2017).

Conviso® is supposed to be active against nearly all economically important weeds in sugar beets (BALGHEIM et al., 2016). Consequently, the solo use of the Conviso® herbicide as an ALS-inhibitor is possible in many cases. The risk to select ALS-resistant weeds in this system is high. For the sustainable use of this system it is important to find strategies to reduce that risk. In this study the following hypothesis were testetd: (i) It is possible to supplement the application of Conviso® with classical sugar beet herbicides in such a way that even ALS-resistant weeds are safely controlled. (ii) The efficacy of Conviso® is comparable to classical herbicides. (iii) The use of an adjuvant ensures the effect of Conviso® under dry conditions. Therefore, an outdoor container test and a field experiment were cultivated in Bingen (Rhein).

5.3 Material und methods

Outdoor container test

The trial was established with 30 containers (0.75 m^2) under outdoor conditions. The sowing of 8 herbicide-tolerant sugar beets (experimental hybrid) was done by hand on 24th March 2016 and 16th March 2017. To require a seed stock of ALS-resitant weeds, seeds of *Echinochloa crus-galli, Stellaria media, Papaver rhoeas* and *Matricaria inodora* were mixed in the sterilized soil of the containers immediately before sowing (Table 1). The seed samples were from the oringal origin, except MATIN seeds. They were multiplied under selection pressure (tribenuron treatment) in 2015. The germination rate was estimated between 50 to 80 % depending on the species. A total of 10 herbicide strategies were tested with three replicates (Table 3). The containers were treated with a one-wheel plot sprayer (Air mix 110-025 Flat Fan, pressure 2.1 bar, water amount 200 l ha^{-1}, speed 4.5 km h^{-1}). The harvest was done on 29th July 2016 and 13th July 2017.

The efficacy of the herbicide applications was evaluated by counting weeds by species two weeks after treatment and by fresh weight determination of weed and sugar beet biomass. The experiences made in the first experimental year led to an adjustment of the seed rates.

Table 1 Characterization of weeds used in the outdoor container test.

weed	EPPO-Code	amount [g per container]		origin, type of ALS-resistance
		2016	**2017**	
Echinochloa crus-galli	ECHCG	0.5	0.75	Thal (A); EMR; Trp574Leu
Matricaria inodora	MATIN	1.5	0.25	Freiburg/Elbe; Pro197Gln
Papaver rhoeas	PAPRH	0.015	0.02	Volkstedt (SA); Pro197Ser
Stellaria media	STEME	0.03	0.1	Selbitz (Bay); Pro197Thr + Trp574Leu

Table 2 Characterization of herbicides

Product	Ingredients	MoA	HRAC
Betanal maxxPro	60 g l^{-1} phenmedipham 27 g l^{-1} lenacil 47 g l^{-1} desmedipham 75 g l^{-1} ethofumesat	inhibition of photosynthesis at PS II inhibition of lipid syhthesis	C 1 N
Conviso®	50 g l^{-1} foramsulfuron 30 g l^{-1} thiencarbazone-methyl	ALS-inhibitor	B
Goltix Gold	700 g l^{-1} metamitron	inhibition of photosynthesis at PS II	C 1
Hasten		adjuvant	
Lontrel SG 720	720 g kg^{-1} clopyralid	Synthetic auxin	O
Mero		adjuvant	
Para Sommer		adjuvant	
Select 240 EC	241,9 g l^{-1} clethodim	inhibition of ACCase	A

Field trial

In field trials, the efficacy and selectivity of different herbicide strategies to ALS-tolerant sugar beets were tested from 2015 to 2017. The trial site was in Bingen (Rhein) with a natural weed infestation. In a randomized block design 12 herbicide programs were tested with 4 replicates (tab. 4). The plot size was 2.5 to 8.0 m. The herbicide application was done by the same plot sprayer as in the container trial.

The investigations included number of weeds, visually assessment of herbicide selectivity, yield and quality determination.

Table 3 Herbicide treatments to control ALS-resistant weed in in sugar beet in outdoor containers in the years 2016 and 2017 (T 2, T 4, T 6 and T 7 were only conducted in 2017).

Treatment	**Product**	**Dose [l ha^{-1} / g ha^{-1}]**		
		early post- 1*	**post- 2**	**post- 3**
T 1	no treatment			
T 2	Conviso®		1.0	
	+ Mero		1.0	
T 3	Goltix Gold	1.25	1.25	1.25
	+ Betanal maxxPro	1.25	1.25	1.25
	+ Hasten		0.5	
T 4	Conviso®		1.0	
	+ Select + Para Sommer			0.75
	+ Lontrel		165	
T 5	Conviso®		0.5	0.5
	+ Betanal maxxPro		1.25	1.25
	+ Goltix Gold		1.25	1.25
T 6	Conviso®		0.5	0.5
	+ Betanal maxxPro	1.25		
	+ Goltix Gold	1.25		
T 7	Conviso®		0.5	0.5
	+ Betanal maxxPro	1.25	1.25	
	+ Goltix Gold	1.25	1.25	
T 8	Conviso®		0.5	0.5
	+ Betanal maxxPro		1.25	1.25
T 9	Conviso®		0.5	0.5
	+ Goltix Gold		1.25	1.25
T 10	Conviso®		0.5	0.5
	+ Mero		1.0	1.0

*early post-1: 6th April 2016, 4th April 2017; post- 2: 20th April 2016, 20th April 2017; post- 3: 10th May 2016, 5th May 2017

Statistical analysis

Data analysis were made with the statistic program “R”, version 3.2.2. Differences between the herbicide programs were compared using an ANOVA with alpha > 0.05, and a Tukey post-hoc test. The investigations were done on beet fresh weight (container trial) and on white sugar yield (field trial).

Table 4 Herbicide programs and dosages used for the field trial from 2015 to 2017 (V 8 to V 12 were only conducted in 2016 and 2017).

treatment	Herbicide variation	dose [l ha^{-1}]	treatment timing
V 1	no treatment		
V 2	mechanical by hand		
V 3	Goltix Gold + Betanal maxxPro + Hasten	3 x 1.25 3 x 1.5 3 x 0.5	NAK 1, 2, 3
V 4	Conviso®	1.0	BBCH 10-14 CHEAL
V 5	Conviso®	2 x 0.5	BBCH 10-14 CHEAL and 14 days later
V 6	Conviso®	2.0	BBCH 10-14 CHEAL
V 7	Conviso®	2 x 1.0	BBCH 10-14 and 14 days later
V 8	Conviso® + Betanal maxxPro	2 x 0.5 2 x 1.25	BBCH 10-14 CHEAL and 14 days later
V 9	Conviso® + Goltix Gold	2 x 0.5 2 x 1.25	BBCH 10-14 CHEAL and 14 days later
V 10	Goltix Gold + Betanal maxxPro	3 x 2.5 3 x 3.0	NAK 1, 2, 3
V 11	Conviso® + Mero	2 x 0.5 2 x 1.0	BBCH 10-14 CHEAL and 14 days later
V 12	Conviso® + Mero	1.0 1.0	BBCH 10-14 CHEAL

5.4 Results

Outdoor container trial

In the containers different weed species showed variation in germination. MATIN developed very fast and became the dominant weed in both years. In 2016, only single individuals of ECHCG weeds occurred in the trial. Nevertheless, the final infestation level differed between the weed species and the herbicide programs. T 1 was an untreated control which included all sown weed species. The best weed control was obtained in treatments T 5 and T 7 (Conviso® plus Betanal maxxPro plus Goltix Gold). Herbicide efficacies ranged between minimum 40 % (PAPRH, 2016) and maximum 100 %. However, numerous individuals of ECHCG (2017) and MATIN (2016) survived in treatment T 5. T 7 achieved a slight improvement in efficiency against ECHCG by an earlier application of classic herbicides in 2017. With the exception of ECHCG, T 3 controlled all weed species well (classic herbicide treatment). For T 6, an insufficient weed control was observed. Compared to a single application (T 2), the splitting treatment of Conviso® plus Mero (T 10) showed a better efficiency. The combination of Conviso® plus one classic herbicide (T 8 and T 9) was less effective against several weeds. In general, the infestation with ALS-resistant MATIN was difficult to eleminate in most herbicide programs.

Table 5 Number of occuring weeds per container and efficacy of herbicide applications in ALS-tolerant sugar beets infested with ALS-resistant weeds two weeks after application in 2016 and 2017.

Treatment	**MATIN**		**STEME**		**PAPRH**		**ECHCG**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
T 1	88*	29	8	42	12	7	0	32
T 2	n.a.	48*	n.a.	38	n.a.	9	n.a.	30
Eff. [%]**		**0**		**0**		**0**		**0**
T 3	3	4	0	11	3	1	5	29
Eff. [%]	**78**	**100**		**100**	**100**	**100**	**66**	**0**
T 4	n.a.	18	n.a.	28	n.a.	4	n.a.	21
Eff. [%]		**67**		**66**		**31**		**100**
T 5	34	5	0	34	3	10	0	28
Eff. [%]	**0**	**100**		**100**	**40**	**100**		**22**
T 6	n.a.	9	n.a.	19	n.a.	4	n.a.	16
Eff. [%]		**0**		**90**		**0**		**0**
T 7	n.a.	3	n.a.	15	n.a.	5	n.a.	21
Eff. [%]		**100**		**100**		**93**		**100**
T 8	28	20	0	41	2	4	0	29
Eff. [%]	**0**	**0**		**98**	**50**	**0**		**100**
T 9	34	5	2	30	9	7	0	38
Eff. [%]	**0**	**69**	**0**	**78**	**21**	**71**		**72**
T 10	72	11	2	35	4	3	0	21
Eff. [%]	**0**	**0**	**0**	**67**	**17**	**0**		**12**

*number of occuring weeds per container

**efficacy of herbicide treatment against occurring weeds; n.a., not available

In 2016 the highest fresh mass of sugar beet leaves were achieved in T 3, T 5 and T 10 (fig. 1). Generally, in 2017 the infestation level was lower than in 2016. It was caused by reduced amount of ALS-resistant MATIN seeds. The values of sugar beet leaf weights varied in this trial extremely. The highest sugar beet yields (beet fresh weight) were found in those herbicide treatments which also had the highest sugar beet leaf masses. As expected T 1 (untreated control) showed the lowest yields in both trial years. In 2016 the highest yield of sugar beet fresh mass was received in T 3 (3 x Goltix Gold + 3 x Betanal maxxPro) with 11.3 kg followed by T 5 (8.1 kg) and T 10 (6.0 kg). In 2017 the significant highest yields were achieved in all treatments where Conviso® was combined with classic herbicides (T 5, T 8 and T 9). All other treatments reached a similar yield level.

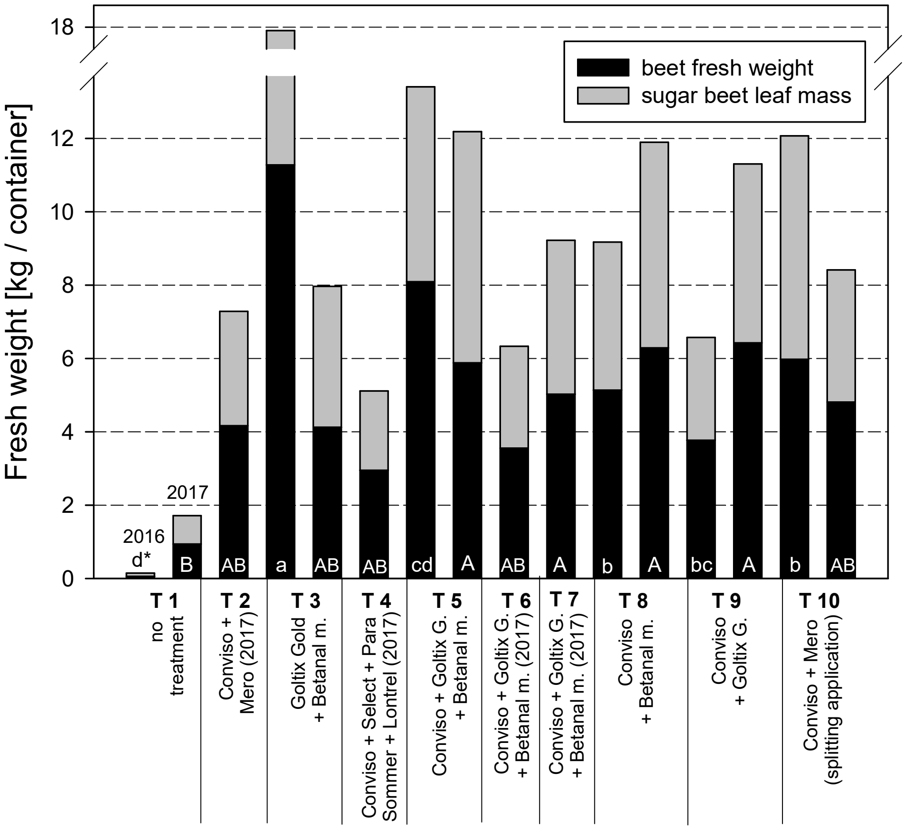

Figure 1 Fresh weight of sugar beet leaves and beet yield depending on herbicide treatment in ALS-tolerant sugar beet (outdoor container trial 2016 and 2017). *Significant differences at p≤0.05 are indicated different small letters (Tukey-HSD) only for beet fresh weight.

Field trial

The field trials confirmed the high tolerance of the hybrid against Conviso®. All treatments including a Conviso® application showed no phenotypic damages in all three years. Only the classic treatment let to chlorosis and growth delay in the standard application (V 3) as well as for the double dose (V 10). Occuring growth retardation disappeared during the growing period. Table 6 displays that V 3 and V 10 achieved fewer yields, however, the yield was not influenced significantly (data not shown).

In 2015 the dominant weeds were *Chenopodium album* (CHEAL), *Galium aparine* (GALAP) and *Solanum nigrum* (SOLNI). In the following year, the weed population comprised of ALS-resistant *Apera spica-venti* (APESV) and susceptible CHEAL. In 2017 there was a wide spectrum of weeds *Amaranthus retroflexus* (AMARE), APESV, CHEAL, ECHCG, volunteer OSR, *Setaria viridis* (SETVI) and SOLNI. The weed population of V 1 (untreated control) covered the plots to 100 % in all years. The splitting applications in V 5 (2 x 0.5 l ha^{-1} Conviso®) and V 7 (2 x 1.0 l ha^{-1} Conviso®) achieved much better efficacies than the single applications. The single application of the double dosage of Conviso® (2.0 l ha^{-1}) was more effective than the splitting application of the double dose rate 2 x 1.0 l ha^{-1}. The use of the adjuvant Mero improved the performance of Conviso® in V 11 (2 x

0.5 l ha^{-1} Conviso® + 1.0 l ha^{-1} Mero). The classic herbicide treatments V 3 and V 10 showed the highest numbers of surviving CHEAL plants. Furthermore, AMARE, ECHCG, SETVI and SOLNI survived, too. In classic herbicide programs, a graminicide would have been used to control grass weeds. Generally, the best herbicide efficacy was obtained in all herbicide treatments which included Conviso®. Moreover, seeds of ALS-tolerant oilseed rape were spread from neighbouring field trials from previous years into this trial site. None of the Conviso® treatments were able to control this ALS-tolerant volunteer OSR. The following molecular analysis confirmed the target site resistance at the position Trp-574 and Ser-653 in the surviving OSR plants. Plots with occuring OSR reached the coverage of minimum 0.5 % and maximum 3.0 %.

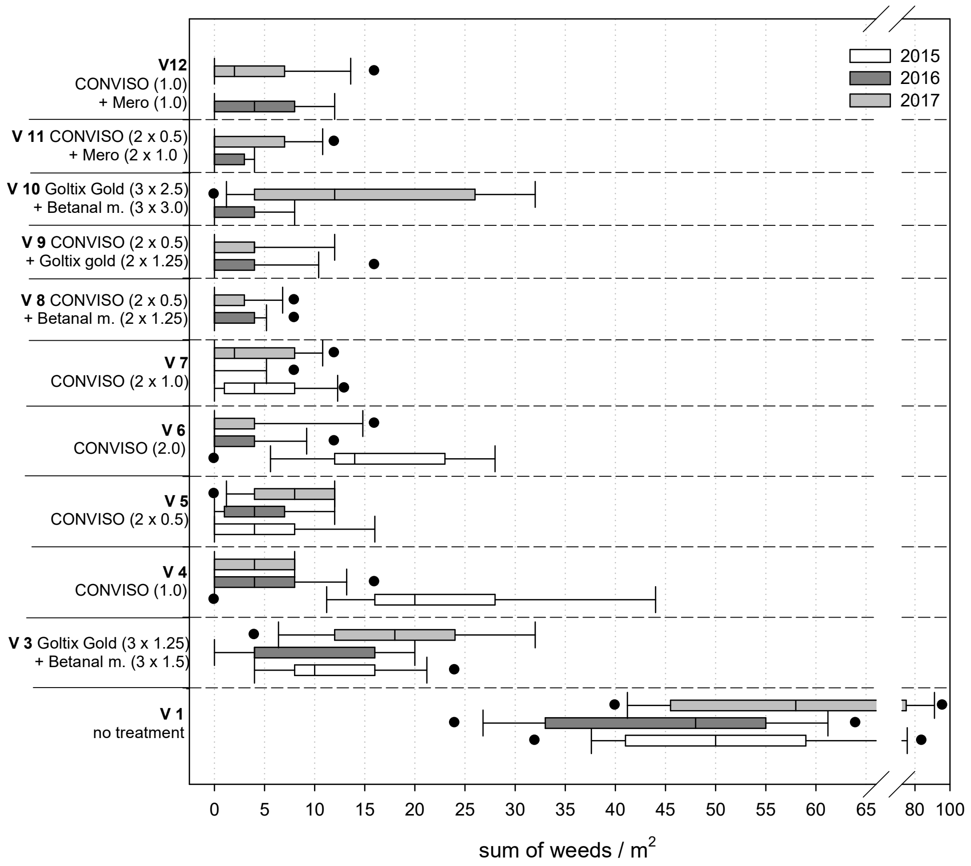

Figure 2 Weed density in ALS-tolerant sugar beet after herbicide treatment at field trial site Bingen (n = 3, 2015 – 2017). Dosages in l ha^{-1}. Box plot with median, 25th-75th quantiles (box) and 5th- 95th quantiles (whiskers). Black circles show outliers.

The white sugar yields in 2015 and 2016 as well as the beet fresh weight in 2017 did not show any differences between the herbicide treatments. Only V 1, the untreated control, achieved significant lower yields. In 2015 the white sugar yields were lower than in 2016. It was caused by drought period during summer.

Table 6 White sugar yield [t ha^{-1}] 2015, 2016 and beet fresh weight [t ha^{-1}] 2017 in ALS-tolerant sugar beet depending on the herbicide treatment.

herbicide treatment	2015 mean value (SD value)	2016 mean value (SD value)	2017 mean value (SD value)
V 1	4.69^{a} *(0.82)	0.51^{a} (0.30)	1.56^{A} (2.06)
V 2	9.49^{b} (0.80)	15.73^{b} (0.49)	88.06^{B} (9.20)
V 3	8.86^{b} (0.39)	14.92^{b} (0.79)	90.54^{B} (8.59)
V 4	9.02^{b} (0.54)	15.03^{b} (0.69)	86.46^{B} (9.96)
V 5	9.57^{b} (0.54)	15.56^{b} (1.01)	86.56^{B} (9.30)
V 6	9.22^{b} (0.79)	15.19^{b} (1.15)	94.31^{B} (6.39)
V 7	8.91^{b} (0.38)	15.42^{b} (0.81)	95.90^{B} (3.02)
V 8		15.55^{b} (0.58)	99.08^{B} (5.41)
V 9		15.91^{b} (0.69)	93.85^{B} (7.79)
V 10		14.45^{b} (0.96)	89.52^{B} (3.67)
V 11		16.13^{b} (0.58)	92.69^{B} (7.29)
V 12		16,11^{b} (1.60)	95.44^{B} (5.15)

*Significant differences at $p \leq 0.05$ (Tukey-HSD) are indicated different small letters.

5.5 Discussion

The aim of this study was to examine the efficacy of herbicide strategies against ALS-resistant weeds using Conviso® plus classic herbicides (container trial). Furthermore, crop selectivity of the CONVISO® SMART hybrids and efficacy of Conviso® under natural weed infestation were investigated (field trial). Resistance to ALS inhibitors is a result of reduced sensitivity of the target ALS enzyme to inhibition by the herbicide (TRANEL and WRIGHT, 2002). The degree of the resistance dominance varies among plant species or alleles (FOES et al., 1999). Thus, in principle, an effect of Conviso® against ALS-resistant weeds was assumed.

In the container trial, the best weed control was obtained in the herbicide treatments including Conviso®, Goltix Gold and Betanal maxxPro (T 5, T 7). Adding just one classic herbicide to the Conviso® treatment led to a decreasing efficacy of the application (T 8, T 9). The herbicide treatment T 2 (1.0 l ha^{-1} Conviso® plus 1.0 l/ha Mero) and the splitting application T 10 (2 x 0.5 lha^{-1} Conviso® plus 2 x 1.0 l/ha Mero) were not effective enough for controlling ALS-resistant weeds. This indicates that Conviso® needs a supplement of classic herbicides for controlling ALS-resistant weeds. In the classic herbicide treatment (T 3), most of the weeds were well controlled. Surviving plants of ECHCG can be explained by the fact that Betanal maxxPro and Goltix Gold are no suitable grass herbicides. The control of ALS-resistant MATIN was a challenge for nearly all herbicide treatments. Owing to the results in most cases the hypothesis can be confirmed that even ALS-resistant weeds are controlled by supplementing Conviso® with classic herbicides (i). The relationship between weed biomass and sugar beet leaf weight corresponded with the number of surviving weeds per container. Low infestation levels favored leaf development and beet growth.

The dominant weed species in field trials were CHEAL, SETVI and SOLNI. In addition to these susceptible species, ALS-tolerant oilseed rape occurred in 2017. These plants came from neighbouring experimental areas and could not be controlled in any herbicide program. In practice, therefore, the cultivation of herbicide tolerant sugar beets and herbicide tolerant oilseed rape in same crop rotation or farm can not be recommended at all. Even for a farm with different crop rotation systems, there might be problems with volunteer OSR. Similar to the container trial, the application of Conviso® plus classic herbicides achieved the best efficacies (V 8, V 9) in the field trial. Investigations on Conviso® in single and in splitting treatments showed a slight benefit of the splitting application. All in all, Conviso® was well active against weeds and an additonal adjuvant ensured the efficacy. Hence Conviso® has a comparable or even better performance in weed controlling like classic herbicides (ii). However, this statement does not apply to the weed control of ALS-resistant species.

The use of Mero improved the performance of Conviso® in the splitting treatment (V 11) in comparison to an application without an adjuvant (V 5). Thus, the hypothesis (iii) can be confirmed. Similar results can be found in the study of BALGHEIM et al. (2016). The number of surviving weeds of the classic herbicides (V 3) was higher than the other herbicide treatments. Comparing the white sugar yields of 2015 and 2017 it is noticeable that there are no significant differences between the herbicide programs.

Compared to classic herbicide programs Conviso® was very selective even with double dose in all three years. No chlorosis or stunting was observed. This may lead to quicker canopy closing and less late developing weeds. Similar results were found by WENDT et al. (2017) in more detailed studies on crop selectivity in ALS-tolerant sugar beets.

6 General Discussion

Weed control is one of the main challenges in preventing yield losses in any crop production system. Herbicides are considered the most effective means of weed control and are the major method of control for economic reasons (Chauvel et al. 2001; Moss 2010; Owen 2016). However, previous EU regulatory actions (Directive 2009/128/EC) and further restrictions due to the latest pesticide approval regulations (EC/1107/2009) have resulted in fewer and fewer herbicides being available for weed control. In addition, only a small number of new herbicides have been discovered in the last 20 years, and no major new MoAs have been marketed for over 30 years (Westwood et al. 2018). Consequently, the efficient use of existing herbicide tools for weed control is of crucial importance. The development of HT crops, whether generated by conventional breeding, mutagenesis or through transgenic techniques, is an approach to maintaining the efficiency of chemical weed control. These plants are tolerant to the already existing effective broad-spectrum herbicides, which expands the opportunities for their use (Mulwa & Mwanza 2006). Growing HT crops can bring significant benefits to farmers by making weed management cheaper (May 2003; Gianessi 2008; Klümper & Qaim 2014), providing more flexible options for weed management, a lower risk for crop injury and being compatible with no-till or reduced-tillage systems (Schütte et al. 2017; Lamichhane et al. 2017). As the cultivation of transgenic HT crops is restricted in Germany, this study focuses on crop tolerance to ALS-inhibiting active ingredients, developed using conventional breeding techniques.

The cultivation of HT crops inherently implies the application of ALS herbicides, and thus alternation between herbicide MoA may be lacking as ALS-inhibiting herbicides are used in many crops. Possible consequences could be increased selection pressure for weeds that are tolerant, or a shift to weed species that are poorly controlled or that develop avoidance mechanisms (Senior & Dale 2002; Knezevic 2010). Today, herbicide resistance in arable weeds is widely known as a result of the adaptive evolution of weed species to the intense selection pressure exerted by herbicides (Beckie 2006; Neve et al. 2009). Therefore, viable management systems are required that ensure the sustainability of HT crops. Although resistance management was not considered to be an issue in HT crop production (Bradshaw et al. 1997; Ghersa et al. 2000), this has changed along with evolved weed resistance to glyphosate in GM glyphosate-resistant crops (Nandula et al. 2005; Powles 2008; Johnson et al. 2009).

Glyphosate as a post-emergence, non-selective herbicide was commercially introduced in 1974 (Duke & Powles 2008). Since then, several factors have driven the increase in glyphosate use. The rapid and widespread adoption of GM-HT maize, cotton and soybeans, which became available in the mid 1990s, had a major impact on herbicide use trends by promoting the use of specific herbicides, most notably glyphosate (Fernandez-Cornejo 2004; Duke & Powles 2008; Green 2012). The increased acreage planted with GM-HT crops, along with the reduction in the price of glyphosate after the patent expired, and the adoption of no-tillage and conservation tillage systems, contributed to the rising use of glyphosate (Vila-Aiub et al. 2008; Benbrook 2016). Thus, the cultivation of GM-HT crops with glyphosate resistance has shifted weed management to a less diverse, more simplified weed management strategy that has predictably resulted in the evolution of glyphosate-resistant weeds. Between 1990 and 2015, 17 different weed species evolved resistance to glyphosate in the U.S. (Kniss 2018). Ironically, the rapid spread of herbicide-resistant weeds is also increasing the use of herbicides because growers´ first reaction to the discovery of herbicide-resistant weeds is to use higher rates or alternative herbicides before changing cultural practices (Green 2014; Peterson et al. 2018).

In general, there is a difference between resistance management and resistance prevention strategies in practice (Niemann 2003). Resistance management includes the alternation of active ingredient, however, alternating the MoA is often not taken into account (Balgheim 2006). Even when changing the MoA, herbicides must be chosen carefully. When selecting herbicides, active ingredients with a low risk of resistance should be included in the herbicide regime (Niemann 2003).

Strategies for preventing or delaying resistance have been highlighted by numerous studies (e.g., Gressel & Segel 1990; Moss & Clarke 1994; Chauvel et al. 2001; Beckie 2006; Moss et al. 2007; Gehring & Thyssen 2014), including measures associated with agronomic practice as well as a combination of different MoAs. However, the cultivation of HT crops has not been considered. To some extent, weed management practices in these crops differ from those of conventional cropping systems. HT crops are designed to rely solely on one herbicide MoA. However, the continuous use or overuse of a herbicide MoA in a crop rotation could increase the potential risk of developing resistant weeds. Therefore, resistance management as well as resistance prevention strategies should be modified when herbicide-resistant weed species occur in crop rotations in which HT crops are cultivated.

6.1 Weed management strategies for HT crop cultivation

An important question is the impact that the introduction of HT crops will have on the development of resistance in weed species. A weed population, once introduced into the seed bank, will gain importance if selection pressure (e.g., herbicides) is exerted that favours this species (Owen & Zelaya 2005). However, there are many factors that affect the frequency, weed density and weed seedbank dynamics of a weed species, such as crop rotation, tillage, environmental conditions and weed management practices (Owen 2008).

6.1.1 Herbicide use

A fundamental question is which factors and measures carried out in field trials lead to the spread of weed populations. In Paper No. 1, herbicide strategies were investigated that included different intensities of ALS-inhibiting herbicides in the crop rotation. At the beginning of the field trials, adequate weed control was achieved in almost all WCS that included an ALS inhibitor application. Whereas *A. myosuroides* became the dominant weed species at the trial site Bingen, *T. perforatum* was the prevalent weed species at the Braunschweig site. However, the selection pressure on ALS-resistant weed species increased within this short-term crop rotation and, consequently, caused sugar beet root yield and wheat grain yield losses at the respective trial sites. Reliance on a single MoA must be avoided to sustain the benefits of the HT varieties in the crop rotation.

In a crop rotation including ALS-tolerant oilseed rape hybrids (Paper No. 2), complementary herbicides with active ingredients with different MoAs were combined. In contrast, winter wheat was treated with an ALS inhibitor only. Unfortunately, the introduction of different MoAs in one crop within a 3-year rotation did not sufficiently reduce the selection pressure on *A. myosuroides*. Thus, the degree of infestation increased dramatically with increasing intensity of ALS inhibitor use. Furthermore, winter OSR was sown in the autumn, allowing *A. myosuroides* to start infestation early in the growing season, whereas sugarbeet varieties were not sown until spring. During the winter months, *A. myosuroides* was able to establish high weed densities, especially when herbicide applications failed due to adverse environmental conditions.

The results of these two field trials show that the use of a single MoA may not be successful, especially when less sensitive or resistant weed species emerge. Rather, herbicide management must include a variety of MoAs that have a low risk of resistance. In general, in a crop rotation with a high proportion of cereals, grass weed control should be achieved with different MoAs. Ideally, ACCase-, ALS- and photosynthetic inhibitors should be omitted or only rarely used due to the high risk of developing resistance in *A. myosuroides*. Alternative active ingredients with a low risk of resistance are diflufenican, flurtamone (inhibition of phytoene desaturase), flufenacet, ethofumesate, prosulfocarb (inhibition of very long-chain fatty acid synthesis) and pendimethalin (inhibition of microtubule assembly) for use in winter wheat cultivation. However, *A. myosuroides* populations with reduced herbicide sensitivity to flufenacet (Hull & Moss 2012; Klingenhagen 2012; Rosenhauer & Petersen 2014) and pendimethalin (Moss 1990; James et al. 1995; Hull et al. 2014; Keshtkar et al. 2015) have been reported.

For sugar beet cultivation only a small number of active ingredients are available. Propaquizafop, fluazifop-butyl and quizalofop-ethyl are approved for grass weed control, however, with all these active ingredients, there is a high risk of developing resistance with regular use. The same applies to clethodim and cycloxydim as they also belong to the ACCase inhibitors. Both can be used in sugar beet as well as winter OSR cultivation. Furthermore, propyzamide (inhibition of microtubule assembly), dimetachlor and metazachlor (inhibition of very long-chain fatty acid synthesis) herbicides are authorized for winter OSR cultivation.

In a field with a high expected weed infestation, a soil-active herbicide should be used in the autumn, followed by a spring treatment. Generally, active ingredients against dicot and monocot weeds should be applied in tank mixtures. If grass weeds such as *A. myosuroides* reach too advanced a stage of development before the winter break, damaging competitive pressure with the crop may result. In addition, herbicide inputs would need to increase significantly in terms of number of applications and amount of herbicides.

Of course, the use of ALS inhibitors is scheduled in the cultivation of HT crops. It is also conceivable that ALS-inhibiting herbicides for imidazolinone-tolerant winter OSR or ALS-tolerant sugar beet varieties will not be used except in another crop within the rotation. In field trials, a possible solution would be to use an alternative MoA for weed control in ALS-tolerant sugar beet and in HT OSR. Accordingly, herbicide application in winter wheat could be done once in a three-year crop rotation with ALS inhibitors.

Another possibility is the use of a complementary ALS-inhibiting herbicide in combination with an alternative MoA to ensure successful weed control. As illustrated by the results reported in Paper No. 4, mixtures of a complementary herbicide and conventional herbicides are suggested to achieve sufficient weed control in HT sugar beet cultivation. Thereby, the selection pressure exerted by the ALS-inhibiting herbicide on ALS-resistant weed species would decrease.

6.1.2 Crop rotation

However, the increasing infestation in the field trials was caused not only by reliance on chemical weed control, but also by other components of cultural control measures. The field trials were conducted in short-term rotations, with a high proportion of winter wheat and unilateral tillage. Crop rotation is an important component of integrated weed management (Swanton & Weise 1991; Clements et al. 1994) that reduces weed density by introducing conditions and practices that are unfavourable to a specific weed species. As a result, both the growth and reproduction of weeds are hindered (Bullock 1992). In addition, crop rotation has been reported to have a negative effect on shifts in the weed population (Doucet et al. 1999).

Jouy and Guilbert (1998, cited in Chauvel et al. 2001) reported a considerable reduction in *A. myosuroides* densities by introducing *Pisum sativum* L. into the crop rotation. Most *A. myosuroides* seeds germinate in the autumn, and thus this weed species is much less of a problem in spring-grown crops (Moss & Clarke 1994; Murphy & Lemerle 2006). Alternating spring and winter crops is thought to be another effective strategy against *A. myosuroides* infestation (Chauvel et al. 2009). Focusing on crop management strategies for the control of herbicide-resistant *A. myosuroides*, Wellhausen et al. (2018) demonstrated that complete control of this grass weed will be very difficult to achieve while maintaining a crop rotation consisting only of winter crops. According to Lutman et al. (2013), the best outcomes were achieved by planting a spring cereal and using a mouldboard plough.

Maize could also be a valuable component for cereal-based crop rotations. Overall, maize cultivation and maize monocultures have increased in Germany, and typical maize weed species have unfortunately increased at the same time (Schröder et al. 2007; DeMol et al. 2012). The emergence of ALS-resistant *T. perforatum* (2009) and *E. crus-galli* (2012), as well as *A. retroflexus* (2012) and *S.*

media (2011), is associated with the frequent use of sulfonylureas (Heap 2021). Some of these weed species can also become problematic in winter wheat or other arable crops. Regarding *T. perforatum*, Paper No. 1 shows that a resistant population can develop rapidly and cause yield losses. The situation is similar for *E. crus-galli*. High weed densities can also become problematic in sugar beet cultivation. German growers are not yet aware of *E. crus-galli* as a problem. As Paper No. 3 shows, the first cases of ALS resistance have been detected in Europe, including in Germany (Heap 2021). ALS application must be carefully calculated with regard to the management of resistance. Furthermore, after the withdrawal of approval for topramezone in Germany, nicosulfuron (ALS inhibitor) has been considered as a substitute active ingredient in grass control (Gehring et al. 2018). Alternatively, Ewert et al. (2014) proposed a dimethenamid-P combination with terbutylazine as a sulfonylurea-free herbicide strategy. Thus, adequate herbicide use would be possible and crop rotation could be expanded with spring crops. Notably, using diverse crop rotations, as illustrated by Andert et al. (2016), is a suitable method to reduce herbicide use intensities. Different crops in a crop rotation provide the opportunity to use different MoAs and therefore, the repeated use of the same MoA can be further reduced (Knezevic 2010).

6.1.3 Cultural measures

Several cultural control measures are also available for grass weed control including delayed drilling, increasing crop competition and non-cropping. Although the benefits of using more competitive varieties may be low, as reported by Lutman et al. (2013), this could be an attractive “no cost“ option. Moss et al. (2007) demonstrated that delayed autumn drilling of winter wheat crops reduced the infestation of *A. myosuroides* in the crop, relative to earlier drilling, by an average of 44 % across 17 field trials. Delayed autumn drilling allows more weed species to emerge and be controlled before sowing.

In the case of extremely large populations of grass weed species, the inclusion of a grass ley (Moss & Clarke 1994) or a fallow year is suggested to be a useful tool for weed management. A study by Hume (1982) found that crop rotation, including a fallow year, was the most important factor in reducing *Setaria viridis* (L.) Beauv. density. Furthermore, Moss & Lutman (2013) evaluated fallowing/ grass leys with a 75% control per year of the *A. myosuroides* seedbank. For these reasons, cultural measures are considered to be important weed management tools.

6.1.4 Tillage

During the field trials, tillage was performed using non-inversion methods with a depth of 10 cm at each site and in each year. Preparation of the stubble and the seedbed was done with a cultivator and a rotary harrow. The one-time use of a plough could have counteracted the increasing *A. myosuroides* populations by burying the most freshly shed seeds to a depth from which seedlings are unlikely to emerge (> 5cm) (Moss & Clarke 1994; Moss 2013). In a study by Lutman et al. (2013) mouldboard ploughing reduced the number of *A. myosuroides* plants m^{-2} in the subsequent crop by 69 % compared with non-inversion tillage. The general influence of primary tillage on weed seeds in the tillage layer is widely recognized (Fay & Olson 1978; Froud-Williams et al. 1983; Ball 1992). Nevertheless, the weed seed bank must be considered in the management of herbicide resistance, with the aim of maintaining a low weed seed bank (Owen 2016; Beckie et al. 2019). Norsworthy et al. (2014) and Barber et al. (2015) even emphasized zero thresholds for herbicide-resistant weeds to keep the seed bank from replenishing.

6.2 Aspects of ALS herbicide resistance

As described above, the selection pressure imposed by herbicides on weed species is expected to lead to the development of resistance. Herbicide resistance is defined as “the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis” (WSSA 1998). Resistance to herbicides has emerged in both grass and dicot weeds and is one of the primary concerns in modern agriculture. Several methods are known for the detection of herbicide resistance, but not all of them differentiate between different mechanisms of resistance (Balgheim 2009). The most widely used but also time-consuming method is the dose-response experiment (Beckie et al. 2000; Deng et al. 2015). Various DNA-based techniques have been developed for resistance diagnostics, including polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) (Kaloumenos et al. 2009), dCAPS (derived cleaved amplified polymorphic sequences) (Délye et al. 2011; Yu et al. 2008) and cleaved amplified polymorphic sequences (CAPS) (Yu et al. 2007).

Evolved TSR is due to point mutations resulting in amino acid changes in a target enzyme that prevent or reduce herbicide binding (Yu & Powles 2014). Target-site based mechanisms, including increased expression of the target gene or structural changes at the herbicide binding site caused by amino acid mutation are mostly responsible for ALS resistance (Délye et al. 2013). As ALS is a nuclear gene and follows normal Mendelian inheritance, resistant ALS alleles are most likely to be expressed by pollen and seeds (Tranel & Wright 2002; Burke et al. 2007). In turn, it was found that mechanisms conferring resistance other than at the target-site were not identifiable when using pollen assays (Richter & Powles 1993; Letouze & Gasquez 2000). Although *A. myosuroides* is propagated solely by seeds (Moss 2013), there is the possibility of spreading the resistance trait via pollen. Petersen et al. (2010) observed inbreeding and outcrossing events within and between their trial plots for the spread of ACCase TSR in *A. myosuroides*. The weed species *T. perforatum* is known to be insect pollinated (Kay 1969). Consequently, the transfer of the resistance trait by both seed and pollen must be considered (Ulber 2014). To date, the potential distance for the spread of the herbicide resistance trait of *T. perforatum* has not been studied, but Bagavathiannan et al. (2013) reported long-distance pollen movement for several weed species, regardless of whether they were spread by insects or wind. Moreover, ALS-resistant weed seeds could have been dispersed over short distances across the trial fields by tillage equipment and harvesting machinery. In view of this, plot distances were as large as possible, although pollen and seed transfer between plots might not have been completely avoided.

6.2.1 Herbicide resistance management

The use of ALS inhibitors in the field trials exerted considerable selection pressure on weed species in a short-term crop rotation. It is the number of applications that drives the evolution of herbicide resistance (Manalil et al. 2011; Hicks et al. 2018). Inevitably, in the presence of ALS-resistant weed species, the aim must be to reduce the selection pressure exerted by herbicides. This should especially be considered when there is resistance to multiple active ingredients. Relying solely on herbicides for weed control is not sustainable in the long term (Moss & Lutman 2013), so non-chemical methods should be integrated in weed management. In general, a selection process takes place whenever an ALS inhibitor or herbicide with another MoA is sprayed. Even the application of a herbicide every 4 or 5 years leads to a certain selection pressure, but just to a lower one.

With respect to glyphosate-resistant GM crops, increased glyphosate use triggered the emergence of weed species that are less sensitive or resistant to glyphosate product labels in GM-HT crops as well as in non-GM-HT crop production systems. As glyphosate-resistant weeds have spread, growers have lost many of the advantages they enjoyed when using glyphosate in glyphosate-resistant crops (Green 2014). In response, the industry has begun working on combined resistance to glyphosate and other herbicide(s) to launch new HT crops with stacked herbicide tolerance traits (Bonny 2016; Schütte et al. 2017; Fartyal et al. 2018). Beckie et al. (2019) summarized the herbicide resistance traits in cultivars of major agronomic crops that are currently available: Crop cultivars with stacked herbicide-resistance traits confer resistance to glyphosate+glufosinate (soybean, maize, cotton), glyphosate+triazine (canola), glyphosate+dicamba (soybean, cotton), glyphosate+isoxaflutole (soybean), glyphosate+2,4-D+aryloxyphen (maize), and glyphosate+glufosinate+dicamba (soybean, cotton). Another three-way stacked soybean system to be released is tolerant to glyphosate, glufosinate and 2,4-D. In Europe, this approach is not an option due to legal restrictions. However, these efforts reflect the importance of managing glyphosate-resistant and other herbicide-resistant weed species. Importantly, this approach is not a long-term solution and will not reduce weed resistance. Managing to reduce weed density is not the same as minimizing herbicide resistance and therefore, weed management must aim to minimize resistance and maximize the efficacy of herbicides (Hicks et al. 2018). Herbicides are efficient at controlling weeds, however this also applies to integrated management practices as discussed earlier. Addidionally, there are long-term economic benefits of preventing resistance by diverse crop rotations, rotating herbicide MoAs and inversion tillage, as calculated by Norsworthy et al. (2012) and Gerhards et al. (2016). A recent study by Adeux et al. (2019) demonstrated a successful cropping strategy containing different sowing periods, occasional ploughing, repeated false seedbed preparations, reduced nitrogen fertilization at the cropping system and crop scale, and a combination of mechanical and chemical weeding. The industry has also renewed efforts towards the discovery and development of novel herbicides. Promising approaches include research with natural phytotoxins (Dayan & Duke 2014), products based on by-products of microorganisms or extracts of plants (Westwood et al. 2017) and the herbicidal properties of many antimalarial drugs (Corral et al. 2017).

6.3 Conclusions and future prospects

This thesis helps extent our knowledge of the relationship between the cultivation of HT crops and the development of herbicide-resistant weed species. The integration of HT crops does not directly impart selection pressure on the weed community (Owen 2008). Indeed, the selection pressure exerted on weed species is increased due to the limited number of herbicides used to control weed species. The selection pressure exerted by ALS inhibitors could lead to an increase in naturally tolerant populations or the development of ALS resistance within the weed population. The study demonstrates that there exists a link between HT crops and increasing herbicide-resistant weed species due to the number of ALS inhibitor applications in the crop rotation. The most influential selective forces are believed to be tillage and herbicide regimes (Owen 2008) and thus, significant impacts on weed population dynamics cannot be separated from the specific effect of the crop rotation (Doucet et al. 1999). This implies that the weed control measures applied by growers are primarily responsible for the ecological selection pressure affecting the weed community. There was no clear evidence or indication of increasing weed densities caused by the cultivation of HT crops. Lamichhane et al. (2017) highlighted that HT crop cultivars require the same diverse management strategies as GM-HT cultivars to address environmental issues, as they present similar advantages and disadvantages. The lessons learned from abroad clearly show the serious problems that can arise from the careless use of GM-HT crops and herbicides, regardless of the cropping system. Growers must proactively maintain the sustainability of the technology by reducing the likelihood of resistance occurring and implementing integrated weed management for these crop production systems. Since there will be no new MoA, ensuring the sustainability of existing herbicides and production systems is a challange.

7 Summary

Herbicides are the most widely used tool for weed management, ensuring and maintaining crop yield and quality. However, intensive herbicide use has exerted selection pressure on weed communities that has inevitably resulted in weed population shifts and the evolution of herbicide resistance. Moreover, strict pesticide authorization regulations in Europe and a significant lack of development of new herbicides with different MoAs have resulted in a limited range of herbicides for weed control in recent years. The introduction of HT crops developed using conventional breeding techniques has expanded the options for chemical weed control in sugar beet and OSR cultivation in Germany. So far, the use of ALS inhibitors has been limited as these crops are sensitive to ALS-inhibiting herbicides. Unfortunately, herbicides targeting acetolactate synthase are among the most commonly used herbicides in arable crops; notably, they are also the most resistance-prone herbicide group. The implementation of HT crop technology could further increase the use of ALS-inhibiting herbicides and thus, reduce the diversity of MoAs used in a crop rotation and increase the risk of resistance.

The present study aimed to analyse in more depth the relationship between the cultivation of HT crops and the development of herbicide-resistant weed species. In Germany, *A. myosuroides* is the most important grass weed in arable fields associated with target-site resistance. In addition, *E. crus-galli* and *T. perforatum* are weed species that have gained attention due to their ALS resistance and their potential importance in the cultivation of HT crops. All investigations carried out during this thesis deal with those three weed species.

The main question was whether there are herbicide strategies that can ensure successful weed control even with a low initial frequency of ALS-resistant weed species in HT sugar beet cultivation? Is it possible to integrate ALS-tolerant sugar beet into a crop rotation without increasing the risk of ALS-resistant weed selection?

The first paper investigated the influence of varying intensities of ALS inhibitor use on the development of herbicide-resistant *A. myosuroides* and *T. perforatum* in a crop rotation including HT sugar beet. During the field trials conducted from 2014 to 2017 at two trial sites in Germany, weed density, genetic resistance background and crop yield were evaluated annually. The results showed that moderate ALS-resistant weed densities can be controlled with an adapted herbicide strategy. However, the herbicide strategy must be extended to include graminicides in sugar beet, and a residual herbicide must be used in winter wheat to ensure weed control. The application of ALS inhibitors promoted the development of ALS-resistant weed populations but the spread of resistant biotypes could not be attributed to the integration of herbicide-tolerant cultivars.

What are the effects of different intensities of ALS inhibitor application on the development of *A. myosuroides* in HT OSR cultivation? Does the combination of ALS inhibitors and alternative herbicides have weed control benefits in HT OSR cultivation?

The second paper addressed the efficacy of herbicide strategies in HT OSR cultivation involving ALS inhibitors in graduated intensities over a 3-year trial period. The parameters of *A. myosuroides* head number, frequency of mutations at the target-site in surviving plants, and crop yield were assessed annually, and the amount of *A. myosuroides* seeds in the soil seed bank was determined at the end of the trial period. The results indicated that the intensity of ALS inhibitor use significantly influenced the density of *A. myosuroides* head number and the development of resistance. The use of an alternative MoA for weed control in HT OSR could not keep *A. myosuroides* infestation to an acceptable level. As a consequence, HT OSR cultivation should not be used if ALS-resistant *A. myosuroides* plants are present in the field. Weed control seems to be difficult when relying only on herbicides. Therefore, weed control must be complemented by additional agronomic methods.

What is the level of resistance of *E. crus-galli* to various ALS-inhibiting herbicides in Europe? How many copies of the *ALS* gene are involved in the expression of target site resistance?

The third study addressed the spatial distribution of ALS-resistant *E. crus-galli* populations in Central Europe. It was demonstrated that several point mutations confer resistance at the positions Ala-122, Pro-197 and Trp-574. Additionally, an Asp-376-Glu mutation in the *ALS* gene and a Ser-653-Tyr amino acid change in the ALS protein were detected in *E. crus-galli*. The study also revealed the first case of an ALS-resistant population (Trp-574-Leu) in the Czech Republic. The level of resistance was evaluated by dose–response experiments. *E. crus-galli* samples showed a high level of resistance depending on the population and the individual active ingredients and mixtures. Using molecular genetic methods, gene copies were examined separately. A single mutation in at least one out of three *ALS* gene copies was sufficient to confer resistance at the positions Pro-197 and Trp-574. At Ala-122, point mutations co-occurring in the *ALS* 1, *ALS* 2 and *ALS* 3 gene copy were identified. The study was able to provide an insight into the occurrence of polyploid *E. crus-galli* in Central Europe and its resistance mechanisms.

Is it possible to supplement the use of the complementary herbicide Conviso® (F+T, foramsulfuron + thiencarbazone-metyhl) with classic sugarbeet herbicides in such a way that even ALS-resistant weeds are reliably controlled? How competitive is the efficacy of F+T compared to classic herbicides? Does the use of an adjuvant ensure the efficacy of F+T under dry conditions?

The fourth study was concerned with the efficacy of the new sugar beet herbicide F+T. The field trials and container trials showed that ALS-resistant weeds were only partly controlled by F+T. The performance of this herbicide was improved by using tank mixtures with other MoAs. In the case of natural weed infestation without the presence of ALS-resistant weeds F+T achieved sufficient weed control. Furthermore, the use of an adjuvant ensured the efficacy of F+T under dry conditions.

8 Zusammenfassung

Herbizide sind das am weitesten verbreitete Instrument für die Unkrautbekämpfung, um den Ertrag und die Qualität von Nutzpflanzen zu sichern und zu erhalten. Der intensive Herbizideinsatz hat jedoch einen Selektionsdruck auf Unkrautgesellschaften ausgeübt, der unweigerlich zu Verschiebungen von Unkrautpopulationen und der Entwicklung von Herbizidresistenzen führte. Darüber hinaus haben die strengen Zulassungsvorschriften für Pestizide in Europa und die ungenügenden Fortschritte bei der Entwicklung neuer Herbizide mit unterschiedlichen MoA in den letzten Jahren zu einer begrenzten Auswahl an Herbiziden geführt. Die Einführung von HT-Pflanzen, die mit konventionellen Züchtungsverfahren entwickelt wurden, hat die Möglichkeiten der chemischen Unkrautregulierung im Zuckerrüben- und OSR-Anbau in Deutschland erweitert. Bisher war der Einsatz von ALS-Hemmern begrenzt, da diese Kulturen sensitiv auf ALS-hemmende Herbizide reagieren. Herbizide, die auf die Acetolactat-Synthase abzielen, gehören zu den am häufigsten eingesetzten Herbiziden im Ackerbau; ferner sind sie die resistenzanfälligste Herbizidgruppe. Die Einführung der HT-Pflanzentechnologie könnte den Einsatz von ALS-hemmenden Herbiziden weiter erhöhen und damit den Wirkstoffwechsel in der reduzieren und das Risiko einer Resistenz verstärken.

Ziel der vorliegenden Studie war es, den Zusammenhang zwischen dem Anbau von HT-Kulturen und der Entwicklung von herbizidresistenten Unkrautarten genauer zu analysieren. In Deutschland ist *A. myosuroides* das bedeutendste Schadgras, das mit Target-Site-Resistenz assoziiert wird. Darüber hinaus sind *E. crus-galli* und *T. perforatum* Unkrautarten, die aufgrund ihrer ALS-Resistenz und ihrer potenziellen Bedeutung für den Anbau von HT-Kulturen an Aufmerksamkeit gewinnen. Alle Untersuchungen, die im Rahmen dieser Arbeit durchgeführt wurden, befassen sich mit diesen drei Unkrautarten.

Die Hauptfrage war, ob es Herbizidstrategien gibt, die auch bei einem geringen Aufkommen von ALS-resistenten Unkrautarten im HT-Zuckerrübenanbau eine erfolgreiche Unkrautbekämpfung sicherstellen können? Ist es möglich, ALS-tolerante Zuckerrüben in eine Fruchtfolge zu integrieren, ohne das Risiko einer ALS-resistenten Unkrautselektion zu erhöhen?

In der ersten Arbeit wurde der Einfluss unterschiedlicher Intensitäten des ALS-Hemmereinsatzes auf die Entwicklung der herbizidresistenten Unkrautpopulationen von *A. myosuroides* und *T. perforatum* in einer Fruchtfolge mit HT-Zuckerrüben untersucht. Während der Feldversuche von 2014 bis 2017 an zwei Versuchsstandorten in Deutschland, wurden die Unkrautdichte, der genetische Resistenzhintergrund und der Ernteertrag jährlich ausgewertet. Die Ergebnisse zeigten, dass moderate ALS-resistente Unkrautdichten mit einer angepassten Herbizidstrategie kontrolliert werden können. Allerdings muss die Herbizidstrategie in Zuckerrüben um eine Graminizidbehandlung erweitert werden und im Winterweizen muss ein Residualherbizid eingesetzt werden, um die Unkrautkontrolle zu gewährleisten. Die Anwendung von ALS-Inhibitoren förderte die Entwicklung ALS-resistenter

Unkrautpopulationen, aber die Ausbreitung resistenter Biotypen konnte nicht auf die Integration herbizidtoleranter Sorten zurückgeführt werden.

Welche Auswirkungen haben unterschiedliche Intensitäten der ALS-Inhibitor-Applikation auf die Entwicklung von *A. myosuroides* im HT-OSR-Anbau? Hat die Kombination von ALS-Inhibitoren und alternativen Herbiziden Vorteile bei der Unkrautbekämpfung im HT-OSR-Anbau?

Die zweite Arbeit befasste sich mit der Wirksamkeit von Herbizidstrategien im HT-OSR-Anbau mit ALS-Inhibitoren in abgestuften Intensitäten über einen dreijährigen Versuchszeitraum. Die Parameter Ährenzahl von *A. myosuroides*, Häufigkeit von Mutationen an der Zielstelle in überlebenden Pflanzen und der Ernteertrag wurden jährlich bewertet, und am Ende des Versuchszeitraums wurde die Menge an *A. myosuroides*-Samen in der Samenbank im Boden bestimmt. Die Ergebnisse zeigten, dass die Intensität des ALS-Hemmstoffeinsatzes die Dichte der *A. myosuroides*-Ährenzahl und die Resistenzentwicklung signifikant beeinflusste. Der Zusatz von alternativen MoA zur Unkrautbekämpfung in HT-OSR konnte den Befall *mit A. myosuroides* auf keinem akzeptablen Niveau halten. Folglich sollte der HT-OSR-Anbau nicht eingesetzt werden, wenn ALS-resistente *A. myosuroides*-Pflanzen auf dem Feld vorhanden sind. Die Unkrautbekämpfung scheint schwierig zu sein, wenn man sich nur auf Herbizide verlässt. Daher muss die Unkrautbekämpfung durch zusätzliche agronomische Methoden ergänzt werden.

Wie hoch ist der Grad der Resistenz von *E. crus-galli* gegen verschiedene ALS-hemmende Herbizide in Europa? Wie viele Kopien des ALS-Gens sind an der Ausprägung der Zielstellenresistenz beteiligt?

Die dritte Studie befasste sich mit der räumlichen Verteilung von ALS-resistenten *E. crus-galli*-Populationen in Mitteleuropa. Es wurde gezeigt, dass mehrere Punktmutationen an den Positionen Ala-122, Pro-197 und Trp-574 Resistenz verleihen. Zusätzlich wurden bei *E. crus-galli* eine Asp-376-Glu-Mutation im *ALS*-Gen und eine Ser-653-Tyr-Aminosäureänderung im ALS-Protein nachgewiesen. Die Studie zeigte auch den ersten Fall einer ALS-resistenten Population (Trp-574-Leu) in der Tschechischen Republik. Der Grad der Resistenz wurde durch Dosis-Wirkungs-Experimente evaluiert. *E. crus-galli* Proben zeigten ein hohes Maß an Resistenz in Abhängigkeit von der Population und den einzelnen Wirkstoffen und Mischungen. Mit molekulargenetischen Methoden wurden die Genkopien einzeln untersucht. Eine einzige Mutation in mindestens einer von drei *ALS*-Genkopien reichte aus, um an den Positionen Pro-197 und Trp-574 Resistenz zu verleihen. An Ala-122 wurden Punktmutationen identifiziert, die in der *ALS* 1, *ALS* 2 und *ALS* 3 Genkopie gemeinsam vorkommen. Die Studie konnte einen Einblick in das Vorkommen der polyploiden *E. crus-galli*-Art in Mitteleuropa und deren Resistenzmechanismen geben.

Ist es möglich, den Einsatz des Komplementärherbizids Conviso® (F+T, Foramsulfuron + Thiencarbazone-methyl) mit klassischen Zuckerrübenherbiziden so zu ergänzen, dass auch ALS-resistente Unkräuter zuverlässig bekämpft werden? Wie konkurrenzfähig ist die Wirksamkeit von F+T im Vergleich zu klassischen Herbiziden? Stellt der Einsatz eines Adjuvants die Wirksamkeit von F+T unter trockenen Bedingungen sicher?

Die vierte Studie befasste sich mit der Wirksamkeit des neuen Herbizids F+T. Die durchgeführten Feldversuche und Kübelversuche zeigten, dass ALS-resistente Unkräuter nur teilweise mit F+T bekämpft wurden. Die Leistung dieses Herbizids wurde durch die Verwendung von Tankmischungen mit anderen MoA verbessert. Bei natürlichem Unkrautbefall ohne das Vorhandensein von ALS-resistenten Unkräutern hat F+T eine ausreichende Unkrautbekämpfung erreicht. Außerdem wurde durch den Einsatz eines Adjuvants die Wirksamkeit von F+T unter trockenen Bedingungen sichergestellt.

9 Bibliography

ADEUX G., MUNIER-JOLAIN N., MEUNIER D., FARCY P., CARLESI S., BARBERI P. & CORDEAU S. (2019) Diversified grain-based cropping systems provide long-term weed control while limiting herbicide use and yield losses. *Agronomy for Sustainable Development* 39: 42.

ALTOP E.K., MENNAN H. (2011) Genetic and morphologic diversity of *Echinochloa crus-galli* populations from different origins. Phytoparasitica 39, 93-102.

ANDERT S., BÜRGER J., STEIN S. & GEROWITT B. (2016) The influence of crop sequence on fungicide and herbicide use intensities in North German arable farming. *European Journal of Agronomy* **77**, 81-89.

ANONYMUS (2020) https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Feldfruechte-Gruenland/Tabellen/ackerland-hauptfruchtgruppen-fruchtarten.html (13.12.2020)

BAGAVATHIANNAN M.V., NORSWORTHY J.K., SCOTT R.C. & BARBER T.L. (2013) The Spread of Herbicide-Resistant Weeds: What Should Growners Know? *Agriculture and National Resources*. FSA2171-PD-6-13N.

BAKER H.G. (1974) The evolution of weeds. *Annual Review Ecology Systems* **5**, 1-24.

BALGHEIM N., WEGENER M., HUMME H., STIBBE C. & HOLTSCHULTE B. (2016) CONVISO®SMART- ein neues System zur erfolgreichen Kontrolle von Ungräsern und Unkräutern in ALS-toleranten Zuckerrüben. *Julius-Kühn-Archiv* **452**, 327-334.

BALGHEIM N. (2009) Investigations on herbicide resistant grass weeds. Dissertation University of Hohenheim.

BALGHEIM R. (2006) Herbizidresistenz vermeiden, Wirkstoffe erhalten- eine Gemeinschaftsaufgabe von Beratung, Forschung und Praxis am Beispiel des Ackerfuchsschwanz (*Alopecurus myosuroides* Huds.). *Journal of Plant Diseases and Protection.* Sonderheft **XX**, 49-56.

BALL D.A. (1992) Weed Seedbank Response to Tillage, Herbicides, and Crop Rotation Sequence. *Weed Science* **40**, 654-659.

BARBER L.T., SMITH K.L., SCOTT R.C., NORSWORTHY, J.K. & VANGILDER A.M. (2015) Zero Tolerance: A Community-Based Program for Glyphosate-Resistant *Palmer Amaranth* Management. University of Arkansas Cooperative Extension Service Bulletin FSA2177: Fayetteville, AR, USA.

BARRETT S.C.H., WILSON B.F. (1981) Colonizing ability in the *Echinochloa crus-galli* complex (barnyard grass). I. Variation in life history. Canadian Journal of Botany 59:10, 1844-1860.

BECKIE H.J. & TARDIF F.J. (2012) Herbicide cross resistance in weeds. *Crop Protection* **35**, 15-28.

BECKIE H.J. (2006) Herbicide-Resistant Weeds: Management Tactics and Practices. *Weed Technology* **20**, 793-814

BECKIE H.J., ASHWORTH M.B. & FLOWER K.C. (2019) Herbicide Resistance Management: Recent Developments and Trends. *Plants* **8**(6), 161.

BECKIE H.J., HEAP I.M., SMEDA R.J. & HALL L.M. (2000) Screening for Herbicide Resistance in Weeds. *Weed Technology* **14**, 428–445.

BENBROOK C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe* 28:3.

BERNASCONI P., WOODWORTH A.R., ROSEN B.A., SUBRAMANIAN M.V. & SIEHL D.L. (1995) A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. *Journal of Biological Chemistry* **270**, 17381-17385.

BEVERSDORF W.D. & KOTT L.S. (1987) Development of Triazine Resistance in Crops by Classical Plant Breeding. *Weed Science* **35**, 9-11

BÖRNER H. (1995) Unkrautbekämpfung. Gustav Fischer Verlag, Jena, Germany.

BOSNIC A.C. & SWANTON C.J. (1997) Influence of barnyardgrass (*Echinochloa crus-galli*) time of emergence and density on corn (*Zea mays*). *Weed Science* **45**, 276-282.

BRADSHAW L.D., PADGETTE S.R., KIMBALL S.L. & WELLS B.H. (1997) Perspectives on Glyphosate Resistance. *Weed Technology* **11**, 189-198.

BUHLER D.D., HARTZLER R.G. & FORCELLA F. (1997) Implications of weed seedbank dynamics to weed management. *Weed Science* **45**, 329-336.

BULLOCK D.G. (1992) Crop rotation. *Critical Reviews in Plant Sciences* **11**, 309-326.

CHAUVEL B., GUILLEMIN J.P., COLBACH N. & GASQUEZ J. (2001) Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroides* Huds.). *Crop Protection* **20**, 127-137.

CHAUVEL B., GUILLEMIN J.P. & COLBACH N. (2009) Evaluation of a herbicide-resistant population of *Alopecurus myosuroides* Huds. in a long-term cropping system experiment. *Crop Protection* **28**, 343-349.

CHAUVEL B., GUILLEMIN J.P., COLBACH N. & GASQUEZ J. (2001) Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroide*s Huds.). *Crop Protection* **20**, 127-137.

CHRISTOFFERS M.J. (1999) Genetic Aspects of Herbicide-Resistant Weed Management. *Weed Technology* **13**, 647-652.

CLEMENTS D.R., WEISE S.F. & SWANTON C.J. (1994) Integrated weed management and weed species diversity. *Phytoprotection* **75**, 1-18.

CORRAL M.G., LEROUX J., STUBBS K.A. & MYLNE J.S. (2017) Herbicidal properties of antimalarial drugs. *Scientific Reports* 7:45871.

COUSENS R., BRAIN P., O´DONOVAN J.T. & O´SULLIVAN P.A. (1987) The Use of Biologically Realistic Equations to Describe the Effects of Weed Density and Relative Time of Emergence on Crop Yield. *Weed Science* **35**, 720-725.

DAYAN F.E. & DUKE S.O. (2014) Natural compounds as next-generation herbicides. *Plant Physiology* **166**, 1090-1105.

DE CAUWER B., ROMBAUT R., BULCKE R., REHEUL D. (2012) Differential sensitivity of *Echinochloa muricate* and *Echinochloa crus-galli* to 4-hydroxyphenyl pyruvate dioxygenase- and acetolactate synthase-inhibiting herbicides in maize. *Weed Science* **52**, 500-509.

DÉLYE C., CAUSSER R., GAUTIER V., PONCET C., MICHEL S. (2015) Using next generation sequencing to detect mutations endowing resistance to pesticides: application to acetolactate-synthase (ALS)-based resistance in barnyardgrass, a polyploid grass weed. *Pest Management Science* **71**, 675–685.

DÉLYE C., CLÉMENT J.A.J., PERNIN F., CHAUVEL B. & LE CORRE V. (2010) High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level. *GfÖ Ecological Society of Germany, Austria and Switzerland. Basic and Applied Ecology* **11**, 504-512.

DÉLYE C. & BOUCANSAUD K. (2008) A molecular assay for the proactive detection of target site-based resistance to herbicides inhibiting acetolactate synthase in *Alopecurus myosuroides*. *Weed Research* **48**, 97-101.

DÉLYE C. (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. *Weed Science* **53**, 728-746.

DÉLYE C., JASIENIUK M. & LE CORRE V. (2013) Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics* **29**, 649-658.

DÉLYE C., PERNIN F. & SCARABEL L. (2011) Evolution and diversity of the mechanisms endowing resistance to herbicides inhibiting acetolactate-synthase (ALS) in corn poppy (*Papaver rhoeas* L.). *Plant Science* **180**, 333-342.

DEMOL F., VONREDEWITZ C., SCHULTE M. & GEROWITT B. (2012) Composition of weed populations in maize as a function of plant or crop management: Results of a nation-wide survey in Germany conducted from 2002 to 2004. *Julius-Kühn-Archiv* **434**, 655-662.

DENG W., LIU M.J., YANG Q., MEI Y., LI X.F. & ZHENG M.Q. (2015) Tribenuron-methyl resistance and mutation diversity of Pro197 in flixweed (*Descurainia sophia* L.) accessions from China. *Pesticide Biochemistry and Physiology* **117**, 68-74.

DEVINE M.D. (2005) Why are there not more herbicide-tolerant crops? *Pest Management Science* **61**, 312-317.

DOUCET C., WEAVER S.E., HAMILL A.S. & ZHANG J. (1999) Separating the effects of crop rotation from weed management on weed density and diversity. *Weed Science* **47**, 729-735.

DUKE S.O. & POWLES S.B. (2008) Glyphosate: a once-in-a-century herbicide. *Pest Management Science* **64**, 319–325.

EWERT K., SCHRÖDER G., MEINLSCHMIDT E. & BERGMANN E. (2014) New weed control strategies in maize considering narrow crop rotations with maize, greater ALS-resistance in common weeds and application restrictions with regard to active substance. *Julius-Kühn-Archiv* **443**, 621-634.

FARTYAL D., AGARWAL A., JAMES D., BORPHUKAN B., RAM B.; SHERI1 V., AGRAWAL P.K., V. ACHARY V.M.M. & REDDY M.K. (2018) Developing dual herbicide tolerant transgenic rice plants for sustainable weed management. *Scientific Reports* 8:11598.

FAY P.K. & OLSON W.A. (1978) Technique for Separating Weed Seed from Soil. *Weed Science* **26**, 530-533.

FERNANDEZ-CORNEJO J. (2004) The Seed Industry in U.S. Agriculture: An Exploration of Data and Information on Crop Seed Markets, Regulation, Industry Structure, and Research and Development. U.S. Department of Agriculture, Economic Research Service, AIB-786, 81 pp.

FOES, M.J., LIU L., VIGUE G., STOLLER E.W., WAX L.M. & TRANEL P.J. (1999) A kochia (*Kochia scoparia*) biotype resistant to triazine and ALS-inhibiting herbicides. *Weed Science* **47**, 20-27.

FROUD-WILLIAMS R.J., CHANCELLOR R.J. & DRENNAN D.S.H. (1983) Influence of cultivation regime upon buried weed seeds in arable cropping systems. *Journal of Applied Ecology* **20**, 199-208.

GEHRING K. & THYSSEN S. (2014) Herbicide treatments for the control of resistant black grass (*Alopecurus myosuroides* Huds.) in winter wheat (*Triticum aestivum*). *Julius-Kühn-Archiv* **443**,311-319.

GEHRING K., THYSSEN S. & FESTNER T. (2018) Efficiency of chemical weed control in maize (*Zea mays*) in Bavaria. *Julius-Kühn-Archiv* **458**, 178-185.

GERHARDS R. & OEBEL H. (2006) Practical experiences with a system for site-specific weed control in arable crops using real-time image analysis and GPS-controlled patch spraying. *Weed Research* **46**, 185-193.

GERHARDS R., DENTLER J., GUTJAHR C., AUBURGER S. & BAHRS E. (2016) An approach to investigate the costs of herbicide-resistant *Alopecurus myosuroides*. *Weed Research* **56**, 407–414.

GHERSA C.M., BENECH-ARNOLD R.L., SATORRE E.H. & MARTÍNEZ-GHERSA M.A. (2000) Advances in weed management strategies. *Field Crops Research* **67**, 95-104.

GIANESSI L.P. (2008) Economic impacts of glyphosate-resistant crops. *Pest Management Science* **64**, 346–352.

GÖTZE P., KENTER C., WENDT M.J. & LADEWIG E. (2018) Survey of efficacy trials for CONVISO®One in sugar beet. *Julius-Kühn-Archiv* **458**, 498-500.

GREEN J.M. (2012) The benefits of herbicide-resistant crops. *Pest Management Science* **68**, 1323–1331.

GREEN J.M. (2014) Current state of herbicides in herbicide-resistant crops. *Pest Management Science* **70**, 1351–1357.

GRESSEL J. & SEGEL L.A. (1990) Modelling the Effectiveness of Herbicide Rotations and Mixtures as Strategies to Delay or Preclude Resistance. *Weed Technology* **4**, 186-198.

GRUBER S. (2004) Genotypische Variation der Überdauerungsneigung von transgenem und konventionell gezüchtetem Raps und Möglichkeiten der Beeinflussung durch Bodenbearbeitung als Beitrag zur Sicherheitsforschung bei transgenen Kulturpflanzen. *Diss. Universität Hohenheim.*

GRUBER S., HÜSKEN A., DIETZ-PFEILSTETTER A., MÖLLERS C., WEBER E.A., STOCKMANN F., THÖLE H., SCHATZKI J., DOWIDEIT K., RENARD M.., BECKER H.C., SCHIEMANN J. & CLAUPEIN W. (2012) Biological confinement strategies for seed- and pollen-mediatedgene flow of GM canola (*Brassica napus* L.). *AgBioForum* **15**, 44-53.

GUO J., RIGGINS C.W., HAUSMAN N.E., HAGER A.G., RIECHERS D.E., DAVIS A.S. & TRANEL P.J. (2015) Nontarget-Site Resistance to ALS Inhibitors in Waterhemp (*Amaranthus tuberculatus*). *Weed Science* **63**, 399-407.

HEAP, I. (2021) The International Survey of Herbicide Resistant Weeds. (available www.weedscience.com).

HICKS H.L., COMONT D., COUTTS S.R., CROOK L., HULL R., NORRIS K., NEVE P., CHILDS D.Z. & FRECKLETON R.P. (2018) The factors driving evolved herbicide resistance at a national scale. *Nature Ecology & Evolution* **2**, 529-536.

HUANG S., GRUBER S. & CLAUPEIN W. (2016) Field history of imidazolinone-tolerant oilseed rape (*Brassica napus*) volunteers in following crops under six long-term tillage systems. *Field Crops Research* **185**, 51-58. https://doi.org/10.1016/j.fcr.2015.10.019

HULL R., TATNELL L.V., COOK S.K., BEFFA R. & MOSS S.R. (2014) Current status of herbicide-resistant weeds in the UK. *Aspects of Applied Biology* **127**, 261-272.

HUME L. (1982) The long-term effects of fertilizer application and three rotations on weed communities in wheat (after 21-22 years at Indian Head, Saskatchewan). *Canadian Journal of Plant Science* **62**, 741-750.

ISAAA (2018) Global Status of Commercialized Biotech/GM Cops: 2018. ISAAA *Brief No. 54*. ISAAA: Ithaca, NY.

ISTA INTERNATIONAL SEED TESTING ASSOCIATION (2015) International Rules for Seed Testing. Vol. 2015, Chapter 6 i-6-26 (30). (available at http://doi.org/10.15258/istarules.2015.06

IWAKAMI S., UCHINO A., WATANABE H., YAMASUE Y., INAMURA T. (2012) Isolation and expression of genes for acetolactate synthase and acetyl-CoA carboxylase in *Echinochloa phyllopogon*, a polyploid weed species. *Pest Management Science* **68**, 1098-1106.

IWAKAMI S., HASHIMOTO M., MATSUSHIMA K., WATANABE H., HAMAMURA K., UCHINO A. (2015) Multiple-herbicide resistance in *Echinochloa crus-galli* var. *formosensis*, an allohexaploid weed species, in dry-seeded rice. *Pesticide Biochemistry and Physiology* **119**, 1-8.

JAMES E.H., KEMP M.S. & MOSS S.R. (1995) Phytotoxicity of Trifluoromethyl- and Methyl-Substituted Dinitroaniline Herbicides on Resistant and Susceptible Populations of Black-grass (*Alopecurus myosuroides*). *Pesticide Science*. **43**, 213-271.

JASIENIUK M., BRÛLÉ-BABEL A.L. & MORRISON I.N. (1996) The evolution and genetics of herbicide resistance in weeds. *Weed Science* **44**, 176-193.

JOHNSON W.G., OWEN M.D.K., KRUGER G.R., YOUNG B.G., SHAW D.R., WILSON R.G., WILCUT J.W., JORDAN D.L. & WELLER S.C. (2009) U.S. Farmer Awareness of Glyphosate-Resistant Weeds and Resistance Management Strategies. *Weed Technology* **23**, 308-312.

JOUY L., GUILBERT F. (1998) Influence des pratiques culturales sur l´évolution de la flore adventice en grandes cultures. In: ANPP (Ed.) 17ième Conférence du COLUMA-Journées Internationales sur la lutte contre les mauvaises herbes. Dijon, France, 79-90.

KALOUMENOS N.S., DORDAS C.A., DIAMANTIDIS G.C. & ELEFTHEROHORINOS I.G. (2009) Multiple Pro_{197} substitutions in the Acetolactate Synthase of Corn Poppy (Papaver rhoeas) Confer Resistance to Tribenuron. *Weed Science* **57**, 362-368.

KAY Q.O.N. (1969) The origin and distribution of diploid and tetraploid *Tripleurospermum inodorum* (L.). *Watsonia* **7** (3), 130-141.

KAY Q.O.N. (1994) *Tripleurospermum inodorum* (L.) SCHULTZ BIP. *Journal of Ecology* **82**, 681-697.

KESHTKAR E., MATHIASSEN S.K., MOSS S.R. & KUDSK P. (2015) Resistance profile of herbicide-resistant *Alopecurus myosuroides* (black-grass) populations in Denmark. *Crop Protection* **69**, 83-89.

KLINGENHAGEN G. (2014) Cruciferous weeds in oil seed rape – appearance and control. *Julius-Kühn-Archiv* **443**, 606-610.

KLINGENHAGEN G. (2012) Comparison of different black-grass populations (*Alopecurus myosuroides* Huds.) in their susceptibility to herbicides under field conditions. *Julius-Kühn-Archiv* **434**, 81-87.

KLÜMPER W. & QAIM M. (2014) A Meta-Analysis of the Impacts of Genetically Modified Crops. *PLOS ONE* **9**, 1-7.

KNEZEVIC S.Z. (2010) Use of Herbicide-Tolerant Crops as Part of an Integrated Weed Management Program. http://extensionpublications.unl.edu/assets/pdf/g1484.pdf

KNISS A.R. (2018) Genetically Engineered Herbicide-Resistant Crops and Herbicide-Resistant Weed Evolution in the United States. *Weed Science* **66**, 260–273.

KOBUSCH H. (2003) Unkrautbekämpfung in Zuckerrüben –Ermittlung der Kritischen Periode. *Diss. Universität Hohenheim.*

KROPFF M.J. (1988) Modelling the effects of weeds on crop production. *Weed Science* **28**, 465-471.

LAMICHHANE J.R., DEVOS Y., BECKIE H.J., OWEN M.D.K., TILLIE P., MESSÉAN A. & KUDSK P. (2017) Integrated weed management systems with herbicide-tolerant crops in the European Union: lessons learnt from home and abroad. *Critical Reviewers in Biotechnology* **37**, 459-475

LAUFER C., SIEBACHMEYER M., GRUBER S., HUANG S., WEBER E.A. & CLAUPEIN W. (2014) Against the current - Clearfield® oilseed rape in Germany. *Julius-Kühn-Archiv* **443**, 720-727.

LETOUZÉ A. & GASQUEZ J. (1999) A pollen test to detect ACCase target-site resistance within *Alopecurus myosuroides* populations. *Weed Research* **40**, 151-162.

LETOUZÉ A. & GASQUEZ J. (2001) Inheritance of fenoxaprop-P-ethyl resistance in a blackgrass (*Alopecurus myosuroides* Huds.) population. *Theoretical and Applied Genetics* **103**, 288-296.

LÖBMANN A., CHRISTEN O. & PETERSEN J. (2019) Development of herbicide resistance in weeds in a crop rotation with acetolactate synthase-tolerant sugar beets under varying selection pressure. *Weed Research* **59**, 479-489.

LOTZ L.A.P., KROPFF M.J. & GROENEVELD R.M.W. (1990) Modelling weed competition and yield losses to study the effect of omission of herbicides in winter wheat. *Netherlands Journal of Agricultural Science* **38**, 711-718.

LUTMAN P.J.W. (2002) Estimation of seed production by *Stellaria media*, *Sinapis arvensis* and *Tripleurospermum inodorum* in arable crops. *Weed Research* **42**, 359-369.

LUTMAN P.J.W., MOSS S.R., COOK S. & WELHAM S.J. (2013) A review oft he effects of crop agronomy on the management of *Alopecurus myosuroides*. *Weed Research* **53**, 299-313.

MANALIL S., BUSI R., RENTON M. & POWLES S.B. (2011) Rapid Evolution of Herbicide Resistance by Low Herbicide Dosages. *Weed Science* **59**, 210-217.

MANLEY B.S., HATZIOS K.K. & WILSON H.P. (1999) Absorption, Translocation and Metabolism of Chlorimuron and Nicosulfuron in Imidazolinone-Resistant and -Susceptible Smooth Pigweed (*Amaranthus hybridus*). *Weed Technology* **13**, 759-764.

MÄRLÄNDER B., HOFFMANN C., KOCH H.J., LADEWIG E., MERKES R., PETERSEN J. & STOCKFISCH N. (2003) Environmental Situation and Yield Performance of the Sugar Beet Crop in Germany: Heading for Sustainable Development. *Journal of Agronomy and Crop Science* **189,** 201-221.

MÄRLÄNDER B. & VON TIEDEMANN A. (2006) Herbizidtolerante Kulturpflanzen – Anwendungspotenziale und Perspektiven. *Schriftenreihe der Deutschen Phytomedizinischen Gesellschaft e.V.* **8**, 32-45.

MARSHALL R., HANLEY S.J., HULL R. & MOSS S.R. (2012) The presence of two different target-site resistance mechanisms in individual plants of *Alopecurus myosuroides* Huds., identified using a quick molecular test for the characterisation of six ALS and seven ACCase SNPs. *Pest Management Science* **69**, 727-737.

MATZENBACHER F.O., BORTOLY E.D., KALSING A., MEROTTO JR A. (2015) Distribution and analysis of the mechanisms of resistance of barnyardgrass (*Echinochloa crus-galli*) to imidazolinone and quinclorac herbicides. *Journal of Agricultural Science* **153**, 1044-1058.

MAUN M.A. & BARRETT S.C.H. (1986) THE BIOLOGY OF CANADIAN WEEDS. 77. *Echinochloa crus-galli* (L.) Beauv. *Canadian Journal of Plant Science* **66**, 739-759.

MAY M.J. (2003) Economic consequences for UK farmers of growing GM herbicide toerant sugar beet. *Annals of applied Biology* **142**, 41-48.

MAZUR B.J. & FALCO S.C. (1989) The development of herbicide resistant crops. *Annual Review Plant Physiology and Plant Molecular Biology* **40**, 441-470.

MCNAUGHTON K.E., LETARTE J., LEE E.A. & TARDIF F.J. (2005) Mutations in ALS confer herbicide resistance in redroot pigweed (*Amaranthus retroflexus*) and Powell amaranth (*Amaranthus powellii*). *Weed Science* **53**, 17-22.

MEHRTENS J., SCHULTE M., HURLE K. (2005) Unkrautflora im Mais – Ergebnisse eines Monitorings in Deutschland. *Gesunde Pflanzen* **57**, 206-218.

MOSS S.R. (1985) The survival of *Alopecurus myosuroides* Huds. seeds in soil. *Weed Research* **25**, 201-211.

MOSS S.R. & HULL R. (2009) The value of pre-emergence herbicides for combating herbicide-resistant *Alopecurus myosuroides* (black grass). *Crop Protection in South Britain, Aspects of Applied Biology* **91**, 79-86.

MOSS S. & LUTMAN P. (2013) Black-grass: the potential of non-chemical control. A Rothamsted Technical Publication. Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

MOSS S. (2013) Black-grass (*Alopecurus myosuroides*): A Rothamsted Technical Publication. http://www.rothamsted.ac.uk (accessed: May 2020).

MOSS S.R. (2010) Integrated weed management (IWM): will it reduce herbicide use? *Communications in Agricultural and Applied Biological Sciences* **75**, 9-17.

MOSS S.R. & CLARKE J.H. (1994) Guidelines for the prevention and control of herbicide-resistant black-grass (*Alopecurus myosuroides* Huds.). *Crop Protection* **13**, 230-234.

MOSS S.R., MARSHALL R., HULL R. & ALARCON-REVERTE R. (2011) Current status of herbicide-resistant weeds in the United Kingdom. *Aspects of Applied Biology* **106**, 1-10.

MOSS S.R., PERRYMAN S.A.M. & TATNELL L.V. (2007) Managing Herbicide-Resistant Blackgrass (*Alopecurus myosuroides*): Theory and Practise. *Weed Technology* **21**, 300-309.

MULWA R.M.S. & MWANZA L.M. (2006) Biotechnology approaches to developing herbicide tolerance/selectivity in crops. *African Journal of Biotechnology* **5**, 396-404.

MURPHY C.E. & LEMERLE D. (2006) Continuous cropping systems and weed selection. *Euphytica* **148**, 61–73.

NAKKA S., THOMPSON C.R., PETERSON D.E. & JUGULAM M. (2017) Target Site-Based and Non-Target Site Based Resistance to ALS Inhibitors in Palmer Amaranth (*Amaranthus palmeri*). *Weed Science* **65**, 681-689.

NANDULA V.K., REDDY K.N., DUKE S.O. & POSTON D.H. (2005) Glyphosate-resistant weeds: current status and future outlook. *Outlooks on Pest Management* 183-187.

NAYLOR R.E.L. (1972) Biological Flora of the British Isles. *Alopecurus myosuroides* Huds. (*A. agrestis* L.). *Journal of Ecology* **60**, 611-622.

NEUHAUSER C., ANDOW D.A., HEIMPEL G.E., MAY G., SHAW R.G. & WAGENIUS S. (2003) Community genetics: expanding the synthesis of ecology and genetics. *Ecology* **84**, 545-558.

NEVE P., VILA-AIUB M. & ROUX F. (2009) Evolutionary-thinking in agricultural weed management. *New Phytologist* **184**, 783-793

NEWHOUSE K., SINGH B., SHANER D. & STIDHAM M. (1991) Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides. *Theoretical and Applied Genetics* **83**, 65-70

NIEMANN P. (2003) Die Wirkstoffe erhalten. *DLG-Mitteilungen* 1/2003.

NORRIS R.F. (1992) Relationship Between Inflorescence Size and Seed Production in Barnyardgrass (*Echinochloa crus-galli*). *Weed Science* **40**, 74-78.

NORSWORTHY J.K., GRIFFITH G., GRIFFIN T., BAGAVATHIANNAN M., GBUR E.E. (2014) In-field movement of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) and its impact on cotton lint yield: evidence supporting a zero-threshold strategy. *Weed Science* **62**, 237-249.

NORSWORTHY J.K., WARD S.M., SHAW D.R., LLEWELLYN R.S., NICHOLS R.L. & WEBSTER T.M. (2012) Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations. *Weed Science* Special Issue, 31–62.

OERKE E.-C. (2006) Crop losses to pests. *Journal of Agricultural Science* **144**, 31-43.

OWEN M.D.K (2008) Weed species shifts in glyphosate-resistant crops. *Pest Management Science* **64**, 377–387.

OWEN M.D.K (2016) Diverse Approaches to Herbicide-Resistant Weed Management. *Weed Science* Special Issue, 570-584.

PANOZZO S., SCARABEL L., TRANEL P.J., SATTIN M. (2013) Target-site resistance to ALS inhibitors in the polyploid species *Echinochloa crus-galli*. *Pesticide Biochemistry and Physiology* **105**, 93-101.

PANOZZO S.; SCARABEL L.; ROSAN V., SATTIN M. (2017) A New Ala-122-Asn AminoAcid Change Confers Decreased Fitness to ALS-Resistant *Echinochloa crus-galli*. *Frontiers in Plant Science* **8**, 2042.

PATZOLD W.L. & TRANEL P.J. (2002) Molecular analysis of cloransulam resistance in a population of giant ragweed. *Weed Science* **50**, 299-305.

PETERSEN, J. (2003) A review on weed control in sugarbeet: from tolerance zero to period threshold. Inderjit (Publ.): Weed biology and Management. Kluwer Academic Publishers, Dordrecht. 467-483.

PETERSEN J., DRESBACH-RUNKEL M. & WAGNER J. (2010) A method to determine the pollen-mediated spread of target-site resistance to acetylcoenzyme A carboxylase inhibitors in black grass (*Alopecurus myosurides* Huds.). *Journal of Plant Diseases and Protection* **117**, 122-128.

PETERSEN J. & KRATO C. (2012) Response of imidazolinone-tolerant volunteer oilseed rape to herbicides and herbicide mixtures used for broad-leaved weed control in sugar beet. *Julius-Kühn-Archiv* **434**, 353-359.

PETERSEN J. & RAFFEL H. (2020) Evolution of herbicide resistance in *Alopecurus myosuroides* and *Apera spica-venti* in German cereal production during the last 15 years. *Julius-Kühn-Archiv* **464**, 326-332.

PETERSON M.A., COLLAVO A., OVEJERO R., SHIVRAIN V. & WALSH M.J. (2018) The challenge of herbicide resistance around the world: a current summary. *Pest Management Science* **74**, 2246–2259.

PETIT C., PERNIN F., HEYDEL J.-M. & DÉLYE C. (2012) Validation of a set of reference genes to study response to herbicide stress in grasses. *BMC Research Notes* **5**:18.

POWLES S.B. & YU Q. (2010) Evolution in action: Plants resistant to herbicides. *Annual Reviews Plant Biology* **61**, 317-347.

POWLES S.B. (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. *Pest Management Science* **64**, 360-365.

PRESTON C. (2003) Inheritance and linkage of metabolism-based herbicide cross-resistance in rigid ryegrass (*Lolium rigidum*). *Weed Science* **51**, 4-12.

R DEVELOPMENT CORE TEAM (2018) *R: A Language and Environment for Statistical Computing.* R foundation for statistical computing, Vienna, Austria. Available at: http://www.R-project.org.

RAY T.B. (1984) Site of Action of Chlorosulfuron- Inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiology* **75**, 827-831.

REDDY K.N. & NANDULA V.K. (2012) Herbicide resistant crops: History, development and current technologies. *Indian Journal of Agronomy* **57**, 1-7.

REY-CABALLERO J., MENÉNDEZ J., OSUNA M.D., SALAS M. & TORRA J. (2017) Target-site and non-target-site resistance mechanisms to ALS inhibiting herbicides in *Papaver rhoeas*. *Pesticide Biochemistry and Physiology* **138**, 57-65.

RIAR D.S., NORSWORTHY J. K., SRIVASTAVA V., NANDULA V., BOND J.A., SCOTT R.C. (2013) Physiological and molecular basis of acetolactate synthase-inhibiting herbicide resistance in barnyardgrass (*Echinochloa crus-galli*). *Journal of Agricultural and Food Chemistry* **61**, 278-289.

RICHTER J. & POWLES S.B. (1993) Pollen Expression of Herbicide Target Site Resistance Genes in Annual Ryegrass (*Lolium rigidum*). *Plant Physiology* **102**, 1037-1041.

RITZ.C., Baty F., Streibig J.C., Gerhard D. (2015) Dose-Response Analysis Using R. PLoS ONE 10(12): e0146021.

RONAGHI M. & ELAHI E. (2002) Discovery of single nucleotide polymorphisms and mutations by Pyrosequencing. *Comparative and Functional Genomics* **3**, 51-56.

ROSENHAUER M. & PETERSEN J. (2015) Bioassay development for the identification of pre-emergence herbicide resistance in *Alopecurus myosuroides* (Huds.) populations. *Gesunde Pflanzen* **67**, 141–150.

ROSENHAUER M., JASER B., FELSENSTEIN F.G. & PETERSEN J. (2013) Development of target-site resistance (TSR) in *Alopecurus myosuroides* (Huds.) in Germany between 2004 and 2012. *Journal of Plant Diseases and Protection* **120**, 179-187.

ROSENHAUER M., SIEVERNICH B. & PETERSEN J. (2016) Impact of imazamox containing herbicides on the development of resistance in black-grass (*Alopecurus myosuroides* Huds.) within an oilseed rape/ wheat crop rotation. *Julius-Kühn-Archiv* **452**, 403-411.

RUNGE F., KEIL S., LÖBMANN A., PETERSEN J., BRÄNDLE F. (2020) Methods for investigating allele frequencies and distribution in the acetolactate-synthase (ALS) gene in target-site-resistant *Echinochloa crus-galli*. *Julius-Kühn-Archiv* **464**, 477-485.

RÜSTNER W.K., KLINK H. & VERREET J.A. (2014) Observation around the Clearfield® Production System in comparison to conventional oilseed rape growing under the special consideration of the existing and developing weed resistance of selected mono- and dicotyledonous weeds under practical conditions in northern Germany. *Julius-Kühn-Archiv* **447**, 164.

RUSU T., GUS P., BOGDAN I., MORARU P.I., POP A.I., SOPTEREAN M.L. & POP L.I. (2010) Influence of infestation with *Echinochloa crus-galli* species on crop production in corn. *Journal of Food, Agriculture & Environment* **8**, 760-763.

SATHASIVAN K., HAUGHN G.W. & MURAI N. (1991) Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var Columbia. *Plant Physiology* **97**, 1044-1050.

SCARABEL L., CARRARO N., SATTIN M. & VAROTTO S. (2004) Molecular basis and genetic characterisation of evolved resistance to ALS-inhibitors in *Papaver rhoeas*. *Plant Science* **166**, 703-709.

SCARABEL L., LOCASCIO A., FURINI A., SATTIN M., VAROTTO S. (2010) Characterisation of *ALS* genes in the polyploid species *Schoenoplectus mucronatus* and implications for resistance management. *Pest Management Science* **66**, 337–344.

SCARABEL L., PERNIN F. & DÉLYE C. (2015) Occurrence, genetic control and evolution of non-target-site based resistance to herbicides inhibiting acetolactate synthase (ALS) in the dicot weed *Papaver rhoeas*. *Plant Science* **238**, 158-169.

SCHÖNHAMMER A. & FREITAG J. (2014) Clearfield®-Clentiga® and Clearfield® Kombi-Pack: Two new herbicides for targeted weed control in winter- and spring oilseed rape. *Julius-Kühn-Archiv* **443**, 543-551.

SCHRÖDER G., MEINLSCHMIDT E., BÄR H., BERGMANN E. & PITTORF I. (2007) Der gezielte Einsatz von reduzierten Aufwandmengen herbizider Tankmischungen im Mais- ein Beitrag zur Umsetzung des integrierten Pflanzenschutzes in der landwirtschaftlichen Praxis. *Gesunde Pflanzen* **59**, 127-139.

SCHÜTTE G., ECKERSTORFER M., RASTELLI V., REICHENBECHER W., RESTREPO-VASSALLI S., RUOHONEN-LEHTO M., WUEST SAUCY A.-G. & MERTENS M. (2017) Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. *Environmental Sciences Europe* 29:5.

SCOTT R.K., WILCOCKSON S.J. & MOISEY F.R. (1979) The effects of time of weed removal on growth and yield of sugar beet. *Cambridge University Press* **93**, 693-709.

SENIOR I.J. & DALE P.J. (2002) Herbicide-tolerant crops in agriculture: oilseed rape as a case study. *Plant Breeding* **121**, 97-107.

SPITTERS C.J.T., KROPFF M.J., DE GROOT W. (1989) Competition between maize and *Echinochloa crus-galli* analysed by a hyperbolic regression model. *Annals of Applied Biology* **115**, 541-551.

STREIBIG J.C. (1988) Herbicide bioassay. *Weed Research* **28**, 479-484.

SWANTON C.J. & WEISE S.F. (1991) Integrated Weed Management: The Rationale and Approach. *Weed Technology* **5**, 657-663.

SWANTON C.J., WEAVER S., COWAN P., VAN ACKER R., DEEN W. & SHRESHTA A. (1999) Weed Thresholds: Theory and Applicability. *Journal of Crop Protection* **2:1**, 9-29.

THIEL H. & VARRELMANN M. (2014) Identification of a new PSII target site *psbA* mutation leading to D1 amino acid Leu$_{218}$Val exchange in the *Chenopodium album* D1 protein and comparison to cross-resistance profiles of known modifications at positions 251 and 264. *Pest Management Science* **70**, 278-285.

THOMPSON J.N. (1998) Rapid evolution as an ecological process. *TREE* **13**, 329-332.

THÖLE H., DIETZ-PFEILSTETTER A. & HÜSKEN A. (2011) Statistical approach to predict abundances of oilseed rape volunteers. *13th International Rapeseed Congress* 312-314.

TRANEL, P.J. &WRIGHT T.R. (2002) Resistance of weeds to ALS-inhibiting herbicides: what have we learnd? *Weed Sccience*. **50**, 700-712.

ULBER L. (2014) Vererbung der Herbizidresistenz gegen ALS-Inhibitoren bei *Tripleurospermum perforatum. Julius-Kühn-Archi*v **443**, 287-292.

ULBER L. (2014) Inheritance of ALS herbicide resistance in *Tripleurospermum perforatum*. *Julius-Kühn-Archiv* **443**, 287-292.

ULBER L., SVOBODA E., JASER B., FELSENSTEIN F. & ZWERGER P. (2012) Deutschlandweites Monitoring zur ALS-Resistenz bei Kamille-Arten. *Julius-Kühn-Archiv* **438**, 318-319.

UMBARGER H.E. (1978) Amino acid biosynthesis and its regulation. *Annual Review Biochemistry* **47**, 533-606.

VASEL E.H., LADEWIG E. & MÄRLÄNDER B. (2012) Weed composition and herbicide use strategies in sugar beet cultivation in Germany. *Journal für Kulturpflanzen* **64**, 112-125.

VELDHUIS L.J., HALL L.M., O´DONOVAN J.T. DYER W. & HALL J.C. (2000) Metabolism-Based Resistance of a Wild Mustard (*Sinapis arvensis* L.) Biotype to Ethametsulfuron-methyl. *Journal of Agricultural and Food Chemistry* **48**, 2986-2990.

VIDOTTO F., TESIO F., TABACCHI M. & FERRERO A. (2007) Herbicide sensitivity of *Echinochloa* spp. accessions in Italian rice fields. *Crop Protection* **26**, 285-293.

VILA-AIUB M.M., VIDAL R.A., BALBI M.C., GUNDEL P.E., TRUCCO F. & GHERSA C.M. (2008) Glyphosate-resistant weeds of South American cropping systems: an overview. *Pest Management Science* **64**, 366–371.

WAGNER J. & BELZ R. (2014) Degree of resistance of hetero- and homozygous resistant genotypes of a target-site resistant blackgrass biotype (haplotype Leu1781) in dose-response experiments with clethodim and cycloxydim. *Julius-Kühn-Archiv* **443**, 106-113.

WALKER A. & ROBERTS H.A. (1975) Effects of incorporation and rainfall on the activity of some soil-applied herbicides. *Weed Research* **15**, 263-269.

WEGENER M., BALGHEIM N., KLIE M., STIBBE C. & HOLTSCHULTE B. (2015) CONVISO®SMART-ein innovativer Ansatz der Unkrautkontrolle in Zuckerrüben. *Sugar Industry* **141**, 517-524.

WELLHAUSEN C., ULBER L. & RISSEL D. (2018) Investigation of crop management strategies for control of herbicide-resistant blackgrass (*Alopecurus myosuroides*). *Julius-Kühn-Archiv* **458**, 82-86.

WENDT, M.J., KENTER C., STIBBE C., LADEWIG E. & MÄRLÄNDER B. (2017) Selectivity of foramsulfuron + thiencarbazone-methyl and classic herbicides in sensitive and non-sensitive sugar beet genotypes. *Weed Research* **57** (4), 267-277.

WESTWOOD J.H., CHARUDATTAN R., DUKE S.O., FENNIMORE S.A., MARRONE P., SLAUGHTER D.C., SWANTON C. & ZOLLINGER R. (2018) Weed Management in 2050: Perspectives on the Future of Weed Science. *Weed Science* **66**, 275–285.

WILSON B.J. & BRAIN P. (1991) Long-term stability of distribution of *Alopecurus myosuroides*

Huds. within cereal fields. *Weed Research* **31**, 367-373.

WISLER G.C. & NORRIS R.F. (2005) Interactions between weeds and cultivated plants as related to management of plant pathogens. *Weed Science* **53**, 914-917.

WOO S.L., THOMAS A.G., PESCHKEN D.P., BOWES G.G., DOUGLAS D.W., HARMS V.L. & MCCLAY A.S. (1991) The biology of Canadian weeds. 99. *Matricaria perforata* MÉRAT (*Asteraceae*). *Canadian Journal of Plant Science* **71**, 1101-1119.

WSSA – WEED SCIENCE SOCIETY OF AMERICA (1998) Official Definitions. *Weed Technology* **12**, 798.

YANG Q., DENG W., LI X., YU Q., BAI L. & ZHENG M. (2016) Target-site and non-target-site based resistance tot he herbicide tribenuron-methyl in flixweed (*Descurainia sophia* L.). *BioMed Central Genomics* **17**:551.

YU Q., AHMAD-HAMDANI M.S., HAN H., CHRISTOFFERS M.J., POWLES S.B. (2013) Herbicide resistance-endowing ACCase gene mutations in hexaploid wild oat (*Avena fatua*): insights into resistance evolution in a hexaploid species. *Heredity* **110**, 220–231.

YU Q., HAN H. & POWLES S.B. (2008) Mutations of the *ALS* gene endowing resistance to ALS-inhibiting herbicides in *Lolium rigidum* populations. *Pest Management Science* **64**, 1229-1236.

YU Q., NELSON J.K., ZHENG M.Q., JACKSON M. & POWLES S.B. (2007) Molecular characterisation of resistance to ALS-inhibiting herbicides in *Hordeum leporinum* biotypes. *Pest Management Science* **63**, 918-927.

YU Q. & POWLES S.B. (2014) Resistance to AHAS inhibitor herbicides: current understanding. *Pest Management Science* **70**, 1340–1350.

ZHAO B., FU D., YU Y., HUANG C., YAN K., LI P., SHAFI J., ZHU H., WIE S. & JI M. (2017) Non-target-site resistance to ALS-inhibiting herbicides in a *Sagittaria trifolia* L. population. *Pesticide Biochemistry and Physiology* **140**, 79-84.

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Affirmation

Declaration under Oath

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

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