Herbicide mixtures for control of herbicide resistant *Apera spica-venti* populations

Herbizidmischungen für die Kontrolle von herbizidresistenten Windhalm-Populationen

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Abstract

Apera spica-venti is a very important weed in cereal crops throughout Central, Northern and Eastern Europe and herbicide resistance to post-emergence herbicides is often present in *A. spica-venti* populations. To improve herbicide efficacy and to prevent further spread of resistance, herbicide mixtures with different mode of action might be a solution. The aim of this study was to investigate potential interactions of ALS- and ACCase inhibiting herbicides on different *A. spica-venti* populations. Mixtures of pinoxaden with met- and thifensulfuron caused antagonism in herbicide performance to all populations tested. Tank mixtures of pinoxaden with flora- and pyroxsulam showed different interactions according to the status of resistance of the tested populations. Populations susceptible to both herbicides showed additive effects indicating that herbicides in the mixture do not influence their respective efficacy. Resistant populations showed not necessarily the same reaction to this herbicide mixture. In target-site resistant populations the efficacy of mixtures was increased with higher portions of the active ingredient still effective on the population. For populations with non-target-site resistance mechanisms the reaction was unpredictable depending on the population and mixture ratio. Synergism, antagonism and additive effects could be observed. However, results showed that these effects cannot be predicted even within different mixtures from the same modes of action.

Keywords: Antagonism, metsulfuron, pyroxsulam, pinoxaden, synergism, thifensulfuron

Zusammenfassung

Windhalm ist ein sehr bedeutendes Ungras im Wintergetreide in Zentral-, Nord- und Osteuropa. Das Vorkommen von herbizidresistenen Population gegenüber blattaktiven Herbiziden ist häufig. Um die Wirksamkeit von Herbizidapplikationen zu verbessern und damit der Verbreitung von Herbizidresistenz entgegen zu wirken, könnten Herbizidmischungen eine Lösung darstellen. Das Ziel dieser Studie war zu prüfen, ob potentielle Interaktionen von ALS- und ACCase-Hemmern bei verschiedenen Windhalm-Herkünften auftreten. Mischungen mit Pinoxaden und Met- und Thifensulfuron verursachten Minderwirkungen gegenüber allen geprüften Herkünften, die auf einen Antagonismus der Wirkstoffe zurückzuführen war. Tankmischungen von Pinoxaden mit Flora- und Pyroxsulam zeigten in der Wirkung Interaktionen mit den verschiedenen Herkünften bzw. Resistenzmechanismen. Während bei sensitiven Herkünften die beiden Herbizide in beliebigen Mischungsverhältnissen ausgebracht werden konnten, ohne die Wirkung negativ zu beeinflussen, war bei resistenten Herkünften keine einheitliche Reaktion zu beobachten. Während Zielortresistenzen gegenüber einer Wirkstoffklasse zunehmend wirksamer kontrolliert wurde je höher der Anteil des noch wirksamen Bestandteils war, war dies bei Nicht-Zielortresistenz nicht unbedingt der Fall. Teilweise gab es Synergismen durch die Tankmischung oder es zeigten sich antagonistische zum Teil auch additive Effekte. Die Tankmischung zweier wirksamer blattaktiver Einzelkomponenten ist demnach nicht immer die Lösung, die eine sichere Bekämpfung von resistenten Windhalmherkünften verspricht.

Stichwörter: Antagonismus, Metsulfuron, Pyroxsulam, Pinoxaden, Synergismus, Thifensulfuron

Introduction

Loose silky bent grass (*Apera spica-venti* (L.) Beauv.; APESV) is an annual grass weed in European winter cereal fields (NORTHAM and CALLIHAN, 1992). APESV is the most important grass weed in winter cereals in many regions of Central, Middle-eastern and Northern Europe (MELANDER et al., 2008; HAMOUZOVÁ et al., 2011). For post-emergence weed control of APESV only a few modes of action are available. Due to high abundance of the weed and frequent use of herbicides, resistant biotypes do occur in all countries where APESV is present (NIEMANN and ZWERGER, 2006; MASSA and GERHARDS, 2011; HEAP, 2017) and resistance in APESV is still spreading in space and strength. Consequently, APESV control strategies must be adapted to the current resistance situation to ensure sustainable arable systems. Next to an implementation of agronomic management practices like crop rotations, soil tillage and others, herbicide application will still be a part of these strategies. However, anti-resistance strategies recommend rotation of herbicide mode of action, use of pre-emergence herbicides and use of herbicide mixtures (WSSA, 1995; DIGGLE et al., 2002; BECKIE et al., 2004; MOSS et al., 2007).

Nevertheless, not all active ingredients may be suitable partners in a certain herbicide mixture. Within herbicide mixtures three scenarios of interactions between the mixture partners may occur. Either the active ingredients herbicides do not influence each other's performance (additivity) or the performance of a herbicide in a mixture is either impaired (antagonism) or promoted (synergism) by the presence of other herbicides in the spray solution (HYDRICK and SHAW, 1994; HATZIOS and PENNER, 1985). Antagonism often occurs between selective broadleaf herbicides and graminicides (MINTON et al., 1989).

The potential for interactions in herbicide tank mixtures is currently not fully understood (DAMALAS, 2004) but knowledge about mixtures can provide important information for farmers as they decide about their weed control programs (HYDRICK and SHAW, 1994). Antagonistic effects in herbicide tank mixture may provoke the development of herbicide resistances. If synergistic effects occur, the herbicide rates can be reduced without increasing the risk of losing efficacy. Should additive effects result from mixing different modes of action, herbicide failure can be prevented in case of resistance if one mode of action remains still effective. However, many selective herbicides for APESV control in cereals contain ALS- or ACCase-inhibitors. For both modes of action resistance in APESV has been described. One idea to improve herbicide efficacy for control of APESV and to prevent spread of herbicide resistance in this species is to mix herbicides from both mode of action at full dose rates.

The aim of our study was to examine the efficacy of two different mixtures of ALS- and ACC-ase-inhibitors on susceptible and resistant APESV populations. Investigations on potential interactions of populations and herbicide mixture performance should allow choosing the right option for farmers in their particular situations without increasing the risk for herbicide resistance development.

Materials and Methods

Apera spica-venti (APESV) populations

Ten different APESV populations form different locations in Austria and Germany were analyzed in the presented study (Tab. 1). Characterization of the herbicide resistance profiles of the populations against pinoxaden and ALS-inhibitors (thifen- and metsulfuron and flora- and pyroxsulam) was conducted in previous bioassays in the greenhouse and subsequent genetic analyses. Two susceptible populations and different herbicide resistant APESV populations were chosen for the experiments. Criteria for choice of populations were different resistance factors and different mechanisms of resistance (NTSR, TSR). Trials with different ALS-inhibitors contained only partly the same populations, because trials with met- and thifensulfuron and pinoxaden showed in general no interactions with APESV but mixtures of pinoxaden with pyroxsulam did. Therefore also populations with resistance to pinoxaden were included into the trials with pyroxsulam and pinoxaden mixtures.

Tab. 1 Resistance factors (based on ED$_{50}$ values; 1.0 = mean of susceptible populations W11-024 and W11-111) and information on target-site-resistance (TSR) of *A. spica-venti* populations used in the experiments (n.i. – not investigated).

***Tab. 1** Resistenzfaktoren (auf Basis der ED$_{50}$-Werte; 1.0 = Mittelwert der sensitive Herkünfte W11-024 und W11-111) und Angaben zur Zielortresistenz (TSR) der eingesetzten A. spica-venti-Herkünfte (n.i. – nicht untersucht).*

				resistance factor			**target-site resistance**	
population-code	**post code**	**location**	**year of sampling**	**pinoxaden**	**thifen- and metsulfuron**	**flora- and pyroxsulam**	**ALS**	**ACCase**
W11-024	A-4773	Eggerding	2011	1.1	1.1	0.8	none	none
W11-111	17495	Klein Bünsow	2011	0.9	0.9	1.2	none	none
W09-157	39362	Meitzendorf	2009	0.9	17.6	11.8	none	none
W11-097	17237	Warbende	2011	0.6	29.2	14.4	Trp-574-Leu	none
W-879	48653	Coesfeld	2008	1.1	34.3	15.6	none	none
W12-062	19386	Granzin	2012	0.8	n.i.	34.0	Pro-197-Thr	none
W10-046	99610	Sömmerda	2010	1.2	22.1	1.4	none	none
Selfkant	52538	Selfkant	2008	9.0	n.i.	0.9	none	Ile-1781-Leu
W12-033	93186	Pettendorf	2012	4.5	n.i.	12.0	none	none
W884	34613	Schwalmstadt	2008	3.4	n.i.	74.4	Pro-197-Thr	none

Herbicides

Herbicides used in the study were pinoxaden (50 g a.i. L^{-1}; Axial 50; EC; Syngenta Agro GmbH), florasulam and pyroxsulam (22.8 + 68.3 g a.i. kg^{-1}; Broadway; WG; Dow AgroSciences GmbH) and met- and thifensulfuron (38.4 + 384.5 g a.i. kg^{-1}; Concert SX; WG; DuPont de Nemours). Herbicides were applied solo and in fixed ratios of pinoxaden and florasulam and pyroxsulam or pinoxaden and met- and thifensulfuron (0.8:0.2; 0.6:0.4; 0.4:0.6; 0.2:0.8) in greenhouse trials (Tab. 2).

Tab. 2 Applied dose rates (g a.i. ha^{-1}) for herbicides applied solo and in fixed ratios (mix) of pinoxaden with flora- and pyroxsulam and pinoxaden with met- and thifensulfuron, respectively.

***Tab. 2** Aufwandmengen (g Wirkstoff ha^{-1}) für die verwendeten Herbizide (solo) und Tankmischungen (mix) von Pinoxaden mit Flora- und Pyroxsulam bzw. Pinoxaden mit Met- und Thifensulfuron.*

Herbicide/Mixture	**Active ingredients**	**Minimum dose**	**Maximum dose**
Axial50 (solo)	pinoxaden	0.14	4.500
Broadway (solo)	florasulam	0.04	294.4
	+pyroxsulam	0.11	887.9
Concert SX (solo)	metsulfuron	0.14	115.2
	+thifensulfuron	1.44	1153.5
Mix: Axial50	pinoxaden	1.13	45
+Concert SX	metsulfuron	0.14	9.22
	+thifensulfuron	1.44	92.28
Mix: Axial50	pinoxaden	0.14	144
+Broadway	florasulam	0.04	9.48
	+pyroxsulam	0.11	28.4

Registrated doses for APESV control: Pinoxaden 45 g ha-1; flora- and pryroxsulam 2.96 + 8.88 g ha^{-1}; met- and thifensulfuron 5.76 + 5.7 g ha^{-1}

The dose ranges were selected to cover the responses of APESV populations from no observable herbicide effect to complete control of the population. The tested herbicide combinations were chosen because it was observed in field trials that pinoxaden with metsulfuron and thifensulfuron showed antagonistic effects (WOLBER and KREYE, 2012). The second combination of pinoxaden with flora- and pyroxsulam was chosen because both herbicides play a very important role in the control of APESV and no observations of possible interactions in tank mixtures of these two herbicides are known up today. For mixtures of pinoxaden with met- and thifensulfuron six dose rates and for mixtures of pinoxaden with flora- and pyroxsulam eight dose rate were used for each mixture ratio. The ED_{50} (the dose that reduces plant growth by 50%) for each herbicide was determined in preliminary experiments to find the most appropriate ratios for the binary mixtures. Used dose rates for herbicides applied solo and in mixtures are given in Table 2.

Bioassays

As soil substrate, a sieved and sterilized (4 h at 70 °C) soil (sandy loam, pH-value 6.5, organic matter content ~ 2%) was used. Seeds of each population were sown on trays, covered with 2 mm soil and trays were placed in the greenhouse with temperatures between 12 and 18 °C. The plants were irrigated manually as required by temporarily flooding of the glasshouse tables. Additional light with an intensity of 300 µE m^{-2} s^{-2} was given from 07:00 to 10:00 and 16:00 to 19:00. Ten days after sowing the seedlings were transplanted into Jiffy pots (8 x 8 cm) filled with the substrate mentioned above. Five seedlings of the same plant size were planted in each pot. Pots were integrated into a randomised block design in the greenhouse. Replications represented the blocks. All in all, three pots per population, dosage and herbicide combination were used as replicates. One week after transplanting at one to two-leaf stage, the herbicides were applied with a laboratory sprayer (Schachtner) nozzle TEE JET 9502EVS, a water volume of 250 l ha^{-1}, a speed of 2.5 km h^{-1} and a pressure of 250 kPa. Twenty-one days after herbicide application the plant biomass was evaluated by cutting and measuring the fresh weight of each surviving plant. The studies were replicated twice under identical glasshouse conditions. Results of the repeated trials were very similar for both mixtures. Consequently, mean data of the two repeated trials are shown.

Statistical analysis

The data obtained from greenhouse trials (fresh biomass) was used to calculate ED_{50} values of herbicides applied solo and in mixtures. The dose-response models were fitted using the statistical software R (R CORE TEAM, 2015) and the drc-package (RITZ and STREIBIG, 2005). The response of fresh biomass (U) on herbicide dose (z) was described by a logistic model (STREIBIG et al., 1998):

U_ij=C+(D-C)/(1+exp[b_i (log(z_ij)-log(ED_50(i)))]

Where U_ij is the fresh biomass at the jth dose of the ith herbicide mixture; D and C are the upper and lower limit of fresh biomass at zero and infinite doses. $ED_{50}(i)$ is the dose required of herbicide i to reduce fresh biomass by half between the upper and lower limit, D and C. The b_i is proportional to the slope of the curve around $ED_{50}(i)$.

In order to achieve a higher practical relevance, all following results will be presented as relative ED_{90} values. Relative ED_{90} values were calculated by dividing the ED_{90} value by the maximum registrated dose rate of regarding herbicide or mixture.

Results

Populations W11-024 and W11-111 were susceptible to all three herbicides tested (Tab. 1). Mean ED_{50}-values of these two populations were used as references for the calculations of resistance factors (RF). Populations W884, W12-033 and Selfkant were identified as pinoxaden resistant on the basis of the conducted bioassays. Selfkant was the only APESV population with ACCase target-site resistant (TSR) used in this experiment. Resistant factor for pinoxaden was 9 in the TSR population 'Selfkant' compared to 3.4 resp. 4.5 in the non-target-site-resistant (NTSR) populations 'W12-033' and 'W884'. Resistance factors to the ALS-inhibitor met- and thifensulfuron were higher compared to flora- and pyroxsulam of the populations W09-157, W12-046, W11-097 and W879. This observation was independent of the resistance mechanism. Resistant factors of flora- and pyroxsulam varied between 11.8 and 74.4. In general resistance factors for flora- and pyroxsulam were higher in populations with TSR. However, also NTSR population W879 showed a resistance factor of 15.6 and was higher than for the TSR population W11-097.

Results of dose-response experiments with different ratios of herbicide mixtures are shown as relative ED_{90}-values in relation to the maximum registered dose. Relative ED_{90}-values below 100 indicate a susceptible plant-response to one herbicide or a mixture while relative ED_{90}-values higher than 100 indicate a reduced effectiveness due to the occurrence of lower susceptibility to herbicides or due to occurrence of herbicide resistance. Results for experiments using pinoxaden and met- and thifensulfuron solo and in mixtures are given in Table 3 and results for pinoxaden and flora- and pyroxsulam are shown in Table 4. Results of the susceptible populations W11-024 and W11-111 are presented as one mean value and described as 'sen' in following tables. The response of the tested populations to herbicides applied solo confirms the described resistance patterns from the preliminary trials reported above.

Tab. 3 Relative ED_{90}-values (% in relation to max. registered dose rate; 100 = registrated dose rate shows 90% efficacy on tested plants) of analyzed APESV populations for pinoxaden, met- and thifensulfuron and different mixtures of both herbicides.

Tab. 3 *Relative ED_{90}-Werte (% in Relation zur max. zugelassenen Aufwandmenge 100 = registrierte Aufwandmenge verursacht 90 % Wirkung) der untersuchten Windhalmherkünfte für Pinoxaden und Met- und Thifensulfuron und deren verschiedenen Tankmischungen.*

fixed ratio		herbicide	sen	W10-046	W-879	W09-157	W11-097
1	-	pinoxaden	13.2	12.8	19.6	19.2	14.7
-	0	met- & thifensulfuron					
0.8	-	pinoxaden	40.7	59.0	37.3	63.3	27.4
-	0.2	met- & thifensulfuron					
0.6	-	pinoxaden	54.2	75.3	50.5	82.3	45.5
-	0.4	met- & thifensulfuron					
0.4	-	pinoxaden	70.4	95.6	71.8	119.9	66.6
-	0.6	met- & thifensulfuron					
0.2	-	pinoxaden	87.6	108.7	175.6	254.6	115.9
-	0.8	met- & thifensulfuron					
0	-	pinoxaden	93.5	>1000	>1000	>1000	>1000*
-	1	met- & thifensulfuron					

*ED_{90} value far outside dose range tested

Tab. 4 Relative ED$_{90}$-values (% in relation to max. reg. dose; 100% = registrated dose cause 90% efficacy) of analyzed *A. spica-venti* populations for pinoxaden with flora- and pyroxsulam and different mixtures of both herbicides.

Tab. 4 *Relative ED$_{90}$-Werte (% in Relation zu max. zugelassenen Aufwandmenge 100 % = registrierte Aufwandmenge verursacht 90 % Wirkung) der untersuchten Windhalmherkünfte für Pinoxaden und Flora- und Pyroxsulam und deren verschiedenen Tankmischungen.*

fixed ratio		herbicide	sen	W09-157	W11-097	W-879	W12-062	W-884	W12-033	Selfkant
1	-	pinoxaden	30.8	42.6	32.6	43.5	29.5	203.3	491.5	>1000*
-	0	flora- & pyroxsulam								
0.8	-	pinoxaden	30.2	213.4	47.1	128.8	19.1	128.6	38.2	32.6
-	0.2	flora- & pyroxsulam								
0.6	-	pinoxaden	27.4	137.1	170.8	280.5	46.0	155.0	47.8	21.1
-	0.4	flora- & pyroxsulam								
0.4	-	pinoxaden	24.7	122.0	147.9	147.6	63.6	116.3	46.8	18.9
-	0.6	flora- & pyroxsulam								
0.2	-	pinoxaden	17.9	84.7	148.2	207.5	>1000	>1000	34.4	18.1
-	0.8	flora- & pyroxsulam								
0	-	pinoxaden	20.9	136.4	267.4	>1000	>1000	>1000	38.1	19.4
-	1	flora- & pyroxsulam								

*ED$_{90}$ value far outside dose range tested

Discussion

Results of mixtures of pinoxaden with met- and thifensulfuron showed in general an increase of relative ED$_{90}$ values with increasing portion of met- and thifensulfuron in the mixture for all populations tested. However, in the ALS resistant populations the relative ED$_{90}$ values increased stronger with higher portion of met- and thifensulfuron in the mixture. This confirms the field observations of WOLBER and KREYE (2012) on mixtures of pinoxaden with met- and thifensulfuron. Data of susceptible and resistant populations showed no difference in herbicide response regarding the ratios. The tested mixture contains two different modes of action for control of APESV but they are losing their potential if they are applied in a tank mixture. Therefore this mixture is not useful for anti-resistance herbicide management.

Results of different herbicide ratios of the mixture of pinoxaden and flora- and pyroxsulam show that there is no general consistent interaction between pinoxaden with flora- and pyroxsulam in a tank mixture. The kind of interaction seems to be depending on the resistance status of the tested population. Results suggest that mixtures of pinoxaden with flora- and pyroxsulam can be a useful tool for the control of ALS resistant APESV populations, but the use of such mixtures requires a profound understanding of the resistance situation (= mechanisms of resistance present in a population) on the certain field where the mixture is to be applied. However, the reasons for this interaction remain unclear and demand further experiments with tank mixtures and resistant populations in the future.

In general, the use of ACCase- and ALS-inhibiting herbicides is considered to implicate a high risk of resistance development (MOSS et al., 2007). Both modes of action are widely used for weed control in cereals and other crops. Besides single nucleotide polymorphism within the two target genes causing resistance, also NTSR to both modes of action can appear. Consequently a mixture of ALS- and ACCase inhibitors bear an inherent risk for selection of multiple resistant biotypes in the long run even if short term efficacy may be increased by mixtures.

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