Efficacy study and resistance detection for pre-emergence herbicides under greenhouse conditions; a method comparison for pendimethalin

Wirksamkeitsprüfung und Resistenzdetektion für bodenaktive Herbizide; Ein Methodenvergleich am Beispiel Pendimethalin

Alexander Menegat[1]*, Bernd Sievernich[2] and Roland Gerhards[1]

[1] Universität Hohenheim, Institut für Phytomedizin, Fachgebiet Herbologie, Otto-Sander-Str. 5, 70599 Stuttgart, Germany

[2] BASF SE, Agrarzentrum Limburgerhof, 67117 Limburgerhof, Germany

*Corresponding author, alexander.menegat@uni-hohenheim.de

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Abstract

Aim of the presented study is to demonstrate the impact of two different irrigation methods, from below and from above, and two different pendimethalin formulations, capsule suspension (CS) and suspension concentrate (SC) on the control efficacy of pendimethalin against Silky bent-grass (*Apera spica-venti*) under greenhouse conditions. We assume that changes regarding the efficacy level of pendimethalin due to methodological factors will affect the reliability of resistance detection.

Five populations of Silky bent-grass were selected according to their various multi-resistance patterns. One population was a standard sensitive reference population while the remaining four populations are characterized by different resistance patterns against herbicides of the HRAC groups A, B, C2, K1 and N. Pendimethalin dose response studies were performed by taking into account the experimental factors (1) irrigation system and (2) pendimethalin formulation.

The results show that a combination of CS-formulation and irrigation from above resulted in a significantly reduced efficacy of pendimethalin independent of the resistance profile of the tested populations. Therefore no differentiation between susceptible and tolerant populations was possible. In contrary the SC-formulation resulted in an overkill situation under irrigation from below even at dosages lower than 20% of the recommended field rate. Thus the differentiation between the resistant and susceptible biotype was impossible as well. For the detection of reduced tolerance against pendimethalin a combination of SC-formulation and irrigation from above was found to be favourable and thus this should become the standard procedure for pendimethalin resistance testing.

Keywords: *Apera spica-venti* capsule suspension, non-target-site resistance, pendimethalin, resistance detection, suspension concentrate

Zusammenfassung

Ziel der vorgestellten Versuche ist es, die Auswirkungen unterschiedlicher Methoden der Bewässerung (Beregnung vs. Anstaubewässerung) und unterschiedlicher Formulierungen von Pendimethalin (Kapselsuspension, CS vs. Suspensionskonzentrat, SC) auf die Wirksamkeit von Pendimethalin unter Gewächshausbedingungen zu untersuchen. Es ist anzunehmen, dass Veränderungen der Wirkung auf Grund von methodischen Faktoren sich auf die Verlässlichkeit der Resistenzdetektion auswirken. Fünf Windhalm Populationen (*Apera spica-venti)* wurden auf Grund ihrer unterschiedlichen Resistenzprofile für die Versuche ausgewählt. Eine der Populationen war eine sensitive Standard-Population. Vier weitere Populationen zeigten Resistenzen gegenüber Wirkstoffen aus den HRAC Gruppen A, B, C2, K1 und N. Für den Wirkstoff Pendimethalin wurden Dosis-Wirkungs-Versuche durchgeführt unter Einbezug der genannten Versuchsparameter (1) Bewässerungsstrategie und (2) Pendimethalin Formulierung.

Die durchgeführten Versuche haben gezeigt, dass eine Kombination aus CS-Formulierung und Beregnung, unabhängig vom Resistenzprofil der getesteten Population, zu einer eingeschränkten Wirksamkeit von Pendimethalin unter Gewächshausbedingungen führt. Demzufolge war keine Unterscheidung zwischen der sensitiven und der resistenten Population möglich. Im Gegensatz dazu führte die Kombination aus SC-Formulierung und Anstaubewässerung bereits bei Dosierungen von weniger als 20% der empfohlenen Feldaufwandmenge zu 100 % Bekämpfungserfolg. Demzufolge war auch hier keine Unterscheidung zwischen der sensitiven und der resistenten Population möglich. Für die Detektion erhöhter Toleranzen gegenüber Pendimethalin hat sich eine Kombination aus SC-Formulierung und Beregnung als vorteilhaft erwiesen. Diese Erkenntnisse sollten bei der Entwicklung eines standardisierten Testverfahrens berücksichtigt werden.

Stichwörter: *Apera spica venti*, Kapselsuspension, metabolische Resistenz, Pendimethalin, Resistenzdetektion, Suspensionskonzentrat

Introduction

The excessive use of the HRAC group A and B herbicides for grass weed control in winter cereals has caused the spread of herbicide resistant grass weed populations all over Europe and has caused the selection of mainly non-target site resistant (NTSR) populations (SIEVERNICH *et al.*, 2013). Therefore non-target-site resistance (NTSR) has become the centre of herbicide resistance research. NTSR causes, in contrary to target-site resistance (TSR), unpredictable multi-resistance patterns across different modes of action. The underlying polygenetic NTSR mechanisms are currently under intensive investigation but, due to their complexity and diversity, only slow progresses in gaining knowledge are expected.

The number of HRAC group K- or N-tolerant grass weed populations is very low (HEAP, 2013) and these compounds are recommended as alternative modes of action, why herbicides like pendimethalin (HRAC group K1) are important compounds for the control of ALS and ACCase resistant weed populations as well as for the prevention of herbicide resistance development. Considering the fact that due to the European Community regulation EC1107/2009 the diversity of compounds will decrease and no new modes of action will be available in the near future, HRAC group K1, K3 and N compounds will become the backbone of weed control in winter cereals.

Pendimethalin is currently approved for use in all European countries over a wide range of crops. The compound is absorbed by roots, coleoptiles of emerging weeds and to a certain extend by leafs. In winter cereals pendimethalin is used as pre-emergence as well as early-post and post-emergence herbicide until weed BBCH 13. For control of silky bent grass pre-emergence and early post-emergence treatments were found to be favourable.

Even if the number of pendimethalin tolerant grass weed populations is low, standardised efficacy testing methods have to be developed to ensure a proactive and reliable monitoring of potential resistance occurrences in the future. However, efficacy studies with soil active herbicides under greenhouse conditions are problematic and deliver controversial data. Results highly depend on soil types used for the trials, temperature conditions in the greenhouse, timing of herbicide application and in the case of pendimethalin even on the type of formulation used.

Several methodological aspects have been discussed before, like the influence of various plant densities on herbicide efficacy, effect of temperature on herbicide efficacy as well as the type of irrigation system (MENNE and WAGNER, 2008; MENNE *et al.*, 2012). Up to now the influence of pendimethalin formulation as well as the combined effect of irrigation system and pendimethalin formulation on resistance detection has not been discussed before.

Material and methods

In addition to a susceptible *Apera spica-venti* standard population four populations with different resistance profiles were selected for the experiments, based on the results of previous herbicide efficacy studies as well as field observations. Seeds of the tested herbicide tolerant populations were collected from locations in Germany, Czech Republic and Poland. The biotypes were selected specifically due to their various multi-resistance profiles against herbicides of the HRAC groups A, B, C2, K1 and N. Single nucleotide polymorphisms (SNP) could be confirmed at the ALS genome for all tested resistant populations. Herbicide resistances profiles for the selected biotypes as well as the respective SNP profiles are shown in Table 2. For the selected herbicide tolerant biotypes a certain level of NTSR (non-target-site resistance) can be assumed, since the SNP's alone cannot explain the multi-resistance profile.

For dose-response studies seeds were sown into 8 cm pots (Jiffy Products International B.V., NL). filled with a soil mixture according to Table 1. Germination tests, realised with the same soil mixture shown in Table 1, were used to estimate the germination capacity and thus for calculation of the seed amount needed per pot for each biotype. Hence a target plant density of 8 plants per pot (+-2) could be realised. The pots were placed in a glasshouse at 15 °C day and 10 °C at night (+-3 °C), 60% humidity and with 12h additional illumination. Herbicides were applied three days after sowing with a standard laboratory track sprayer (8002 EVS TeeJet nozzle, pressure 320 kPa, water amount 200 l/ha). The two tested formulations of pendimethalin were sprayed in five dosages ranging from 2000 g a.i./ha to 250 g a.i./ha and an untreated control. Three repetitions per treatment were realized.

The herbicide efficacy assessment took place 21 days after treatment by a colour threshold based binary image analysis. A detailed description of the binary image analysis method in conjunction with herbicide dose response pot experiments can be found in JÄCK *et al.* (2011) as well as in MASSA and GERHARDS (2011).

For method comparison the experiment contained the following variables:

1) Irrigation conditions – from above and from below
2) Formulation of pendimethalin – capsulated suspension (Stomp Aqua®, BASF SE) and suspension concentrate (Stomp SC®, BASF SE)

Pots treated with Stomp Aqua® and irrigated from below were once watered from above 24 h after treatment for activation of the herbicide.

Tab. 1 Soil texture and organic matter content of the substrate used for experiments.

Tab. 1 *Korngrößenzusammensetzung und organische Substanz des verwendeten Bodensubstrates.*

Soil texture	**%**
Clay	14,9
Sand	58,3
Silt	26,8
C_{org}/organic matter	1,29/2,23

Tab. 2 Resistance profile of the tested Silky bent grass populations. Resistance classification according to MOSS (1999).

Tab. 2 *Resistenzprofil der getesteten Windhalm- Populationen. Resistenzklassifikation nach MOSS (1999).*

	10-174-STD	**10-139**	**12-134**	**12-215**	**12-259**
cycloxydim	S	S	S	S	S
pinoxaden	S	S	S	S	R?
mesosulfuron + iodosulfuron	S	RR	RR	RR	RR
pyroxsulam	S	R?	RR	S	RR
isoproturon	S	RR	S	S	S
pendimethalin	S	S	S	S	R?
prosulfocarb	S	S	R?	R?	R?
NTSR	-	x	x	x	x
SNP ALS	-	Pro197	Pro197Thr	Pro197ThrTrp574Leu	Pro197Ser

A dose response analysis was performed, using the non-linear regression model after STREIBIG (1988), with the statistical language R (R DEVELOPMENT CORE TEAM, 2010) and the R add-on package drc (RITZ and STREIBIG, 2005). ED50 and ED90 values were calculated and analysed for statistical significant differences between the biotypes.

Results

For analysis of the effects of formulation and irrigation, data of pendimethalin susceptible populations were pooled (population 10-174-STD, 10-139, 12-134 and 12-215). Dose response parameters of the pooled populations were not significantly different at p = 0.05. Dose response curves for the two tested formulations (CS and SC) were compared by testing the ED50 and ED90 values for significant differences.

The type of irrigation significantly influenced the efficacy of the CS-formulation (Fig. 1, A). ED50 and ED90 values were significantly different at p = 0.05. As can be seen in Figure 1, irrigation from above resulted in a faster release of pendimethalin from the capsules and hence in a lower ED50 value of 257.43 g a.i./ha compared to irrigation from below with an ED50 value of 567.2 g a.i./ha. Nevertheless the ED90 value of the irrigation from above was significantly higher compared to the irrigation from below. Under irrigation from above the pendimethalin dosage has to be 2.3 times higher to reach an efficacy level of 90% compared to the irrigation from below. Both ED90 values for the CS-formulation are much higher than the maximum recommended dosage of pendimethalin under field conditions (2000 g a.i./ha).

The ED50 as well as ED90 values of the SC-formulation (Fig. 1, B) for the tested irrigation systems were not significantly different (ED50 values: irrigation from above 188.7 g a.i./ha; irrigation from below 51.2 g a.i./ha). The irrigation system did not affect the herbicide efficacy. Unless no significant differences were found it could be observed that the efficacy at dosage below 100 g a.i./ha was higher under irrigation from below compared to from above.

The observed effects of the tested irrigation systems and pendimethalin formulations influenced the detection of the pendimethalin tolerant population. Considering the SC-formulation und irrigation from below, no significant differences were found comparing population 12-259 (subsequently named tolerant) and the susceptible populations (Fig. 2, A). The low ED90 values indicate that the herbicide efficacy under irrigation from below was high enough to affect susceptible and tolerant populations to the same extend. Under irrigation from above ED90 values of the susceptible and the tolerant population were significantly different at p = 0.05.

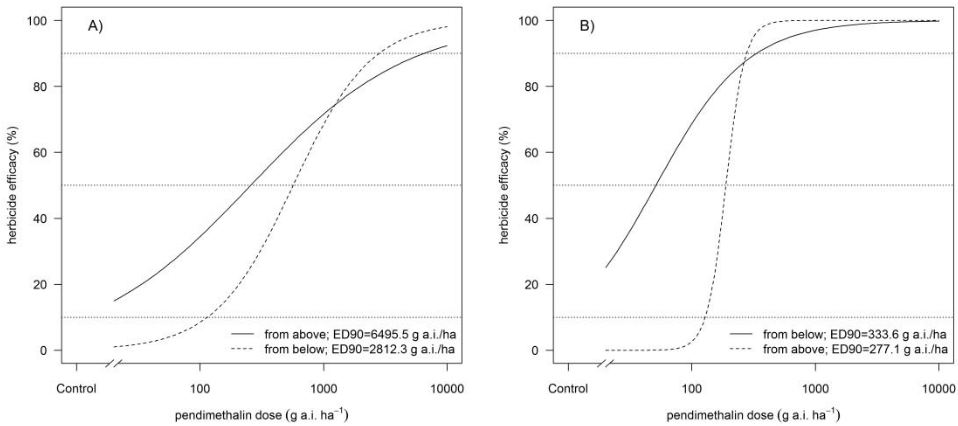

Fig. 1 Dose response curves and ED90 values of susceptible populations for pendimethalin CS-formulation (A) and SC-formulation (B) under irrigation from above and from below.

Abb. 1 *Vergleich der Dosis-Wirkungs Beziehung und ED90-Werte sensitiver Populationen für Pendimethalin CS-Formulierung (A) und SC-Formulierung (B) unter Bewässerung von oben und Anstaubewässerung.*

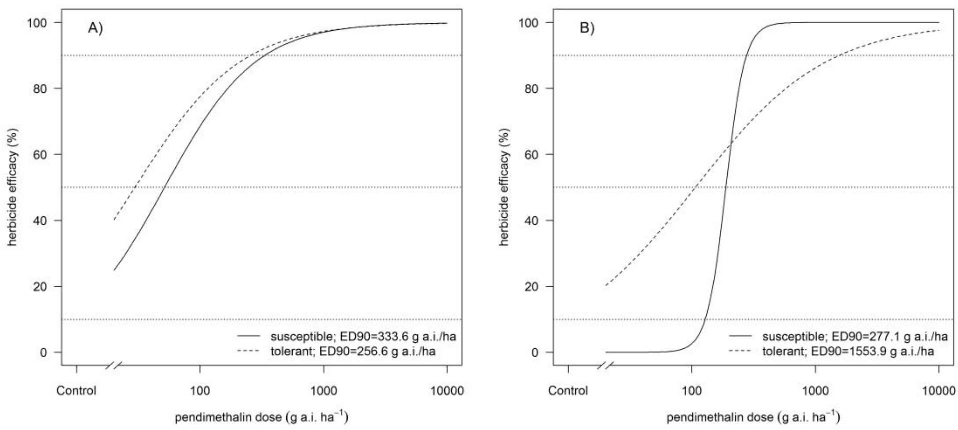

Fig. 2 Dose response curves and ED90 values of susceptible and tolerant populations for pendimethalin SC-formulation under irrigation from below (A) and from above (B).

Abb. 2 *Vergleich der Dosis-Wirkungs-Beziehung und ED90-Werte sensitiver und toleranter Populationen für pendimethalin SC-Formulierung unter Anstaubewässerung (A) und Bewässerung von oben (B).*

A resistance factor of 5.6 was found for the tolerant population, which corresponds with previous greenhouse efficacy studies for this population. Differentiation between susceptible and tolerant populations for the CS-formulation was not possible since a dose response analysis for the tolerant biotype was not possible due to a very low herbicide efficacy under both tested irrigation system. This finding corresponds with the high ED90 values found even for the susceptible populations (Fig. 1).

Discussion

For herbicide resistance detection purposes it is essential to avoid a false positive or false negative classification of suspicious weed populations. Therefore the methods used in the experiments have to be selected carefully, especially when dealing with soil-acting compounds like pendimethalin. So far a standard procedure for resistance tests with soil active herbicides is still lacking.

The presented results could show that under greenhouse conditions the use of a pendimethalin CS-formulation is accompanied by several difficulties. Release of active substance requires physical stress due to soil water and temperature depending swelling and shrinking of the capsules. Under greenhouse conditions, where environmental conditions are kept uniform and target plants germinate and grow much faster than under field conditions, the compound has no chance to develop its full efficacy.

Furthermore the artificial soil substrates used for greenhouse experiments are characterised by a high proportion of coarse pores and a low proportion of fine pores especially when sand contents are higher than 30% allowing a fast development of the root system within a short period after emergence. Under these conditions, irrigation from above also forces an active transport of the capsules by the water stream within the top layer of the soil, diluting the concentration of the active substance at the soil surface. Summarized these effects lead to a severe underestimation of the herbicide activity. In terms of resistance detection this may cause a high proportion of false positive detections.

Treatment with the SC-formulation combined with irrigation from below resulted in much higher efficacy of pendimethalin at lower dose rates. The irrigation from below is probably hindering a particle bound transport of pendimethalin within the top soil layer and most of the compound concentrates at the soil surface where it is highly effective against germinating plants. This resulted in an overkill situation were even a pendimethalin tolerant population was controlled by 100% at dosages far below the recommended field dose. For herbicide resistance detection this means a high proportion of false negative detections, especially when dealing with populations with an only slightly increased tolerance against the compound.

Summarised the following points should be considered for the development of standard procedures for greenhouse trials with pendimethalin:

- SC-formulation of pendimethalin should be used instead of a CS-formulation, since the release of the compound from the capsules is reduced and is following a different release pattern under greenhouse conditions.
- Irrigation from above has to be preferred, since the dose response curve is more gradual with a better corresponding behaviour likewise as under field conditions.
- In case of dose response studies, ED90 values have to be preferred for resistance factor determination since increased tolerances due to enhanced metabolism only tend to become significant above the ED50 dose.

Beside the presented results other aspects have to be considered for the development of a standard method, for example target plant density and temperature regime as discussed in MENNE *et al.* (2012) and MENNE and WAGNER (2008). Furthermore, transferability of greenhouse data to field conditions for soil residual compounds needs to be further investigated to allow for a more precise and reliable description of the susceptibility of tested biotypes. Smaller differences observed under greenhouse conditions may not translate into a noticeable distinction of product performance under field conditions.

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