Control of *Chenopodium album* L. utilizing two plant pathogenic fungi in combination with reduced doses of nicosulfuron

Bekämpfung von Chenopodium album L. mit Hilfe von zwei pflanzen-pathogenen Pilzen in der Kombination mit reduzierten Aufwandmengen von Nicosulfuron

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Summary

Although biological control agents provide satisfactory weed control in solely few cases, they should be considered as a part of integrated weed management (IWM) strategy in most cases. Using herbicides in reduced doses became an important strategy within the concept of IWM. Therefore, a combination of biological control agents and reduced herbicide doses could be considered as a good strategy for controlling some important weeds. Studies were carried out in 2008-09 to investigate the efficacy of a combination of Motivell (nicosulfuron) in reduced doses and two plant pathogenic fungi (*Ascochyta caulina* and *Stagonospora vitensis*) for the control of *Chenopodium album*. First, ED_{50} dose of Motivell for *C. album* was determined by four dose-response experiments conducted in pots. In the second step of the study nicosulfuron tolerance of fungi was determined in three experiments in petri dishes. At the last step six different pot experiments were carried out on *C. album* to evaluate the efficacy of reduced herbicide rates and fungi spores alone or in combination. The studies showed that the dose corresponding ED_{50} was about 12.5 % of the recommended dose. Doses up to 50 % of the recommended did not affect fungi growth under laboratory conditions, so that a combination was possible. Fungi treatments alone provided no effect in most cases. In 3 of 6 experiments, herbicide at 12.5 %-dose in combination with fungi treatments provided higher effects than 12.5 %-dose alone. Application of 25 % nicosulfuron dose alone was highly effective so that there is no need for a combination with biological control agents. It can be concluded that the combination of biological control agents with reduced herbicide doses could be an effective alternative in suppressing *C. album* under optimal climatic conditions.

Keywords: *Ascochyta caulina,* biological control, *Chenopodium album,* Motivell, nicosulfuron, *Stagonospora vitensis*

Zusammenfassung

Die biologische Unkrautbekämpfung könnte ein wichtiges Verfahren sein, aber ihr Erfolg war bei den bisherigen Versuchen durch einige Faktoren begrenzt. Obwohl biologische Methoden in einigen Fällen eine ausreichende Unkrautbekämpfung bewirkten, sollten sie eher als ein Teil der Integrierten Unkrautbekämpfung (IWM) betrachtet werden. Die Anwendung reduzierter Herbizidaufwandmengen ist ein fester Bestandteil des IWM, so dass deren kombinierte Anwendung mit spezifischen Pflanzenpathogenen als künftige Bekämpfungsstrategie gegenüber wichtigen Unkrautarten zu untersuchen ist. Mit diesem Ziel wurden 2008-2009 Untersuchungen zur Wirkung von reduzierten Motivell-Aufwandmengen in Kombination mit zwei Pflanzenpathogenen (*Ascochyta caulina* und *Stagonospora vitensis*) auf *Chenopodium album* durchgeführt. Zuerst wurde die ED_{50} von Motivell unter Anwendung von vier Aufwandmengen in Topfversuchen ermittelt. Im zweiten Schritt wurde die Toleranzgrenze der untersuchten Pilze gegenüber Nicosulfuron in drei Untersuchungen auf Nährboden in der Petrischale festgestellt. In insgesamt sechs Topfversuchen wurde dann die alleinige und kombinierte Anwendung von Motivell und Suspensionen der Pilzsporen gegenüber *C. album* geprüft. Als ED_{50} wurden 12,5 % der zugelassenen Aufwandmenge von Motivell festgestellt. Da erst Motivell-Aufwandmengen über 50 % das Pilzwachstum beeinflussten, waren Kombinationen bis zu 25 % der Herbizidkonzentration ohne Einfluss auf die Pilze. Die 12,5 %ige Aufwandmenge plus Pilzsporen zeigte in drei von sechs6 Versuchen eine bessere Wirkung als das Herbizid allein. Die alleinige Anwendung von Motivell 25 % hatte jedoch eine stärkere Wirkung als die 12,5 %ige plus Pilz, womit durch diese Ergebnisse der Sinn einer Kombination in Frage gestellt werden kann. Daraus schlussfolgernd sollte man trotzdem für die Zukunft die Möglichkeit einer Kombination reduzierten Herbizidaufwands mit geeigneten Pflanzenpathogenen nicht ausschließen und die Untersuchungen fortführen.

Stichwörter: *Ascochyta caulina,* biologische Kontrolle, *Chenopodium album,* Motivell, Nicosulfuron, *Stagonospora vitensis*

1. Introduction

Using plant pathogenic microorganisms for weed control became an important alternative weed control strategy which has been investigated since the last 30 years in Europe. In some studies the possibilities for using microorganisms for selective weed control have been investigated (EGGERS and THUN, 1988; KEMPENAAR, 1995; KEMPENAAR and SCHEEPENS, 1996; NETLAND et al., 2001). Results of these studies showed that *C. album* could be effectively suppressed by *Ascochyta caulina* under controlled conditions, while the success of this control was lower under field conditions. This could be attributed to climatic conditions (EINHORN, 2002) as well as the formulation and application techniques (CHARUDATTAN, 2001).

Based on this information, it can be concluded that biological weed control could fail in effectiveness when applied alone, but still microorganisms should be considered as an important factor within the frame of integrated weed management strategy (IWM). Using herbicides in reduced doses has been widely investigated so far and as a result it was approved that low dose treatments may provide satisfactory weed control in most cases. However, efficacy of reduced herbicide doses is also dependent on some factors, such as weed species sensitivity, growth stage as well as climatic conditions (DOGAN and HURLE, 1998; KUDSK and STREIBIG, 2003).

Therefore, it is reasonable to evaluate the efficacy of a combination of these two methods for weed control. *Chenopodium album* L. is a worldwide important weed species which is difficult to control with herbicides. In some previous studies carried out on the biological control of this weed in Europe, the fungus *Ascochyta caulina* has been found to be promising for the control of this weed (KEMPENAAR, 1995; NETLAND et al., 2001; VURRO et al., 2001). However, the efficacy of this fungus was variable depending on the conditions named above. Thus, the aim of the studies here presented was to establish a control strategy of *C. album* by means of *Ascochyta caulina* in combination with reduced doses of nicosulfuron. Furthermore another potential biocontrol agent fungus, namely *Stagonospora vitensis* has been used in the studies, which was isolated from *C. album* and used in some previous experiments *(*EINHORN, 2002*)*.

2. Materials and methods

Experiments were carried out at the Department of Agronomy of the Faculty of Agriculture and Horticulture, Humboldt-University Berlin, between September 2008 and March 2009. Studies were conducted in three steps: 1) dose-response experiments to determine the appropriate herbicide dose for mixture with fungi, 2) evaluation of fungi (*Ascochyta caulina* and *Stagonospora vitensis*) tolerance to herbicide, and 3) assessment of the effect of herbicide-fungi mixture on *C. album*.

2.1 Dose-response experiments

These studies were conducted as pot experiments under greenhouse conditions. Seeds of *C. album* were sown into trays containing a growing medium (turf, soil, perlite). After emergence, three *C. album* seedlings at cotyledon stage were transferred into each pot containing the same medium. The pots were placed in a greenhouse with a light regime of 15/9 h (daytime and night, respectively) in which average temperature was 22 °C. The *C. album* plants were grown under these conditions until herbicide application and pots were watered when required. At 2-4 leaf stage of *C. album*, the pots were sprayed with eight different doses of nicosulfuron as formulated herbicide Motivell. Treatments were done by hand operated sprayer with a water volume of 200 l/ha. The doses applied were 0.0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.0 l/ha Motivell, each with four replications.

To assess the efficacy of different herbicide rates, as well as to describe dose-response relationship, above ground part of weeds were harvested two weeks after application. ED_{50} values of herbicide were determined based on visual observation, fresh and dry weed biomass via four parameter logistic non linear regression analysis by using sigma Plot Package (MICHEL et al., 1999). These experiments were replicated four times.

2.2 Determination of fungi tolerance to herbicide

As the second step of the study, the tolerance of two fungi species to nicosulfuron was determined. With this aim, laboratory experiments were conducted in petri plates (9 cm) with four replications for each treatment to test if nicosulfuron has an adverse effect on fungi growth. The growing medium was potato dextrose agar, PDA, containing Motivell. First PDA was prepared and then the herbicide was mixed into the medium when it was cooled down to 60 °C. Motivell was mixed to the PDA medium at two different concentrations corresponding the recommended and half dose. After the PDA medium with nicosulfuron became solid, discs (each 5 mm in diameter) of each fungus were placed in the centre of the petri plates. As a control treatment, petri plates containing PDA without herbicide were used. All petri plates were incubated at 20 °C and UV light (12 hours light, 12 hours dark) and checked weekly for the presence of mycelial growth, and at each observation the diameter of fungi colonies were measured and recorded. Final evaluation was done three weeks after the beginning of experiment. Colonial diameters were submitted to analysis of variance (one way ANOVA) by using Statgraphics package.

2.3 Effect of the plant pathogenic fungi in combination with nicosulfuron

To evaluate the efficacy of reduced nicosulfuron rates and biocontrol agents as well as their combination on *C. album*, six different pot experiments were conducted under greenhouse conditions (Tab. 1). *C. album* plants were grown as described for dose-response experiments (chapter 2.1). In order to assess the influence of weed growth stage, *C. album* plants were treated at 2-4 leaf stages in three experiments and at 4-6 leaf stages in further three experiments.

Tab. 1 Motivell (nicosulfuron) and fungi treatments as well as the combinations.

Tab. 1 *Behandlungsvarianten mit den Pilzen und Motivell (Nicosulfuron).*

1	Control
2	*Ascochyta caulina* alone
3	*Ascochyta caulina* + Motivell 12.5 %
4	*Ascochyta caulina* + Motivell 25 %
5	*Stagonospora vitensis* alone
6	*Stagonospora vitensis* + Motivell 12.5 %
7	*Stagonospora vitensis* + Motivell 25 %
8	Motivell 12.5 % alone
9	Motivell 25 % alone
10	Motivell 50 % alone

Motivell was used at three different doses in the experiments. Since results from dose-response experiments showed that the ED_{50} values for 2-4 leaf stage plants were between 10-15 % of the recommended dose, 12.5 % of the recommended dose has been decided as a mixing partner with fungi. Also the efficacy of quarter dose alone or in combination with fungi was assessed. Half herbicide dose alone was used in the experiments for comparison.

Spores of *Ascochyta caulina* and *Stagonospora vitensis* were used in the experiments for inoculation, which were derived from two weeks old PDA and wheat bran medium, respectively. To obtain spores of *A. caulina* 10 ml sterile distilled water containing 0.05 % Tween 80 was given to petri plates. After flooding for 3 hours, the PDA surface was incised by a spatula and suspended spores were collected. Spores of *S. vitensis* were obtained by suspending wheat bran by sterile distilled water containing Tween 80 (0.05 %) and the bran medium mixture was broken by using a mixer. Spore numbers were counted by using THOMA chamber under light microscope. Fungi spore concentrations for the experiments were adjusted to 2.2×10^6 spores/ml for *A. caulina* and 1.6×10^6 spores/ml for *S. vitensis.*

Herbicide and fungi treatments have been conducted as mentioned in dose-response experiments (chapter 2.1). All treatments were replicated with two pots, each containing three *C. album* plants. Since it was to estimate that the fungi activity would be better on affected plants, the herbicide mixtures were applied three hours before the fungi spore treatments. After spraying fungi spores, pots were kept under plastic sheets for 24 hours to maintain optimal humidity condition for spore germination and plant infection. After this period plants were grown under normal greenhouse conditions. After 11 days, above ground parts of all plants were harvested and their fresh and dry weights were determined.

To be able to compare all experimental results with each other, both fresh and dry weight data were converted to percent in relation to control and analysis of variance was performed. Data was analyzed by using GLM, and means were separated by using Duncan-test. Only results based on percent dry weight data are reported here.

3. Results and Discussion

3.1 Dose-response experiments

These experiments were conducted to determine the herbicide dose causing moderate weed damage (50 %) that would be considered as the mixing partner with fungi. Since the analysis of variance showed that the experiment factors and related interactions were not significant, data from four experiments were pooled and jointly submitted to dose-response analysis.

Tab. 2 ED_{50} of Motivell (nicosulfuron) for *Chenopodium album* depending on observation parameters.

Tab. 2 *ED_{50} von Motivell (Nicosulfuron) für Chenopodium album je nach Messparameter.*

Observation parameter	**ED_{50} (ml/ha)***	**b**
Visual observation	149.8 ± 6.9	1.64 ± 0.13
Fresh weight	103.0 ± 1.9	2.6 ± 0.14
Dry weight	114.0 ± 1.5	1.53 ± 0.03

* Average of 4 dose-response experiments, ± Standard error of estimated parameters

As shown in Table 2, the ED_{50} doses of Motivell for *C. album* were defined between 100 and 150 ml/ha depending on the observation corresponding about 10-15 % of the recommended Motivell dose. Therefore, 12.5 % of the recommended dose was chosen for the further studies, in which the effect of herbicide fungi concentrations was investigated.

3.2 Determination of fungi tolerance to herbicide

The mycelia growth of the fungi as affected by different Motivell treatments was evaluated by comparing colonial diameters of the fungi on PDA media with and without herbicide supplementation (Tab. 3). Results showed that nicosulfuron had no significant effect on the growth of *A. caulina*, so that the herbicide can be mixed with these fungus spores or can be sprayed before *A. caulina* spore application respectively even at the recommended dose. Although not statistically significant, it is noteworthy that the growth of this fungus was even higher (by 20 %), when grown on medium having received half herbicide dose. In the case of *S. vitensis*, addition of half dose of Motivell to the growing medium stimulated fungus growth significantly (by 39 %). However when the growing medium was supplemented with the recommended dose, the growth of *S. vitensis* was significantly inhibited, so that the colonial diameter of the fungus was 61 % lower as compared to the untreated control.

These results suggest that the Motivell concentrations used in these experiments (12.5 and 25 % of the recommended dose) as mixing partner with potential biocontrol fungi had no negative effect on the fungi, so that both components were compatible.

Tab. 3 Mycelial growth of *Ascochyta caulina* and *Stagonospora vitensis* on PDA medium containing Motivell.

Tab. 3 *Myzelwachstum von Ascochyta caulina und Stagonospora vitensis auf PDA-Medium mit Motivell.*

	Diameter (mm)			Diameter (mm)		
Treatments	***A. caulina***	**%****		***S. vitensis***	**%****	
Untreated	69.75 ± 0.63*	100	a	57.00 ± 6.35*	100	b
Nicosulfuron 50 %	85.00 ± 0.00	122	a	79.50 ± 2.10	139	a
Nicosulfuron 100 %	75.00 ± 0.82	108	a	22.25 ± 4.85	39	c

*Standard error, **% growth of *A. caulina* and *S. vitensis* mycelia growth as compared to control

3.3 Effect of the plant pathogenic fungi in combination with nicosulfuron on *C. album*

In Table 4 relative biomass of *C. album* plants affected by different treatments are shown. Since statistical analysis showed that the experimental factors were significant, results from all experiments are presented separately. When the fungi were applied alone (without herbicide) they provided in most experiments no effect on the weed. *A. caulina* alone reduced weed biomass by about 45-50 % only in the two experiments and *S. vitensis* reduced relative weed biomass by 48 % only in one experiment. Motivell at 12.5 % of the recommended dose provided different weed control scores ranging between 0 and 76 %. Combination of fungi with this dose improved the control in most experiments, however, that was especially apparent in the case of the experiments 3, 4 and 6 when 12.5 % dose treatment alone provided poor weed control. Statistically significant improvement of weed control was observed only in two experiments with *A. caulina* (experiments 4 and 6) and with *S. vitensis* (experiments 2 and 6). Quarter dose of Motivell provided in most experiments (except for 6th experiment) high weed control efficacy comparable to the effect achieved by half dose of herbicide. So fungi addition did not improve the weed control by this dose. In all cases there were no statistically significant differences among weed control scores obtained at this dose alone and in combination with fungi. Although it was not statistically significant there was still improved weed control of this dose with *A. caulina* addition in the 4th experiment and *S. vitensis* addition in the 3th and 6th experiments.

Tab. 4 Efficacy [%] of *A. caulina* and *S. vitensis* alone or in combination with Motivell on *C. album*.

Tab. 4 *Wirksamkeit [% Trockenmasse] von A. caulina und S. vitensis allein oder in Kombination mit Motivell auf* C. album.

	Experiment number					
% dry weight	**1**	**2**	**3**	**4**	**5**	**6**
	treated at 4-6 leaf stage			**treated at 2-4 leaf stage**		
Untreated	100 ab	100 b	100 ab	100 a	100 a	100 abc
Nicosulfuron 12.5 %	47 bc	33 cd	80 abc	98 a	24 b	130 ab
Nicosulfuron 12.5 % + *A. caulina*	38 bc	20 d	28 cde	10 b	34 b	47 cde
Nicosulfuron 12.5 % + *S. vitensis*	34 bc	14 d	67 bcd	128 a	27 b	34 de
Nicosulfuron 25 %	11 c	6 d	15 de	15 b	13 b	54 cde
Nicosulfuron 25 % + *A. caulina*	21 c	11 d	19 de	5 b	25 b	43 cde
Nicosulfuron 25 % + *S. vitensis*	34 bc	14 d	4 e	21 b	12 b	13 e
A. caulina alone	102 ab	56 c	101 ab	125 a	52 b	143 a
S. vitensis alone	163 a	138 a	132 a	97 a	52 b	79 bcd
Nicosulfuron 50 %	12 c	10 d	5 e	5 b	17 b	53 cde

The main objective of this study was to evaluate the performance of two phytopathogenic fungi species in suppressing *C. album*, alone or in combination with reduced herbicide doses. Thus, the study was conducted at three steps: The first step concerned the determination of the herbicide rate to be used, the second step the evaluation of the fungi tolerance to herbicides and the third step was related to the main part of the study. Results of our studies showed that both fungi alone provided no or no sufficient efficacy in suppressing the target weed in all experiments. Although it is stated that *A. caulina* is an important potential mycoherbicide for *C. album* control (KEMPENAAR et al., 1996; NETLAND et al., 2001), they pointed out that the efficacy of this fungus is dependent on wetness period, spore numbers applied, plant development stages and temperature. Since all these factors are more related to the penetration ability of fungi into healthy plant tissues, it can be assumed that a combination of the fungi with reduced herbicide rates would be able to improve the efficacy of fungi, because penetration might be easier into affected plant tissues. With this aim herbicide and fungal spores were not sprayed simultaneously in a mixed formulation as in the studies of KEMPENAAR and SCHEEPENS (1996) and MÜLLER-SCHÄRER (2002). To have a synergistic effect between herbicide and fungal spores, the herbicide was sprayed in our studies 3 hours prior to fungal spore treatment. MITCHELL et al. (2008) found in a similar study for control of *Sorghum bicolor* with *Colletotrichum* species in combination with glyphosate that the herbicide treatment should be done prior to spraying spores to optimize the effectiveness of the combination.

Our results showed that combination treatments have increased weed control efficacy as compared to sole herbicide treatment, and in few cases this increase was significant. This was especially apparent with 12.5 % of the recommended Motivell dose. In some other studies concerning the control of *C. album* with fungi species, it was also found that a combination of potential biocontrol agents and herbicides provided higher weed control efficacy, as compared to sole herbicide treatments (NETLAND et al., 2001; VURRO et al., 2001). Since the increase in the efficacy of 12.5 % dose via addition of fungi treatments was especially observed in cases where this dose alone provided no weed control (experiments 3, 4 and 6), it seems that such combination could support the herbicide efficacy under conditions in which the herbicide fails. In cases where the herbicide effect is high, it can be possible that high efficacy masks the stimulatory effect of the fungi.

It can be concluded that the combination of two specific pathogenic fungi with reduced herbicide doses could be an effective alternative in suppressing *C. Album*. However, further studies are needed to make a concrete decision.

Based on the results here and other studies published in the literature, following points should be also considered in further studies:

- using fungi with different herbicide doses,
- using pathogen spores in higher concentrations,
- prolonging wetness period for improved pathogen penetration,
- broadening the interval between herbicide application and spore treatments to spray spores on more effected plants.

To achieve more realistic results, such experiments should also be carried out under field conditions.

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