# The effects of different herbicide strategies on the genetic composition of *Apera spica-venti* (L.) P. Beauv.

*Die Effekte von verschiedenen Herbizidstrategien auf die genetische Zusammensetzung von Apera spica-venti (L.) P. Beauv.*

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## Summary

Herbicide resistance in weeds is an increasing problem in crop production systems. Resistance to acetolactat synthase (ALS) inhibitors is common worldwide. In Germany, there is recently an increase in the number of ALS resistant biotypes of *Apera spica-venti*.

The effects of different herbicide strategies on the weed species *A. spica-venti* were analyzed by a field trial. Two fields with a previous detected ALS target site resistance were chosen for a three year experiment. Four different herbicide strategies were applied: An ALS inhibitor each year, a soil-acting herbicide each year, an alternation between ALS inhibitor and soil-acting herbicide and a combination of several herbicidal mode of action groups. To assess the spatial distribution of resistant biotypes, plant samples were taken on fixed grid points before and after the herbicide treatment. The plant samples were analyzed for SNPs (Single Nucleotide Polymorphism) on the ALS codon P197. The results show that the different herbicide strategies exert an influence on the dynamics of resistance development.

**Keywords:** ALS target site resistance, field trials, Germany, herbicide resistance, loose silky-bent

## Zusammenfassung

Herbizidresistenz ist ein immer stärker werdendes Problem in der Getreideproduktion. Resistenzen gegenüber Acetolactatsynthase (ALS)-Hemmern sind weltweit verbreitet. In Deutschland gibt es eine steigende Zahl an ALS-Resistenzfällen bei *Apera spica-venti*-Populationen.

Die Effekte von verschiedenen Herbizidstrategien auf die Ungrasart *A. spica-venti* wurden durch Feldversuche untersucht. Zwei Felder mit einer nachgewiesenen ALS-Wirkortresistenz wurden ausgewählt um einen 3-jährigen Feldversuch durchzuführen. Vier verschiedene Herbizidstrategien wurden angewandt: Ein ALS-Hemmer in jedem Jahr, ein Bodenherbizid in jedem Jahr, Wechsel zwischen ALS-Hemmer und Bodenherbizid und eine Kombination aus unterschiedlichen Herbizidgruppen. Um die Verteilung der resistenten Biotypen zu bestimmen wurden Pflanzenproben an einem festen Raster vor und nach den Herbizidbehandlungen entnommen. Die Pflanzenproben wurden dann auf SNPs (Single Nucleotide Polymorphism) auf dem ALS-Kodon P197 untersucht. Die Ergebnisse zeigen das unterschiedliche Herbizidstrategien einen Einfluss auf die Resistenzdynamik ausüben.

**Stichwörter**: ALS-target-site Resistenz, Deutschland, Feldversuch, Herbizidresistenz, Windhalm

## 1. Introduction

Resistance to herbicides inhibiting acetolactate synthase (ALS) is the most common type of herbicide resistance worldwide (HEAP, 2011). Most of these cases are caused by various single-nucleotide polymorphisms (SNPs) on the ALS gene. ALS is the target site of many herbicides including sulfonylureas, imidazolinones and triazolopyrimidines (CORBET and TARDIF, 2006). The inhibition of the ALS leads to plant death due to reduced quantities of the amino acids leucine, valine and isoleucine. The first reported case of an ALS resistant *A. spica-venti* population in Germany was in 2005 (HEAP, 2011). Loose silky bent, also called windgrass (*Apera spica-venti* (L.) P. Beauv.), is commonly found on light and sandy soil. This annual, monocotyledonous plant species occurs in winter cereals and winter oilseed rape in Germany. Seeds germinate mostly at or near the surface in late summer and autumn, with a germination peak in late September to early October (WARWICK et al., 1985; WALLGREN and AVHOLM, 1978). Seeds do not have a long lifespan and exhibit little primary dormancy. Their longevity ranges from one to seven years, but mostly did not exceed two years (WALLGREN and AVHOLM, 1978; ZEMANEK, 1980) Flowering occurs mainly during summer and plants can produce up to 2000 seeds (WARWICK et al., 1985). There are also reports about up to 16000 seeds per plant (SOUKUP et al., 2006). Due to strong tillering and a long stem, *A. spica-venti* has a high competitive ability against the crop. Field trials were conducted to investigate the tillage effects on the seed bank (CARDINA et al., 1991; BALL and MILLER, 1990) or the impact of different types and times of tillage and herbicide treatments on the weed flora (MELANDER, 1995; BOSTRÖM, 1999). The objectives of this paper are to investigate the impact of different herbicide strategies on the density of the *A. spica-venti* population and also on their genetic composition.

## 2. Materials and methods

### 2.1 Site description

Two commercial fields with a prior detected resistance to ALS inhibitors were chosen for a three year field trial. The two fields are situated in the North (Lower Saxony) and in the East (Saxony) of Germany. The field in Lower Saxony (from here on called site A) is characterized by a sandy soil and ploughing was conducted frequently. The eight year field history showed a crop rotation consisting of triticale and maize. ALS-inhibitors respectively sulfonylureas were used frequently over at least a period of eight years prior to our trial. The second field in Saxony (from here on called site B) is characterized by a loamy sand and the last ploughing operation occurred at least 15 years ago. The crop rotation is almost only consisting of winter wheat and winter oilseed rape. To control *A. spica-venti* treatments with ALS inhibitors occurred three years in a row prior to our field trial. The ten year field history showed no further treatments with an ALS inhibitor.

### 2.2 Experiment design

In the three years of the trial, from 2008 to 2011, winter wheat was grown. An area of approximately one hectare was divided into four plots. In the first year a ten meter grid was laid over the area. From the second year the grid was scaled down to six meters to get a more detailed resolution. On the four plots, different herbicide treatments were applied. Plot one was treated with an ALS inhibitor for the whole three year trial period. On plot two, a soil-acting herbicide, with an active ingredient from the HRAC group F1 and K3, was applied for three years. On plot 3, the herbicides from plot 1 and 2 were alternated. In the first and third year the ALS inhibitor was used and in the second year the herbicide treatment was conducted with the soil-acting herbicide. On plot 4, a hundred percent control of *A. spica venti* was tried to be achieved. Therefore, two herbicide applications with different mode of action (MoA) were used each year. In the first and third year one herbicide from the HRAC group C2 was applied and approximately four weeks later a herbicide from HRAC group A was used. In the second year the treatment consisted of a soil-acting herbicide with the mode of action F1 and K3 in autumn and an ACCase inhibitor in spring.

At the grid points, *A. spica-venti* seedling counts were obtained by placing a 0.1 $m^2$ quadrat and counting all seedlings within the square. The numbers were converted to weed seedlings per 1 $m^2$. The counting was carried out before and six weeks after the herbicide application. At the same time plant samples were taken at each grid point. In the first year before herbicide application we took five plants. After that we took two samples. These *A. spica-venti* samples were analyzed using pyrosequencing to see if there is a single nucleotide polymorphism (SNP) on the ALS gen at position 197. Pyrosequencing is a method based on sequencing by synthesis. From the preprocess plant material a DNA fragment is amplified via PCR (Polymerase chain reaction). The purified PCR samples are analyzed by real-time monitoring of the DNA synthesis (AHMADIAN et al., 2006; MÜLHARDT, 2006).

## 3. Results

### 3.1 Apera spica-venti density

In Figure 1, the *A. spica-venti* density at study site A is shown. The density had a homogeneous distribution over the plots in the beginning of the field trial. The densities in plots 1 and 3 remained roughly the same from the first to the second year. In the other two plots a slight decrease from the first to the second year was seen. In the third year there was a dramatic increase in the number of plants per m² in plots 1 to 3. In Plot 4 was a minor increase in the third year was observed. In the case of the application of an ALS inhibitor the plant density stayed the same or decreased only slightly. If another herbicide than an ALS inhibitor was applied the density declined substantially after the treatment.

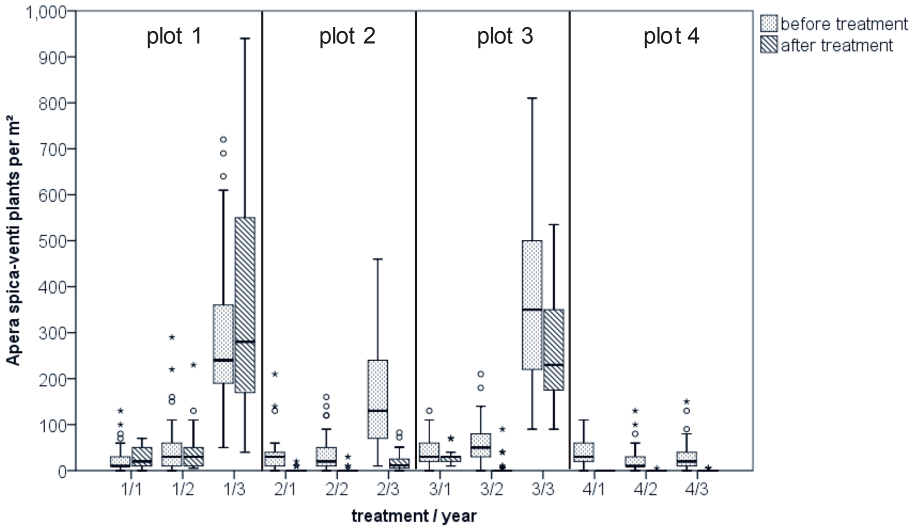


**Fig. 1** *A. spica-venti* density per m² at site A (treatments: 1, ALS inhibitor each year; 2, soil-acting herbicide each year; 3, ALS inhibitor year 1+3 and soil-acting herbicide year 2; 4, combination of herbicide groups).

***Abb. 1*** *A. spica-venti-Dichte pro m² am Standort A (Behandlungen: 1, ALS-Hemmer in jedem Jahr; 2, Bodenherbizid in jedem Jahr; 3, ALS-Hemmer 1.+3. Jahr und Bodenherbizid 2. Jahr; 4, Kombination von Herbizidgruppen).*

The density of *A. spica-venti* during the 3-year field trial at study site B is shown in Figure 2. In the beginning of the experiment the density was nearly the same in the plots 1, 3 and 4. In plot 2 the density was slightly higher than in the other plots. In all plots there was an increase in the density from the first to the second year. An unexpected low density in all plots was observed in year three. The number of surviving plants after the treatment was very low in all plots apart from the cases when the ALS inhibitor was used, here only a small decline in the density of *A. spica-venti* was seen.

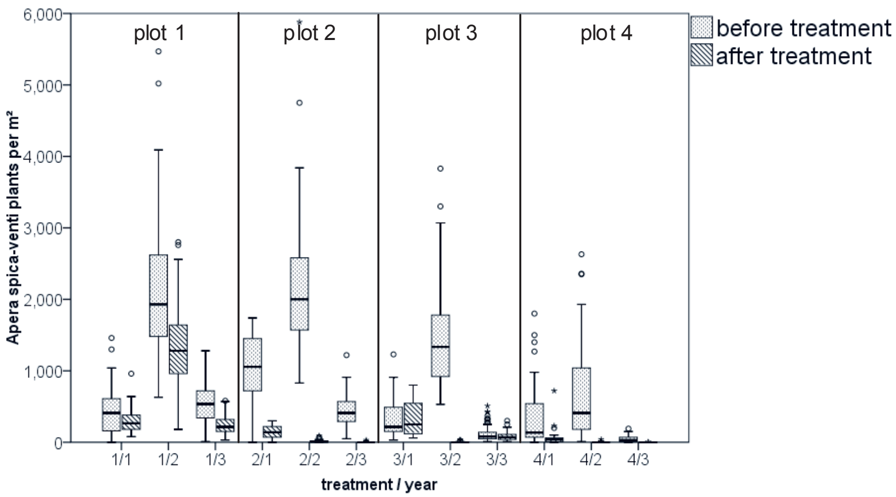


**Fig. 2** *A. spica-venti* density per m² at site B (treatments: 1, ALS inhibitor each year; 2, soil-acting herbicide each year; 3, ALS inhibitor year 1+3 and soil-acting herbicide year 2; 4, combination of herbicide groups).

***Abb. 2*** *A. spica-venti-Dichte pro m² am Standort B (Behandlungen: 1, ALS-Hemmer in jedem Jahr; 2, Bodenherbizid in jedem Jahr; 3, ALS-Hemmer 1.+3. Jahr und Bodenherbizid 2. Jahr; 4, Kombination von Herbizidgruppen).*

### 3.2 Efficacy

Figure 3 shows the efficacies of the treatments on both fields. Therefore the *A. spica-venti* densities before and after the treatment (shown above) were compared. The ALS inhibitor showed low efficacies on both fields in the three years. The effects of the treatment had also a wide range, so that there were grid points with efficacies of 80 % as well as grid points where there was no effect on the *A. spica-venti* density. The slightly lower efficacy in plot 2 at site A in the third year and on site B in the first year was probably caused by unfavorable conditions at the time of application.

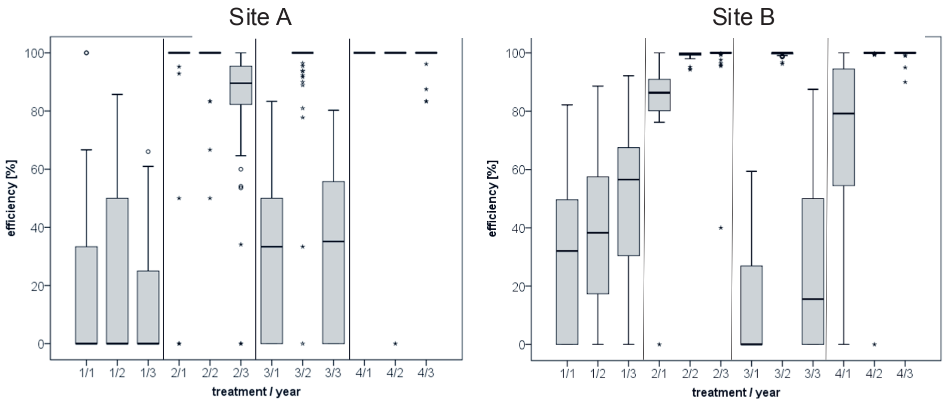


**Fig. 3** Herbicide efficiencies on sites A and B (treatments: 1, ALS inhibitor each year; 2, soil-acting herbicide each year; 3, ALS inhibitor year 1+3 and soil-acting herbicide year 2; 4, combination of herbicide groups).

***Abb. 3*** *Herbizidwirkungsgrade an den Standorten A und B (Behandlungen: 1, ALS-Hemmer in jedem Jahr; 2, Bodenherbizid in jedem Jahr; 3, ALS-Hemmer 1.+3. Jahr und Bodenherbizid 2. Jahr; 4, Kombination von Herbizidgruppen).*

### 3.3 Proportion of resistant and susceptible plants

When *A. spica-venti* plants were found at a grid point, samples were taken before and after the herbicide application. The numbers of samples taken in each plot are shown in Table 1 and 2. These samples were analyzed by pyrosequencing to detect a mutation on the ALS gene, allowing to decide whether the sampled plant was susceptible or resistant against an ALS inhibiting herbicide. In Figure 4 and 5 the proportion of susceptible and resistant samples is shown for the first two years. The sample analysis from the third year was not completed at the time of writing. Plot 1 of field A showed a decreasing number of susceptible plants from the first to the second year. But in plot 3 with the same treatment this development was not seen. Plot 2 shows only slight fluctuations between the years and time of the sample taking. A trend in either direction was not seen. Propositions regarding the development of composition of the population are quite difficult for plot 4 because of the few surviving plants and lesser number of seedlings in the second year. In the first year, only two samples were taken after the treatment on plot 4 and in the second year there was only one sample.

**Tab. 1** Sample size at site A.

***Tab. 1*** *Probenumfang am Standort A.*

| | 1. year before treatment | 1. year after treatment | 2. year before treatment | 2. year after treatment |
|---|---|---|---|---|
| plot 1 | 135 | 66 | 130 | 130 |
| plot 2 | 160 | 15 | 108 | 6 |
| plot 3 | 160 | 64 | 130 | 22 |
| plot 4 | 160 | 2 | 123 | 1 |

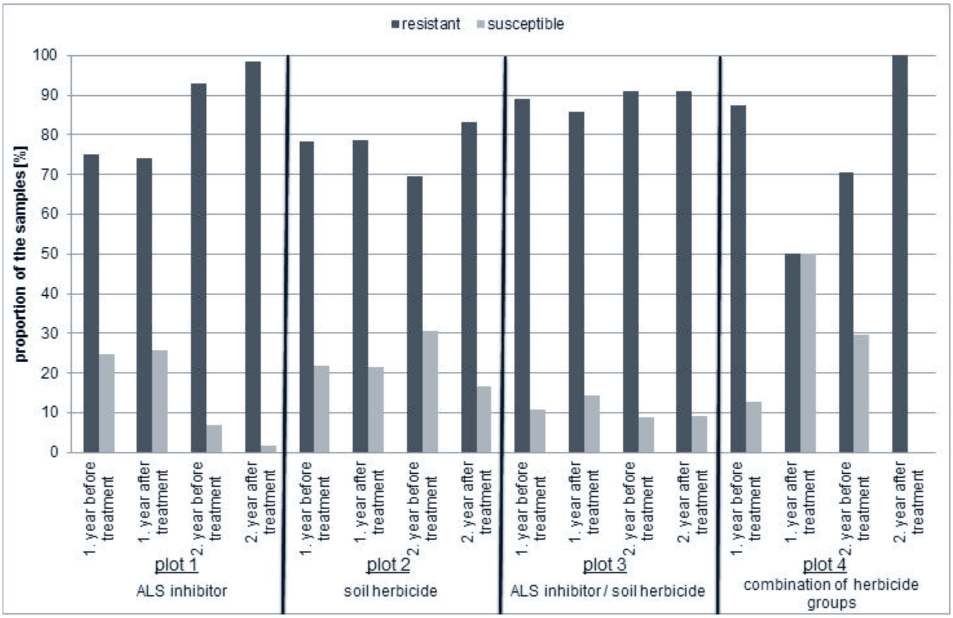


**Fig. 4** Proportion of susceptible and resistant *A. spica-venti* samples on site A.

***Abb. 4*** *Anteil an sensitiven und resistenten A. spica-venti-Pflanzen am Standort A.*

On field B there was an increase in resistant plant samples in plot 1 and 3. Also plot 4 showed an increase of resistant plants from the first to the second year. Similar to field A, there was no trend seen in either direction on plot 2.

**Tab. 2** Sample size at site B.

***Tab. 2*** *Probenumfang am Standort B.*

| | 1. year before treatment | 1. year after treatment | 2. year before treatment | 2. year after treatment |
|---|---|---|---|---|
| plot 1 | 160 | 64 | 132 | 134 |
| plot 2 | 159 | 64 | 130 | 76 |
| plot 3 | 120 | 48 | 131 | 47 |
| plot 4 | 155 | 58 | 130 | 18 |

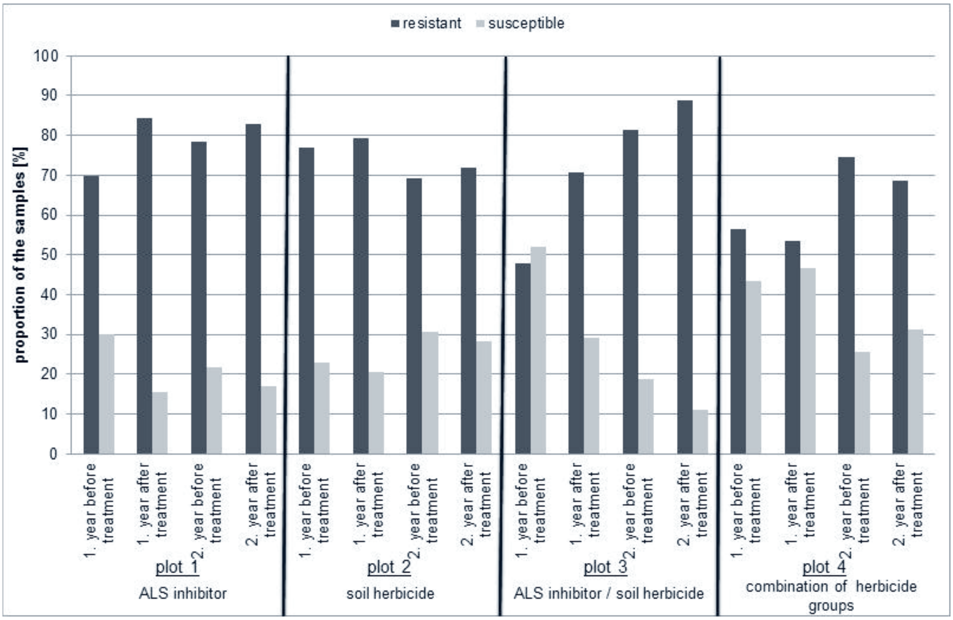


**Fig. 5** Proportion of susceptible and resistant *A. spica-venti* plants on site B.

***Abb. 5*** *Anteil an sensitiven und resistenten A. spica-venti-Pflanzen am Standort B.*

## 4. Discussion

The results of the field experiments show that the different treatments had an impact on the density of *A. spica-venti* and also on the genetic composition of the population. So an increase in weed density was seen on both fields on plots where an ALS inhibitor was used. Also a decrease in weed density was seen if no ALS inhibitor was used. But the density is not only influenced by the treatment. Other factors also have a great impact. On field A there was an increase in weed density over all plots in the third year even in the plots were there was a good weed control in the years before. On the other field we observed the opposite. Here, the density declined over all plots in the third year even if there was a low weed control level in the years before. It is important to identify the other factors to give accurate predictions for future weed infestations. These factors could be weather conditions, application date and conditions or the drilling date. Changes in the composition of susceptible and resistant plants were observed on both fields but longer observation periods are needed to identify clear trends.

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