

Results from two years of *Matricaria inodora* L. and *Matricaria chamomilla* L. monitoring (2012 + 2013) – greenhouse efficacy trials with Tribenuron and Florasulam and ALS target site resistance test at Pro 197 and Thr 574

Ergebnisse aus zwei Jahren (2012 + 2013) eines Matricaria inodora- und Matricaria chamomilla-Monitoring – Biotest mit Tribenuron und Florasulam und ALS-Target-Site-Analyse an den Stellen Pro 197 und Thr 574

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Abstract

The first ALS resistant *Matricaria chamomilla* (MATCH) biotype was found in 2006 in Schleswig-Holstein in Witzwort (SCHLEICH-SAIDFAR *et al.*, 2011). Since 2006 ALS resistant *Matricaria chamomilla* and *Matricaria inodora* (MATIN) were found at other locations near the North Sea coastline in Schleswig-Holstein and Lower Saxony (ULBER *et al.*, 2012). As there were more and more cases of reduced efficacy of sulfonylureas on mayweed, Dow AgroSciences decided in 2012 and 2013 to test MATCH and MATIN samples in Germany. In 2012 seed samples collected by Dow AgroSciences were planted in greenhouse environment and treated with tribenuron or florasulam. As a second step plants were checked for mutations of the ALS gene at positions Pro 197 and Thr 574 via Pyrosequencing. In Europe there is no known case of metabolic ALS-resistance (ULBER *et al.*, 2012). It was found that mayweed plants showed a mutation at position Pro 197 only. In 2013 leaf samples were taken only and investigated for mutations of ALS position Pro 197 and Thr 574.

Keywords: Dow AgroSciences, Germany, mayweed, resistance

Zusammenfassung

Die erste nachgewiesene ALS-resistente Kamille (*Matricaria chamomilla/recutita*) stammt aus dem Jahr 2006 aus Witzwort in Schleswig-Holstein (SCHLEICH-SAIDFAR *et al.*, 2011). Seit 2006 gab es einige weitere Kamille-Standorte, bei denen eine ALS-Resistenz nachgewiesen wurde (ULBER *et al.*, 2012). Diese befanden sich in erster Linie an der Nordseeküste Schleswig-Holsteins und Niedersachsens. Da sich die ALS-resistente Kamille weiter ausbreitete, wurden 2012 und 2013 im Auftrag von Dow AgroSciences deutschlandweit Kamille-Proben (MATIN und MATCH) gesammelt und untersucht. In 2012 wurden Samenproben gesammelt, die dann im Biotest mit Tribenuron bzw. Florasulam behandelt und danach mit Pyrosequencing an den Stellen Pro 197 und Thr 574 auf Mutationen untersucht wurden. Da bei ALS-Resistenz bei zweikeimblättrigen Unkräutern europaweit keine metabolische Resistenz bekannt ist (ULBER *et al.*, 2012), wurden 2013 deutschlandweit Blattproben gesammelt und an den Stellen Pro 197 und Thr 574 auf Mutation untersucht.

Stichwörter: Deutschland, Dow AgroSciences, Kamille, Resistenz

Introduction

The first ALS resistant *Matricaria chamomilla* (MATCH) biotype was found in 2006 in Schleswig-Holstein in Witzwort (SCHLEICH-SAIDFAHR *et al.*, 2011). During the following years ALS resistant MATCH and MATIN plants were found at other locations close to the coastline of the North Sea in Schleswig-Holstein and Lower Saxony. There were increasing incidents of reduced efficacy of sulfonylurea herbicides on mayweed. A mayweed herbicide resistance monitoring program was carried out by the Julius-Kühn-Institut in 2011 and results were published in 2012 (ULBER *et al.*, 2012).

Due to the fact that florasulam (a triazolopyrimidine herbicide from Dow AgroSciences) always showed full efficacy in greenhouse trials and in the field the intention was to compare the efficacy of triazolopyrimidines and sulfonylureas on ALS target site resistant mayweed species.

Material and Methods

In 2012, 34 *Matricaria* seed samples were randomly collected, i.e. the *Matricaria* samples came from fields without any anticipated ALS resistance. From these collections, 19 seed samples were identified as *Matricaria inodora* and 15 seed samples as *Matricaria chamomilla*. The seed samples were investigated by EpiLogic GmbH for greenhouse efficacy testing and the ALS target site resistance analysis via pyrosequencing at positions Pro 197 and Thr 574. Therefore the DNA from the *Matricaria* leaves were extracted and amplified via PCR.

The greenhouse trials were carried out under the following conditions:

- 5 plants per pot
- the herbicides (40 g/ha Pointer SX or 100 ml/ha Primus) were applied at BBCH 12
- 3 repetitions per pot/variety
- 2 *Matricaria* standards – to compare efficacy- one ALS resistant standard and one susceptible standard
- visual efficacy control after 28 days
- 14 hours day light
- light intensity equals a cloudy day
- temperature at night: 12 °C
- temperature day time: 14 – 18 °C

The target-site resistance analysis was carried out with plants from the greenhouse trials (untreated variety) via PCR and pyrosequencing at Pro 197 and Thr 574. Therefore the DNA from the *Matricaria* leaves were extracted and amplified via PCR.

A mutation of the ALS gene caused reduced efficacy by an ALS inhibitor herbicide (SCHLEICH-SAIDFAR *et al.*, 2011).

In 2013, 70 leaf samples of *Matricaria inodora* and *Matricaria chamomilla* were randomly collected in the fields. Each sample consisted of 1 leaf from each of 8 different plants. The leaves were sent in a paper envelope to IdentXX GmbH. ALS target site resistance analysis was conducted for each leaf per plant at Pro 197 and Thr 574. In total 560 different *Matricaria* plants (70 samples x 8 plants) were analyzed.

Results

Results 2012 – greenhouse trial and target site resistance analysis

In 2012, investigations did not reveal any target site resistance or reduced efficacy of tribenuron and florasulam on *Matricaria inodora*.

However, there were two samples of *Matricaria chamomilla* and *Matricaria recutita* that showed reduced susceptibility to tribenuron at 88% control (sample from Schleswig-Holstein) and 13% efficacy (sample from Nordrhein-Westfalen). In both samples target site resistance at position Pro 179 was detected. The Schleswig-Holstein sample showed a mutation from Pro 197 to Thr 197 with a frequency of 50% of the plants from that location, the sample from Nordrhein-Westfalen had a mutation from Pro 197 to Ser 197 and 71% of the plants from that location were concerned.

There were two samples with mutations at Pro 197 but with no reduced tribenuron or florasulam efficacy. One sample from Sachsen-Anhalt had a frequency of 19% Pro 197 to Ser 197 and one sample from Brandenburg had a frequency of 17% Pro 197 to Ser 197. There was no cross resistance between tribenuron (sulfonylurea) and florasulam (triazolopyrimidin) (Fig. 1 and 2).

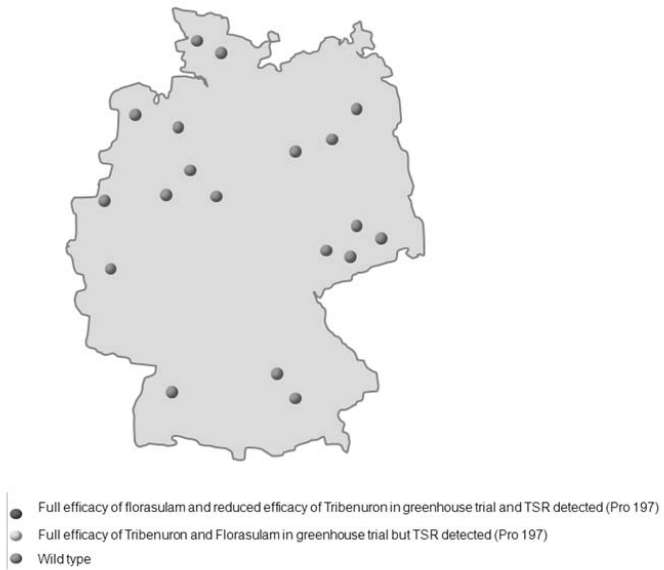


Fig. 1 Seed samples of *Matricaria inodora*.

Abb. 1 Samenproben von *Matricaria inodora*.

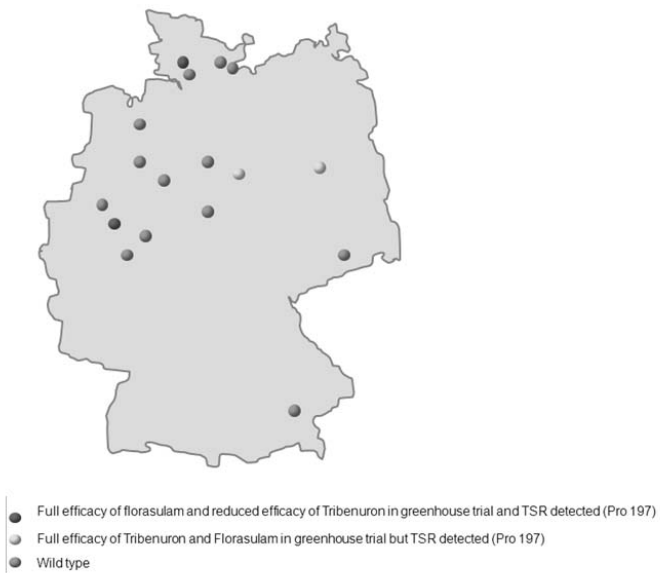


Fig. 2 Seed samples of *Matricaria chamomilla*.

Abb. 2 Samenproben von *Matricaria chamomilla*.

Results 2013 – target site analysis

ALS resistant *Matricaria* biotypes were found. From the 70 samples, 52 samples showed no mutation at position Pro 197 and Thr 574. 18 samples showed mutation at Pro 197 (Fig. 3). In addition, 7 out of 18 samples showed a mutation to Thr 197 and 11 samples to Ser 197.



Fig. 3 Results of sampling program 2013.

Abb. 3 Ergebnisse der Probenahme 2013.

Discussion

The results from the 2012 and 2013 sampling program indicated occurrence of ALS resistant *Matricaria* at the North Sea coastline and further south in the region of Hannover – Bielefeld – Dortmund. Not all samples where ALS target site resistance was identified showed reduced tribenuron efficacy. Especially in eastern Germany and Bavaria *Matricaria* samples with mutation at position Pro 197 were found and they were susceptible to tribenuron. As resistance continues to develop in these regions there is an expectation that resistance to tribenuron will increase. The only mutation with *Matricaria* found in 2012 and 2013 was at position Pro 197 of the ALS gene. Cross resistance between florasulam and tribenuron in case of mutation at Pro 197 was not found because greenhouse trials confirmed full florasulam efficacy. Further investigations are needed to determine if the use of clopyralid (in Ariane C and Primus Perfect), an active ingredient belonging to the HRAC class O, can help to prevent development of resistant *Matricaria* in cereal crops.

References

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