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Target site resistance to metamitron in *Chenopodium album* L.

Target-site Resistenz gegenüber Metamitron in *Chenopodium album* L.

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Summary

Fat-hen (*Chenopodium album* L.) is becoming more difficult to control in sugar beet. Seeds of *C. album* were collected in different European countries (Belgium, France, Germany, The Netherlands and Sweden) where the control with metamitron had failed. Greenhouse bioassays with metamitron pre-plant incorporated, revealed that most populations are resistant to metamitron, a key herbicide in sugar beet.

The expected cross-resistance for atrazine was found in all metamitron-resistant populations except for the Swedish one, which is highly resistant to metamitron but not to atrazine. DNA sequence analysis confirmed the presence of a Ser-264-Gly mutation on the *psbA* gene for all populations that are both metamitron- and atrazine-resistant. This mutation on the *psbA* gene encoding the D1 protein of photosystem (PS) II is well known for atrazine-resistant biotypes with resistance to other PS II inhibitors in many weed species. The Ala-251-Val substitution identified in the Swedish population constitutes the first case in which this mutation conferring resistance to certain PS II inhibitors is reported in a higher plant from the field.

Key words: atrazine, bioassay, photosystem II, *psbA* gene, sugar beet, triazinones, triazines

Zusammenfassung

Der weiße Gänsefuß (*Chenopodium album* L.) lässt sich in Zuckerrüben immer schwieriger bekämpfen. In verschiedenen europäischen Ländern (Belgien, Deutschland, Frankreich, Niederlande und Schweden) wurden Samen von *C. album* in Feldern nach unzureichender Bekämpfung mit Metamitron gesammelt. In Gewächshausversuchen wurde nach Behandlung mit Metamitron im Vorauflauf bei den meisten Populationen eine Resistenz gegenüber wichtigen Herbiziden im Zuckerrübenanbau nachgewiesen.

Eine Kreuzresistenz gegenüber Atrazin wurde bei allen metamitronresistenten Populationen mit Ausnahme der schwedischen festgestellt. Letztere war höchst resistent gegenüber Metamitron jedoch sensitiv gegenüber Atrazin. Eine DNA-Sequenzierung bestätigte das Vorkommen einer Ser-264-Gly Mutation auf dem *psbA* Gen bei allen Populationen mit Resistenz gegenüber Metamitron und Atrazin. Diese Mutation auf dem *psbA* Gen, welche für das D1-Protein im Photosystem-II-Komplex kodiert, ist wohl bekannt bei vielen Unkrautbiotypen mit Resistenz gegenüber Atrazin und Kreuzresistenz gegenüber anderen PS-II-Hemmern. Bei der schwedischen Population wurde eine Ala-251-Val Mutation identifiziert. Das Vorkommen dieser Mutation in Feldpopulationen höheren Pflanzen wurde bisher nicht beschrieben.

Stichwörter: Atrazin, Biotest, Photosystem II, *psbA* Gen, Triazinone, Triazine, Zuckerrübe

1 Introduction

Resistance to Photosystem (PS) II inhibitors was firstly reported in 1970 (Ryan 1970) and currently is the second most prevalent type of herbicide resistance found in weeds (Heap 2007). In fat-hen (*Chenopodium album* L.) resistance to atrazine and cross-resistance to many other PS II inhibitors has been reported (Arntzen et al. 1981; Gasquez 1981; Bulcke et al. 1985; Fuerst et al. 1986). So far, four mutations conferring resistance to triazines have been identified in higher plants from the field. All of them are located on helices IV and V and their connecting loop of the D1 protein that is encoded by the *psbA* gene (Schwenger-Erger et al. 1993). The first and still most frequent mutation detected was a substitution of Ser-264-Gly (Gronwald 1994); it was also found in *C. album* (Naber et al. 1990). Later, three other mutations of the *psbA* gene have been identified: Ser-264-Thr in *Portulaca oleracea* L. (Masabni and Zandstra 1999), Val-219-Ile in *Poa annua* L. (Mengistu et al. 2000) and Asn-266-Thr in *Senecio vulgaris* L. (Park and Mallory-Smith 2006). In algae, mutations at other positions of the *psbA* gene causing herbicide resistance have been described (Oettmeier 1999).

Recently, unsatisfactory control of *C. album* has been reported in several sugar beet fields in various European countries. Greenhouse bioassays with *C. album* populations originating from problematic fields revealed that most of them were resistant (R) to metamitron, a key herbicide in sugar beet. All Belgian and French populations were also R to atrazine. However, one Swedish population displayed a high degree of resistance to metamitron but not to atrazine (Mechant et al. 2005; Mechant and Bulcke 2006; Mechant et al. 2007).

Given the importance of target site resistance based on a mutation in the *psbA* gene, it is necessary to look at the DNA sequence of these populations. The objectives of this study were (1) to select a number of *C. album* populations based on their response to metamitron and atrazine in bioassays and (2) to verify whether a mutation can be found between position 211 and 275 of their *psbA* gene where all mutations found so far are located (Naber et al. 1990; Trebst 1991; Gronwald 1994; Oettmeier 1999).

Tab. 1: Response of Belgian (B) and Swedish (S) Chenopodium album populations to metamitron and atrazine.
Tab. 1: Reaktion belgischer und schwedischer Chenopodium album Populationen gegenüber Metamitron und Atrazin.

Population	Plants per pot (% of untreated)		Foliage fresh weight per plant (% of untreated)	
	metamitron[1]	atrazine	metamitron[1]	atrazine
EXP 1				
Herbiseed-S[2]	43	0	16	0
Melle[2]	149	51	83	111
B Boutersem I	117	94	48	47
B Kortessem	170	130	77	79
EXP 2				
Herbiseed-S[2]	104	0	35	0
Melle[2]	92	63	77	89
B Ciplet	104	127	82	69
B Lubbeek I	41	29	101	147
B Héron	44	0	41	0
EXP 3				
Herbiseed-S[2]	75	1	37	1
Melle[2,3]	144	100	42	46
S Hammarlunda	66	37	118	10

[1] Mean value of 2 and 4 mg/kg metamitron PPI
[2] Reference populations: Herbiseed-S = herbicide sensitive; Melle = atrazine-resistant
[3] Due to poor germination of Melle in EXP 3 the response of this population is not that expected from an atrazine-resistant reference population (cf. response of Melle in EXP 1 and 2)

2 Materials and methods

2.1 C. album *populations*

During the 2003 and 2005 growing seasons, seeds from *C. album* populations were collected in sugar beet fields at different locations in Belgium, France, Germany, The Netherlands and Sweden. During the 2004–2007 period, these populations were examined in several bioassays comparing their response to metamitron and atrazine with that of reference populations. The atrazine-resistant reference, Melle, came from a plot repeatedly treated with atrazine in a long-term experiment at Melle (Belgium). The herbicide-sensitive (S) references were Herbiseed-S (Herbiseed, Twyford, UK) and Schriek (from a field under organic farming for over 25 years at Schriek, Belgium). All bioassays were implemented as described by Mechant and Bulcke (2005) in a protocol for the detection of resistance to metamitron in *C. album*. Generally, herbicides were applied pre-plant incorporated (PPI) at following rates: 2 and 4 mg metamitron/kg air dry soil and 1.5 mg atrazine/kg. Each pot was seeded with 200 (EXP 1) or 80 (EXP 2 and 3) seeds at 2 to 3 mm depth. Four to five weeks after sowing the number of surviving plants per pot and the foliage fresh weight per plant were determined. These data were expressed as a percentage of the values recorded in the corresponding control. (Mechant et al. 2005; Mechant and Bulcke 2006; Mechant et al. 2007).

Based on their responses to both herbicides in three bioassays (EXP 1, 2 and 3) (Tab. 1), the following *C. album* populations were selected for DNA sequence analysis: three reference populations (Herbiseed-S, Schriek and Melle), one Swedish population (Hammarlunda), four Belgian metamitron-R populations (Boutersem I, Ciplet, Kortessem and Lubbeek I) and one Belgian S population (Héron). As seeds collected in the field might have come from R and S plants, some populations were rather heterogeneous. Therefore, all resistant *C. album* populations, including the atrazine-R reference Melle, were treated with metamitron (6 or 12 mg a.i./kg PPI) and/or atrazine (1.5 mg a.i./kg PPI) (Tab. 2) to ensure that only R plants would be analysed, being transferred the survivals to a seed production field at the Experimental farm of Ghent University at Melle. All S populations were transferred to the seed production field without any record of treatment. To ensure 100 mg of fresh plant material, about 20 leaves per plant were collected from 5 plants per R and 10 plants per S *C. album* population and were kept at -18 °C awaiting DNA sequence analysis.

2.2 DNA sequence analysis

The method described by Naber et al. (1990) was used for DNA sequence analysis of the PS II *psbA* gene. This method sequences only this part of the D1 protein where all mutations causing resistance to PS II inhibitors are located, i.e. between amino acid residues Phe-211 and Leu-275 (Naber et al. 1990; Trebst 1991; Gronwald 1994; Oettmeier 1999).

3 Results

3.1 Bioassays

In the greenhouse bioassays, the S references Herbiseed-S and Schriek responded less sensitive than expected to metamitron. Therefore, it was decided to classify a given population as R to metamitron and/or atrazine provided it outyielded the S reference by 20% or more. The percentage of survival in metamitron and atrazine treated pots was quite variable among populations. So, where a population such as Lubbeek I had a low percentage of surviving plants, a very high percentage of foliage fresh weight per plant could be recorded due to less competition (Tab. 1).

All of the nineteen Belgian populations tested but two, could be classified as R to metamitron and atrazine. Similar results were obtained with the French populations: nine out of ten populations were found to be R to metamitron and also to atrazine. Although germination of the Dutch and German populations was very low, there was an indication that the first was R to both herbicides whereas the second appeared to be S. The Swedish population, Hammarlunda, was classified as R to metamitron but S to atrazine.

3.2 DNA sequence analysis

The method used gave a clear sequence of the *psbA* gene of *C. album* from codon 202 up to 275, thus covering the whole region where mutations causing resistance to PS II inhibitors could be expected. The sequence of the reference populations, both S and atrazine-R, fully corresponded with the sequence of the respective wild-type and triazine-R biotype of *C. album* found by Naber et al. (1990).

The results are summarised in table 2, together with their response to metamitron and atrazine and the selected treatment to avoid heterogeneity. The S population, Héron, had no mutation as might be expected from a population S to both herbicides. In all Belgian metamitron- and atrazine-R *C. album* populations sequenced, the mutation Ser-264-Gly was found. In the population Hammarlunda (Sweden), a substitution Ala (from GCT)-251-Val (from GTT) was detected.

4 Discussion

In the S populations, Herbiseed-S and Héron, a considerable number of plants with still reasonable amount of biomass production survived metamitron-treatment. Mengistu et al. (2000) pointed out that the lack of mutation in the herbicide-binding region of *P. annua* L. biotypes resistant to the triazinone metribuzin, indicates that resistance to triazinones can also be conferred by other mechanisms, such as metabolism. Therefore, it would be worthwhile to look for the presence of a metabolism based tolerance like it is well known in sugar beet, also belonging to the Chenopodiaceae family (Schmidt and Fedtke 1977).

DNA sequence analysis of the Belgian *C. album* populations confirm the bioassay results: all populations R to metamitron and atrazine have a mutation Ser-264-Gly of the *psbA* gene. Normally, target site resistance to atrazine results in target site cross-resistance to triazinones (Trebst 1991). However, the Swedish population Hammarlunda with altered target site has a high degree of resistance to metamitron but not to atrazine. Resistance to the triazinone metribuzin and the susceptibility to the s-triazine prometryn have been reported by Eleftherohorinos et al. (2000). Unfortunately, no DNA sequence information of this biotype is available. Further, Gray et al. (1995) reported absence of cross-resistance to metribuzin in *Abutilon theophrasti* Medikus with metabolism based resistance to atrazine (Gray et al. 1996). In the case of Hammarlunda, DNA sequence analysis provides an explanation for the absence of cross-resistance to atrazine: resistance to metamitron is caused by an alternation of Ala-251-Val and it is the type of mutation that determines the cross-resistance profile (Oettmeier et al. 1991; Trebst 1991). This mutation, previously found only in unicellular green algae such as *Chlamydomonas* (Oettmeier 1999) and as a double mutation (Ala-251-Val and Val-219-Ile) in cell cultures of *Chenopodium rubrum* (Schwenger-Erger et al. 1993), has been reported to result in a high degree of resistance to triazinones. To our knowledge, in higher plants from the field, this is (1) the first report on this substitution at position 251 and (2) the fifth mutation reported conferring resistance to PS II inhibitors.

The occurrence of *C. album* biotypes with resistance to metamitron but different cross-resistance profiles could raise the question which herbicide(s) did select for the resistance. In Sweden, having no history of atrazine use, the triazinones metamitron, used in sugar beet, and metribuzin, used in rotational potato, could have selected for resistance. In Belgium three different herbicides and/or crop rotations could have contributed to resistance development: (1) a record of continuous use of atrazine in maize resulting in triazine-resistant *C. album* in the seedbank, (2) metamitron use in sugar beet and (3) metribuzin use in potato.

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Tab. 2: Mutations in *Chenopodium album* populations with different resistance profile and selection background.
Tab. 2: Mutationen in Chenopodium album *Populationen mit unterschiedlichem Resistenzprofil und Selektionshintergrund.*

Population	Response[1] to		Selection by	Mutation
	metamitron	atrazine		
Herbiseed-S[2]	S	S	None	none
Schriek[2]	S	S	None	none
Melle[2]	R	R	atrazine: 1.5 mg/kg	SER264→GLY
B Boutersem I	R	R	atrazine: 1.5 mg/kg	SER264→GLY
B Ciplet	R	R	atrazine: 1.5 mg/kg	SER264→GLY
B Kortessem	R	R	atrazine: 1.5 mg/kg	SER264→GLY
B Kortessem	R	R	metamitron: 6 mg/kg	SER264→GLY
B Lubbeek I	R	R	metamitron: 6 mg/kg	SER264→GLY
B Héron	S	S	None	none
S Hammarlunda	R	S	metamitron: 12 mg/kg	ALA251→VAL

[1] Based on bioassay results (see also Tab. 1); S = sensitive and R = resistant
[2] Reference populations: Herbiseed-S, Schriek = herbicide sensitive; Melle = atrazine-resistant

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