THE PRESENT STATE OF HERBICIDE RESISTANCE OF 
WEED POPULATIONS IN THE CZECH REPUBLIC

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Abstract: In 1985–2002 thirteen weeds resistant to atrazine were selected by a repeated application of triazine herbicides on arable land, in orchards, non-agricultural land and at railways in the Czech Republic. Recently *Digitaria sanguinalis* biotypes resistant to atrazine have been found at three railway junctions. Long-lasting application of the active ingredient imazapyr at railways caused selection of resistant *Kochia scoparia* biotypes. High resistance to chlorsulfuron has been discovered in five *Apera* spica-venti biotypes originating in winter cereals fields. The molecular basis of resistance to atrazine has been identified in the following weeds: *Kochia scoparia*, *Solanum nigrum*, *Senecio vulgaris*, *Conyza canadensis*, *Digitaria sanguinalis*, *Amaranthus retroflexus* and *Chenopodium album*. The resistance was conferred by a glycine for serine substitution at residue 264 of the D1 protein in all of those weeds. The resistance to imazapyr in Czech *Kochia scoparia* biotypes was conferred by a mutation at codon 574 of the ALS gene. Analysis of the results of DNA sequencing indicated, that the mutation induced a leucine for tryptophane substitution. There was excellent correspondence between the phenotypic resistance to herbicides of individual plants and the presence of mutations.

Key words: triazines, ALS inhibitors, psbA gene, ALS gene, mutation, PCR, sequencing

INTRODUCTION

Thirteen weed species resistant to atrazine have been developed in the Czech Republic (Mikulka and Chodová 2002) as a result of long-term application of triazine herbicides. Most of resistant weeds were found at railways. Triazine herbicides were used at the Czech railways in the 1980s and 1990s and then from 1997 to 1999. Long-term use of imazapyr at the Czech railways led to selection of 4 kochia [*Kochia scoparia* (L.) Schrad.] biotypes resistant to acetolactate synthase (ALS)-inhibiting herbicides

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Recently, resistance to chlorsulfuron has been detected in silky bent-grass (*Apera spica-venti* (L.) P. Beauv) by Nováková *et al.* (2006) and a biotype of large crabgrass (*Digitaria sanguinalis*) resistant to atrazine has been detected by Salava *et al.* (2006a, 2006b).

The aim of the work presented is to summarize information on the occurrence and research on the resistance of weeds to herbicides in the Czech Republic.

**MATERIALS AND METHODS**

A number of methods are used for identification of weeds resistant to herbicides in the Czech Republic.

**Whole-plant response assay**

The plants arisen from seeds of unknown susceptibility to the herbicide are grown to the stage of 1–4 right leaves and they are treated with graduated doses of the herbicide. Phytotoxic symptoms are rated individually according to the European Weed Research Society (EWRS) classification scheme for plant tolerance (Anonymous 1992).

**Chlorophyll fluorescence assay**

The method is based on the fact that the function of photosystem 2 in weeds susceptible to triazine herbicides is disturbed in the presence of triazines and fluorescence parameters change (Chodová *et al.* 1995; Nováková *et al.* 2005; Salava *et al.* 2006a, 2006b, 2006c). Fully expanded leaves are incubated in atrazine solutions, fluorescence induction curves as well as the fluorescence parameters are recorded with a chlorophyll fluorometer (Ahrens *et al.* 1981).

**Photochemical activity of isolated chloroplasts**

The photochemical activity of chloroplasts of susceptible plants is disturbed in the presence of triazines (Kočová *et al.* 1988; Körnerová *et al.* 1998). The photochemical activity is measured polarographically as the Hill reaction activity and characterized as the amount of oxygen formed by the chloroplast suspension in defined conditions after the white light irradiation and addition of the electron acceptor (Holá *et al.* 2004).

**In vivo acetolactate synthase activity**

The specific activity of ALS is measured by *in vivo* ALS assays (Sprague *et al.* 1997). This assay is based on detection of acetoin accumulation in plant tissue treated with either a KARI (ketolacid reductoisomerase) inhibitor alone or a KARI inhibitor (CPCA – 1,1-cyclopropanedicarboxylic acid) plus an ALS inhibitor. The accumulated acetolactate is converted to acetoin, which can be quantified colorimetrically.

**Molecular analysis**

DNA is extracted from the apical portion of individual plants using the DNeasy Plant Mini Kit (QIAGEN) according to manufacturer’s instructions. The herbicide binding region of the *psbA* gene and two regions of the ALS gene (Domain A and B), respectively, are PCR-amplified and sequenced for detection of mutations. Sequencing is performed directly on PCR products. Sequence editing and analysis is done.
The present state of herbicide resistance of weed populations

using the program BLAST (Altschul et al. 1997). The ExPASy translate tool (Gasteiger et al. 2003) is used to determine the peptide sequences.

RESULTS AND DISCUSSION

Fourteen weeds resistant to atrazine were selected by a long-term application of triazine herbicides on arable land, in orchards, non-agriculture land and at railways in the Czech Republic: Amaranthus retroflexus, Chenopodium album, Amaranthus powelli, Polygonum lapathifolium, Conyza canadensis, Senecio vulgaris, Echinochloa crus-galli, Polygonum persicaria, Setaria viridis, Chenopodium strictum, Chenopodium pedunculare, Poa annua, Solanum nigrum (Mikulka and Chodová 2002; Chodová et al. 2004) and Digitaria sanguinalis (Salava et al. 2006 a, 2006b).

The effect of atrazine on susceptible and resistant plants is presented in Table 1. All tested plants from the resistant biotypes remained green and undamaged after the treatment with atrazine. The resistance was verified by measuring the Hill reaction activity in the presence of atrazine. Susceptible plants showed 0–17% of the photochemical activity of chloroplasts of the control, resistant plants did 62–89%.

Table 1. Comparison of Czech atrazine resistant and susceptible biotypes of seven weed species with respect to results of whole-plant response assay, Hill reaction activity, chlorophyll fluorescence assay and the presence of mutation in psbA gene

<table>
<thead>
<tr>
<th>Species</th>
<th>Whole-plant response assay*</th>
<th>Hill reaction activity*</th>
<th>Chlorophyll fluorescence assay*</th>
<th>Mutation at codon 264 of psbA gene*</th>
<th>literature cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum nigrum</td>
<td>9</td>
<td>1</td>
<td>not tested</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kochia scoparia</td>
<td>9</td>
<td>1</td>
<td>16.9</td>
<td>88.8</td>
<td>+</td>
</tr>
<tr>
<td>Senecio vulgaris</td>
<td>9</td>
<td>1</td>
<td>8.1</td>
<td>81.3</td>
<td>+</td>
</tr>
<tr>
<td>Conyza canadensis</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>65.2</td>
<td>+</td>
</tr>
<tr>
<td>Amaranthus retroflexus</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>80.3</td>
<td>+</td>
</tr>
<tr>
<td>Digitaria sanguinalis</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>61.7</td>
<td>+</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>62.1</td>
<td>+</td>
</tr>
</tbody>
</table>

S – susceptible biotype, R – resistant biotype

* EWRS classification scheme for plant tolerance (1–9): 1 = resistant plants (showing no symptoms/healthy plants), 9 = susceptible plants (heavy damage to total kill)

* % of control

* after application of atrazine: changes compared to control (+), not changed (–)

* mutation: detected (+), not detected (–)

1) Salava et al. 2004a
2) Chodová and Salava 2004a
3) Nováková et al. 2005
4) Salava and Chodová 2006b
5) Salava et al. 2006c
6) Salava et al. 2006a, 2006b
7) Salava et al. (not published)
Triazine resistance of the weed biotypes was also confirmed by in vivo measurement of chlorophyll fluorescence emitted by leaves treated with atrazine (Table 1). The results obtained for *Digitaria sanguinalis* are presented in Figure 1.

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Curves of slow chlorophyll fluorescence induction of *Digitaria sanguinalis* atrazine susceptible biotype ($F_o = 2597$, $F_p = 4503$, $F_t = 3492$) and resistant biotype ($F_o = 2886$, $F_p = 4921$, $F_t = 3756$) in the untreated control and after application of $10^{-2}$M atrazine (susceptible biotype $F_o = 4158$, $F_p = 6520$, $F_t = 6396$) and resistant biotype ($F_o = 3227$, $F_p = 5480$, $F_t = 4434$). The value $F_o$ was recorded in 0 sec, $F_p$ in 10 sec and $F_t$ in 20 sec. [$F_o$-origin (basic) relative fluorescence, $F_p$-peak fluorescence, $F_t$-terminal fluorescence]

After atrazine treatment, leaves from the resistant biotypes did not exhibit any changes in the fluorescence curve pattern, when compared with untreated control leaves, and points of the peak and terminal fluorescence could be clearly distinguished. Leaves from the susceptible biotypes showed typically changed fluorescence curve patterns without distinguishable points of the peak and terminal fluorescence.

In almost all cases, a Ser$_{264}$ to Gly mutation in the D1 protein is responsible for conferring resistance to photosystem 2 (PS 2) inhibitors in weed biotypes (Gronwald 1994). Therefore a region of the *psbA* gene that encodes for amino acids 163 to 329 of the D1 protein was sequenced. Sequences from atrazine susceptible and resistant biotypes differed by a single nucleotide substitution at the variable Ser codon (AGT
to GGT) at position 264, predicting a Ser in the susceptible but a Gly in the resistant biotype. This amino acid change causes a decreased binding affinity of triazine herbicides to the D1 protein of PS 2, and thus assures an uninterrupted electron transport from PS 2 to the quinone pool in the presence of the herbicide. A Ser to Gly substitution has previously been shown to encode a resistant D1 protein in many species (Hirschberg and McIntosh 1983; Goloubinoff and Edelman 1984; Blyden and Gray 1986; Foes et al. 1998, 1999). The DNA sequence and the deduced amino acid sequence of the psbA gene fragment from Digitaria sanguinalis atrazine susceptible biotype are shown in Figure 2.

![DNA sequence and amino acid sequence comparison](image)

Fig. 2. Comparison of nucleotide sequence of the 459 bp fragment from resistant and susceptible biotypes of Digitaria sanguinalis using susceptible (S) as a reference. Dots in the resistant (R) sequence indicate matches to the reference sequence; differences indicated by A, C, G or T. Bold print in the amino acid sequence indicates the site where the mutation confers atrazine resistance. The boxed codon is the location of the single nucleotide substitution (GGT) encoding Gly in the resistant sequence and is the only amino acid difference predicted between the two sequences.

Resistance of kochia to imazapyr was proved by biological, in vivo ALS assays and by a mutation in the ALS gene (Table 2). The two Czech kochia biotypes (Praha-Bubny and Praha-Libeň) displayed a high level of resistance to ALS-inhibiting herbicides in in vivo ALS enzyme assays, indicating that resistance to these herbicides was site-of-action mediated. Two regions of the ALS gene (Domain A and B) were PCR-amplified
and sequenced for detection of mutations. There was one nucleotide polymorphism between the alleles from the susceptible and resistant biotypes. The polymorphism conferred a substitution of leucine in the resistant biotype for tryptophan in the susceptible biotype at position 574. This mutation has been shown previously to confer resistance to ALS inhibitors in kochia (Foes et al. 1999) and in other species (Woodworth et al. 1996; Foes et al. 1998; Boutsalis et al. 1999; Patzoldt et al. 2002). Restriction enzyme analysis of the polymerase chain reaction products was used to confirm results of the sequence analysis (data not shown). All kochia biotypes involved in this study had excellent correspondence between the presence of the mutation and herbicide resistance (Table 2). Rapid and reliable assays based on restriction analysis of PCR products for the detection of mutations in the \textit{psbA} and ALS genes in populations of weeds were developed. These assays are less labour-intensive and much faster than conventional field or greenhouse testing.

Table 2. Comparison of Czech imazapyr resistant and susceptible biotypes of kochia with respect to results of whole-plant response assay and presence of mutation in ALS gene

<table>
<thead>
<tr>
<th>Locality</th>
<th>Whole-plant response assay</th>
<th>Mutation at codon 574 of ALS gene$^{a,b,1)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prague-Bubny</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Prague-Libeň</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Prague-Vršovice</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Olbramovice</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Prague-Invalidovna</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Prague-Karlín</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Prague-Žižkov</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Jihlava</td>
<td>1</td>
<td>+</td>
</tr>
</tbody>
</table>

$^a$ EWRS classification scheme for plant tolerance

1–9: 1 – resistant plants (showing no symptoms/healthy plants)

9 – susceptible plants (heavy damage to total kill)

$^b$ mutation: detected (+), not detected (–)

$^{1)}$ Salava et al. 2004

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REFERENCES


**POLISH SUMMARY**

**OBECNY STAN ODPORNOŚCI NA HERBYCYDY POPULACJI CHWASTÓW W REPUBLICE CZECH**