Co-Resistance of Atrazine-Resistant *Chenopodium* and *Amaranthus* Biotypes to other Photosystem II Inhibiting Herbicides

P. Solymosi* and E. Lehoczki**

* Plant Protection Institute, Hungarian Academy of Sciences, P.O. Box 102, H-1525 Budapest, Hungary
** Department of Botany, Hungarian Academy of Sciences, H-6722 Szeged, Hungary

Z. Naturforsch. 44c, 119—127 (1989); received August 22, 1988

Atrazine-Resistance. Atrazine and Diuron, Fenuron, Lenacil, Phenmedipham Co-Resistance. *Amaranthus bouchonii*, *A. hybridus*, *A. retroflexus*, *Chenopodium album*

Biotypes of *Amaranthus retroflexus* L., *A. hybridus* L., *A. bouchonii* Thell. and *Chenopodium album* L., insensitive to atrazine were collected from maize monoculture where atrazine had been applied extensively. Atrazine-resistant biotypes of *A. retroflexus* and *A. hybridus* showed phenmedipham and lenacil co-resistance and atrazine-resistant biotype of *C. album* showed fenuron co-resistance. An atrazine-resistant biotype of *A. bouchonii* with co-resistance to diuron was not resistant to fenuron, lenacil and phenmedipham.

Introduction

The phenomenon of cross-resistance or co-resistance of pests to pesticides having the same mode of action was summarized in Georghiou and Saito [1] for insecticides and for fungicides in Dekker and Georgopoulos [2]. However, some weed species have evolved multiple-resistances to herbicides considered to have different sites of action [3].

Co-resistance of triazine-resistant weed species to other photosystem II inhibiting herbicides has been reported [4—9]. Different levels of co-resistance among triazine, triazinone, pyridazinone, phenylurea as well as uracil-type herbicides were found [9]. A mutation in the chloroplast *psb A* gene controls this resistance [10]. There is a difference in the wild type (herbicides-susceptible) codon at the normally mutated position (amino acid 264) between higher plants and algae. Single point mutations in weeds can only yield transversion from serine to glycine and in algae from serine to alanine. In the herbicide resistant algae and in the cyanobacterium have been observed [11—16] also other amino acid substitutions (Ala251 → Val, Gly256 → Asp). The resistance in weeds is predominantly to atrazine and that in algae to diuron. As the weeds have always remained diuron sensitive, it was thought that weeds could not have diuron tolerance [4].

Co-resistance to atrazine and chloridazon (pyrazon) was described [17—21]. Recently, a sequentially evolved multiple resistance between atrazine and paraquat was found [22]. There have been no reports, to the best of our knowledge of weeds resistance to diuron, a commonly used phenylurea herbicide.

We report below on some recently discovered co-resistance cases in Hungary.

Materials and Methods

Plant material

Atrazine-resistant plants (*A. bouchonii*, *A. hybridus*, *A. retroflexus* and *C. album*) were collected from maize-monocultures (Bábolna, Gyermely, Ipolytarnócs, Mako and Sopronhorpács) where atrazine had been applied continuously for about 12 yr. Susceptible plants were collected from Kápolnásnyék and Rum where no herbicides had been applied. The plants were grown in the greenhouse at 25±5 °C and 70 ±5% relative humidity. One hundred seeds were sown at 1 cm depth in each 30 × 30 × 10 cm plastic containers filled with sterilized greenhouse potting soil.

Herbicide treatments

Sample populations of four weed species were pre-treated with the following commercially formulated herbicides: atrazine (Aktion); lenacil (Adol 80 WP); diuron (Diuron 80 WP); fenuron (Falisilvan) and...
phenmedipham (Betanal 50 EC). Atrazine, diuron, fenuron, lenacil were applied pre-emergence and phenmedipham was applied post-emergence at the 2 to 4 leaf-stage. Atrazine-resistant *A. bouchonii* plants were grown in an experimental field (in Nagykovács) where 2.4 kg/ha diuron had been used for three years.

Treatments with lenacil and phenmedipham were carried out again one month before the fluorescence induction assays at 8 to 12 leaf-stage. The herbicides were applied at 0.15 to 4 kg/a.i. ha.

**Fluorescence measurements**

The activity of the photosynthetic electron transport chain was established with susceptible test plants having 8 to 12 leaves. Herbicide solutions, 10 μM and 100 μM in 0.1 M sodium phosphate buffer (pH 6.5) were prepared by 500-fold dilution of the unformulated herbicide dissolved in 96% ethanol.

Chlorophyll fluorescence measurements were carried out (as described in [23, 24]) with intact leaves after herbicide-treatments for 1, 12 and 24 h. We made rapid (0.1s) and slow (100s) chlorophyll fluorescence induction measurements.

**Hill-reaction measurements**

Photosystem-II-dependent electron transport was assayed with isolated thylakoid membranes as described in [24, 25]. The 2 ml reaction medium contained 50 mM K-phosphate buffer (pH 6.8), 10 mM NaCl, 5 mM MgCl₂, 100 mM sorbitol, 1 mM NH₄Cl, 0.1 μM gramicidin-D and 30 μM 2,6-dichlorophenol-indophenol (DCPIP). Technical grade herbicide was added to the reaction mixture using methanol as the carrier solvent. The methanol concentration did not exceed 0.3% and at this concentration had no effect on photoreduction of DCPIP. Photoreduction of DCPIP was monitored at 580 nm under saturating light conditions with a dual beam spectrophotometer. Actinic light was filtered through a red filter (Corning-type 2-58).

There were seven replication of each treatment. Herbicide rates required for 50% injury were estimated using linear regression of logittransformed observations [26]. Resistance ratios were then estimated by dividing the *I₅₀* for the resistant biotype by the *I₅₀* of the susceptible biotype. Thus, the resistance ratio is a measure of the degree of resistance.

**Growth measurements**

Ten replicates of one hundred seeds each of atrazine-resistant *A. retroflexus* were sown in the soil at 1 cm depth of plastic containers. Containers were filled with sterilized greenhouse potting soil. Treatments were with unformulated herbicide (atrazine 2 kg/ha; lenacil 1 kg/ha; atrazine and lenacil 1 + 1 kg/ha) by preemergence application. Shoots of these plants were harvested 35 days after sowing. Dry weight was measured after drying for 24 h at 104 °C.

**Results**

Chlorophyll fluorescence curves after 1, 12 and 24 h herbicide treatment were identical for all studied weed species. This is shown (Fig. 1) in case of *A. hybridus*. These co-resistances are chloroplast-level resistances because the kinetics of fluorescence curves were unchanged at different times after herbicide applications.

The atrazine-resistant biotypes of *C. album* from Ipolytarnóc (Fig. 2, 3) and Makó (data not shown) had chlorophyll fluorescence induction curves with fenuron similar to the atrazine-resistant biotype. This *C. album* had fluorescence induction curves for 10 μM diuron similar to the atrazine-sensitive biotype (Fig. 2, 3).

Atrazine-resistant *A. bouchonii* is naturally diuron-sensitive (Fig. 4). Diuron-resistant plants were found in an atrazine-resistant population from Sopronhorpács that had previously been treated with 2.4 kg/ha diuron, for three years. One percent of the atrazine-resistant seeds survived the diuron treatment (Table I). These survivors were still somewhat inhibited by diuron, producing only about 2500 seeds per plant, whereas normal plants produce 20 times more seeds. After these plants grown on diuron for 3 generations they were still partially co-resistant to 10 μM diuron (Fig. 5). We also noted among these plants a few individuals (2–3 individuals of ten) had total diuron-resistance (Fig. 5 C, D), but the majority of the plants had only partial-resistance. These atrazine-resistant and partially diuron-co-resistant biotypes of *A. bouchonii* were susceptible to fenuron, lenacil and phenmedipham (Table II).

The fluorescence induction curves for whole leaves of *A. retroflexus* biotypes from Bábolna and *A. hybridus* biotypes from Gyermely showing co-resistance to phenmedipham and lenacil, are shown in Fig. 6 and Fig. 7. Both *A. retroflexus* and *A. hybridus*...
Fig. 1. Evidence that resistance is immediate and not a function of herbicide degradation. Chlorophyll fluorescence induction of atrazine-resistant intact A. hybridus leaves (Bábolna population) infiltrated with atrazine, diuron, phenmedipham and lenacil after 1, 12 and 24 h.

Fig. 2. Chlorophyll fluorescence induction of atrazine-resistant C. album. The intact leaves (from population of Ipolytarnóc) were infiltrated with atrazine. This population had a normal atrazine resistance (A. rapid chlorophyll fluorescence curves; B. slow chlorophyll fluorescence curves).
Fig. 3. Fenuron co-resistance with atrazine. Chlorophyll fluorescence induction of atrazine and fenuron co-resistant *C. album* leaves (Ipolytarnóc population) infiltrated with fenuron or diuron (A. rapid chlorophyll fluorescence curves; B. slow chlorophyll fluorescence curves).

Fig. 4. Normal diuron sensitivity of atrazine-resistant *A. bouchonii*. Chlorophyll fluorescence induction of atrazine-resistant *A. bouchonii* leaves (Sopronhorpaics population) infiltrated with atrazine or diuron (A. rapid chlorophyll fluorescence curves; B. slow chlorophyll fluorescence curves).

Fig. 5. Partial tolerance and total resistance of diuron-selected atrazine-resistant *A. bouchonii*. Chlorophyll fluorescence induction of atrazine and diuron co-resistant *A. bouchonii* leaves (from experimental field of Nagykovács) infiltrated with diuron. These plants derived from seeds grown on 2.4 kg/ha diuron, for three years (A.-C. rapid chlorophyll fluorescence curves; B./D. slow chlorophyll fluorescence curves). There are significant differences in A.- and C.- curves. C.-curves showed total diuron resistance. This diuron resistance is news in literature.
Fig. 6. Phenmedipham co-resistance with atrazine. Chlorophyll fluorescence induction of atrazine and phenmedipham co-resistant A. hybridus leaves (Babolna population) infiltrated with atrazine and phenmedipham (A. rapid chlorophyll fluorescence curves; B. slow chlorophyll fluorescence curves). Phenmedipham co-resistance is a new case in literature.

Table I. Biotypes of A. bouchonii response to diuron treatments, during 3 years. Biotypes of A. bouchonii were treated with diuron one week after germination. In the years 1985–1987 the seeds were sown which had survived the diuron treatments in previous year. Three diuron treatments and an untreated control were each replicated four times. These data are in average of four replications.

<table>
<thead>
<tr>
<th>Diuron dose [kg/ha]</th>
<th>1985</th>
<th>1986</th>
<th>1987</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>AR</td>
<td>S</td>
</tr>
<tr>
<td>Untreated control</td>
<td>930/1000</td>
<td>900/1000</td>
<td>941/1000</td>
</tr>
<tr>
<td>0.5</td>
<td>0/1000</td>
<td>9/1000</td>
<td>0/1000</td>
</tr>
<tr>
<td>1.0</td>
<td>0/1000</td>
<td>3/1000</td>
<td>0/1000</td>
</tr>
<tr>
<td>2.4</td>
<td>0/1000</td>
<td>1/1000</td>
<td>0/1000</td>
</tr>
</tbody>
</table>

S = Sensitive (from Rum); AR = atrazine-resistant (from Sopronhorpács).

Table II. $I_{50}$ value and resistance ratios in atrazine-resistant and susceptible biotypes of four weed species using Hill reaction. Herbicide rates required for 50% injury were estimated using linear regression of observations. Resistance ratios were then estimated by dividing the $I_{50}$ for the resistant biotype by the $I_{50}$ of the susceptible biotype. Thus, the resistance ratio is a measure of the degree of resistance.

<table>
<thead>
<tr>
<th>Place of isolation</th>
<th>Primary field resistance</th>
<th>Atrazine R</th>
<th>Diuron R</th>
<th>$I_{50}$ concentration [μg]</th>
<th>Fenuron R</th>
<th>Lenacil R</th>
<th>Phenmedipham R</th>
</tr>
</thead>
<tbody>
<tr>
<td>bridus</td>
<td>Kápolnásnyék</td>
<td>none</td>
<td>0.3</td>
<td>0.04</td>
<td>0.45</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Bábolna</td>
<td>atrazine</td>
<td>310</td>
<td>1033</td>
<td>0.052</td>
<td>0.13</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>Gyermely</td>
<td>atrazine</td>
<td>290</td>
<td>966</td>
<td>0.042</td>
<td>0.10</td>
<td>0.042</td>
</tr>
<tr>
<td>triflexus</td>
<td>Kápolnásnyék</td>
<td>none</td>
<td>0.3</td>
<td>0.04</td>
<td>0.45</td>
<td>0.25</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Bábolna</td>
<td>atrazine</td>
<td>330</td>
<td>1100</td>
<td>0.051</td>
<td>1.3</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>Gyermely</td>
<td>atrazine</td>
<td>265</td>
<td>950</td>
<td>0.041</td>
<td>1.0</td>
<td>0.041</td>
</tr>
<tr>
<td>backonii</td>
<td>Rum</td>
<td>none</td>
<td>0.14</td>
<td>0.42</td>
<td>0.02</td>
<td>0.025</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>Sopronhorpács</td>
<td>atrazine</td>
<td>120</td>
<td>857</td>
<td>340</td>
<td>809</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>(Fig. 5C, D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rum</td>
<td>Kápolnásnyék</td>
<td>none</td>
<td>0.12</td>
<td>0.06</td>
<td>0.26</td>
<td>0.051</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Ipolytarnóc</td>
<td>atrazine</td>
<td>150</td>
<td>1250</td>
<td>0.079</td>
<td>1.3</td>
<td>550</td>
</tr>
<tr>
<td></td>
<td>Makó</td>
<td>atrazine</td>
<td>130</td>
<td>1083</td>
<td>0.076</td>
<td>1.3</td>
<td>440</td>
</tr>
</tbody>
</table>

R = Resistance ratios.
from Bábolna and Gyermely were co-resistant to phenmedipham and lenacil at the chloroplast level (Table II). When these atrazine-resistant biotypes were treated with phenmedipham or lenacil (2.5 kg/ha and 1.6 kg/ha, respectively) one month before the chlorophyll fluorescence induction measurements, they became more susceptible to atrazine (Fig. 8, 9).

The leaf-fluorescence induction measurements were supplemented with Hill-reaction study. These data show the isolated thylakoid-membranes from the leaves of atrazine-resistant plants can tolerate herbicide concentrations many times greater than sensitive wild types (Table II). High resistance ratios (857—2115) were observed to the atrazine (in case of A. hybridus, A. retroflexus and A. bouchonii), fenuron (in case of C. album), lenacil and phenmedipham, evaluated in studies on chloroplast-level. Resistance to diuron was observed in atrazine-resistant A. bouchonii (in experimental field). The degree of diuron-resistance was also at a high level (resistance ratio = 809).

One month after growing on phenmedipham or lenacil, 100 μm unformulated atrazine caused a near-complete inhibition in A. hybridus and A. retroflexus biotypes (Fig. 7, 9). When atrazine-resistant A. retroflexus was treated with 1 kg/ha lenacil and 1 kg/ha atrazine in greenhouse conditions, the A. retroflexus plants survived but showed a 87.5% inhibition of growth (Table III). If however A. retroflexus plants previously treated with 1 kg/ha each of lenacil and atrazine were exposed to 1 kg/ha bromofenoxim, 100% of the plants perished within 3 days, as did atrazine-resistant control plants, not pre-treated with atrazine together with lenacil (Fig. 10).

Seeds were collected from plants of each species grown on atrazine, diuron, fenuron, lenacil and phenmedipham. They all retained the resistance of the mother plants, except diuron-tolerant A. bouchonii plants (Table IV).

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Rate [kg/ha]</th>
<th>Dry weight of A. retroflexus [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible control without herbicides</td>
<td>36.7 ± 2.1 (34.2—41.6)</td>
<td></td>
</tr>
<tr>
<td>Untreated R control</td>
<td>27.3 ± 0.7 (25.4—30.2)</td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>2</td>
<td>27.4 ± 0.7 (26.0—29.4)</td>
</tr>
<tr>
<td>Lenacil</td>
<td>1</td>
<td>23.1 ± 0.9 (21.3—31.2)</td>
</tr>
<tr>
<td>Lenacil + atrazine</td>
<td>3.4 ± 0.3 (1.3—3.5)</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Dry weight of atrazine-resistant A. retroflexus grown in presence of herbicides. Plants (R from Gyermely population; S from Kápolnásnyék population) were pre-emergence sprayed with unformulated atrazine and/or lenacil. Shoots of these plants (20 plants/repetition) were harvested 35 days after sowing and dry weights were determined. Results are given ± SE, with 95% confidence limits in parentheses. This experiment was made in 10 repetitions.
A. RETROFLEXUS

--- Atrazine R from same batch that were not pre-treated with Lenacil (control)

--- Treated with 1.6 kg/ha Lenacil 1 month before measurements (control)

--- 100 μM Atrazine (Atrazine R)

Fig. 9. Atrazine sensitivity of atrazine-resistant A. retroflexus. Chlorophyll fluorescence induction of atrazine-resistant A. retroflexus leaves (Gyermely population) infiltrated with atrazine. These plants were treated once with 1.6 kg/ha lenacil 1 month before fluorescence induction measurements. This experiment was made in four repetitions.

Fig. 10. The lack of growth atrazine and lenacil co-resistant A. retroflexus. Atrazine-resistant A. retroflexus seeds were treatment with 1 + 1 kg/ha lenacil and atrazine. This sample of A. retroflexus (Gyermely population) was killed within 3 days with 1 kg/ha bromofenoxim.

Table IV. Herbicide susceptibility of seeds from isolates. Seeds and seedlings (Phenmedipham) were treated with formulated herbicide. Atrazine, diuron, fenuron, lenacil were pre-emergence applied. Phenmedipham was post-emergence applied. These data are in average of ten replications with 100 seeds each replicates.

<table>
<thead>
<tr>
<th>Place of isolation</th>
<th>Atrazine</th>
<th>Diuron</th>
<th>Fenuron</th>
<th>Lenacil</th>
<th>Phenmedipham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Käpolnsnýek (S)</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
</tr>
<tr>
<td>Babolna (AR)</td>
<td>0 0</td>
<td>0 96</td>
<td>0 98</td>
<td>0 97</td>
<td>0 99</td>
</tr>
<tr>
<td>Käpolnsnýek (AR)</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
</tr>
<tr>
<td>Rum (S)</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
</tr>
<tr>
<td>Sopronhorps (AR)</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
</tr>
<tr>
<td>Käpolnsnýek (S)</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
</tr>
<tr>
<td>Ipolytarnoc (AR)</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
<td>0 100</td>
</tr>
</tbody>
</table>

S = Susceptible; AR = atrazine-resistant; DT = diuron-tolerant; DR = diuron-resistant.
Discussion

The photosynthesis of the resistant *Amaranthus* and *Chenopodium* plants after 24 h herbicide treatment have remained unchanged. Atrazine-diuron, atrazine-fenuron, atrazine-lenacil and atrazine-phenmedipham co-resistances are not dependent from the recovery time in *Amaranthus* species and *Chenopodium album*. Detoxification of atrazine can be observed in case of maize [27] and atrazine-resistant *Abutilon theophrasti* [29], where the atrazine will breakdown by a glutathione-s-transferase.

All PS II herbicide-resistant weed biotypes sequenced to date have the same amino acid substitution at the same site in the *psb A* gene product. Substitution at other places in the sequence would be expected to lead to different resistances and co-resistances. Unfortunately, data on the co-resistances of all these biotypes have not been published and it is not clear if they are identical. For this reason the same species with differing co-resistances should be sequenced. In addition to the modification of the D-1 quinone binding protein (the product of the *psb A* gene controlling resistance) other factors may play a role in the expression of herbicide resistance [3].

A large number of chloroplast mutants lacking the *psb A* genes coding for D-1 have been isolated in *Chlamydomonas reinhardtii* [32]. Although these mutants are able to synthesize and to integrate the other PS II polypeptides in the thylakoid membrane, these proteins turn over and no stable functional PS II complex is assembled [33].

The atrazine-resistant biotypes may be genetically unstable, due to the presence of a newly coded plastom mutator gene [10]. The presence of such mutator gene in the population increases the mutation rate to chloroplast DNA mutations, quickly giving rise to further mutations. The plastom mutator should increase mutation frequency for all plastom mutants including resistance to phenylureas and uracil herbicides. Thus, enrichment for triazine-resistance should also carry enrichment for resistance to other photosystem-II-inhibiting herbicides. They are already co-resistant to others.

Not only is atrazine-resistance spreading steadily but there is also an increasing number of cases of co-resistances [21, 20].

*Amaranthus bouchonii* is the first vascular plant to have diuron-resistance. Diuron-resistance has been successfully selected only in green algae under laboratory circumstances [30]. The data from algae show that triazines and diuron plastid-resistance are inherited on different alleles of the same gene. The effect of phenmedipham and lenacil treatments epigenetically abolishing atrazine resistance are new cases in literature.