JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 48, No. 3 (2008)

DOI: 10.2478/v10045-008-0043-4

POPULATIONS OF *POLYGONUM* SPP. RESISTANT TO PHOTOSYSTEM II INHIBITING HERBICIDES IN SOUTH-WESTERN POLAND

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Received: June 5, 2008
Accepted: August 8, 2008

Abstract: The aim of conducted research was the identification of resistance and cross-resistance of *Polygonum aviculare, P. convolvulus* and *P. persicaria* to photosystem II inhibiting herbicides (atrazine, simazine, metribuzine, metamitron, linuron, lenacil, bentazone and chloridazone).
The research was conducted as monitoring tests. During eight years (2000–2007) seeds of weeds were collected from 243 fields in South-Western Poland.
Resistance of biotypes was diagnosed by biological tests (evaluation of phytotoxicity, measurement of fresh plant mass and calculating of resistance index) and fluorescence method.
Identified biotypes showed, in most cases, high level of resistance (IR > 6). Most of *Polygonum* biotypes were resistant to herbicides from triazine group (artazine, simazine, metribuzine and metamitron), lenacil and chloridazone. Resistant biotypes of *Polygonum* were identified on 15–20% of monitored fields. Participation of resistant biotypes, for all monitored fields, in *Polygonum* communities did not exceed 40%.
On monitored fields also several cases of cross-resistance was determined. *Polygonum* biotypes were resistant to atrazine and other triazines (simazine, metribuzine and metamitron), atrazine–lenacil and lenacil–chloridazone.

Key words: resistance, cross-resistance, *Polygonum* spp., herbicide, photosynthesis inhibitors

INTRODUCTION

Herbicides play an inportant role in weed control in many crops. The evolution of herbicide-resistant weed biotypes is an increasing concern for the growers of today and the future. Some factors influencing the development of resistance include con-

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tinuous use of a herbicide, use of single site-of-action herbicides and genetic diversity of weed species (Meartens *et al.* 2004). In the U.S.A. the first reported case of a weed resistant to the triazine herbicides was reported in the mid-1960's (Ryan 1970). In 1999 there were more than 210 documented herbicide-resistant weed biotypes reported worldwide (Heap 1999). Of these 210 cases, 65 weed species had evolved resistance to the triazine herbicides. Selection pressure on weeds by herbicides have resulted in 319 herbicide-resistant biotypes (Heap 2008).

Herbicides containing active ingredients such as: derivatives of triazine, phenylurea, carbamate acid and diazine block the photosynthetic electron transport chain at the reducing site of photosystem II (PS II). Triazine resistant *P. persicaria* biotype was reported, the first time, in France in 1980 (Heap 2008).

In Poland, the first cases of resistance were recorded in the mid-1980's, in the areas with intensive weed control. Persistent herbicides (atrazine, simazine) with the same control mechanism (inhibition of photosystem II) were applied for many consecutive years to maize monocultures and orchards (Gadomski *et al.* 1996; Lipecki 1988; Lipecki and Szwedo 1988; Rola *et al.* 1988). On these fields weed biotypes of: *Chenopodium album, Amaranthus retroflexus* and *Echinochloa crus-galli* exhibited resistance to atrazine, simazine, cyanazine and prometryne.

Cross-resistance is a problem in view of the control of resistant weed populations. The plants in which resistance was caused by one herbicide are resistant to other herbicides with the same or similar control mechanism. Hence they are resistant to a broad spectrum of herbicidal products. The cross-resistance of *C. album* to all triazines in use (atrazine, simazine, terbutryn, prometryn, terbuthylazine and cyanazine) and to herbicides lenacil and chloridazon was demonstrated in Czech Republic (Mikulka and Chodowa 2002). Cross-resistance plays a very important role in weeds resistant to triazines as well as to other herbicides controlling photosystem II: lenacil, chloridazon, metamitron, phenmedipham, fenuron, diuron, linuron, isoproturon, chlortoluron, monolinuron, bromacil, metribuzin, metabenzthiazuron. Cross-resistance makes protection from these resistant populations very difficult (Mikulka and Chodowa 2000).

The aim of the research was identification of resistance and cross-resistance of *P. aviculare, P. convolvulus* and *P. persicaria* to photosystem II inhibiting herbicides (atrazine, simazine, metribuzine, metamitron, linuron, lenacil, bentazone and chloridazone).

MATERIALS AND METHODS

Source of plant and seed materials

The research was conducted as the monitoring tests. During eight years (2000–2007) seeds of weeds were collected from 243 fields in South-Western Poland (*P. aviculare* – 52 fields, *P. convolvulus* – 118 fields and *P. persicaria* – 73 fields). On these fields farmers cultivated through 3–8 years, usually in monoculture, maize, sugar beet, potato and cereals (mainly winter wheat) and intensive chemical weed control by herbicides, containing active ingredient such as: atrazine, metribuzine, metamitron, linuron, chloridazone and lenacil were used. All information was obtained from survey of farmers.

Resistance of *Polygonum* biotypes was determined by two methods. Seeds of weeds were collected from all fields and analyzed by a biological test and fluorescence mea-

surement. For analysis about 100 plants were collected from each field. Samples were taken from an area of 2500 m^2 (square 50 x 50 m) of each monitored field.

Identification of herbicide resistant weeds by biological test

The seeds of weed were planted at a depth of 0.5 cm in 320 ml plastic pots (5 seeds per pot) containing 1: 1 (by volume) mixture of peat and sand. Seedlings were grown in the greenhouse at 25/15 ± 3°C day/night temperature. Natural sunlight was supplemented with light from sodium lamps that provided a photosynthetic photon flux density of 500 μmol/m^2·s at plant height during a 16-h photoperiod.

Herbicides were applied to the plants at the four-leaf stage of development. The herbicides and application doses used were as showed in table 1. Doses of herbicide corresponded to 0.25, 0.5, 1, 2, 4 and 8 multiplication of recommend dose applied in the field. Herbicides were applied using a stationary chamber sprayer with mobile nozzle type TeeJet XR11003-VS and calibrated to deliver 250 l/ha at a pressure of 200 kPa. Treatments were evaluated for ph ytotoxicity to plants 14 days after treatment. Visual observations were recorded using a scale of 0–100, with 100 indicating no injury and 0 equal to plant death. Moreover ED_{50} (effective dose of herbicide caused plant weight reduction about 50% in comparison with untreated object), 3–4 weeks after treatment, fresh plant weight was evaluated. Degree of resistance (RI = Resistance Index) was quantified by calculating the ratio of ED_{50} for resistant and susceptible populations. The experiment was conducted using a completely randomized design with four replications. A similar method of analyses was described in Maertens *et al.* (2004).

Table 1. Herbicides and their doses used in tests

Active substance (a.s.)	Doses [kg a.s./ha]					
	0.25N	0.5N	1N	2N	4N	8N
Atrazine	0.22	0.45	0.90	1.80	3.60	7.20
Simazine	0.37	0.75	1.50	3.00	6.00	12.0
Metribuzine	0.18	0.35	0.70	1.40	2.80	5.60
Metamitron	0.70	1.40	2.80	5.60	11.2	22.4
Linuron	0.25	0.50	1.00	2.00	4.00	8.00
Lenacil	0.20	0.40	0.80	1.60	3.20	6.40
Bentazone	0.24	0.48	0.96	1.92	3.84	7.68
Chloridazone	0.65	1.30	2.60	5.20	10.4	20.8

N – recommend dose (used in field conditions)

Fluorescence measurement

For chlorophyll fluorescence analysis, the method of Ducruet and Gasquez (1978) was used with the following modifications [based on works according to De Prado *et al.* (1998) and Fraga and Tasende (2003)]: five detached fresh, least leaves per plant were analysed (untreated plants, three leaves were incubated in Petri dishes in a 100 μm solution of atrazine, simazine, metribuzine, metamitron, linuron, lenacil, ben-

tazon and chloridazone and the other two leaves in Petri dishes with distilled water as controls. All dishes were placed into a growth chamber under continuous light for 3 h (350 μmol/m^2·s). Prior to chlorophyll fluorescence measurements, the leaves were kept in the dark for 1h. The fluorescence measurements were carried out in Fluorescence Spectrophotometer Hitachi, model F-2500 having a solid sample holder attachment. Leaf samples were exited with 470-nm light and the 685-nm fluorescence emitted at 90° was recorded.

RESULTS

The susceptible (S) and resistant (R) character of weed biotypes were verified in greenhouse tests. As shown in Figure 1, the S biotypes of *P. aviculare* were killed by doses of 2.80 kg/ha (1 N) of metamitron (ED_{50} = 1), respectively whereas the R biotypes survived the recommended and higher doses of metamitron (ED_{50} more than 8).

The slow fluorescence induction curves for leaves were recorded during 1 min. As shown in fig. 2, in untreated samples the onset of illumination caused a rapid rise in chlorophyll fluorescence to a maximum level M followed by decay to a steady-state fluorescence level L. In S leaf samples treated with herbicides chlorophyll fluorescence raised immediately to a maximum level higher than M and remained constant at this maximum level thereafter (Fig. 2A). R leaf samples treated with atrazine or metamitron behaved as the untreated samples whereas those treated with linuron behaved as herbicide treated S leaf samples (Fig. 2B). Since the amount of fluorescence emitted by chlorophyll gives information on the reduced state attained by the first stable electron acceptor of PS II (Q_A), the above results indicate that, whereas in S plants atrazine, metamitron and linuron caused a complete inhibition of photosynthetic electron transport beyond Q_A. In R plants only linuron caused a similar inhibitory action.

Based on the both methods resistant biotypes of *Polygonum* collected from fields in South-Western Poland were identified. Results from monitoring researches are shown in table 2.

P. aviculare biotypes were resistant to atrazine, metamitron and lenacil. Positive results were determined on 8–15% of monitored fields. Moreover, the *P. aviculare* biotypes were resistant to simazine, metribuzine and chloridazone (4–8% of fields). On monitored fields also a few cases of cross-resistance were obtained: atrazine–enacil (2 fields), atrazine–metamitron (3 fields) and lenacil–chloridazone (1 field). *P. convolvulus* biotypes were resistant to atrazine, simazine, lenacil and chloridazone. Positive results were determined on 5–20% of monitored fields. Moreover, the *P. convolvulus* biotypes were resistant to metribuzine, bentazone and linuron (2–4% of fields). On monitored areas also a few cases of cross-resistance were obtained: atrazine–simazine (5 fields), atrazine–simazine–metribuzine (2 fields) atrazine–lenacil (fields) and atrazine–lenacil–chloridazone (1 field). *P. persicaria* biotypes were resistant to atrazine, simazine, metamitron and lenacil. Positive results were determined on 8–17% of monitored fields. Moreover, the *P. persicaria* biotypes were resistant to metribuzine and chloridazone (4–5% of fields). On monitored fields also a few cases of cross-resistance were obtained: atrazine–lenacil (1 field), atrazine–metamitron (2 fields), atrazine–simazine–metribuzine (1 field) and lenacil–chloridazone (1 field).

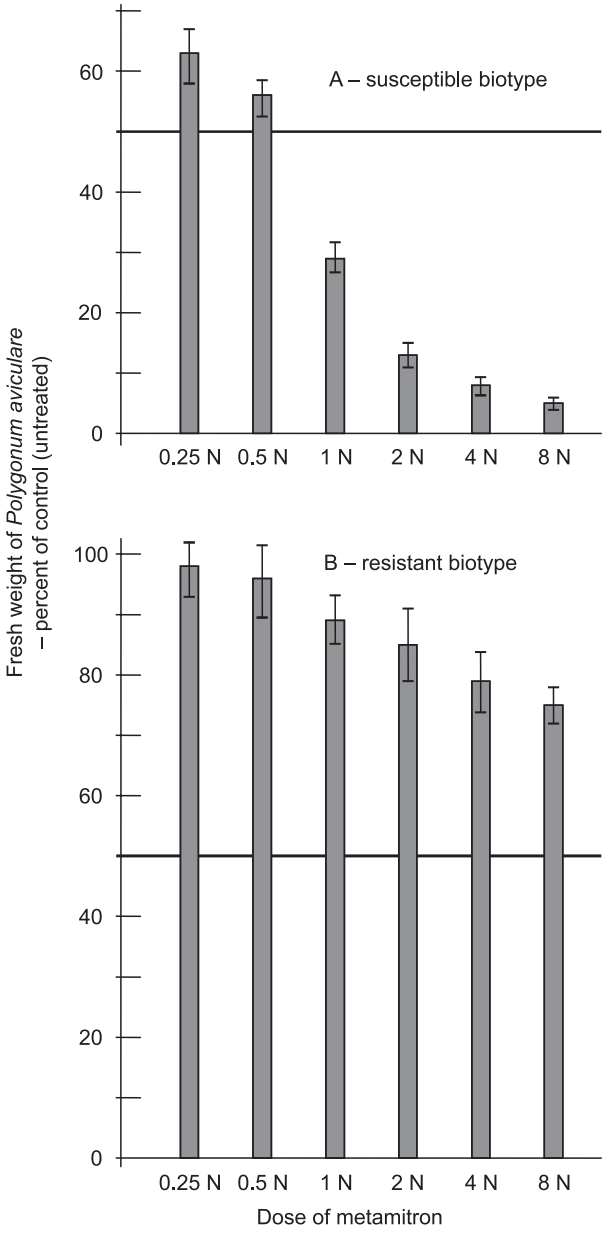

Fig. 1. Evaluation of *Polygonum aviculare* susceptibility to metamitron (N - recommended dose) Vertical bars represent standard errors of the mean value for 4 replications

Table 2. Identification of herbicide resistant biotypes of *Polygonum* spp.

Active ingredien of herbicide	Number of fields with resistant biotypes	Resistance Index (IR)	Participation* [%]
Polygonum aviculare			
Atrazine	8	≥ 6	17–32
Simazine	4	≥ 4	8–21
Metribuzine	2	4–6	6–18
Metamitron	7	≥ 6	12–29
Bentazone	0	–	0
Chloridazone	2	6–8	15–20
Lenacil	4	≥ 6	10–28
Linuron	0	–	0
Polygonum convolvulus			
Atrazine	23	≥ 8	21–40
Simazine	11	≥ 6	10–32
Metribuzine	5	≥ 4	5–20
Bentazone	3	4–8	5–9
Chloridazone	6	≥ 6	9–22
Lenacil	12	≥ 6	19–32
Linuron	2	4–6	4–7
Polygonum persicaria			
Atrazine	12	≥ 6	15–36
Simazine	6	≥ 6	12–27
Metribuzine	4	≥ 4	9–23
Metamitron	9	≥ 6	20–37
Bentazone	0	–	0
Chloridazone	3	≥ 6	12–22
Lenacil	6	≥ 6	9–30
Linuron	0	–	0

*participation of resistant biotypes in communities

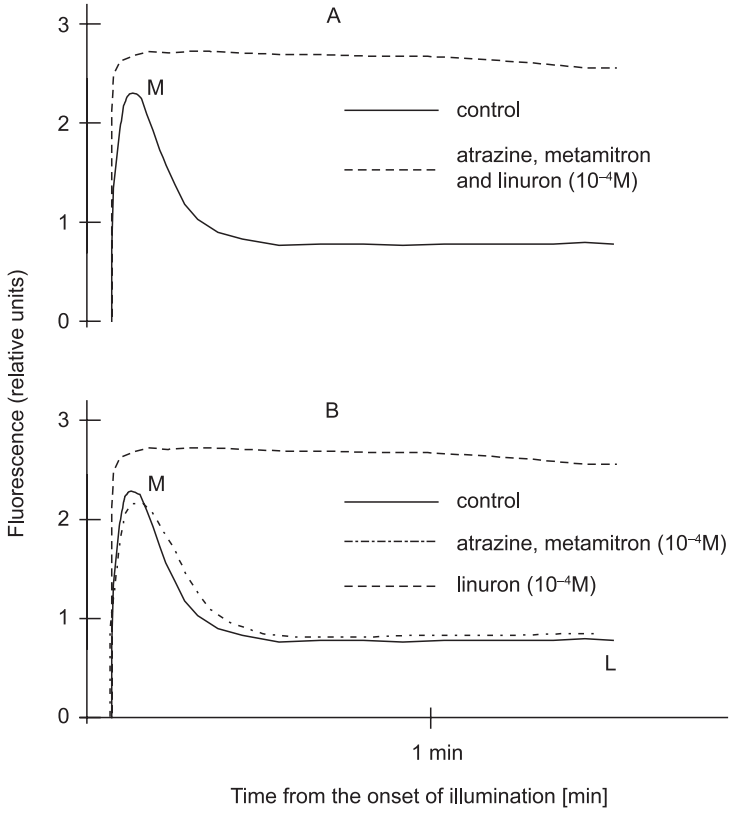

Fig. 2. Slow fluorescence induction curves of *Polygonum persicaria* leaves after atrazine, metamitron and linuron treatment
A – susceptible biotypes, B – resistant biotypes

DISCUSSION

Both used methods allow the identification and confirmation of the occurrence of *Polygonum* biotypes resistant to photosystem II inhibiting herbicides.

Identified biotypes showed, for majority cases, high level of resistance (IR > 6). Most of *Polygonum* biotypes were resistant to herbicides from triazine group (artazine, simazine, metribuzine and metamitron), lenacil and chloridazone. Resistant biotypes of *Polygonum* were identified on 15–20% of monitored fields. Participation of resistant biotypes, for all monitored fields, in *Polygonum* communities did not exceed 40%.

On monitored fields also several cases of cross-resistance were determined. *Polygonum* biotypes were resistant to atrazine and other triazines (simazine, metribuzine and metamitron), atrazine–lenacil and lenacil–chloridazone.

Based on information contents on internet pages (Heap 2008), resistant to triazines and other substances from PSII group biotypes of Polygonum were reported in Czech Republic, France, USA and New Zealand (Rahman and Patterson 1987; Mikulka *et al.* 1988; DePrado *et al.* 1995). Similar results of resistance and cross-resistance to tri-

azines and other inhibitors of photosystem II occurred in other weeds as described by Furest *et al.* (1986), Mikulka and Chodova (2000, 2002), Gadomski *et al.* (1996).

Since 1988, in the Institute of Soil Science and Plant Cultivation, monitoring of the occurrence of resistant weeds on fields of Lower Silesia is being carrying out. Each year shows the new species of weeds, which are resistant to different herbicides.

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POLISH SUMMARY

BIOTYPY *POLYGONUM* SPP. ODPORNE NA HERBICYDY Z GRUPY INHIBITORÓW FOTOSYNTEZY PSII NA POLACH POŁUDNIOWO-ZACHODNIEJ POLSKI

Celem badań była identyfikacja odporności prostej i krzyżowej *Polygonum aviculare, P. convolvulus,* i *P. persicaria* na substancje aktywne herbicydów z grupy inhibitorów fotosyntezy PSII takich, jak: atrazyna, symazyna, metrybuzyna, metamitron, linuron, lenacil, bentazon i chlorydazon.

Badania monitoringowe prowadzono w latach 2000–2007. Nasiona chwastów pobrano z 243 pól uprawnych położonych w południowo-zachodniej Polsce. Odporność biotypów *Polygonum* oznaczano metodą biotestu (ocena fitotoksyczności, pomiar świeżej masy chwastów i obliczenie indeksu odporności - IR) oraz metodą pomiaru fluorescencji liści.

Identyfikowane biotypy *Polygonum* wykazywały, w większości przypadków, wysoki poziom odporności (IR > 6). Analizowane chwasty z rodziny *Polygonum* wykazywały odporność w stosunku do substancji z grupy triazyn (atrazyna, symazyn, metrybuzyna i metamitron) oraz lenacilu i chlorydazonu. Biotypy odporne wykryto na 15–20% analizowanych pól. Udział osobników odpornych w zbiorowiskach *Polygonum* nie przekraczał 40%. Na monitorowanych polach stwierdzono także kilka przypadków występowania odporności krzyżowej. Biotypy *Polygonum* były odporne na atazynę i inne substancje z grupy triazyn (symazyna, metrybuzyna i metamitron) jak również atrazynę i lenacil oraz lenacil i chlorydazon.