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Biotypes of scentless chamomile *Matricaria maritima* (L.) ssp. *inodora* (L.) Dostal and common poppy *Papaver rhoeas* (L.) resistant to tribenuron methyl, in Poland

*Kazimierz Adamczewski, Roman Kierzek, Kinga Matysiak**

Institute of Plant Protection – National Research Institute, Department of Weed Science,
Władysława Węgorka 20, 60-318 Poznań, Poland

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Abstract: Scentless chamomile *Matricaria maritima* (L.) ssp. *inodora* (L.) Dostal and common poppy *Papaver rhoeas* (L.) are species which very often infest winter cereal and winter rape crops. Inhibitors of acetolactate synthase (ALS) are commonly used for control of these weeds. The herbicides are characterised by a single site of action in the plant, which has an influence on selection of the weed population and may result in a rapid development of resistance. In 2012, five seed samples of scentless chamomile and five samples of common poppy were collected from five winter wheat crop fields in Żuławy Gdańskie where the weed species were very poorly controlled. Results of greenhouse experiments showed that two biotypes of scentless chamomile and common poppy were resistant to tribenuron methyl. It was not possible to control resistant biotypes even after use of tribenuron methyl at a dose four times higher than recommended in Poland, it is the first reported case of scentless chamomile and common poppy biotypes' resistance to herbicides. It is not of economic importance but it does prove the growing problem of weed resistance in the country.

Key words: common poppy, resistance, scentless chamomile, tribenuron methyl

Introduction

Results of many research papers show that use of herbicides with the same mechanism of action, results in elimination of sensitive biotypes from the population. Less sensitive biotypes remain uncontrolled and transfer the trait to the next generations. The process of selection occurring for many years, results in dominance of resistant biotypes in the population. It is a mechanism of weed resistance development to herbicides. Those species exhibiting very high fertility are the most susceptible to the process. Such species include scentless chamomile and common poppy.

Sulfonylurea herbicides due to their low cost and high biological activity are the most popular acetylolactate synthase (ALS) inhibitors and they are used throughout the world. Prevalence of sulfonylurea herbicide use resulted in the development of resistance to that herbicide group in some weed species i.e. silky bentgrass, blackgrass, and wild oat (Cavan *et al*. 1999; Hull and Moss 2007; Hamouzova *et al*. 2010). In Poland, this group of herbicides currently plays a crucial role in the chemical weed control of winter cereals. Herbicides of the group of sulfonylurea derivatives, such as tribenuron methyl, chlorsulfuron, mezosulfuron + iodosulfuron and iodosulfuron, have been used for weed control in winter cereal cultivation for many years.

The mechanism of weed resistance to ALS is quite well known. Most cases of resistance to ALS inhibitors occur by point mutations. This type of resistance (target-site) is connected with Pro-197 mutation and as a result of this mutation, amino acid proline at the position 197 is replaced with other amino acids (Yu *et al*. 2008; Krysiak *et al*. 2011a; Adamczewski *et al*. 2013). The mutation in position 197 always conferred high (more the 10-fold) resistance to sulfonylureas but resistance to other ALS inhibitors depended on the particular weed species (Krysiak *et al*. 2011a).

Scentless chamomile *Matricaria maritima* (L.) ssp. *inodora* (L.) Dostal and common poppy *Papaver rhoeas* (L.) are the most often found weeds in winter cereals and winter rape. Both species are very competitive especially in cultivation of winter wheat and winter rape, and germinate at the same time as the cultivated plants. In thinned crops, scentless chamomile and common poppy develop very abundant mass which considerably affects yielding. Due to their mass occurrence, they are some of the most troublesome weeds and may be considered ultimate weeds. Their thick stalks dry very slowly, which hinders harvesting of the cultivated plants, especially of winter rape and winter wheat. Cereal grains and rape seeds collected from fields infested with scentless chamomile and common poppy are more contaminated and damp. The economic threshold of harmfulness of scentless chamomile in cereals is 2–5 plants per m². A density of 25 annual

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*Corresponding address:
ior.poznan.kinga@gmail.com

plants per m^2 of this weed species can result in a 55% reduction in spring wheat yield (Woo *et al*. 1991). In winter wheat, the same density of spring emerging *M. maritima* caused a slight reduction of yield only in a cool, moist year. Yield losses in infested farm fields seeded to wheat, ranged between 30 and 80% at 25 plants per m^2 (Douglas 1989). In the case of high weed prevalence, the stand is very shadowed, which additionally causes plant lodging.

The economic threshold of common poppy harmfulness is estimated at 4–6 plants per 1 m^2 (Heap 2014). In Poland, the economic thresholds of harmfulness for *P. rhoeas* in winter wheat ranged between 6–10 plants per m^2 (Kapeluszny 1988).

Scentless chamomile does not have any special habitat requirements. It is most frequently found and is most abundant in lightly acid sandy and loamy soils which are at least periodically humid or poorly ventilated yet rich in nutrients, especially nitrogen. Scentless chamomile is a polymorphic entomophilous species, however, self-compatibility (self-fertility) is often observed; it can be diploid or tetraploid. Common poppy is nitrophilous, favors loamy soils, alluvial soils and calcareous soils; it can also be found in ruderal areas. Common poppy is entomophilous, its flowers are self-sterile, i.e. self-incompatibility is observed – inability to self-pollinate. There is a fast dissemination of common poppy biotypes resistant to herbicides, because, as it was demonstrated, the gene of resistance to sulfonylurea herbicides is located in the pollen (Cavan *et al.* 1999; Busi *et al.* 2009; Adamczewski and Matysiak 2009).

In some crops, in areas of intensive winter wheat and winter rape cultivation, scentless chamomile and common poppy are predominant weeds in the population and often outgrow stands of these crops. Hence, programs of plant protection against weeds include use of herbicides to control these species. A several-year use of herbicides with the same mechanism of action, has an influence on the development of resistance. Admittedly, the Herbicide Resistance Action Committee (HRAC) list included four biotypes of scentless chamomile resistant to herbicides i.e. France, Germany, Great Britain, but in the last few years, scientific papers confirmed new cases of herbicide resistance of this species (Tiede *et al*. 2014). The first biotype of common poppy resistant to 2,4-D and tribenuron methyl was discovered in 1993 in Spain. At present, biotypes of common poppy resistant to ALS were found in crops of winter wheat of seven European countries (Denmark, France, Greece, Spain, Germany, Italy, Great Britain). Most cases (as many as three) were noted in Italy. In addition, two biotypes were characterised by multiple resistance to tribenuron methyl and synthetic auxins (Heap 2014). Among the European countries, herbicide resistant biotypes of common poppy are widely described i.e. by Kaloumenos and Eleftherohorinos (2008), Marshall *et al*. (2010), Kaloumenos *et al*. (2011), and Moss *et al*. (2011).

The first aim of the study was to collect seeds of scentless chamomile and common poppy from the fields where, in spite of herbicide use, the weeds were very poorly controlled. The second aim was to determine under greenhouse conditions, if the lack of the effective control of these species is associated with resistance.

Materials and Methods

Sample collection

In 2012, seed samples of scentless chamomile and common poppy were collected in Żuławy Gdańskie (Northern Poland, the surrounding region of the city of Gdańsk) from winter wheat crops. Samples of mature plants were collected from 30 ha fields where poor effectiveness of herbicides was noted. The samples were collected from many places so that they represented the whole field or site where poor herbicide effect was observed. One or two flower heads (scentless chamomile) or capsules (common poppy) were collected from each plant. A sample of about 50–60 plants was collected from each field. During the sample collection, particular attention was given to not gathering plants from underlaps and headlands. Five samples of scentless chamomile and five samples of common poppy from five fields were collected.

Greenhouse experiments

After drying, seeds were cleaned under laboratory conditions and then kept for one week at a temperature of ca. –5°C in a fridge, to interrupt the dormancy period. In this way, the prepared seeds were examined under greenhouse conditions to assess resistance to herbicides. In the first stage of the research, five herbicides at recommended doses were used for scentless chamomile i.e. tribenuron methyl, chlorosulfuron, isoproturon + diflufenican, metazachlor + chinomerak, isoproturon, and two for common poppy i.e. tribenuron methyl and isoproturon + diflufenican. On this basis, resistant biotypes were selected. In the second experiment, several doses (2.34; 4.69; 9.38; 18.75; 37.5; 75, and 150 g/ha) of tribenuron methyl were used for both scentless chamomile and common poppy to plot the regression curve and calculate the resistance index. Sensitive biotypes (S) were used as standards: for scentless chamomile 2/2012, collected in Gołuski near Poznań, and for common poppy 2/2009 – collected near railway tracks in Poznań–Podolany.

Greenhouse experiments were carried out in four repetitions in 0.5 l plastic pots. The diameter of the pots were 9 cm. Garden soil mixed with sand at a ratio of 3 : 1 was used for the experiments. About 25 seeds were sown into each plot, and after emergence seedlings were thinned so that 10 plants were left in each pot. The greenhouse temperature was 20–25°C, and day : night length was 16 : 8 h. Herbicide spraying was made with a greenhouse sprayer at the stage of 3–4 leaves. The employed sprayer was TeeJet TT 11002 with a pressure of 3 bars. Water use for the treatment was 250 l/ha. An assessment of herbicide effect was carried out four weeks after the treatment by evaluating the fresh mass of the above-ground plant parts.

Statistical analysis

The percentage of plant fresh-mass reduction was determined in relation to the control (untreated object). The results were statistically analysed with analysis of variance. A regression curve was plotted for each biotype at

Table 1. Influence of tribenuron methyl on resistance parameters of *M. maritima* ssp. *inodora* and *P. rhoeas* biotypes

Species	Resistance parameters	Herbicide dose for susceptible (S) and resistant (R) biotypes [a.i./ha]		
		Standard S	R1	R2
M. maritina ssp. *inodora*	ED50	2.3 (1.9–2.6)	56.5 (43.9–69.1)	59.6 (50.7–68.1)
	Resistance index	1	24.8	26.1
P. rhoeas	ED50	2.2 (1.7–2.6)	57.8 (48.4–67.3)	53.3 (43.9–63.6)
	Resistance index	1	26.5	24.5

the confidence level of 0.05. The Polo Plus software was used for the calculation of an effective dose (ED50) causing 50% reduction of green mass (Robertson *et al*. 2002). On this basis, the resistance index was determined, which is a ratio of a dose causing 50% reduction of green mass of resistant biotype plants to a dose having a similar effect on sensitive biotype plants.

Results and Discussion

Results of the greenhouse experiments from the first stage of the research demonstrated that among the five examined samples, two biotypes of scentless chamomile and two biotypes of common poppy were poorly controlled by tribenuron methyl. Further research was conducted on these biotypes to plot the curve of control and calculate the resistance index. Table 1 presents the results of the research concerning ED50 and resistance indexes for resistant biotypes of scentless chamomile and common poppy. Figures 1 and 2 show the curve of weed control with tribenuron methyl, respectively, for scentless chamomile (Fig. 1) and common poppy (Fig. 2). To obtain 50% control of scentless chamomile, from 56.5 g/ha of tribenuron methyl for biotype R1 to 59.6 g/ha for biotype R2 should be used. In the case of the sensitive biotype, the same effect was obtained when only 2.3 g/ha of this herbicide was used. However, for resistant biotypes of common poppy, the values ranged from 53.3 g/ha (biotype R2) to 57.8 g/ha (biotype R1) of tribenuron methyl. Obtaining 50% control of the sensitive biotype of common poppy required using 2.2 g/ha of tribenuron methyl. The curves of weed control (given in figure 1 for scentless chamomile and figure 2 for common poppy) with the examined herbicide for the both weed species, are very similar. There was a similar

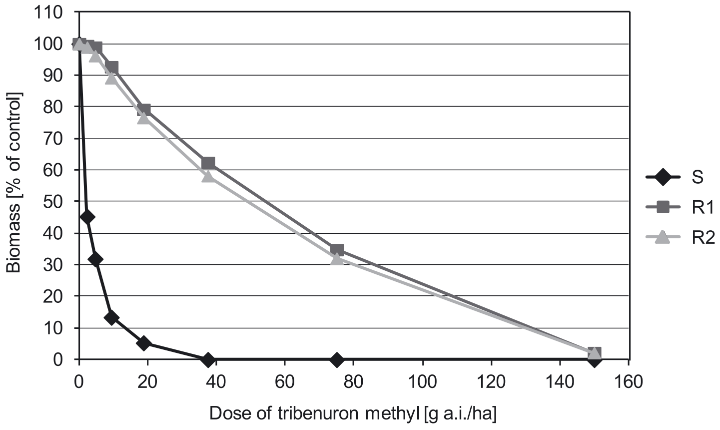

Fig. 1. Influence of tribenuron methyl on fresh mass reduction of resistant (R1, R2) and susceptible (S) biotypes of *M. maritima* ssp. *inodora*

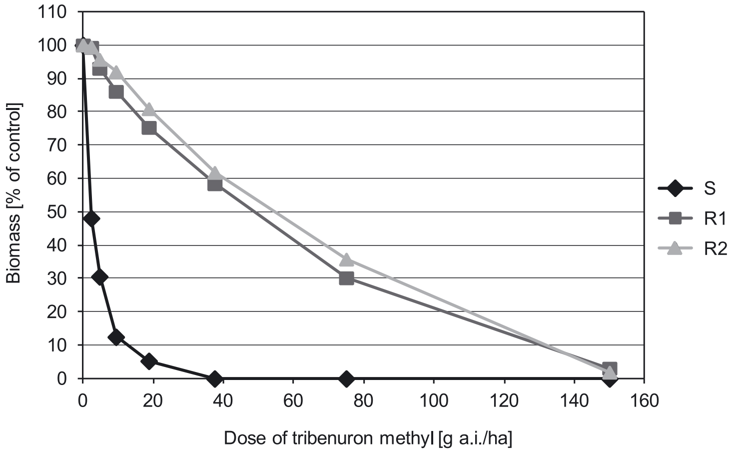

Fig. 2. Influence of tribenuron methyl on fresh mass reduction of resistant (R1, R2) and susceptible (S) biotypes of *P. rhoeas*

Fig. 3. Influence of tribenuron methyl on the susceptible (S) and resistant (R) biotype of *M. maritima* ssp. *inodora*: 1–3 – biotype S; 4–6 – biotype R; 1 and 4 – untreated (control); 2 and 5 – recommended dose; 3 and 6 – 4-times higher dose

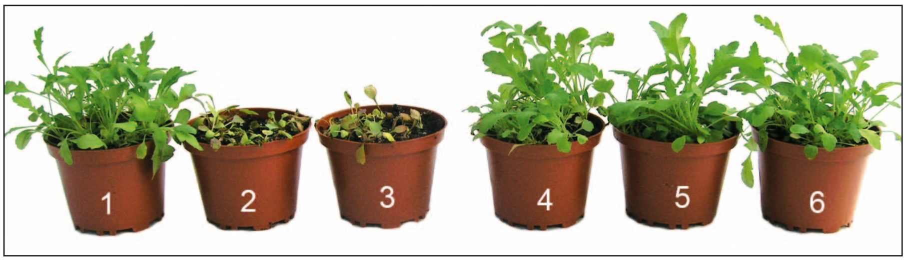

Fig 4. Influence of tribenuron methyl on the susceptible (S) and resistant (R) biotype of *P. rhoeas*: 1–3 – biotype S; 4–6 – biotype R; 1 and 4 – untreated (control); 2 and 5 – recommended dose; 3 and 6 – four-times higher dose

response of the two species to tribenuron methyl. The resistance indexes given in table 1, ranging from 24.8 to 26.1 for scentless chamomile and from 24.5 to 26.5 for common poppy, are also very similar. The resistant biotype of scentless chamomile was not controlled even after use of a four-times higher dose than recommended (Fig. 3). A similar effect of tribenuron methyl was noted for common poppy. The use of the four-times higher dose of the examined herbicide had little influence on effective control of the resistant biotype of common poppy (Fig. 4).

Literature on resistance of scentless chamomile and common poppy is very diverse. Most scientific papers concern common poppy, while scentless chamomile, so far, has not been a subject of a paper. In southern European countries, common poppy is considered one of the most important weeds of cereal crops. However, scentless chamomile is a weed that is not predominant in those European regions. Available scientific reports shows lack of research papers on scentless chamomile biotypes resistant to herbicides. However, there are a dozen or so papers on common poppy resistance to herbicides. The papers largely concern molecular aspects of the resistance (Claude *et al.* 1998; Cirujeda *et al.* 2001; Duran-Prado *et al.* 2004; Marshall *et al.* 2010; Torra *et al.* 2010; Delye *et al.* 2011). These studies indicate that resistance of common poppy to ALS inhibitors results from Pro197 mutation. Proline amino acid is replaced at position 197 with such amino acids as: arginine, histidine, leucine, serine or threonine, while tryptophan (Trp574) is replaced with leucine (Beckie and Tardif 2012). The findings of Kaloumenos *et al.* (2009) support the hypothesis that substitution of Pro197 by amino acids (Ser, Thr, Ala, Arg, or Leu) resulted in an altered secondary structure, which stabilises an ALS tertiary conformation that prevents tribenuron methyl binding and thus confers resistance to this herbicide. The number of scientific papers on resistance of common poppy proves the importance of the problem in southern European countries. Our research was focused on obtaining information about whether resistance of scentless chamomile and common poppy to herbicides can be observed in Poland. No molecular analyses were carried out concerning the mechanism of the resistance. It can be noted, though, that the high resistance indexes obtained both for scentless chamomile and common poppy proved a high probability of the existence of resistance at the target site to acetolactate synthase inhibitors, i.e. mutational resistance. Due to the slightly different blooming biology of common poppy and scentless chamomile, a growth of common poppy resistance to acetolactate synthase-inhibiting herbicides can be anticipated in Poland.

In Poland, the molecular basis of resistant biotypes of blackgrass to sulfonylurea herbicides are widely described by Krysiak *et al.* (2011b), whereas Adamczewski *et al.* (2013) described wild oat biotypes resistant to herbicides (acetolactate synthase and acetyl-CoA inhibitors). The researchers followed Jander *et al.* (2003), Hull and Moss (2007), Yuan *et al.* (2007), Yu *et al.* (2010) and use sulfometuron on ALS-resistant weeds and via this rapid test determined the mechanism of resistance (target-site or non-target-site). Sulfometuron is a non-selective herbicide controlling all weeds. But Sulfometuron is not effective in the case of mutations in position Pro-197. Among the other monocotyledonous weed species resistant to sulfonylureas in Poland, there were also found resistant biotypes of silky bentgrass and blackgrass to sulfonylureas (Adamczewski and Kierzek 2007; Adamczewski *et al.* 2009).

The resistance to sulfonylura herbicides (chlorosulfuron) and cross-resistance was also identified in broadleaf species i.e. cornflower (Marczewska and Rola 2005;

Marczewska-Kolasa and Rola 2008; Adamczewski and Kierzek 2010). Moreover, Marczewska *et al*. (2007) and Marczewska-Kolasa *et al*. (2011) describe some biochemical changes (free amino acids) in weeds resistant to ALS inhibitors. The authors, on the basis of their owns studies, also indicate gas chromatography and isothermal colorimetry as important and helpful methods to identify herbicide weed resistance.

Conclusions

Two out of five examined biotypes, both of scentless chamomile and common poppy, turned out to be resistant to tribenuron methyl. In Poland, it is the first such case of the resistance of the two weed species to herbicides. Admittedly, at present it is of no considerable economic importance, yet it is more proof of the growing problem of weed resistance to herbicides, in Poland. The preliminary study described in this paper, confirms only the resistance to tribenuron-methyl. Identification biotypes of scentless chamomile and common poppy resistant to other active ingredients of herbicides, in Poland, is desired.

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