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REVIEW

Significant increase of weed resistance to herbicides in Poland

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Abstract

Between 2004 and 2017, multiple studies on the herbicide resistance of weeds were conducted by the Institute of Plant Protection – National Research Institute in Poland. Weed seeds, collected from fields located in various regions of Poland where herbicide use was ineffective, were used in studies conducted under greenhouse conditions. A total of 261 loose silky bent (*Apera spica-venti* L.) samples were found to be herbicide resistant, which translates to 52.4% of the fields under study. Nearly 50% of the analyzed samples exhibited resistance to sulfonylurea herbicides. Resistance to acetyl CoA carboxylase (ACCase) inhibitors was found in 18 fields, whereas resistance to photosystem II (PSII) inhibitors (isoproturon) was found in 12 fields. Herbicide resistance of blackgrass (*Alopecurus myosuroides* Huds.) occurred in 26 of the fields under study. In addition, resistance of wild oat (*Avena fatua* L.) to acetyl CoA carboxylase inhibitors occurred in 10 spring cereal crops. In the case of winter wheat, resistance of cornflower (*Centaurea cyanus* L.) to tribenuron-methyl occurred in 23 fields. Scentless chamomile (*Matricaria inodora* L.) and field poppy (*Papaver rhoeas* L.) were resistant to tribenuron-methyl in four and three fields, respectively, of winter wheat. In the case of sugar beet, three biotypes of fat hen (*Chenopodium album* L.) and two biotypes of redroot amaranth (*Amaranthus retroflexus* L.) were resistant to metamitron. Horseweed (*Conyza canadensis* L.), which grows on railway tracks, exhibited resistance to glyphosate. This paper reviews all studies conducted in Poland on weed resistance. Based on the results, maps of weed resistance in Poland were created.

Keywords: ACCase inhibitors, ALS inhibitors, dicot weeds, grass weeds, PSII inhibitors, resistance coefficient

Introduction

Weed resistance to herbicides is one of the major problems that weed scientists have been dealing with for approximately the last dozen years. The first cases of weed resistance were documented in those countries (the United States, Austarlia, Canada, France) where intense chemical protection against weeds had been in use for many years, in combination with constraints to crop cultivation and simplified crop rotation systems. It is also in these countries where studies on weed resistance to herbicides are the most advanced (Gressel 2009; Heap 2014; Neve *et al.* 2014). However, when the first resistance cases appeared, they were not considered to be a serious problem or to present a major threat. Currently, the growing weed resistance problem has attracted the attention of the scientific community, governments, chemical companies and farmers (Gressel 2009; Davis and Frisvold 2017; Davies *et al.* 2019; Moss *et al.* 2019). No new major mode of action of a commercial herbicide active ingredient has been introduced in the last 20 years. Before that, a new mode of action was introduced approximately every 3 years (Duke 2014).

According to the International Survey of Herbicide Resistant Weeds the highest number of resistant weed biotypes has been observed in the USA (556), Australia (142), Canada (103), France (52), Brazil (49), China (42), Spain (36), Israel (31), Japan (37), Germany (31), and Great Britain (23). The number in parentheses is the number of resistant biotypes in a given country as of January 18, 2018. As of now, weed resistance to herbicides has been observed among 251 species, including 146 dicotyledons and 105 monocotyledons. The problem has now been observed in 91 crop plants in 68 countries. Thus far, resistance to 162 herbicides and to 23 out of 26 modes of action has been observed (Heap 2018). According to the data provided by the Institute of Plant Protection – National Research Institute (IPP – NRI) (recommendations for 2016–2017), in Poland, 149 out of 675 registered herbicides are of the sulfonylurea type, with their mode of action based on 19 active substances. This type constitutes more than 20% of all herbicides used in Polish agriculture.

A similar situation has been observed in other countries with intense farming. Sulfonylurea herbicides are very popular and are widely used; consequently, resistance to acetolactate synthase (ALS) inhibitors is the most common, with 160 cases observed (Norsworthy *et al.* 2014; Mahmood *et al.* 2016; Heap 2018). Such a high number of weed species resistant to that type of herbicide probably results from the fact that sulfonyurea herbicides affect the plant at the beginning of its metabolic pathway. Moreover, the resistance trait is transmitted to this group of herbicides through pollen (Adamczewski and Matysiak 2009; Busi *et al.* 2009). In recent years, an increase in the resistance to glyphosate has been observed. As of now, as many as 32 weed species have been found to be resistant to this specific herbicide. The highest number of resistant species was found in the USA in regions where transgenic soy, cotton and corn are cultivated (Duke and Powles 2008).

The weed mechanism of resistance to ALS has been very well described. The target-site resistance of these herbicides is most often connected with the Pro197 mutation. As a result of this mutation, the amino acid proline in position 197 can be replaced by other amino acids. The Pro197–Ser mutation is the most common mutation in resistant weed biotypes. Many studies (Jander *et al.* 2003; Hull and Moss 2007; Yu *et al.* 2010; Adamczewski and Matysiak 2012) show that by using sulfometron to control the weed biotypes resistant to ALS herbicides, it is possible to determine whether it is mutational or non-mutational resistance. Sulfometuron is a non-selective sulfonylurea herbicide that combats all types of weeds but is not effective against ALS-resistant weeds with Pro197 mutations.

Studies on the herbicide resistance of weeds have been conducted at the Institute of Plant Protection – National Research Institute in Poland (IPP – NRI) for many years. In these studies, researchers assessed the occurrence of resistance in several species, such as loose silky-bent (*Apera spica-venti* L.), blackgrass (*Alopecurus myosuroides* Huds.), scentless chamomile (*Matricaria inodora* L.), wild oat (*Avena fatua* L.), cornflower (*Centaurea cyanus* L.), fat hen (*Chenopodium album* L.), redroot amaranth (*Amaranthus retroflexus* L.), field poppy (*Papaver rhoeas* L.) and horseweed (*Conyza canadensis* L.). The purpose of those just mentioned studies was to draw a map of the occurrence of herbicide resistance of the most dangerous weeds in Poland and to assess the risk of such resistance spreading. This publication was aimed to summarize the studies on weed resistance to herbicides that were conducted in Poland at the IPP – NRI between 2004 and 2017.

Weed resistance identification

Collection of samples

Weed seeds were collected from across Poland between 2004 and 2017. The seeds were collected from fields where herbicides were ineffective with regard to the control of certain weed species. The samples were collected by the employees of the IPP – NRI, manufacturers of plant protection chemicals, such as Syngenta and Dow AgroSciences, and distributors of plant protection chemicals, such as Procam and Agrii. The samples were collected from multiple locations to represent the whole field or the site where herbicides were found to be ineffective.

Panicles of loose silky-bent were collected in mid-July from fields where, despite the application of herbicides, this species was not controlled effectively, and where crops were cultivated in a monoculture or with crop rotation with the dominance of winter crops. One panicle was collected from each plant. One sample contained more than 50 panicles of loose silky-bent. During the study, almost 500 samples of loose silky-bent were collected. Samples of blackgrass were collected at the beginning of July in selected fields of winter wheat. During the study, 58 blackgrass seed samples were collected. One ear was collected from each plant (50–60 plants in total). Wild oat was collected in mid-July from 34 fields of spring barley or spring wheat from sites where chemical herbicides were ineffective. Forty-eight samples of cornflower were collected from a winter wheat plantation. Twenty-eight samples of fat hen and 22 samples of redroot amaranth were collected from a sugar beet plantation. Twenty-three samples of scentless chamomile and 13 samples of field poppy were collected from a winter wheat plantation, whereas horseweed was collected from railway tracks.

Greenhouse studies

The greenhouse studies consisted of two or three types of experiments. The first stage consisted of the application of one (recommended) dose of herbicides with different mechanisms of control. The purpose of the study was to select samples that did not respond or responded poorly to the applied herbicides and to then use these selected samples in further studies. In the experiments, the resistance of weeds to the following sulfonylurea herbicides was assessed: chlorsulfuron, mesosulfuron + iodosulfuron, sulfosulfuron, pyroxsulam, propoxycarbazone-Na, and tribenuron-methyl. In the first stage of the study, only the recommended dose of the herbicide was applied. In the second stage of the study, the herbicides were applied at four doses: the recommended dose, and double, triple and quadruple doses. For the purpose of calculating the herbicide resistance index, eight doses: 1/4N, 1/2N, 1N, 2N, 4N, 8N, 16N, 32N (N means recommended – normal dose) of the herbicides were applied. In the studies that aimed to determine the type of resistance mechanism to sulfonylurea herbicides, sulfometuron and imazapyr were applied. Both sulfometuron and imazapyr are non-selective herbicides, i.e., they control all types of weeds but are not effective against weeds resistant to sulfonylurea herbicides. This is why these substances are used for the selection of resistant biotypes in molecular studies (Hull and Moss 2007; Yu *et al.* 2010; Adamczewski and Kierzek 2011). Sulfometuron is not effective if a plant contains the Pro197 mutation, whereas imazapyr does not work on weeds with the Trp574 mutation. This is why the application of the above active substances allows us to determine whether we are dealing with a mutational resistance or some other type of resistance. However, using this method, we cannot determine which amino acid was replaced by proline and tryptophan. For that purpose, we needed to conduct molecular studies.

In these studies, the following acetyl CoA carboxylase (ACCase) inhibitors were used: fenoxaprop-P-ethyl, pinoxaden, and a photosystem II (PSII) inhibitor, i.e., isoproturon. A sensitive biotype, collected from non-agricultural sites where no herbicide treatments had been performed, was used as a standard for each weed species. In the case of loose silky-bent, between 2004 and 2007, a sensitive biotype from Switzerland was used as a standard. The methodological details are specified in the description of the study results for the different weed species.

Overview of the results

Loose silky-bent

This is one of the most common weeds in winter cereal crops. Due to its mass occurrence, which occurs frequently in more humid years, the weed significantly affects crop yield. It has been estimated that loose silky-bent constitutes a problem in 45–55% of winter crops, especially winter wheat and winter triticale. This particular weed is especially important for Polish agriculture from an economic point of view and that is why it has received so much attention in the studies on weed resistance conducted at the IPP – NRI. In Poland, ALS and ACCase inhibitors are commonly used to control loose silky-bent. In addition, though to a lesser degree, PSII inhibitors, such as isoproturon and chlortoluron, are also used.

During a 14-year period (2004–2017), studies on the resistance of loose silky-bent to herbicides were conducted on 498 samples collected from 491 fields. The results presented in Table 1 show that the resistance of loose silky-bent to herbicides was observed in 261 samples, which translates to 52.4% of the fields under study (Table 1). The studies show that the biotypes of loose silky-bent that are resistant to sulfonylurea herbicides are the most common. They were found in more than 50% of the fields under study. In addition to resistance to ALS inhibitors, resistance to ACCase inhibitors was observed in 18 fields, and resistance to PSII inhibitors was found in 12 fields, which translates to 3.6 and 2.4%, respectively, of the fields under study. Most of the studied samples (14) that were resistant to the latter group of herbicides exhibited resistance also to other groups of herbicides (multiple resistance). Four out of 18 samples were resistant to only ACCase inhibitors. In the case of PSII inhibitors, only five out of 12 samples were resistant just to isoproturon. Seven biotypes exhibited multiple resistance, i.e., to both PSII and acetolactate synthase inhibitors. All of the biotypes resistant to ALS inhibitors exhibited multiple resistance. No evidence of simple resistance to this chemical group of herbicides has been found. Resistance to chlorsulfuron was the most common (Table 2). Such a high number of fields with loose silky-bent biotypes resistant to this herbicide probably results from the fact that for years, farmers have been using chlorsulfuron to control weeds, as it is the cheapest herbicide available on the market. Somewhat fewer samples of loose silky-bent were resistant to mesosulfuron + iodosulfuron and sulfosulfuron – 168 and 159 samples, respectively. Among the resistant biotypes of loose silky-bent, 101 samples were resistant to pyroxsulam and 104 were resistant to propoxycarbazone-Na (Table 2).

As can be observed on the map (Fig. 1), the biotypes of loose silky-bent that are resistant to herbicides can be found across Poland. However, herbicide resistance is especially common in the northern regions of Poland, in Warmian-Masurian, Pomeranian and West Pomeranian voivodeships (voivodeship is an administrative region of Poland). It was from these regions that we obtained the most information about the low efficiency of herbicides and thus collected the highest number of samples. Somewhat fewer fields with herbicide-resistant loose silky-bent were observed in Lower Silesia, Silesia, Lubusz, Greater Poland, Masovia and Lublin regions. We can safely assume that herbicide-resistant biotypes of loose silky-bent can also be found in other regions of the country. In Lubusz voivodeship,

Table 1. Seed samples of loose silky-bent (*Apera spica-venti* L.) collected from 2004 to 2017 and resistance to herbicides in Poland

Specification	Seed samples		Biotypes resistant to herbicides		
	collected	resistant	ALS group	ACCase group	PSII group
Numbers	498	261.0	252.0	18 (14 + 4)	12 (7 + 5)
%	100	52.4	50.6	3.6	2.4

ALS – acetolactate synthase; ACCase – acetyl CoA carboxylase; PSII – photosystem II

Table 2. Number of loose silky-bent (*Apera spica-venti* L.) biotypes resistant to four doses of five acetolactate synthase (ALS) herbicides in Poland

Herbicides	Number of resistant *Apera spica-venti*				
	1N	2N	3N	4N	Total
Chlorosulfuron	36	40	46	74	196
Sulfosulfuron	31	38	42	48	159
Iodosulfuron + Mesosulfuron	30	35	50	53	168
Pyroxysulam	25	21	25	30	101
Propoxycarbazone-Na	23	22	28	31	104

N – normal (recommended) dose

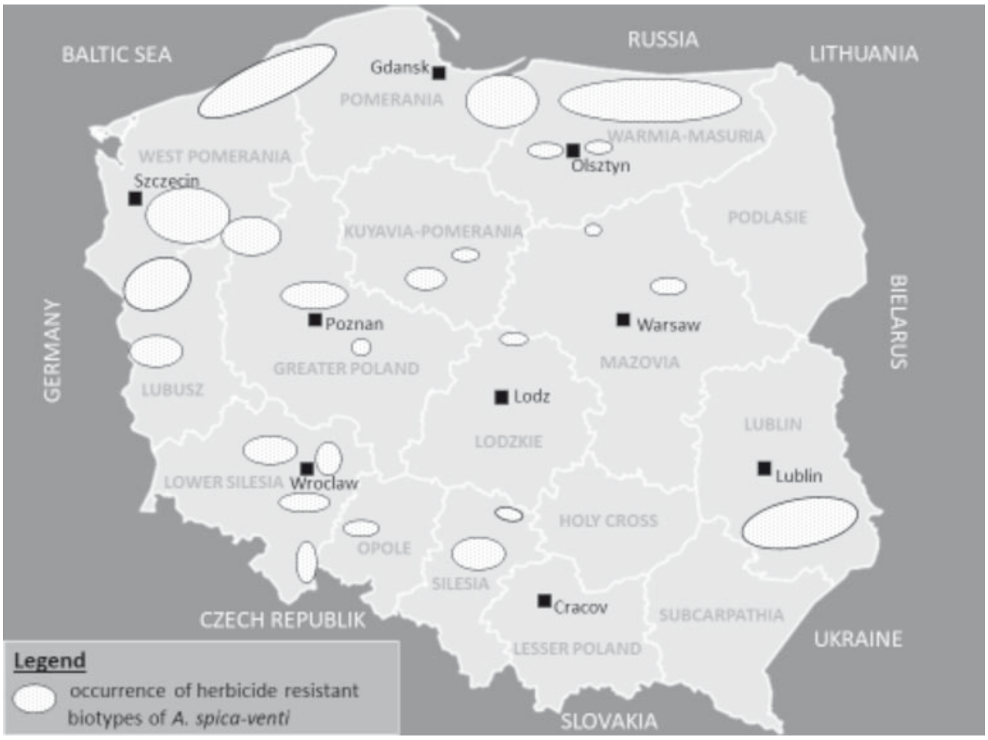

Fig. 1. Distribution of loose silky-bent (*Apera spica-venti* L.) resistant biotypes to herbicides in Poland (the bigger the circle, the more cases of resistance)

there are many fields with herbicide-resistant biotypes of this weed, especially in winter wheat fields with a small area of up to 1 ha.

To determine in which regions of the country the herbicide resistance index of loose silky-bent was the highest and whether the resistance mechanism varied, we had to conduct appropriate studies. For this purpose, we selected 10 biotypes from different regions of Poland that showed the highest resistance to sulfonylurea herbicides in the first stage of the studies.

In the experiments, three herbicides: chlorosulforon, sulfometuron and imazapyr were applied at eight doses from 1/4N to 16N where N means recommended – normal dose. The analysis of the results in Table 3 clearly shows that the resistance index of loose silky-bent to chlorosulfuron depended on the place of origin. The biotypes of loose silky-bent from the Lublin region, Silesia and Greater Poland exhibited the lowest resistance index. The biotypes of loose silky-bent from the Lubusz region and Lower Silesia were slightly more resistant. The most resistant biotypes of loose silky-bent were found in Western Pomerania, Pomerania and Warmia-Masuria. Such a high level of resistance in those regions probably resulted from the extensive use of sulfonylurea herbicides in combination with monocultural cultivation of wheat or a very high percentage of winter cereal crops in rotation.

Our studies on the resistance of loose silky-bent to sulfonylurea herbicides, conducted between 2004 and 2017, show that this is the most common type of resistance (Adamczewski and Kierzek 2007), whereas resistance to ACCase inhibitors and isoproturon is less common (Adamczewski and Matysiak 2010; Adamczewski *et al*. 2017). Such a high number of fields with herbicide-resistant biotypes of loose silky-bent in the northern parts of Warmian-Masurian and Pomeranian voivodeships, as well as in the southern parts of West Pomeranian voivodeship, probably results from the fact that in these regions, there are many large farms that focus mainly on cereal crops and which have been using chlorsulfuron, i.e., the cheapest herbicide, for many years, in combination with the simplification of cultivation methods. The results also show that the resistance of loose silky-bent to sulfonylurea herbicides in Poland is not observed in individual fields but rather, throughout certain regions. This dangerous phenomenon is also widely described by our neighbouring countries (Soukup *et al.* 2006; Massa and Gerhards 2011).

Blackgrass

This plant is one of the major weeds in many western European countries. In Poland, a growing infestation of this weed has been observed for years. The weed prefers good, compact, clayey soil, as well as redzinas that are rich in nutrients and moderately moist. In Poland, blackgrass infests winter and spring cereal crops, as well as winter rape, sugar beet and legumes. Its mass occurrence has been observed in the Pomeranian

Table 3. Detection parameters of susceptible (S) and resistant (R1–R10) biotypes of loose silky-bent (*Apera spica-venti* L.) to three active ingredients in Poland

Biotypes (Locality)	Chlorosulfuron		Sulfometuron		Imazapyr	
	ED_{50}*	R/S**	ED_{50}	R/S	ED_{50}	R/S
S (standard)	8.78 (7.12–10.42)***	1	28.78 (25.09–32.69)	1	22.31 (19.63–25.07)	1
R1 (Biłgoraj – Lublin voivodeship)	76.21 (73.13–79.32)	8.68	32.23 (29.64–34.83)	1.12	24.32 (22.32–26.34)	1.09
R 2 (Sośnicowice – Silesia voivodeship)	89.67 (87.12–92.28)	10.21	31.62 (29.98–33.26)	1.10	25.26 (23.89–26.63)	1.13
R3 (Szczepanów – Lower Silesia voivodeship)	163.48 (159.36–167.60)	18.62	694.17 (689.36–698.94)	24.12	25.66 (23.48–27.85)	1.20
R4 (Ośno Lubuskie – Lubusz voivodeship)	154.89 (152.25–157.51)	17.64	525.24 (522.68–527.84)	18.25	26.10 (24.28–27.92)	1.17
R5 (Szczepankowo – Greater Poland voivodeship)	112.21 (109.06–115.35)	12.78	31.08 (28.36–33.86)	1.08	24.76 (22.16–27.36)	1.08
R6 (Darskowo – West Pomerania voivodeship)	262.61 (258.98–266.24)	29.91	806.13 (803.21–809.15)	28.01	26.77 (24.17–29.38)	1.13
R7 (Karwice – West Pomerania voivodeship)	328.46 (325.12–329.80)	37.41	34.82 (30.98–38.56)	1.21	25.21 (22.68–27.75)	1.13
R8 (Mareza – Pomerania voivodeship)	225.21 (223.36–227.08)	25.65	608.81 (605.62–611.21)	21.15	25.66 (23.46–27.86)	1.15
R9 (Galiny – Warmia-Masuria voivodeship)	262.96 (258.75–267.18)	29.95	32.12 (30.56–33.68)	1.12	798.56 (796.14–800.95)	35.79
R10 (Gęsie Góry – Warmia-Masuria voivodeship)	353.40 (349.16–357.64)	40.25	985.72 (980.36–991.18)	34.25	810.74 (806.75–814.74)	36.35

*50% effective dose
**resistance coefficient
***lower and upper bound at 95% level of confidence

region, the northeastern part of the Warmian-Masurian voivodeship, the Lubusz region, as well as in Lower Silesia and Opole voivodeships. It has been estimated that in Poland, blackgrass infests between 250 and 350 thousand ha of land where its population should be controlled.

Both ALS and ACCase inhibitors are commonly used to control blackgrass. In Europe, the first herbicide-resistant biotypes of blackgrass were recorded in 1982 in Great Britain. It has been estimated that in England, herbicide-resistant populations of blackgrass occur across an area of approx. 1 million ha (Moss and Cussans 1985; Délye *et al.* 2003; Moss *et al.* 2003; Délye 2005; Délye *et al.* 2007; Moss *et al.* 2007; Moss *et al.* 2011). The occurrence of sulfonylurea-resistant blackgrass has also been observed in Poland (Adamczewski *et al.* 2010; Adamczewski *et al.* 2016). In countries where herbicide resistance is highly problematic, many experiments have aimed to develop a programme for the control of resistant blackgrass biotypes (Cavan *et al.* 2000; Chauvel *et al.* 2001; Moss *et al.* 2007; Petersen and Rosenhauer 2014).

During the eight years of the study, 58 blackgrass seed samples were collected from fields where farmers used herbicides and obtained poor or very poor results in terms of weed control. Experiments conducted under greenhouse conditions showed that some biotypes could not be controlled using iodosulfuron + mesosulfuron and exhibited very high resistance (Adamczewski *et al.* 2010). From studies (Hull and Moss 2007; Yu *et al.* 2010) which show that by using sulfometuron (a nonselective herbicide that does not work on plants with Pro197 mutations), one can establish the ALS herbicide resistance mechanism and determine whether it is mutational or metabolic resistance.

The results obtained between 2010 and 2014 have already been published (Adamczewski *et al.* 2016). The above-mentioned publication also included a map of the occurrence of blackgrass biotypes resistant to herbicides. The aim of our publication is to summarize the results of an 8-year study conducted between 2010 and 2017. Figure 2 shows the occurrence of resistant biotypes of blackgrass in Poland. The map shows that blackgrass resistance occurred mostly in the northern and western parts of the country. During the 8 years of study, herbicide-resistant populations were found in 26 fields. In comparison with the period between 2010 and 2014, a significant increase in the population of herbicide-resistant blackgrass biotypes is clearly visible. Such rapid growth is probably due to the application of a no-till system, as well as the maturation biology of blackgrass. The seeds of this weed are relatively large and mature very rapidly, falling to the ground during the dough and milk growth stages of wheat, so that during harvest,

Fig. 2. Distribution of blackgrass (*Alopecurus myosuroides* Huds.) resistant biotypes to herbicides in Poland (the bigger the circle, the more cases of resistance)

the ears do not contain any grains. Due to the no-till system, the only grains that germinate are those that fall to the ground.

In western Europe, resistance to ACCase inhibitors is the dominant type of resistance (Délye *et al.* 2007; Petersen and Rosenhauer 2014). In Poland, according to our studies, there are no differences in the resistance to ALS and ACCase inhibitors (Adamczewski *et al.* 2016). In some regions of the country, we have observed multiple resistance. This means that there are no herbicides in Poland that would be able to combat the biotypes of blackgrass that exhibit such resistance.

According to our information, there are three regions of Poland: the western part of West Pomerania, the northern part of Warmia-Masuria, and northeastern Pomerania, where there are a few hundred hectares of fields where farmers face significant difficulties in regard to controlling resistant blackgrass biotypes. This region is marked on the map with a circle. It would be beneficial to verify the above-described English system under Polish conditions, especially in the three regions where multiple resistance occurs.

Wild oat

Wild oat, one of the most thoroughly described weeds in literature, can be found in all parts of the world, including Alaska and Iceland. This weed occurs extensively in many regions of Poland, most frequently on nutrient-rich and moderately humid soils. The weed infests mostly spring cereal crops, sugar beets and legumes in the eastern part of Pomeranian voivodeship, as well as winter crops in the northwestern part of the country. For the past approximately dozen years, we have observed a growing infestation of wild oat. This may be because some farmers find it difficult to recognise the weed properly. The characteristic parts of wild oat are its leaves, which are convoluted to the left, i.e., counter-clockwise, which makes it easy to distinguish it from other plants.

Chemical control of wild oat, especially in the case of cereal crops, is one of the most expensive treatments due to the high price of herbicides (Petersen and Rosenhauer 2014). A proper assessment of the occurrence of herbicide-resistant wild oat biotypes is further complicated by the fact that there are several botanical varieties of wild oat that differ in terms of herbicide sensitivity. This is a clear signal that the problem of herbicide-resistant wild oat biotypes is real, and we may expect it to increase in the future. Wild oat belongs to a group of weeds that are most prone to develop resistance to herbicides. This is caused by the following properties of the species: high genetic variability, high fertility, prevalence and very high competitiveness against crops. Moreover, wild oat is most often controlled using ACCase inhibitor herbicides. This type of herbicide affects only a single site in a plant, which further affects the selection process in a weed population. Such a combination of the herbicide control mechanism and the biological properties of the weed, prone to developing resistance, leads to rapid population selection, which may result in a rapid increase in the herbicide resistance of wild oat. According to the list published by Heap (2018), there are currently 52 herbicide-resistant biotypes of wild oat documented around the world, with most cases being observed in the USA and Canada. Resistance of wild oat is most frequently observed in response to ACCase and ALS inhibitors. Studies on the resistance of wild oat to herbicides have also been conducted at the University of Agriculture in Krakow (Lesser Poland) (Stokłosa and Kieć 2006). The results of these studies show that in southeastern Poland, there are biotypes resistant to fenoxaprop-P-ethyl and diclofop-methyl.

The resistance of wild oat to herbicides was studied at the IPP – NRI on the basis of 34 samples collected during the 2008–2016 period. Some of the results of the studies have already been published (Adamczewski *et al.* 2013). In this publication, we present a map (Fig. 3) of the occurrence of herbicide-resistant wild oat biotypes in Poland. Our studies have shown that in Poland, the resistance of wild oat manifests itself through the development of biotypes resistant to both ACCase and ALS inhibitors. Experiments conducted under controlled conditions revealed that herbicide-resistant wild oat biotypes occurred in 10 fields. In recent years, we have observed an increase in the infestation of crops by wild oat. Based on the information obtained by plant protection services, a decrease in the effectiveness of herbicides to control wild oat has been observed. This could mean that wild oat is developing resistance to herbicides. The rapid spread of wild oat herbicide resistance also results from the fact that in this case, resistance is transmitted through pollen (Cavan *et al.* 1998).

Consequently, studies on wild oat should be extended to cover the herbicide resistance problem as well. For this purpose, monitoring of the occurrence of herbicide-resistant wild oat biotypes is needed.

Cornflower

This common weed can be found across Poland, mainly in winter crops and winter rape, but less so among spring crops and root crops (potato and beet). Cornflower is an annual plant with spring and winter forms. One plant in a winter crop may sometimes yield more than one thousand achenes that fall extremely rapidly, usually before harvest. The achenes germinate in the fall, at the same time as the winter crop and hibernate in the form of a rosette. Cornflower is a melliferous plant pollinated by insects. In recent years, growing

Fig. 3. Distribution of wild oat (*Avena fatua* L.) resistant biotypes to herbicides in Poland (the bigger the circle, the more cases of resistance)

infestation of winter crops by this weed species has been observed. Cornflower is a relatively tall plant, often reaching as high as 80 cm, which is equal to the height of winter wheat.

Those species of weeds that exhibit high fertility, i.e., those that easily reproduce, are the most prone to develop herbicide resistance. Cornflower belongs, without a doubt, to this group. The first information on the resistance of cornflower to chlorsulfuron in Poland was provided in 2008 (Marczewska-Kolasa and Rola 2008).

Due to its common and massive occurrence, cornflower needs to be controlled using chemical substances. For many years, weeds infesting winter crops have been controlled with sulfonylurea herbicides, such as tribenuron-methyl, chlorsulfuron, mesosulfuron + iodosulfuron and iodosulfuron.

Between 2006 and 2017, 48 samples of cornflower were collected from a winter wheat plantation. The samples were then used in a series of greenhouse experiments using several herbicides with different mechanisms of action. According to the results of our studies, cornflower biotypes resistant to tribenuron-methyl occurred in 23 fields. In addition, one biotype of cornflower resistant to dicamba was also found in the eastern part of West Pomeranian voivodeship. Some of the results of these studies have already been published (Adamczewski and Kierzek 2010; Adamczewski and Kierzek 2011). In this publication, we present a map (Fig. 4) of the occurrence of cornflower resistance in Poland. The highest number of resistant cornflower biotypes was found in northern Poland, in Warmia-Masuria, the eastern part of Pomerania and Lower Silesia. In addition, resistant cornflower biotypes were also found in Greater Poland. In general, the herbicide-resistant biotypes of cornflower occurred on farms that controlled dicotyledonous weeds with herbicides containing tribenuron-methyl, used minimal cultivation methods, and cultivated wheat in a monoculture or in short rotations.

The data obtained on the basis of these experiments show a difference in the resistance of cornflower biotypes to the studied herbicides. The biotypes resistant to ALS inhibitors were slightly more resistant to tribenuron-methyl than to chlorsulfuron. The studies also showed the occurrence of cross resistance to sulfonylurea herbicides among the resistant cornflower biotypes. The results of our studies confirm a scientific thesis that focused on the occurrence of chlorsulfuron-resistant biotypes in Poland (Marczewska-Kolasa and Rola 2008). It is highly probable that the herbicide-resistant biotypes of cornflower also occur in other regions of Poland and will constitute a significant difficulty for farmers in the coming years.

Scentless chamomile and field poppy

Other weed species suspected of developing resistance to herbicides are field poppy and scentless chamomile. According to the information obtained from DowAgro-Science, Syngenta and ProCam, in some winter wheat fields in Pomerania and Masuria, both field poppy and scentless chamomile responded poorly to the application of tribenuron-methyl. Between 2010 and 2016, 23 samples of scentless chamomile and 13 samples of field poppy were collected. The samples were later used in greenhouse experiments using tribenuron-methyl and MCPA + dicamba herbicides. Some of the results of these studies have already been published (Adamczewski *et al.* 2014). The greenhouse experiments conducted using these samples showed that four biotypes of scentless chamomile were resistant to tribenuron-methyl. Two samples of scentless chamomile were collected in the eastern part of Pomerania and another two in Warmia-Masuria. Tribenuron-methyl was not effective against the two samples of field poppy from Pomerania and the one sample from Warmia-Masuria. The data obtained from these experiments show that the resistance of the two weed species to tribenuron-methyl, although without any economic significance, constitutes evidence that herbicide resistance of weeds is a growing problem in Poland.

Herbicide resistance of field poppy and scentless chamomile has not received much attention in the available literature. This may result from the fact that scentless chamomile is a dangerous weed only in some parts of Europe (Poland, Germany, Great Britain) (Moss *et al.* 2011; Ulber *et al.* 2012; Tiede *et al.* 2014). German studies indicate the occurrence of an ALS mutation at positions Pro197 and Thr 574 in resistant biotypes of this weed (Ulber *et al.* 2012). Field poppy, on the other hand, is one of the most important crop weeds in southern and western Europe; consequently, there are more publications available on this subject. Most of these publications pertain to the molecular aspects of resistance (Claude *et al.* 1998; Cirujeda *et al.* 2001; Durán-Prado *et al.* 2004; Marshall *et al.* 2010; Torra *et al.* 2010). The above studies also show that herbicide-resistant biotypes of field poppy constitute a problem in Spain and Great Britain.

Fat hen and redroot amaranth

The herbicide most commonly used to control weeds in sugar beet plantations is metamitron. This substance has been in use in Poland for almost 40 years. According to the information obtained from sugar beet plantations, the effectiveness of control of some species of dicotyledonous weeds, especially fat hen and redroot amaranth, has decreased in recent years. Consequently, between 2007 and 2016, 28 samples of fat hen and 22 samples of redroot amaranth were collected from a sugar beet plantation in Greater Poland. The collected seeds were used for greenhouse experiments to determine whether these biotypes were resistant to herbicides. In the first stage of the study, the following herbicides were applied at recommended doses: metamitron, chloridazon and phenmedipham + desmedifam. The experiments conducted with the collected samples

Fig. 4. Distribution of cornflower (*Centaurea cyanus* L.) resistant biotypes to herbicides in Poland (the bigger the circle, the more cases of resistance)

allowed us to select those biotypes against which metamitron was not effective. Four biotypes of fat hen and two biotypes of redroot amaranth qualified for further study. In the second stage of the study, metamitron was applied at the dose of 0.625–20.0 g · ha^{-1}.

Some of the results of the study on the resistance of fat hen and redroot amaranth to metamitron have already been published (Adamczewski and Kierzek 2011). The level of resistance to metamitron varied across all four biotypes of fat hen. The data on the effective dose (ED_{50}) show that in order to achieve 50% effectiveness in control of biotype 1 7,100 g · ha^{-1} of herbicide is required; for biotype 2 it was 4,600 g · ha^{-1} and for biotype 3 it was 3,800 g · ha^{-1}. The resistance index for these biotypes equalled, respectively, 4.4, 2.9 and 2.4. In the case of the herbicide-sensitive biotype, only 1,600 g · ha^{-1} of herbicide had to be applied to achieve 50% effectiveness. Biotype 4 was the least resistant, with ED_{50} of 2,900 g · ha^{-1} and a resistance index of 1.8. In order for a biotype to be considered herbicide resistant, the resistance index should exceed 2; consequently, this biotype was not deemed to be herbicide resistant. The results obtained in the studies show that fat hen is becoming increasingly resistant to metamitron. Studies conducted in Belgium (Mechant *et al.* 2007; Mechant *et al.* 2008) also point to a varied level of resistance to metamitron among the biotypes of fat hen in sugar beet plantations.

Only two out of 22 samples of redroot amaranth collected from a sugar beet plantation showed resistance to metamitron. These samples were collected in Greater Poland voivodeship. The remaining samples exhibited sensitivity to metamitron. In the case of resistant redroot amaranth biotypes, between 5,600 and 8,100 g · ha^{-1} of metamitron had to be applied to achieve 50% effective control, whereas in the case of herbicide-sensitive biotypes, this amount was only 1,100 g · ha^{-1}. The resistance index of the herbicide-resistant biotypes ranged from 5.1 to 7.4.

Metamitron disturbs the photosynthetic process of a plant at the photosystem II level. The mechanism of metamitron is quite similar to the mechanism of atrazine. Multiyear corn weed treatments using atrazine resulted in the development of biotypes of fat hen and redroot amaranth resistant to this herbicide (PSII inhibitor). After atrazine was banned, it was expected that resistant biotypes would no longer be a problem. However, the occurrence of metamitron-resistant biotypes of these species in sugar beet plantations shows that the gene responsible for their resistance to PSII inhibitors is still present in the environment and becomes active under favourable conditions. This result is related to the fact that the resistance mechanism of these weed species to atrazine and metamitron is similar.

Horseweed

Glyphosate has become the world's most widely used herbicide since it was first introduced in 1974. Glyphosate resistance in weeds was first reported in a population of rigid ryegrass (*Lolium rigidum* Gaud.) in 1995 in Australia. Currently in the USA, the glyphosate resistance of weeds is related to the cultivation of transgenic plants (Duke and Powles 2008). In Poland, the occurrence of horseweed resistant to glyphosate has only been found on railway tracks (Adamczewski *et al.* 2011).

Conclusions

In recent years, an increase in the occurrence of herbicide-resistant weed biotypes has been observed. Not only has the number of resistant species increased, but the area where such biotypes can be found has expanded. The occurrence of some resistant biotypes, such as loose silky-bent, blackgrass and cornflower, constitutes a significant economic problem for agriculture. A biotype of loose silky-bent resistant to sulfonylurea herbicides has been observed across the country. Resistance of loose silky-bent was found in 261 samples, i.e., in 52.4% of the fields under study. Resistance to ACCase inhibitors was observed in 18 fields, and resistance to PSII inhibitors was found in 12 fields, which translates to 3.6 and 2.4%, respectively, of the fields under study. It has been estimated that the herbicide-resistant biotypes of loose silky-bent can be found across an area of approx. 0.5 m million ha.

The area where resistant biotypes of blackgrass occur is also increasing. The fast-growing multiple resistance of blackgrass to ACCase and ALS inhibitors is a very dangerous phenomenon. As a consequence, there are no herbicides that can be used to control the resistant biotypes of this weed in both winter wheat and winter rape. The only way to effectively reduce the population of resistant biotypes of blackgrass would be through the discontinuation of no-till farming and reversion to ploughing. The herbicide resistance of wild oat has not received sufficient attention. This may result from the fact that farmers tend to ignore this type of weed in their fields. The studies confirmed the occurrence of wild oat biotypes that are resistant to ACCase and ALS inhibitors.

In the case of cornflower, there are also some biotypes that exhibit cross resistance to ALS inhibitors, i.e., to chlorsulfuron and tribenuron-methyl. The level of cornflower resistance to ALS inhibitors is very high, which indicates a very high risk for winter crops that are often treated with sulfonylurea herbicides. In north-eastern Poland, the occurrence of herbicide-resistant biotypes of scentless chamomile and field poppy was observed. The biotypes were found to be resistant to tribenuron-methyl. This is the first recorded case of herbicide resistance for these two weed species in Poland. Although this finding does not have any current economic significance, it is more evidence of the growing problem of herbicide resistance of weeds in Poland.

Metamitron (PSII inhibitor)-resistant fat hen and redroot amaranth biotypes have been found in sugar beet plantations. This is because the mechanism of resistance to metamitron and atrazine, which have been used in corn fields in Poland for decades, is similar. This finding indicates that the gene responsible for weed resistance to PSII inhibitors is present in the environment and may become active under favourable conditions.

The increasing occurrence of weed populations worldwide with resistance to multiple site-of-action herbicides will inevitably require more knowledge, planning, costs, time and risk by growers than in the past. Many researchers provide some crucial herbicide-resistant weed management practices (Shaner and Beckie 2014; Beckie and Harker 2017). In the coming years, herbicide-resistant weeds will be a growing challenge that scientists, advisors and farmers in Poland and other areas will have to face.

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