Polish Journal of Agronomy
2017, 30, 3–9

Response of several coniferous shrubs to the application of herbicide containing foramsulfuron and methylsodium iodosulfuron

Andrzej Pacholczak, Karolina Nowakowska, Dorota Gromadzka

Department of Ornamental Plants, Faculty of Horticulture, Biotechnology and Landscape Architecture
Warsaw University of Life Sciences – SGGW (WULS), Nowoursynowska 166, 02-787 Warszawa, Poland

Abstract. The chemical method is the most popular method of weed control due to its efficiency and low costs. A broad assortment of herbicides allows to choose right preparations to control weeds in field production of ornamental shrubs. The experiment on the use of the foramsulfuron- and methylsodium iodosulfuron--containing herbicide was carried out in 2015. Plants of coniferous: *Picea pungens* f. *glauca*, *Pinus mugo* subsp. *mugo* and *Thuja occidentalis* 'Smaragd' were planted in the field and their height was measured. When plant growth ceased they were measured again (in mid-September) and plant material for biochemical analyses was then sampled. The results show that spraying plants with the herbicide in the dosage of 0.15 kg·ha^{-1}, in April, before growth started, was not toxic for conifers *Picea pungens* f. *glauca* and *Pinus mugo* subsp. *mugo* while *Thuja occidentalis* 'Smaragd' proved sensitive to the treatment. The preparation applied together with the recommended adjuvant efficiently controlled most of the weeds. The weeds resistant to the herbicide were: *Convolvulus arvensis, Equisetum arvense* and *Cerastium holosteoides*. In two insensitive taxa the herbicide foliar application of the herbicide resulted in an increase in total soluble sugars, free amino acids and polyphenolic acids as well as an increased peroxidase activity in plant shoots while the content of chlorophyll and catalase activity were decreased. In the sensitive *Thuja occidentalis* 'Smaragd' the content of free amino acids and polyphenolic acids as well as activities of peroxidase and catalase increased while the levels of chlorophyll, total soluble sugars and hydrogen peroxide fell.

Key words: organic compounds, ornamental trees and shrubs, reactive oxygen species (ROS), weeds

INTRODUCTION

The most frequent method used to control weeds in field nursery production is mechanical method or hand weeding, both needing a high financial input, therefore chemical weed control is gaining popularity (Altland et al., 2003). However, possibilities to use chemicals in agriculture, including ornamental nurseries are under the EU legal restrictions. What more, a number of pesticides registered on the European market is decreasing, especially of those with a harmful impact on the environment. A relatively small acreage of ornamental nurseries as compared to other horticultural or agricultural crops as well a huge number of species and cultivars of ornamentals make the chances of chemical weed control in the Polish nurseries rather small as the big chemical companies are not interested in registration of herbicides suitable for use in the production of ornamental trees and shrubs (Falkowski, Matysiak, 2010).

Herbicides are widely used in forests and nurseries, with great control effectiveness and cost-efficiency (Radosevich et al., 2007). Chemical weed control is the best technique for the removal of competing vegetation on forest and nursery sites where the newly germinated tree seedlings are densely and, in most cases, irregularly spaced (Willoughby et al., 2003).

Herbicides, when properly used in ornamental nurseries, are selective in weed control and do not damage ornamental plants. There are, however, many factors which increase sensitivity of crops to herbicides, such as plant age (the younger plants the more sensitive they are), time after potting (freshly potted plants are more sensitive than those well established in containers), developmental phase (plants are more sensitive in a phase of intensive growth than during dormancy), herbicide dosage and temperature (the higher temperature the more probable damage) (Altland et al., 2003; South, Carey, 2005).

Use of herbicides may create a risk of damage for the crops. In the case of trees and shrubs the herbicide injuries do not result in dying of the entire plant but only in lesser injuries or functional disorders, such as dying of shoots or growth retardation. A degree of injuries depends on many factors, such as atmospheric conditions during and after a treatment, plant age and its developmental phase. For herbaceous plant, a herbicide application results in a stress

Corresponding author:

Andrzej Pacholczak
e-mail: andrzej_pacholczak@sggw.pl
phone/fax: +48 22 5932268

which is manifested by biochemical changes, inhibition or retardation of shoot and root growth or plant death. Due to a stress the reactive oxygen species (ROS) are produced intensively and their excess may damage proteins, lipids and nucleic acids (Štajner et al., 2003).

The herbicide sulfometuron is effective for removal of herbaceous weeds and improves the growth of newly planted conifer seedlings. However, there are reports that it stunts the growth of newly planted tree (Barnes et al., 1990) which is not surprising as it is known to reduce cell division in herbaceous plants.

The negative results on shoot growth have led to speculation that sulfometuron methyl applications may retard root development. In the northwestern USA, the value of sulfometuron for weed control in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) has been established. However, phytotoxicity research is limited, and usually restricted to coastal Douglas-fir and ponderosa pine (*Pinus ponderosa* Laws) (Busse et al., 2004). These studies confirm differences in species sensitivity, and also suggest the application of smaller doses than recommended by the label. According to Burney and Jacobs (2009) Coastal Douglas-fir, western red-cedar (*Thuja plicata* Donn Ex. D. Don), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) treated with label recommended doses showed significant decreases in root system development during the growing season following site preparation treatment. The authors hypothesized that the too high concentrations of herbicide would result in decreased growth and life parameters and suggest that any phytotoxic effect of sulfometuron may be alleviated by higher substrate moisture and lower substrate pH hastening residue breakdown.

Sulfonylureas act by inhibiting the enzyme acetolactate synthase, which catalyzes the synthesis of the three branched-chain amino acids valine, leucine, and isoleucine. The accumulation of acetolactate synthase substrates (e.g., a-ketobutyrate) in leaves may be responsible for the cessation of plant growth. Soon after herbicide application, plant cell division stops quickly and death occurs within one to three weeks (LaRossa, Van Dyk, 1987; Brown, 1990; Boutin et al., 2000).

The aim of this work was to evaluate the efficiency of the herbicide for weed control and its phytotoxicity for conifers produced outdoors. Spraying crops aimed as well to check the herbicide phytotoxicity in case of an accidental plant contact with it, which may also have practical implications for the growers of ornamental shrubs. The effect of the preparation on contents of several organic compounds in plant shoots and activity of enzymes involved in defense system against oxidative stress was also determined.

MATERIALS AND METHODS

The experiment was carried out in 2015. On March 25, 3-year old shrubs of *Picea pungens* f. *glauca, Pinus mugo* subsp. *mugo* and *Thuja occidentalis* 'Smaragd', produced in P9 containers, were planted out in the field into sand-clay soil, spaced 40 x 50 cm. Each experimental plot had the area of 100 m^2. The plots were laid out as randomized block design. Plants were 20–25 cm high and had well developed root balls. After they were planted into the field their height was measured. The experiment included 4 treatments, each in three replicates (plots) with 24 plants which gave 288 plants of each taxon.

Two weather parameters (monthly mean temperature and total monthly rainfall) during plant growth and the herbicide treatments are presented in Table 1. The weather conditions might have affected plant development and herbicide efficiency.

The foramsulfuron and methylsodium iodosulfuron-containing herbicide was used as recommended by a producer, i.e. 0,15 kg·ha^{-1} plus adjuvant Mero 842 EC 2 l·ha^{-1}. The treatments with the herbicide were done on three dates: on 15 (T1) and 29 (T2) of April and May 13 (T3). The control treatment was the plot sprayed with water and not weeded during vegetation. Spraying was done with a pressure sprayer of 20 L volume and a lance with 4 nozzles. The soil surface and plants were covered with the herbicide and this was done during rainless and windless day, with temperature 20–22^{o}C.

After the treatment, the appearing weeds were counted, a degree of weed cover was visually evaluated, the effectiveness of herbicide was determined, its effect on shrub growth was described and the damage to conifers was described and documented. The evaluation of the herbicide effectiveness was based on identification of weeds: tolerant to the treatment or damaged by the herbicide: deformed, discolored or chlorotic. The successive observations were done every 4 weeks. The final analyzes and measurements were performed after growth cessation (in mid September) when also damage resulting from the herbicide application was evaluated (i.e. the herbicide phytotoxicity).

Table 1. Rainfall and mean temperatures in 2015 (Experimental Station SGGW, Warsaw).

Characteristics	April	May	June	July	August	September
Mean monthly temp. [^{o}C]	7.9	13.3	16.8	18.2	17.4	13.3
Rainfall [mm]	33	53	67	71	58	43

Visual evaluation of the weed sensitivity to the herbicide allowed to classify weeds as:
– non sensitive – with normal growth (without any leaf color changes);
– moderately sensitive – responding with an evident growth retardation and leaf yellowing;
– sensitive – loosing turgidity, and showing leaf yellowing, gradually drying and finally dying.

Also the herbicide effect on conifers' growth and damage on the shrubs as well as contents of several organic compounds and enzymatic activities in plants was determined. The material for biochemical analyses was randomly sampled from control and treated plants in mid-August when the plant growth had ceased. On that day the plant height was measured again to calculate a length of new growth.

Biochemical analyses

For biochemical analyses the middle parts of the main shoot were sampled. One shoot from every of 24 shrubs from each treatment and each replication was taken at the beginning and at the end of the experiment. The shoot parts were finely chopped, mixed, and 0.5 g samples were used for the measurements. Triplicate extracts were prepared for each analysis and three measurements were done for each extract producing nine readings for each data point.

The total chlorophyll content (chlorophyll a+b) was analyzed according to Lichtenthaler and Wellburn (1983). Total soluble sugars were determined according to Dubois et al. (1956), free amino acids were measured by the method of Rosen (1957). Polyphenolic acids were measured by the colorimetric method with the Arnow's reagent according to the Polish Norm PN-91/R-87019. The hydrogen peroxide content was measured by the method of Zhang et al. (2013). The catalase activity was analyzed according to Goth (1991), and the peroxidase activity – by the method of Nakano and Asada (1981).

Statistical analyses

Arcsine transformation was performed for all data taken in percentages (Snedecor, Cochran, 1967). All data – shoot length and the results of biochemical analyses were subjected to the one-factorial ANOVA followed by Newman-Keuls test at $\alpha = 0.05$ (Wójcik, Laudański, 1989).

RESULTS

The weed sensitivity to the herbicide tested

The herbicide containing foramsulfuron and methylsodium iodosulfuron, used together with a recommended adjuvant, efficiently controlled most of the weeds in the field production of conifers. Regardless of the treatment date the following species: *Convolvulus arvensis*, *Cerastium holosteoides* and *Equisetum arvense* were tolerant to the herbicide (Table 2). A medium sensitivity to the herbicide was found in *Cirsium arvense*, *Digitaria sanguinalis* and *Polygonum aviculare* whose young plants were slightly damaged but later recovered during the season. In several weeds, i.e. *Taraxacum officinale* or *Chenopodium album* physiological changes such as leaf discoloration were observed.

Table 2. Weed sensitivity to the herbicide.

Weed susceptibility	Weed species
Sensitive weeds	*Amaranthus retroflexus* L. *Anagallis arvensis* L. *Artemisia vulgaris* L. *Calendula officinalis* L. *Capsella bursa-pastoris* L. (Med.) *Chenopodium album* L. *Echinochloa crus-galli* L. *Euphorbia helioscopia* L. *Galinsoga parviflora* Cav. *Geranium pusillum* L. *Impatiens parviflora* DC. *Lactuca virosa* L. *Lamium purpureum* L. *Matricaria chamomilla* L. *Oxalis corniculata* L. *Oxalis stricta* L. *Phacelia tanacetifolia* Benth. *Plantago lanceolata* L. *Plantago major* L. *Poa annua* L. *Poa arvensis* L. *Rorippa silvestris* L. *Rumex crispus* L. *Senecio vulgaris* L. *Sonchus asper* L. *Sonchus oleraceus* L. *Stellaria media* L. *Taraxacum officinale* L. *Thlaspi arvense* L. *Trifolium pratense* L. *Trifolium repens* L. *Veronica arvensis* L. *Vicia cracca* L. *Vicia sativa* ssp. *nigra* L.
Moderately sensitive weeds	*Cirsium arvense* L. *Digitaria sanguinalis* L. *Polygonum aviculare* L.
Non sensitive weeds	*Convolvulus arvensis* L. *Cerastium holosteoides* Fr. em. Hyl. *Equisetum arvense* L.

The herbicide effect on length of new growth

The analysis of variance did not show a significant effect of the herbicide on the new growth in *Picea pungens* f. *glauca*. Plants from all the treatments had the new growth of comparable length (Table 3), however, the treatment carried out on the third date (T3, 13.05.) resulted in

Table 3. Increase in shoot length [cm] in conifers treated with the herbicide.

Species	Control	T 1	T 2	T 3	Standard error
Picea pungens f. *glauca*	4.6 a	4.7 a	4.5 a	4.8 a	0.327
Pinus mugo subsp. *mugo*	4.5 b	4.2 b	4.3 b	3.2 a	0.322
Thuja occidentalis 'Smaragd'	7.0 c	2.1 a	2.1 a	3.2 b	0.234

Means in each row followed by the same letter do not differ significantly at α = 0.05
date of herbicide application: T1 –15 April, T2 – 29 April, T3 – 13 May

Table 4. Effect of herbicide on total chlorophyll, total soluble sugars, polyphenolic acids, free amino acids, hydrogen peroxide, catalase and peroxidase activity in shoots of coniferous shrubs.

Content/activity	Species	Control	T 1	T 2	T 3	Standard error
Chlorophyll [mg·g^{-1} d.m]	*Picea pungens* f. *glauca*	1.9 b	2.0 b	2.1 b	1.2 a	0.06
	Pinus mugo	2.2 b	2.1 b	1.9 a	1.8 a	0.04
	Thuja occ. 'Smaragd'	2.3 c	2.1 b	1.8 a	1.9 ab	0.06
Total soluble sugars [mg·g^{-1} d.m]	*Picea pungens* f. *glauca*	38.6 a	40.9 b	45.9 c	41.8 b	0.445
	Pinus mugo	26.5 a	30.1 b	32.7 c	28.9 b	0.610
	Thuja occ. 'Smaragd'	19.7 c	15.2 a	16.8 b	16.3 ab	0.365
Free amino acids [µM of leucine g^{-1} d.m.]	*Picea pungens* f. *glauca*	120.8 a	151.0 b	250.6 c	316.3 d	6.74
	Pinus mugo	140.3 a	193.0 b	183.9 b	259.5 c	7.63
	Thuja occ. 'Smaragd'	158.9 a	429.8 c	341.7 b	342.2 b	17.41
Polyphenolic acids [mg g^{-1} d.m.]	*Picea pungens* f. *glauca*	11.5 ab	10.7 a	12.8 bc	13.4 c	0.431
	Pinus mugo	13.8 a	14.3 b	14.9 bc	15.5 c	0.148
	Thuja occ. 'Smaragd'	9.9 a	12.9 b	12.9 b	13.1 b	0.409
Hydrogen peroxide [µg H_2O_2 g^{-1} d.m.]	*Picea pungens* f. *glauca*	30.6 a	31.3 a	32.6 a	32.4 a	0.899
	Pinus mugo	39.3 ab	38.8 a	41.3 b	40.5 ab	0.739
	Thuja occ. 'Smaragd'	87.3 c	83.1 b	78.3 a	78.1 a	0.998
Catalase activity [mkat g^{-1} d.m.]	*Picea pungens* f. *glauca*	6559.1 b	5629.1 a	5213.3 a	5282.3 a	88.24
	Pinus mugo	6760.5 b	5619.1 a	5652.2 a	5500.3 a	79.56
	Thuja occ. 'Smaragd'	5516.6 b	6688.5 d	6096.4 c	5110.7 a	38.86
Peroxidase activity [nkat min^{-1} g^{-1} d.m.]	*Picea pungens* f. *glauca*	0.09 a	0.12 b	0.11 b	0.10 ab	0.005
	Pinus mugo	0.05 a	0.09 b	0.07 b	0.08 c	0.003
	Thuja occ. 'Smaragd'	0.09 a	0.10 a	0.12 b	0.13 b	0.006

Means in each row followed by the same letter do not differ significantly at α = 0.05
date of herbicide application: T1 –15 April, T2 – 29 April, T3 – 13 May

a slight yellowing of new growth. The new pine shoot parts in plants treated on T1 and T2 were as long as the control ones, however, those sprayed on T3 were over by ¼ shorter.

Thuja occidentalis 'Smaragd' sprayed with the herbicide produced new growth shorter than in the control treatment – even by 70% in case of the first and second date, i.e. in April. Generally, treatments with the herbicide negatively affected shrubs which were growing poorly and their shoot tips were getting brown and dying.

Results of biochemical analyses

In spruce plants sprayed with the herbicide on T1 and T2 the chlorophyll content was comparable to that in control treatment while in plants treated on T3 its level was

37% lower (Table 4). The pine plants sprayed on T2 and T3 had the chlorophyll content decreased as compared to T1 and the control. In arborvitae the chlorophyll content was lowered by the treatment done on all 3 dates.

The total soluble sugar content increased in spruce shoots sprayed with the herbicide (Table 4), the highest having been found in shrubs sprayed on T2 – 19% higher than in control plants. Also in pine plants the treatments – regardless of their date – increased the sugar content relative to the control. In plants sprayed on T2 the sugar content was by 23% higher than in control plants. In arborvitae the treatments with the herbicide resulted in decreased amounts of sugars due probably to the preparation toxicity for this species.

Regardless of the treatment date the levels of free amino acids in conifers' shoots were elevated (Table 4). Spruce plants sprayed on T2 and T3 had two- and 2.6-fold amount of amino acids, respectively, as compared to the control. In pine plants sprayed on T1 and T2 the amino acid content was by 37% higher than in untreated plants while in those sprayed on T3 – almost twice as high. The amount of free amino acids increased over twofold in arborvitae plants sprayed on T2 and T3 and almost threefold in those treated on T1. The levels of polyphenolic acids in plants treated with the herbicide were increased, especially in plants sprayed on T2 and T3 (Table 4).

The contents of H_2O_2 in shoots of spruce and pine treated with the herbicide remained on the level of the respective control treatments (Table 4). In arborvitae the herbicide application resulted in its small decrease: 5% in T1 to 10% in T3, due probably to the phytotoxicity of the preparation for this species. Catalase activity in shoots of spruce and pine treated with the herbicide decreased by approx. 20% while in arborvitae it increased by 21% in T1 and 10% in T2, i.e. in the treatments where the important damages were observed due to the herbicide application (Table 4). Peroxidase activity increased in all the plants from the treatments where the herbicide application was involved, regardless of its date (Table 4).

DISCUSSION

Presence of weeds may be dangerous for crops. Young plants are particularly sensitive to weeds, especially during dry and hot summers when they are threatened by water deficit and less light access. The most frequent methods to control weeds are hand weeding and herbicide applications (Altland et al., 2003).

The experiment confirmed the efficiency of the herbicide under study against weeds enlisted in its label for example: *Amaranthus retroflexus, Capsella bursa-pastoris, Chenopodium album, Echinochloa crus-galli, Galinsoga parviflora, Poa annua, Poa arvensis, Sonchus asper*, *Stellaria media, Taraxacum officinale, Thlaspi arvense, Veronica arvensis, Vicia cracca*. On experimental plots the sensitivity to the herbicide of such weed species as *Artemisia vulgaris*, *Euphorbia helioscopia*, *Geranium pusillum, Lamium purpureum, Matricaria chamomilla, Oxalis stricta*, *Plantago major, Rorippa silvestris, Senecio vulgaris, Trifolium repens* was also evaluated. Studies on the application the same active substances – foramsulfuron and methylsodium iodosulfuron – were also carried out by Falkowski and Matysiak (2010). Their experiment revealed a medium sensitivity to the herbicide of *Chenopodium album* and *Rumex crispus*. Damalas et al. (2011) tried to control weeds of *Echinochloa* species by sulfonylurea herbicides. Foramsulfuron was more efficient when applied on weeds in the phase of 2–3 leaves while the addition of sulcotrion increased its efficiency even by 20%. Efficiency of the above substances against *Amaranthus retroflexus, Echinochloa crus-galli* and *Chenopodium album* was confirmed in the experiments of Kir and Doğan (2009).

A poor assortment on the Polish market of chemicals for weed control in the ornamental plant production may be related to a relatively small acreage of nurseries and the large number of taxa under culture. Usually, herbicides used in horticultural and agricultural production act selectively therefore they should not affect the crops. The studies show however, that in some plants toxicity symptoms may occur such as shortening of new growth (Falkowski, Matysiak, 2010). In this experiment this was visible in arborvitae 'Smaragd'. Similarly, Robertson and Davis (2012) reported a considerable shortening of new growth in *Larix occidentalis*, *Pseudotsuga menziesii* and *Pinus monticola* after the application of another active substance from the group of ALS inhibitors, i.e. sulfmeturon. The authors of this work report the new growth in pine plants treated with the herbicide on date 1 and 2 to be comparable to the control untreated plants. In spruce – regardless of the treatment date – no differences in new shoot length was observed between treated and control plants. Neither the use of glyphosate in the experiment of Nilsson and Örlander (2004) on spruce nor that of Coll et al. (2007) on poplar affected the length of new growth.

In all the conifers under study the chlorophyll content fell due to the herbicide application. Similar observation was reported by Matsumoto et al. (2002) in *Echinochloa oryzicola* treated with pirazol. However, no differences in chlorophyll amounts in corn leaves were found between control and treated plants after application of a herbicide containing foramsulfuron and methyl iodosulfuron (Idziak et al., 2014). Relative to the respective controls all the treated taxa contained more free amino acids in their shoots which is in line with the results of Marczewska et al. (2006) who studied the effect of chlorsulfuron on *Apera spica-venti*. Such an accumulation was also observed by Zabalza et al. (2006) in soybean leaves after the use of a herbicide from the ALS inhibitors' group (imazethapyr). Zabalza et al. (2004) observed an increase in total soluble sugars in pea leaves after treatment with herbicides containing chlorsulfuron and imazethapyr. Similar response was observed in this experiment in spruce and pine while in arborvitae the sugar content decreased probably due to phytotoxicity of the preparation for this taxon. Polyphenolic acids participate in the reduction of oxidative stress and their increased content means a start of the defense response of an organism against stress factors. It was proved that treating cucumber with caffeic acid mitigated the effects of cold stress, limited growth inhibition and increased production of polyphenolic acids while reducing free radicals (Wan et al., 2015). In this experiment the contents of polyphenolic acids were increased in all the conifers sprayed on T2 and T3. Similarly, the increase in the amounts of phenolic compounds in response to a herbicide treatment was observed in potato (Zarzecka, Gugała, 2011). Different results were reported by Kjaer et al. (2001) in leaves of *Fallopia convolvulus* where herbicides from the group of the inhibitors of amino acid synthesis (methsulfuron and chlorsulfuron) decreased the contents of phenolic compound, caffeic acid including.

In spruce and pine plants treated with the herbicide the catalase activity was decreased. This was also reported by Song et al. (2007) who used chlortoluron in wheat (*Triticum aestivum*) production. According to Lü et al. (2009) catalase may have a lesser impact on oxidative stress induced by herbicides. Such an opinion is not in line with our results on arborvitae where the increase in catalase activity after the first and second date of treatment was observed, concomitant with damages resulting from the herbicide application. Browning and dying of shoot tips was observed due probably to a grave phytotoxicity of the preparation for this taxon. Hydrogen peroxide as one of the reactive oxygen species is produced continuously in plant cell as a by-product of metabolic paths. Increased reactive oxygen species (ROS) production occurs in defense response to wounding (Apel, Hirt, 2004). In this experiment the H_2O_2 level in spruce and pine plants treated with the herbicide remained on a level of control untreated plants. In arborvitae a decrease in the hydrogen peroxide content occurred due to the treatment with the herbicide – which may be associated with phyotoxicity of this preparation for this taxon. A herbicide application induces stress in herbaceous plants – both crops and weeds – which results in inhibition of seed germination, weakened growth of roots and shoots or dying of entire plants (Štajner et al., 2003). Under stress conditions the oxygen reactive species are intensively produced. They appear due to the escape of electrons from the electron transport path in membranes of chlorophyll tylacoids. The excess of ROS may damage proteins, lipids and nucleic acids what can be mitigated by antioxidant enzyme activity such as peroxidase. In corn and broad bean an increase in peroxidase activity was observed after the application of herbicides containing fluometuron, atrazine or rimsulfuron (Hassan, Nemat-Alla, 2005). In this experiment the increase in peroxidase activity in all the conifers under study resulted from the herbicide use, regardless of the treatment date. In conclusion, choosing a right date of herbicide application is essential for the success of the treatment. They should be carried out in early spring before the new vegetation starts. A preparation used at the beginning of April may efficiently control weeds without damaging crops. The symptoms of phytotoxicity might have been lesser if the treatment had been done in early spring and some covers had been fixed on booms or anti-splash nozzles were used to protect the shoots of conifers.

CONCLUSIONS

1. Foliar application of the herbicide (0,15 kg ha^{-1}) done in April before the start of growth was not toxic for conifers *Picea pungens* f. *glauca* and *Pinus mugo* subsp. *mugo*.
2. Shrubs of *Thuja occidentalis* 'Smaragd' were sensitive to the herbicide showing foliage yellowing, browning and drying what contributed to growth inhibition.
3. The herbicide applied together with a recommended adjuvant controlled most weeds in the field production of conifers. The weeds resistant to the herbicide were: *Cerastium holosteoides*, *Convolvulus arvensis* and *Equisetum arvense*. Moderate sensitivity to the herbicide showed the following: *Cirsium arvense*, *Digitaria sanguinalis* and *Polygonum aviculare*.
4. In shrubs resistant to the herbicide (spruce and pine) its application resulted in the elevated levels of total soluble sugars, free amino acids, polyphenolic acids and in the increased activity of peroxidase while the chlorophyll content and catalase activity decreased. In the sensitive arborvitae 'Smaragd' the levels of free amino acids and polyphenolic acids as well as activities of catalase and peroxidase increased, while the contents of chlorophyll, total soluble sugars and hydrogen peroxide diminished.

REFERENCES

Apel K., Hirt H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Plant Biology, 55: 373-399.

Altland J., Gilliam Ch., Wehtje G., 2003. Weed control in field nurseries. HortTechnology, 13: 9-14.

Barnes A.D., Zedaker S.M., Feret P.P., Seiler J.R., 1990. The effects of sulfometuron on the root growth of loblolly pine. New Forests, 3: 289-295. doi:10.1007/BF00030038.

Boutin C., Lee H., Peart T., Batchelor S., Maguire R.J., 2000. Effects of the sulfonylurea herbicide metsulfuron methyl on growth and reproduction of five wetland and terrestrial plant species. Environmental Toxicology and Chemistry, 19(10): 2532-2541.

Brown H.M., 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Journal of Pesticide Science, 29: 263-281.

Burney O.T., Jacobs D.F., 2009. Influence of sulfometuron methyl on conifer seedling root development. New Forests, 37: 85-97.

Busse M.D., Fiddler G.O., Ratcliff A.W., 2004. Ectomycorrhizal formation in herbicide treated soils of differing clay and organic matter content. Water, Air & Soil Pollution, 152: 23-34.

Coll L., Messier Ch., Delagrange S., Berninger F., 2007. Growth, allocation and leaf gas exchanges of hybrid poplar plants in their establishment phase on previously forested sites: effect of different vegetation management techniques. Annals of Forest Sciences, 64: 275-285.

Damalas Ch., Lithourgidis A., Eleftherohorinos I., 2011. *Echinochloa* species control in maize (*Zea mays* L.) with sulfonylurea herbicides applied alone and in mixtures with broadleaf herbicides. Crop Protection, 34: 70-75.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28: 350-356.

Falkowski G., Matysiak B., 2010. Skuteczność chwastobójcza związków z grupy pochodnych sulfonylomocznika w szkółkach roślin iglastych. XIV Ogólnopolska Konferencja Szkółkarska: Nowości w nawożeniu i ochronie roślin szkółkarskich. ISiK, Skierniewice, pp. 47-53.

Goth L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta, 196: 143-152.

Hassan N., Nemat-Alla M., 2005. Oxidative stress in herbicide-treated broad bean and maize plants. Acta Physiologiae Plantarum, 27: 429-438.

Idziak R., Woźnica Z., Szulc P., 2014. Efficacy of foramsulfuron + iodosulfuron applied with adjuvants and zinc fertilizer. Progress in Plant Protection, 54: 407-411. [in Polish]

Kir K., Doğan M., 2009. Weed control in maize (*Zea* mays L.) with effective minimum rates of foramsulfuron. Turkish Journal of Agriculture and Forestry, 43: 601-610.

Kjaer C., Elmegaard N., Pedersen M.B., Damgaard C., Nielsen J.K., 2001. Phytochemical responses to herbicide exposure and effects on herbivorous insects. Pesticides Research, 55: 20-25.

LaRossa R.A., Van Dyk T.K., 1987. Metabolic mayhem caused by 2-ketoacid imbalances. BioEssays, 7: 125-130.

Lichtenthaler H.K., Wellburn A.R., 1983. Determinations of total carotenoids and chlorophylls a and b leaf extracts in different solvents. Biochemical Society Transaction, 603: 591-592.

Lü Z., Sang, L., Li Z., Min H., 2009. Catalase and superoxide dismutase activities in a *Stenotrophomonas maltophilia* WZ2 resistant to herbicide pollution. Ecotoxicology and Environmental Safety, 72(1): 136-143.

Marczewska K., Sadowski J., Rola H., 2006. Changes in branched chain amino acids content in leaves of *Apera spica-venti* biotypes resistant and susceptible to chlorsulfuron. Journal of Plant Protection Research, 46(2): 191-198.

Matsumoto H., Mizutani M., Yamaguchi T., Kadotani J., 2002. Herbicide pyrazolate causes cessation of carotenoids synthesis in early watergrass by inhibiting 4-hydroxyphenylpyruvate dioxygenase. Weed Biology and Management, 2: 39-45.

Nakano S., Asada K., 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiology, 22: 867-880.

Nilsson U., Örlander G., 2004. Response of newly planted Norway spruce seedlings to fertilization, irrigation and herbicide treatments. Annals of Forest Sciences, 60: 637-643.

Polskie Towarzystwo Farmakologiczne 1999. Farmakopea Polska V. [Polish Pharmacopoeia V]. PTF, Warsaw, 5: 445-446. [in Polish]

Radosevich S.R., Holt J., Ghersa C.M., 2007. Ecology of weeds and invasive plants. 454. Relationship to Agriculture and Natural Resource Management (3rd ed). John Wileyand-Sons, New York, USA.

Robertson N., Davis A., 2012. Sulfometuron methyl influences seedling growth and leaf function of three conifer species. New Forests, 43: 185-195.

Rosen H., 1957. A modified ninhydrin colorimetric analysis for amino acids. Archives of Biochemistry and Biophysics, 67: 10-15.

Snedecor G.W., Cochran W.G., 1967. Statistical methods. 593. The Iowa State University Press, Ames, Iowa USA.

Song N.H., Yin X.L., Chen G.F., Yang H., 2007. Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. Chemosphere, 68(9): 1779-1787.

South D.B., Carey W.A., 2005. Weed control in bareroot hardwood nurseries. pp. 34-38. Dumroese, R.K.; Riley, L.E.; Landis, T.D. (eds.). National Proc.: Forest and Conservation Nursery Associations 2004: USDA Forest Service, Rocky Mountain Research Station RMRS-P-35. Fort Collins, CO.

Štajner D., Popović M., Štajner M., 2003. Herbicide induced oxidative stress in lettuce, beans, pea seeds and leafs. Biologia Plantarum, 47: 575-579.

Wan Y., Zhang Y., Zhang L., Zhou Z., Li X., Shi W., Wang X., Bai J., 2015. Caffeic acid protects cucumber against chilling stress by regulating antioxidant enzyme activity and proline and soluble sugar content. Acta Physiologiae Plantarum, 37: 1706.

Willoughby I., Clay D., Dixon F., 2003. The effect of pre-emergent herbicides on germination and early growth of broadleaved species used for direct seeding. Forestry, 76(1): 83-94.

Wójcik A.R., Laudański Z., 1989. Planowanie i wnioskowanie statystyczne w doświadczalnictwie. Państwowe Wydawnictwo Naukowe, Warszawa, p. 130.

Zabalza A., Orcaray L., Gaston S., Royuela M., 2004. Carbohydrate accumulation in leaves of plants treated with the herbicide chlorsulfuron or imazethapyr is due to decrease in sink strength. Journal of Agricultural and Food Chemistry, 52: 7601-7606.

Zabalza A., Gaston S., Ribas-Carbó M., Orcaray I., Igal M., Royuela M., 2006. Nitrogen assimilation studies using ^{15}N in soybean plants treated with imazethapyr, an inhibitor of branched-chain amino acid biosynthesis. Journal of Agricultural and Food Chemistry, 54: 8818-8823.

Zarzecka K., Gugała M., 2011. The effect of herbicides and soil tillage systems of the content of polyphenols in potato tubers. Polish Journal of Environmental Studies, 20: 513-517.

Zhang Q., Fu S., Li H., Liu Y., 2013. A novel method for the determination of hydrogen peroxide in bleaching effluents by spectroscopy. BioResources, 8(3): 3699-3705.

received – 23 February 2017
revised – 24 July 2017