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EFFECT OF HALOXYFOP AND ALLOXYDIM APPLIED SEPARATELY AND IN COMBINATION WITH SALICYLIC ACID, DIPHENYLAMINE, OR NORFLURAZON ON THE ROOT GROWTH AND FATTY ACID COMPOSITION OF THE SELECTED SPECIES OF GRASSES AND DICOTYLEDONOUS PLANTS

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Abstract. Eight species from the grass family were studied: wheat, rye, triticale, barley, oats, maize, couch grass, rice, and two dicotyledonous species: field pea and common flax. Seedlings of the tested species were grown in hydroponic cultures with haloxyfop or alloxydim alone (graminicides) and with haloxyfop or alloxydim plus one of the tested antagonists (diphenylamine, salicylic acid, or norflurazon). All investigated species of the grass family showed similar susceptibility to the tested graminicides (measured with their effect on root growth). The tested dicotyledonous species were completely unsusceptible. Except in maize, the addition of antagonistic substances to the medium with herbicide weakened considerably the inhibitory effect of both haloxyfop and alloxydim. The tested graminicides also had a significant effect on the fatty acid composition of susceptible species (except maize, where the effect was low). In the lipids of the apical parts of roots, a significant decrease in the content of oleic and linoleic acids and a considerable increase in the content of linolenic acid were observed.

Keywords: graminicides, graminicides mechanism of action, unsaturated fatty acids, antagonistic substances

INTRODUCTION

Graminicides are herbicides that selectively destroy species from the grass family and have no significant effect on the majority of dicotyledonous plants. Aryloxy-phenoxy-

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-propionic acid derivatives (for example haloxyfop), as well as 1.3-cyclohexanedione derivatives (alloxydim), belong to this herbicide group [Nestler 1982, Cobb 1992]. Graminicides are the active substances of numerous herbicides (for example Illoxan, Fusilade, Targa, Perennal, and Agil), traditionally referred to as "graminicides". They were introduced into agricultural practice in the mid-1980s but in spite of the widespread application, there are still controversies regarding their mode of action [De Prado *et al*. 1999, Shimabukuro *et al.* 2001, Lout *et al.* 2004]. At present, there are two hypotheses that attempt to explain the causes of graminicide phytotoxicity. The first one assumes that the *de novo* biosynthesis of fatty acids is restrained and this is the reason for their phytotoxicity. It was proved that the majority of species sensitive to graminicides contain in their plastids an eukaryotic form of acetyl-CoA carboxylase (homodimeric form) susceptible to these herbicides [Lichtenthaler 1990, Tardif *et al.* 1996, Heap and Morrison 1996, Shukla *et al.* 1997]. The second hypothesis assumes that graminicides cause phytotoxic effects through free-radical reaction stimulation (causing oxidative stress) in susceptible plants [Banaś *et al.* 1993a, Shimabukuro and Hoffer 1996, Shimabukuro *et al.* 2001, Luo *et al.* 2004]. This hypothesis was proposed, inter alia, due to the results of studies with the use of antagonistic substances in relation to the discussed herbicides. Banaś *et al.* [1993a, b] showed that wheat root growth inhibition caused by haloxyfop or alloxydim was reversed by genistein (flavonoid), norflurazon (compound from the pyridazinone herbicide group), salicylic acid, salicylhydroxamic acid, propyl gallate, monophenylobutazon, or diphenylamine. Shimabukuro *et al.* [2001] demonstrated an antagonistic effect of vitamins E and 2,4-D on diclofop-methyl, and Luo *et al.* [2004] showed an antagonistic effect of vitamin E and ethoxyquin (antioxidant) on fluazifop-butyl. Common trait of most of these compounds is the fact that they may act as free radical scavengers and/or as lipoxygenases inhibitors. Poly-unsaturated fatty acids may be the generators of free radicals in plants treated with graminicides. Banaś *et al.* [1990, 1993a, b] demonstrated a strong increase in the percentage content of linolenic acid (18:3) in the apical parts of wheat roots treated with haloxyfop or alloxydim and in the young, developing parts of wheat and oats seedlings treated with haloxyfop-etoxyethyl. Similar results were obtained by Shimabukuro *et al.* [2001] in relation to oats seedlings and plants *Euphorbia esula* treated with diclofop-methyl.

The amount of studies on the effect of graminicides on the composition of the fatty acids of plant lipids is, however, relatively low. This also concerns the substances antagonistic to graminicides. However, this kind of research seems to be crucial for the understanding of the mechanism of action of these herbicides. Therefore, in this study, the effect of haloxyfop and alloxydim applied separately and jointly with salicylic acid, norflurazon, and diphenylamine (substances of an antagonistic effect) on the root growth of selected grass species and dicotyledonous plants was performed. Also, the effect of haloxyfop and alloxydim on the fatty acid composition of roots' lipids of the tested species was investigated. In order to find out whether cultivar differences may have an effect on the response of the studied species to the treatment with the tested active substances, in the case of some species, more than one cultivar was used for the research.

MATERIAL AND METHODS

The following eight grass species were studied: wheat (*Triticum aestivum* L., cultivars Rosa, Hemika, Gama, and Almari), rye (*Secale cereale* L., cultivars Motto, Dańkowskie Złote), triticale (*Triticale*, cultivar Presto), barley (*Hordeum vulgare* L., cultivar Rudzik), oats (*Avena sativa* L., cultivar German), maize (*Zea mays* L., cultivars Zenit, Dea, Helga, Concord, and Złota Karłowa), couch grass (*Agropyron repens* L.) rice (*Oryza sativa* L., cultivar Bahia), and two dicotyledonous species: field pea (*Pisum sativum* L., cultivar Vreta) and common flax (*Linum usitatissimum* L., cultivar Concurrent). Seeds of the tested species (except couch grass) were soaked for about 18 hours in distilled water, rinsed, and put in rows on a Petri dish with a double layer of moist filter paper. Dishes with seeds were placed in a thermostat at the temperature of 21^{o}C (maize at 25^{o}C) and were left for germination in darkness for the period of two (wheat, maize, triticale, rye, and common flax) or three (oats, rice, barley, and pea) days. After that period, seedlings with the central root of the length of 20-26 mm were selected and put into the holes of plastic or cork discs (the length of the central root of every seedling was recorded beforehand). Discs with seedlings were placed in the beakers containing Hoagland solution [Nilsson 1977] and the tested substances (Tables 1, 2, 3). Breakers with seedlings were subsequently moved to the dark thermostat set at the temperature of 21^{o}C (maize at 25^{o}C). After 24 hours, plants were taken out of the medium and central root length was measured again. The growth of the roots from mediums with the tested substances (Tables 1, 2, 3) was compared with the root growth from the control mediums. Every experiment was repeated independently three times, and in each case a dozen or so seedlings of the tested species were grown in every variant of the medium.

Couch grass seedlings obtained from an organic vegetable farm were grown in pots with garden soil. When their offshoots reached about 15 cm, they were carefully taken out from the soil, rinsed, and moved (by five pieces) into proper mediums (Table 3).

Fatty acid composition was determined in the apical parts of the tested roots according to the modified Bligh and Dyer method [1959]. A dozen or so apices of about 10 mm in length were used for a single analysis. They were ground in a glass homogenizer with the addition of 3.75 cm^3 of chloroform and methanol mixture (ratio 1 : 2) and 1 cm^3 0.15 M acetic acid. Homogenate was moved into a test tube and 1.25 cm^3 of chloroform and 1.25 cm^3 of distilled water was added. The mixture was shaken intensively and centrifuged at circa 1000 g for three minutes. After centrifugation, chloroform layer (lower, containing lipids) was aspirated with a Pasteur pipette and moved into a methylation test tube. Chloroform was completely vaporized on a heated sand bath (about 60^{o}C) under a nitrogen stream, and the sediment was poured over with 2 cm^3 of methylation mixture (2% sulphuric acid in methanol). Methylation was carried out at 90^{o}C for one hour. After cooling down, internal standard (17 : 0 – Me – methyl ester of heptadecanoid acid; Sigma), as well as 3 cm^3 of hexane and 3 cm^3 of distilled water were added. The mixture was shaken intensively and centrifuged (1000 g for three minutes). Hexane fraction was retrieved, completely vaporized, and dissolved in a small amount of hexane. The analysis of methyl esters of the fatty acids contained in the extract was carried out on a Shimadzu gas chromatograph with a flame ionisation detector and a glass column (2.5 m × 3 mm) filled with 3% SP-2300 on Supelcoport 100/120 mesh (Sigma-Aldrich). Fatty acids were identified on the basis of the standard (Sigma) retention times. Determination of their content was carried out through the comparison of their peak areas with the area of the internal standard peak (done automatically by an integrator coupled with a gas chromatograph).

Table 1. Effect of haloxyfop and alloxydim (treatment time – 24 hrs) on the root growth of the selected species/cultivars of grasses and dicotyledonous plants
Tabela 1. Wpływ haloksyfopu i aloksydymu (czas traktowania 24 godz.) na wzrost korzeni wybranych gatunków/odmian traw i roślin dwuliściennych

Species Gatunek	Cultivar Odmiana	Increase in root length – Przyrost długości korzeni								
		K	H, 10^{-8}M	H, 10^{-7}M	H, 10^{-6}M	H, 10^{-5}M	A, 10^{-8}M	A, 10^{-7}M	A, 10^{-6}M	A, 10^{-5}M
		mm	%K	%K	%K	%K	%K	%K	%K	%K
Wheat Pszenica zwyczajna	Rosa	22.9 ± 2.3	89.1^{a} ± 4.8	62.0^{a} ± 5.2	21.8^{a} ± 2.2	20.9^{a} ± 6.4	87.2^{a} ± 5.4	45.5^{a} ± 4.1	24.8^{a} ± 4.1	20.7^{a} ± 4.1
	Hemika	15.3 ± 2.5	–	–	–	–	85.0^{a} ± 7.8	58.8^{a} ± 8.5	39.2^{a} ± 7.2	33.3^{a} ± 7.8
	Gama	19.4 ± 2.5	77.8^{a} ± 20.1	58.2^{a} ± 10.8	29.9^{a} ± 3.6	21.6^{a} ± 3.1	–	–	–	–
Rye Żyto zwyczajne	Motto	27.8 ± 3.8	90.3^{a} ± 11.5	51.1^{a} ± 10.2	25.9^{a} ± 2.5	23.4^{a} ± 1.8	81.2^{a} ± 5.4	63.2^{a} ± 4.9	30.9^{a} ± 5.4	21.9^{a} ± 5.4
	Dańkowskie Złote	26.2 ± 3.9	80.5^{a-} ± 14.5	44.5^{a} ± 7.6	27.1^{a} ± 3.8	24.0^{a} ± 3.1	–	–	–	–
Triticale Pszenżyto	Presto	22.9 ± 1.6	91.7^{a} ± 7.8	55.9^{a} ± 9.6	26.6^{a} ± 2.6	24.5^{a} ± 2.2	–	–	–	–
Oats Owies zwyczajny	German	23.6 ± 3.8	76.7^{a} ± 7.2	47.5^{a} ± 8.1	30.5^{a} ± 3.8	25.4^{a} ± 3.8	–	–	–	–
Barley Jęczmień zwyczajny	Rudzik	21.8 ± 2.8	68.8^{a} ± 4.2	57.8^{a} ± 6.4	40.4^{a} ± 3.7	24.3^{a} ± 2.3	80.1^{a} ± 7.9	52.3^{a} ± 7.9	39.7^{a} ± 5.3	33.^{8a} ± 4.6
Maize Kukurydza zwyczajna	Zenit	24.3 ± 3.2	82.3^{a} ± 7.0	49.4^{a} ± 5.3	39.1^{a} ± 4.5	32.1^{a} ± 3.3	80.3^{a} ± 6.2	66.0^{a} ± 5.8	31.3^{a} ± 6.6	23.6^{a} ± 5.0
	Dea	32.8 ± 4.4	74.4^{a} ± 9.7	32.9^{a} ± 7.3	29.3^{a} ± 8.5	28.4^{a} ± 3.3	–	–	–	–
	Helga	34.8 ± 1.1	–	–	–	–	79.7^{a} ± 5.6	46.8^{a} ± 4.7	18.8^{a} ± 2.4	15.6^{a} ± 2.6
Field pea Groch siewny	Vreta	18.5 ± 0.6	102.4 ± 6.1	101.8 ± 6.1	102.4 ± 4.8	101.7 ± 5.5	102.7 ± 5.4	101.1 ± 7.6	104.3 ± 9.7	102.2 ± 8.6
Common flax Len zwyczajny	Concurent	24.3 ± 2.2	101.2 ± 7.0	102.9 ± 7.0	102.5 ± 8.2	99.2 ± 9.5	94.9 ± 8.1	102.1 ± 6.4	105.9 ± 8.5	96.2 ± 5.9

K – control – kontrola, H – haloxyfop – haloksyfop, A – alloxydim – aloksydym
± – standard deviation – odchylenie standardowe
a – significant difference between the control and haloxyfop- or alloxydim-treated plants in "mean difference two-sided test" at P = 0.05 – statystycznie udowodniona różnica pomiędzy kontrolą a roślinami traktowanymi haloksyfopem lub aloksydymem w dwustronnym teście różnic średnich przy P = 0,05

Table 2. Effect of haloxyfop and alloxydim (treatment time – 24 hrs) applied individually and together with antagonistic substances on the root growth of selected grass species

Tabela 2. Wpływ haloksyfopu i aloksydymu (czas traktowania 24 godz.), zastosowanych oddzielnie i łącznie z substancjami o działaniu antagonistycznym, na wzrost korzeni wybranych gatunków traw

Species Gatunek	Cultivar Odmiana	Increase in root length – Przyrost długości korzeni								
		K	H	H + SAL	H + DPA	H + SAN	A	A + SAL	A + DPA	A + SAN
		mm	%K	%K	%K	%K	%K	%K	%K	%K
Wheat Pszenica zwyczajna	Almari	19.8 ± 1.6	48.0^{a} ± 10.6	89.0^{b} ± 8.3	98.8^{b} ± 11.5	77.0^{b} ± 6.4	43.8^{a} ± 8.7	118.0^{b} ± 17.5	108.2^{b} ± 3.6	89.2^{b} ± 8.2
	Gama	22.9 ± 1.6	38.2^{a} ± 12.4	78.4^{b} ± 10.4	86.0^{b} ± 21.0	72.1^{b} ± 16.1	34.6^{a} ± 5.9	88.5^{b} ± 5.9	91.5^{b} ± 4.3	87.2^{b} ± 16.2
	Rosa	23.1 ± 1.9	61.5^{a} ± 5.2	86.1^{b} ± 4.8	93.9^{b} ± 6.9	81.0^{b} ± 6.9	54.1^{a} ± 2.3	68.2^{b} ± 2.3	75.0^{b} ± 3.6	81.8^{b} ± 2.7
Rye Żyto zwyczajne	Motto	28.4 ± 3.8	47.5^{a} ± 7.7	78.5^{b} ± 10.2	72.9^{b} ± 5.6	78.5^{b} ± 2.8	46.4^{a} ± 4.1	81.6^{b} ± 3.6	81.6^{b} ± 3.6	76.0^{b} ± 3.6
	Dańkowskie Złote	28.4 ± 2.6	52.8^{a} ± 6.3	83.1^{b} ± 12.3	83.1^{b} ± 11.3	74.3^{b} ± 7.0	–	–	–	–
Triticale Pszenżyto	Presto	25.4 ± 1.5	50.4^{a} ± 5.1	77.6^{b} ± 9.8	75.2^{b} ± 5.1	82.3^{b} ± 5.5	–	–	–	–
Oats Owies zwyczajny	German	23.0 ± 3.2	53.5^{a} ± 9.1	75.6^{b} ± 3.5	86.5^{b} ± 9.1	70.0^{b} ± 5.2	–	–	–	–
Barley Jęczmień zwyczajny	Rudzik	21.3 ± 2.9	50.7^{a} ± 7.0	99.1^{b} ± 9.4	96.7^{b} ± 8.9	59.2^{b} ± 7.0	52.9^{a} ± 4.0	86.1^{b} ± 3.9	86.1^{b} ± 3.9	80.1^{b} ± 6.0
Maize Kukurydza	Zenit	23.8 ± 4.5	48.7^{a} ± 6.7	50.1 ± 4.2	53.4^{b} ± 5.4	48.7 ± 4.6	–	–	–	–
	Dea	32.5 ± 4.2	36.9^{a} ± 4.9	44.9^{a} ± 5.2	42.5^{a} ± 3.7	37.2^{a} ± 4.3	–	–	–	–
	Helga	32.9 ± 7.4	38.6^{a} ± 6.4	46.2^{b} ± 9.4	44.7^{b} ± 9.4	40.1 ± 5.5	57.9^{a} ±3.3	60.9 ± 5.0	63.2 ± 3.9	60.7 ± 2.8

K – control – kontrola, H – haloxyfop [10^{-7}M] – haloksyfop [10^{-7}M], A – alloxydim [10^{-7}M] (rye 5x10^{-7}M) – aloksydym [10^{-7}M] (żyto 5x10^{-7}M)

SAL – salicylic acid [3x10^{-5}M] – kwas salicylowy [3x10^{-5}M], DPA – diphenylamine [10^{-5}M] – difenyloamina [10^{-5}M], SAN – norflurazon [3x10^{-5}M] – norflurazon [3x10^{-5}M]

± – standard deviation – odchylenie standardowe

a, b – significant difference between the control and haloxyfop- or alloxydim-treated plants (a) and between haloxyfop- or alloxydim-treated plants and plants treated with haloxyfop or alloxydim + antagonistic substance (b) in "mean difference two-sided test" at P = 0.05 – statystycznie udowodniona różnica pomiędzy kontrolą a roślinami traktowanymi haloksyfopem lub aloksydymem (a) i pomiędzy roślinami traktowanymi haloksyfopem lub aloksydymem a roślinami traktowanymi haloksyfopem lub aloksydymem + substancja antagonistyczna (b) w dwustronnym teście różnic średnich przy P = 0,05

Table 3. Effect of haloxyfop and alloxydim (treatment time – 24 hrs) on the composition of the fatty acids in the root apices of the selected species of grasses and dicotyledonous plants

Tabela 3. Wpływ haloksyfopu i aloksydymu (czas traktowania 24 godz.) na skład kwasów tłuszczowych lipidów wierzchołków korzeni wybranych gatunków traw oraz roślin dwuliściennych

Species Gatunek	Cultivar Odmiana	Fatty acids, mol% – Kwasy tłuszczowe, %mol														
		16:0			18:0			18:1			18:2			18:3		
		K	H	A	K	H	A	K	H	A	K	H	A	K	H	A
Wheat Pszenica	Gama	24.9 ± 1.1	25.3 ± 3.0	22.8 ± 2.4	2.0 ± 0.5	1.7 ± 1.1	1.5 ± 0.3	8.7 ± 1.2	3.6[a] ± 0.1	5.2[a] ± 0.1	40.6 ± 2.3	24.4[a] ± 2.2	24.9[a] ± 1.1	23.8 ± 2.4	44.9[a] ± 4.4	47.7[a] ± 4.4
zwyczajna	Rosa	25.5 ± 3.4	–	22.3 ± 3.1	0.9 ± 0.3	–	0.5 ± 0.4	4.9 ± 1.1	–	2.2[a] ± 0.9	41.8 ± 2.2	–	26.8[a] ± 1.9	27.0 ± 2.3	–	48.0[a] ± 3.3
Barley Jęczmień zwyczajny	Rudzik	26.3 ± 4.9	22.2 ± 1.2	–	traces ślady	traces ślady	–	3.6 ± 1.0	1.4[a] ± 1.1	–	40.9 ± 5.0	34.9[a] ± 5.2	–	29.0 ± 2.0	34.9[a] ± 5.2	–
Couch grass Perz właściwy	–	23.1 ± 1.3	21.9 ± 2.6	–	2.1 ± 0.7	1.2 ± 0.7	–	8.3 ± 3.9	4.2[a] ± 1.0	–	41.7 ± 3.5	41.7 ± 2.6	–	23.8 ± 3.5	29.6[a] ± 3.2	–
Rice – Ryż	Bahia	18.9 ± 1.3	21.2 ± 2.3	–	1.3 ± 0.7	1.2 ± 0.5	–	9.9 ± 1.2	3.9[a] ± 1.1	–	31.2 ± 2.1	30.9 ± 1.9	–	24.4 ± 1.8	28.2[a] ± 2.0	–
Maize Kukurydza	Concord	24.6 ± 1.1	21.7[a] ± 0.7	20.4[a] ± 0.7	0.7 ± 0.1	0.4[a] ± 0.1	0.3[a] ± 0.1	4.5 ± 0.8	1.4[a] ± 0.3	1.2[a] ± 0.3	65.7 ± 1.2	69.0[a] ± 0.6	68.7[a] ± 1.1	3.8 ± 0.3	6.9[a] ± 0.7	7.9[a] ± 0.5
	Złota Karłowa	20.7 ± 0.9	15.7[a] ± 1.0	–	0.7 ± 0.1	0.3[a] ± 0.1	–	3.5 ± 0.4	0.5[a] ± 0.1	–	64.7 ± 1.9	69.1[a] ± 0.9	–	4.0 ± 0.1	5.9[a] ± 0.3	–
Pea – Groch	Vreta	19.5 ± 0.3	20.3 ± 0.6	20.0 ± 1.0	3.0 ± 0.4	2.7 ± 0.1	3.0 ± 0.3	5.9 ± 1.3	4.1 ± 0.3	4.9 ± 0.5	56.2 ± 1.7	58.4 ± 0.2	37.8 ± 1.2	11.3 ± 1.0	11.6 ± 0.3	12.0 ± 0.3
Common flax – Len	Concurent	24.2 ± 1.3	23.1 ± 0.4	23.9 ± 0.8	4.0 ± 0.4	3.7 ± 0.5	4.4 ± 0.3	5.9 ± 1.1	4.9 ± 0.7	4.5 ± 0.9	35.8 ± 1.6	35.6 ± 2.2	37.8 ± 1.2	26.2 ± 1.2	29.5 ± 1.5	27.8 ± 1.3

16:0 – palmitic acid – kwas palmitynowy, 18:0 – stearic acid – kwas stearynowy, 18:1 – oleic acid – kwas oleinowy, 18:2 – linoleic acid – kwas linolowy, 18:3 – linolenic acid – kwas linolenowy

K – control – kontrola, H – haloxyfop [10^{-7}M] (maize Concord 3x10^{-8}M, pea and common flax 10^{-5}M) – haloksyfop [10^{-7}M] (kukurydza odmiany Concord 3x10^{-8}M, groch i len 10^{-5}M),

A – alloxydim [10^{-7}M] (maize 5x10^{-7}M, pea and common flax 10^{-5}M) – aloksydym [10^{-7}M] (kukurydza 5x10^{-7}M, groch i len 10^{-5}M)

± – standard deviation – odchylenie standardowe

a – significant difference between the control and haloxyfop- or alloxydim-treated plants in "mean difference two-sided test" at P = 0.05 – statystycznie udowodniona różnica pomiędzy kontrolą a roślinami traktowanymi haloksyfopem lub aloksydymem w dwustronnym teście różnic średnich przy P = 0,05

In order to picture the repetitiveness of the obtained results, standard deviations have been given with the mean values. When the results were presented as a percentage of control values, first the arithmetic mean and standard deviation were calculated, and then the values were given as a percent of average control values. In order to determine whether the effect of the tested active substances on the studied physiological and biochemical processes was statistically significant, the obtained results were analysed in "mean difference two-sided test" with the significance level of P = 0.05. The test evaluates whether there is a statistically proven difference between two populations; in the case of the present research, a difference between the control population and a chosen population (Tables 1, 2, 3) and, additionally, between two other populations (Table 2).

RESULTS

The study began with testing the effects of various concentrations of haloxyfop and alloxydim on the root growth of selected grass and dicotyledonous plant species. The lowest concentration of tested graminicides was 10^{-8}M, and the highest 10^{-5}M. All of the studied grass species responded in a similar way to the increase in the graminicide concentration in the growth medium. With the increase in haloxyfop or alloxydim concentration, root growth decreased. Susceptibility of the particular species in relation to the studied herbicides differed relatively little. However, certain differences in susceptibility were observed. They occurred even between the cultivars of a given species (for example between the particular wheat or maize cultivars). Some of the studied species/cultivars were somewhat more susceptible in relation to haloxyfop (rye cultivar Motto, maize cultivar Zenit), other in relation to alloxydim (wheat cultivar Rosa, barley cultivar Rudzik). Inhibition of the root growth of the tested grass species during 24 hours of growing with the studied graminicides used at the lowest concentration (10^{-8}M) oscillated between 10% and 26% in comparison with the control. Graminicides applied at the concentration of 10^{-7}M caused root growth inhibition ranging from 34% to 67%, at the concentration of 10^{-6}M from 60% to 81%, and at the concentration of 10^{-5}M from 66% to 84%. At the same time, neither haloxyfop nor alloxydim had any effect on the root growth of the tested dicotyledonous plant species, namely field pea and common flax (Table 1).

In the studies concerning the antagonistic effect of salicylic acid, diphenylamine, and norflurazon, the applied concentrations of haloxyfop and alloxydim inhibited the growth of roots of the studied species from about 40% to about 65% in comparison with the control during 24hour-incubation. Except maize, the remaining species responded in a similar way to the addition of an antagonistic substance to the medium with the tested graminicide. Addition of salicylic acid, diphenylamine, or norflurazon caused a significant (sometimes total) reduction in the inhibitory effect of haloxyfop or alloxydim. In contrast, growth inhibition of maize roots was hardly weakened by the tested antagonistic substances. Among the tested antagonistic substances, the strongest effect (in most cases) was shown by diphenylamine and the lowest by norflurazon (Table 2).

In the lipids of the apical parts of the roots of the tested wheat cultivars treated with haloxyfop or alloxydim, a significant decrease was observed in the percentage content of oleic and linoleic acids and an almost double increase in the content of linolenic acid.

Similar response was observed also in the tested barley cultivar. Changes in the fatty acid composition of couch grass and rice roots were not so pronounced. However, a significant decrease was observed in the content of oleic acid and a slight, although statistically significant, increase in the content of linolenic acid. Changes in the percentage content of palmitic and stearic acids in the discussed species were not statistically significant. In the roots of the tested maize cultivars treated with haloxyfop or alloxydim, a decrease in the percentage content of oleic acid was also found (from around 4% in the control plants to around 1% in the treated plants) and a slight increase in the content of linoleic (from around 65% to around 69%) and linolenic (from around 4% to around 7%) acids. In the roots of the treated plants, also a statistically significant decrease in the percentage content of palmitic and stearic acids was found (Table 3).

In the case of the tested dicotyledonous plants, no changes in the composition of fatty acids in the root lipids were found after haloxyfop or alloxydim treatment, in spite of the application of many times higher concentrations of those herbicides in comparison with the concentrations used in the study with the tested grass species (Table 3).

DISCUSSION

Species from grass family used in the studies, except couch grass, are not controlled by graminicides in agricultural practice. These species were treated as model plants. The majority of species from the grass family controlled with the tested herbicides produce very small seeds. These seeds usually germinate irregularly, and the roots formed by them are very thin and difficult to grow in water cultures. Roots of the tested species are, on the other hand, thicker and develop well in water cultures. Therefore, they are a good material for studies aiming to explain the mode of action of graminicides.

Studied grass species differed only insignificantly in their sensitivity to the tested graminicides (determined by their effect on root growth). Moreover, there were no major differences in the sensitivity of the particular cultivars of the tested species. Therefore, it might be assumed that the effect of antagonistic substances and changes in root metabolism will be similar if the mechanism of action of graminicides is the same in relation to all susceptible species. Present research carried out with the use of antagonistic substances suggests, however, the possibility of the existence of somewhat different mechanisms of action of graminicides in different susceptible species. In the case of wheat, rye, triticale, oats, and barley, the addition to the medium containing haloxyfop or alloxydim of such substances as salicylic acid, diphenylamine, or norflurazon, significantly reduced the inhibitory effect of these herbicides on the growth of their roots. These substances did not restrain, however, maize root growth inhibition caused by these herbicides.

Species in which the studied antagonistic substances significantly reduced the inhibitory effect of the tested graminicides were characterized by a notable content of linolenic acid in the lipids of the apical parts of their roots. Moreover, haloxyfop or alloxydim treatment caused a further increase in its content. In maize, also an increase in the percentage content of linolenic acid was observed under the influence of the treatment with the tested graminicides, but even in this case its content did not exceed 8% of all fatty acids.

Effect of the tested antagonistic substances is not fully understood. Diphenylamine is assumed, for example, to have the ability to bind free radicals [Bors *et al*. 1989]. Norflurazon inhibits, among others, desaturase Δ15 [Fedtke 1982]. One of the properties of salicylic acids is inhibition of oleic acid desaturation into linoleic acid and linoleic acid into linolenic acid [Banaś *et al*. 1993b, Banaś *et al*. 1997]. Previous assumption that graminicides may act by promoting the overproduction of free radicals (created on the basis of linolenic acid; Banaś *et al*. 1993, 1993a) seems to be confirmed in this study. This hypothesis was put forward in relation to wheat but may also concern other susceptible species in which, like in wheat, a significant increase in the content of linolenic acid occurs under the influence of graminicide treatment. Conducted research suggests, however, that the mechanism of action of graminicides may be different in various species. Maize studied in the present research did not respond like the other tested species to the applied antagonistic substances. In this case, neither of the tested antagonistic substances weakened the inhibitory effect of haloxyfop or alloxydim on the root growth. Maize contains, however, a scarce amount of linolenic acid in the lipids of root apices, and therefore the obtained results appear to confirm the hypothesis that free radicals that are generated on the basis of linolenic acid may be of key importance in explaining the phytotoxic effect of graminicides. The results also suggest that such mechanism of action may be the major cause of graminicide phytotoxicity only with reference to some of the susceptible species. For example, in the case of maize (tested in the present study), other mechanisms may play a crucial role in their mode of action.

CONCLUSIONS

The studied graminicides (haloxyfop and alloxydim) inhibited the root growth of sensitive species at very low concentrations. Diphenylamine (substance able to bind free radicals), salicylic acid, and norflurazon (substances that have, among others, the ability to restrain the formation of poly-unsaturated fatty acids) may significantly reduce the inhibitory effect of tested graminicides on the root growth of most of the investigated grass species (except maize). Apart from inhibition of the root growth, haloxyfop and alloxydim also had a significant effect on fatty acids composition of the lipids of tested susceptible species (except maize, where the effect was negligible). In the lipids of the apical parts of roots, a significant decrease in the content of oleic and linoleic acids, as well as an increase in linolenic acid content were observed. This leads to a conclusion that in the case of the species that respond to graminicides and their antagonists in a similar way to the most of the tested ones (except the maize), free radicals generated from linolenic acid may be involved in the graminicides mode of action, while in other species (which respond in a similar way to maize), different mechanisms may play the crucial role.

Cultivar's traits of the tested species appear not to have a significant effect on their physiological and biochemical responses to the treatment with the tested active substances.

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WPŁYW HALOKSYFOPU I ALOKSYDYMU STOSOWANYCH ODDZIELNIE I ŁĄCZNIE Z KWASEM SALICYLOWYM, DIFENYLOAMINĄ LUB NORFLURAZONEM NA WZROST KORZENI WYBRANYCH GATUNKÓW TRAW I ROŚLIN DWULIŚCIENNYCH ORAZ SKŁAD ICH KWASÓW TŁUSZCZOWYCH

Streszczenie. Badaniami objęto osiem gatunków z rodziny traw (pszenicę zwyczajną, żyto zwyczajne, pszenżyto, jęczmień zwyczajny, owies zwyczajny, kukurydzę zwyczajną, perz właściwy, ryż siewny) oraz dwa gatunki dwuliścienne (groch zwyczajny i len zwyczajny). Siewki testowanych gatunków hodowano w kulturach wodnych z dodatkiem haloksyfopu lub aloksydymu (graminicydy) oraz z dodatkiem testowanego graminicydu i jednej z badanych substancji o działaniu antagonistycznym (difenyloaminy, kwasu salicylowego, norflurazonu). Wszystkie testowane gatunki z rodziny traw wykazywały zbliżoną wrażliwość (mierzoną ich wpływem na przyrost długości korzeni) w stosunku do haloksyfopu i aloksydymu, zaś testowane gatunki dwuliścienne były niewrażliwe. Oprócz kukurydzy, dodatek do pożywki z haloksyfopem lub aloksydymem substancji o działaniu antagonistycznym osłabiał znacznie ich inhibitorowe działanie. Zarówno haloksyfop, jak i aloksydym wywierały również znaczny wpływ na skład kwasów tłuszczowych większości gatunków wrażliwych (oprócz kukurydzy, gdzie wpływ ten był niewielki). W lipidach z wierzchołkowych partii korzeni stwierdzono znaczne obniżenie się zawartości kwasu oleinowego i linolowego oraz podwyższenie zawartości kwasu linolenowego.

Słowa kluczowe: graminicydy, mechanizm działania graminicydów, nienasycone kwasy tłuszczowe, substancje antagonistyczne

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