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MODIFICATION OF IAA-OXIDASE ACTIVITY BY FACTOR PRESENT IN LEAVES OF *CHENOPODIUM ALBUM* AND *ECHINOCHLOA CRUS-GALLI* IN RELATION TO SUSCEPTIBLE-RESISTANT PLANT RESPONSE TO TRIAZINE

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Abstract. Content of total phenols in not treated plants of *Chenopodium album* biotype susceptible to atrazine was 40% higher compared to resistant one. Atrazine applied at 1 kg per hectare increased the amount of these compounds in both biotypes, especially as regards on the level of monophenols in susceptible biotype, which was 3 times higher than in untreated plants. Amount of monophenols in atrazine-resistant biotype increased slightly due to atrazine application. After application of atrazine the polyphenols/monophenols ratio decreased from 4.2 to 1.1 in susceptible biotype, while it was not much changed in resistant biotype.
Atrazine increased the content of flavanols in S biotype eight-times compared with untreated plants, while in resistant one this phenomenon was not observed. IAA-oxidase activity measured *in vitro* was modified by leaf extracts from *C. album* and *Echinochloa crus-galli*. Than the extracts from triazine-treated resistant biotype were more capable of protecting the indoloacetic acid (IAA) against enzyme activity. It may suggest that the mechanism susceptibility or resistance to herbicide is not only involved with PS II complex, but consists also in formation in plant the systems which favour or protect the auxin degradation, respectively.

Key words: *Chenopodium album*, *Echinochloa crus-galli*, resistance to triazine, phenols, IAA-oxidase

INTRODUCTION

Weed resistance to herbicides is an increasing problem in plant protection. Atrazine was first herbicide for which this phenomenon was reported. There are about 60 atrazine resistant biotypes of weeds. The most common type of resistance to atrazine consists on target site modification (Kelly et al. 1999). However, other

type of resistance has been observed. For R-biotype of velvet leaf this is due to over-production of two GST isoenzymes, but for R-biotype of annual reygrass resistance to atrazine may results from cytochrome P450 mechanism (Lamoureux and Rusness 1995). Maize and some weed monocotyledonous species are capable to detoxify by GSH conjugation.

During our study the metabolism of atrazine resistant biotype of fat-hen (*Chenopodium album* L.) we paid attention to phenolic compounds. It is well known that these substances display a high biological activity. Therefore, they could play an important role in plant response to pests, environment pollution and other stress caused by abiotic and biotic agents (Cohen et al. 1994; Eckey-Kaltenbach et al. 1994; Feucht and Treutter 1989; Giebel 1970; 1982). It is also assumed that the level of phenols in plants can change due to herbicide action (Falco et al. 1989; Kumar and Rans 1994; Yamaguchi et al. 1986). Buhmann et al. (1987) found also that the phenol compounds interact with protein D_1. On the other hand, atrazine inhibits electron transport associated with the PS II complex, consequently it causes damage to protein D_1 (Gronwald 1994). Therefore, it may be assumed that specific phenolics synergise with herbicides. Phenols display also another activity. They can modify the indoleacetic acid (IAA)-oxidase *in vitro* as well as *in vivo* systems. Usually monophenols as cofactors activate this enzyme. On the contrary, polyphenols prevent the destruction effect of oxidase (Billot 1986; Grambow 1986, Henderson and Nitsch 1962). Plants contain these two phenolic groups, and their action in an oxidative system is demonstrated as an effect of both mono- and polyphenol activities. It is not unlikely that dramatic decrease in the IAA level can lead to plant death, and hence it assists herbicidal effect. Therefore, we focus on indoleacetic acid (IAA)-oxidase activity systems of selected weeds.

MATERIALS AND METHODS

Plant materials

The plant materials chosen for biochemical studies were obtained from seeds collected from atrazine-resistant population (R) of fat-hen (*C. album* L). found in the maize field near Poznań (the western part of Poland) and barnyard grass [*E. crus-galli* (L.) Roem et Schult.] planted from seeds obtained from University of Agriculture in Warsaw. Seeds from biotype susceptible to atrazine (S) of both species were collected from plants grown in the field not previously treated with atrazine. The homogenesity of plant materials was checked by spraying them with atrazine at 2 kg a.i. per hectar. Additionally, the maize was included in the study in order to find out whether the biochemical changes in cultivated plant resistant to atrazine occur.

The type of resistance to atrazine of R-biotypes was determined on the molecular level at the University of Agriculture in Warsaw with application of PCR technique. R-biotype of *E. crus-galli* revealed the mutation in psbA gene which determines the resistance through inhibition an electron transport in PS II complex. This mutation was not detected in R-biotype of *C. album*.

All tested plants were grown in 14 cm diameter pots in a greenhouse under controlled conditions, i.e. 20/16°C day/night and 16 hs photoperiod. When weeds

reached 3–4 leaf growth stage and maize 2–3 leaf stage, they were sprayed with atrazine at 1 kg a.i. per hectar. A week after treatment the leaves from each plant group were collected and used for biochemical analyses.

Biochemical analyses

The leaf extracts were using for quantification of phenolics and measure their effect on the IAA-oxidase activity altered by plant substances. These extracts were prepared from 100 plants of each plant group using Schmid and Feucht methode (1986) with acetone, then evaporated on a Rinco Vacuum Evaporator at 40°C to give 1 ml of extract equivalent to 1 g of fresh leaf weight.

The phenols were determined colorimetrically using Folin reagent (Walter and Purcel 1979). Chlorogenic acid was used as a standard. Monophenols were separated from polyphenols on the neutral Al_2O_3 column which adsorbed the polyphenols only (Zucker and Ahrens 1958). The quantity of polyphenols was calculated from the difference between total phenols and monophenols. The ratio of poly- to monophenols (p/m.) was calculated for each plant group.

Flavanols were determined in acetone extracts spectrometrically after Swain and Hillis (1959) with vanillin reagent, (+)-catechin as an internal standard and the absorbance at 500 nm. The presence of acetone and chlorophyll in leaf extracts disturb the analysis. Therefore, we were obliged to remove these substances by evaporation of the extracts and resolved the residue by sonification with water and hexane. Water fraction contained the flavanols and other not defined hydrophylic substances.

The activity of IAA-oxidase modified by leaf extracts of *C. album, E. crus-galli* and maize was measured *in vitro* as the amount of IAA decomposed during 30-minutes reaction. Here we adapted and modified the methods of Galston and Dalberg (1954) and Galdacre et al. (1953). Peroxidase from horseradish (activity 60 U/mg) obtained from Sigma was used as IAA-oxidase. The IAA that was not decomposed and remained in reaction mixtures was further determined with Salkowski reagent.

The reaction mixture (10 ml) contained: 6 ml 1/25 M phosphate buffer (pH 6,1), 1 ml 10^{-3} M Mn Cl_2 solution, 1 ml 10^{-3} M p-dichlorophenol solution, 1 ml peroxidase solution (0.1 mg in 10 ml of buffer) and suitable extract from the leaves of tested plants at the dosage rate correspondent to 20 mg fresh weight of the leaves. The check had no leaf extract.

The level of phenolics and IAA were determined from three spectrophotometric measurements of each sample. No difference between these measurements and a large number of plants (100 plants of each plant group) prepared for extracts caused that statistical analysis were unnecessary.

RESULTS AND DISCUSSION

The *in vitro* tests proved that leaves of *C. album, E. crus-galli* and maize contain factor which slows down the destruction of IAA in the peroxydase system (Figs 1, 2, 3). This inhibition was large, but it diminished when the extracts from plants treated with atrazine were added to the peroxydase (IAA-oxydase) systems. The lag time of IAA breakdown estimated from curve's destruction for untreated R and S

biotypes was between 8 and 13 minutes. It decreased for plants treated with atrazine, but it was different for R and S-biotypes, i.e. 7 and 5 minutes or 2 and 3 minutes, respectively. The value of lag time for maize was 5 minutes and it did not changed under herbicidal treatment.

Plant phenolic compounds can modify the IAA-oxidase activity *in vitro* as well *in vivo* systems. Therefore we decided to measure the level of these compounds in leaves of *C. album*.

The amount of phenols increased in both biotypes due to the application of atrazine, especially when the level of mono- and polyphenols is taken into consideration. The content of monophenols in susceptible biotype treated with atrazine was 3 times higher than in untreated plants. Consequently the polyphenols/monophenols ratio decreased from 4.2 to 1.1 (Tab. 1). However, this aspect requires some further research. It may also be of great importance while considering the modification of the activity of IAA-oxidase *in vitro* as well as *in vivo* systems.

The changes of p/m ratio mentioned above can exert an influence on the plant growth physiology. Monophenols act as IAA-oxidase activators (Billot 1986; Grambow 1986; Henderson and Nitsch 1962). This enzyme is responsible for catabolism of indol-3-yl acetic acid (IAA) which disturbs metabolic processes and can lead even to the death of a plant. The action of monophenols can be completely or partially reversed by polyphenols which are usually IAA-oxidase inhibitors. Thus, the biological activity of IAA in plants will depend on the mono- and polyphenols interaction, i.e. on the p/m ratio.

The level of monophenols in *C. album* resistant to atrazine biotype increased slightly due to atrazine application. Furthermore, the p/m ratio was not much changed compared with untreated plants. Therefore, the extracts from leaves of the R biotype were capable of protecting IAA against destructive action of peroxidase (Fig. 1).

The level of phenols was not determined in *E. crus-galli* and maize. However, leaf extracts from these plants introduced into the peroxidase system were able to modify the IAA-oxidase activity similarly to extracts of *C. album* (Figs 2, 3). Thus the extract of *E. crus-galli* S-biotype, contrary to the resistant one treated with atrazine lost its own ability to inhibition the IAA destruction (Fig. 2). The maize, as a plant resistant to atrazine, did not lose the ability to the restrain of IAA breakdown after herbicide treatment (Fig. 3).

It is interesting that atrazine also influenced the level of flavanols in atrazine-susceptible biotype of *C. album* while it was not observed in the biotype resistant

Table 1. Phenolics content (μg per 1 g fresh weight) in *Chenopodium album* leaves of biotype susceptible (S) and resistant (R) to atrazine

C. album biotype	Phenols			p/m ratio	Flavanols
	total	mono (m)	poly (p)		
S – untreated	403	77	326	4.2	23
S – atrazine treated	480	226	254	1.1	175
R – untreated	290	68	222	3.3	21
R – atrazine treated	380	95	285	3.0	36

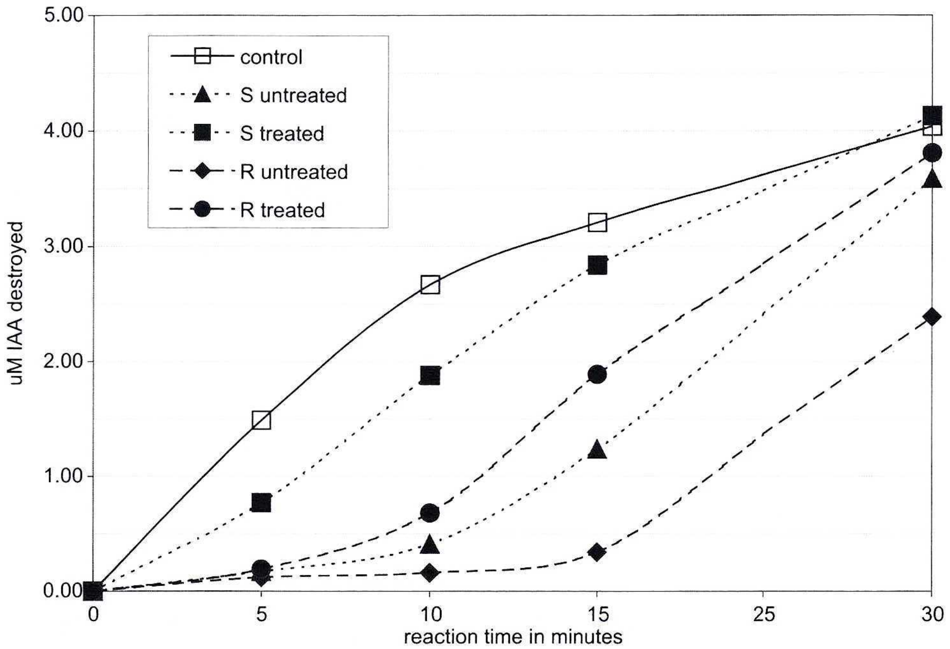

Fig. 1. The breakdown of indole-3-acetic acid (IAA) in peroxidase system with extracts from leaves of R and S biotypes of *C. album* treated with atrazine or untreated

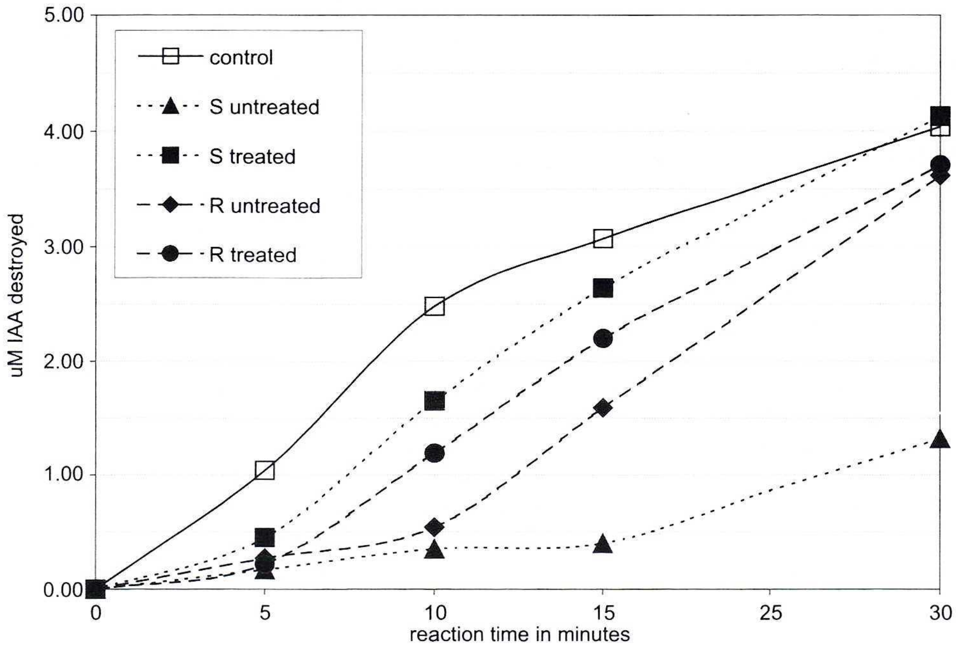

Fig. 2. The breakdown of indole-3-acetic acid (IAA) in peroxidase system with extracts from leaves of R and S biotypes of *E. crus-galli* treated with simazine or untreated

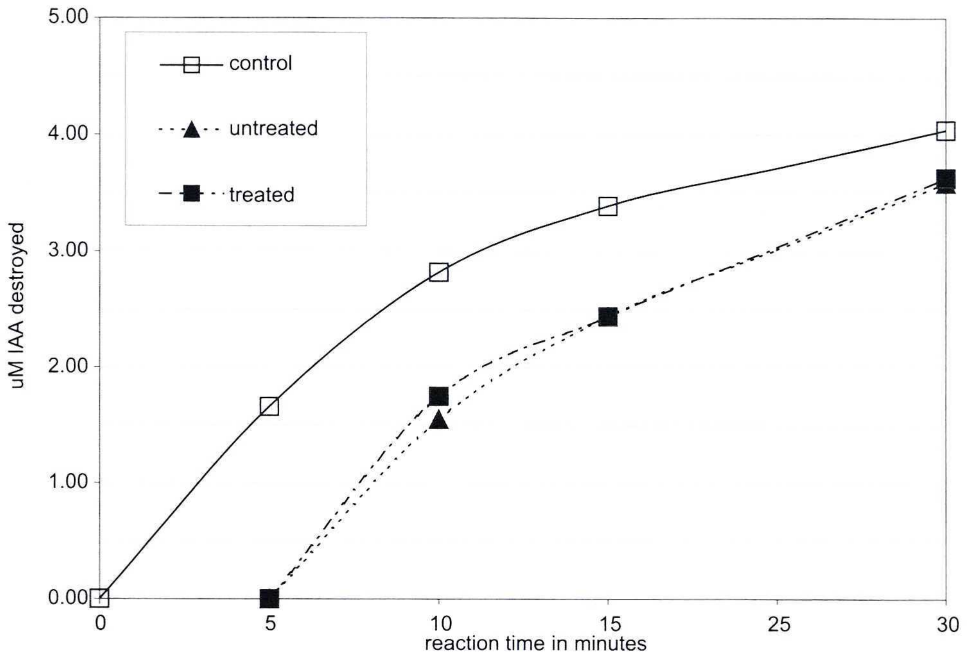

Fig. 3. The breakdown of indole-3-acetic acid (IAA) in peroxidase system with extracts from leaves of *Zea mays* treated with atrazine or untreated

to atrazine. The data shown in table 1 indicate that the application of atrazine increased the content of flavanols in S biotype eight-times compared with untreated plants. Flavanols can take part in the mechanism of herbicidal action of atrazine because they are known as very reactive compounds and they were usually found in high concentration in withered plants (Schmidt and Feucht 1986; Saunders and McClure 1976). Catechine and other flavanols are able to inactivate the phosphorylation and proteinkinase (Paliyath and Poovaiah 1984) as well as glucansynthase (Mason and Wasserman 1987). Stenlid (1963) found that some flavanols have a destructive effect on the plasma membrane potential and they reduce ATP level in plant cells. Thus they stimulate ethylen production. Moreover, flavanoids activate IAA-oxidase in plants and they also can catch free radicals.

It is known that herbicide detoxification may occur via GSH conjugation. Besides, IAA and cinnamic acid (co-factor of IAA-oxidase) may also be conjugated with GSH. The oxidative breakdown of plant auxines can be protect *in vivo* by this way. Not GSH-conjugated substrates, i.e. IAA and cinnamic acid (or other phenylpropanoic acid) will able take part in oxidative reaction (Marrs 1996).

We let an idea that in S-biotypes of tested plants the application of triazine herbicides induce biosyntesis of IAA-oxidase activator which may have a monophenolic character.

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POLISH SUMMARY

MODYFIKOWANIE AKTYWNOŚCI OKSYDAZY IAA PRZEZ CZYNNIKI OBECNE W KOMOSIE BIAŁEJ (*CHENOPODIUM ALBUM*) I CHWASTNICY JEDNOSTRONNEJ (*ECHINOCHLOA CRUS GALLI*) W ODNIESIENIU DO PODATNO-ODPORNOŚCIOWEJ REAKCJI ROŚLIN NA ATRAZYNĘ

Całkowita zawartość fenoli w liściach komosy biotypu wrażliwego (S) na atrazynę była o 40% wyższa w porównaniu do ich zawartości w biotypie odpornym (R) na ten herbicyd. Atrazyna zastosowana w dawce 1 kg/ha powodowała wzrost poziomu fenoli u obu biotypów. Szczególnie duży, bo trzykrotny wzrost stwierdzono w przypadku monofenoli u biotypu S. Stąd w roślinach tych zmniejszył się stosunek polifenoli do monofenoli z 4,2 do 1,1. Stwierdzono ponadto, że po zastosowaniu atrazyny u biotypu S nastapił 8-krotny wzrost poziomu flawanoli. Takie zależności nie występowały u roślin komosy należących do biotypu R.

Aktywność oksydazy IAA mierzona *in vitro* była modyfikowana przez wprowadzone do układów ekstrakty z liści komosy białej i chwastnicy jednostronnej. Ekstrakty z biotypów odpornych na triazyny traktowane odpowiednim herbicydem nadal hamowały rozkład auksyny (IAA), podczas gdy ekstrakty pozyskane z biotypów wrażliwych takiego działania nie wykazywały.

Uzyskane wyniki sugerują, iż mechanizm wrażliwości/odporności roślin na atrazynę związany jest nie tylko z systemem PS II, ale także ze systemem katabolizujacym auksynę.