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COMPARISON OF SUSCEPTIBILITY OF CROP OAT CV. AKT AND WILD OAT LEAVES TO DICURAN 80 WP IN MIXTURE WITH TWO ADJUVANTS, MEASURED BY CHLOROPHYLL FLUORESCENCE*

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Abstract. The results of leaf chlorophyll fluorescence measurements of crop oat cv. Akt and wild oat subjected to Dicuran 80 WP (a.s. chlortoluron) herbicide with two adjuvants (Adpros 85 SL, Atpolan 80 EC) are presented. Chlortoluron, as a phenyl-urea herbicide, is an inhibitor of photosynthetic electron transport in photosystem II, which can be measured by means of the special pulse PAM-210 fluorometer as ETR (electron transport rate) parameter. Dicuran 80 WP, applied alone in concentration of 0.5 μmol dm^{-3}, caused a decrease in ETR value in the wild oat leaves, whereas it did not affect the crop oat. The adjuvants Adpros 85 SL and Atpolan 80 EC added to Dicuran amplified the action of this herbicide.

Keywords: *Avena fatua, Avena sativa*, chlortoluron, oat, photosynthesis inhibitor, photosynthetic electron transport, photosystem II, wild oat

INTRODUCTION

Chlortoluron {3-(3-chloro-p-tolyl)-1,1-dimethylurea}[1] is a phenyl-urea herbicide used to control broadleaf and annual grass weeds in cereal fields. It is a pre- and post-emergence grass and broad-leaved weed killer for use on winter wheat, barley, triticale and durum wheat. One of such preparations is Dicuran 80 WP

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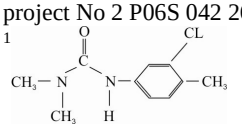
1 

which includes 80% of chlortoluron as active substance. The mechanism of chlortoluron metabolism in plants has been explained. Gonneau *et al.* (1988) showed that at least two distinct enzymatic systems could participate in the metabolism of chlortoluron in the plant tolerant species. It was principally detoxified by hydroxylation of ring methyl or by N-dealkylation. Cytochrome P-450 has been suggested to be involved in both N-demethylation and ring-methyl hydroxylation of chlortoluron, and enhancement of P450 monooxygenase activity related to chlortoluron metabolism in plants may result in tolerance to the herbicide (Gorinova *et al.* 2005). In winter wheat, barley and cotton tolerant to chlortoluron, the ring-methyl group was rapidly oxidised firstly to the non-phytotoxic benzyl alcohol compounds 3 and 4 and subsequently to benzoic acid derivatives 5, 6 and 7 that form glucose conjugates. In susceptible blackgrass and wild oat the N-demethylation pathway is favoured, and the resultant metabolites retain phytotoxicity (Ryan *et al.* 1981). Dicuran 80 WP, as other herbicides, can be used with some adjuvants which may be purchased separately and added to a tank mix prior to use (Tu and Randall 2003). Adjuvants may also improve an herbicide efficacy so that the concentration or total amount of herbicide required to achieve a given effect is reduced, sometimes as much as five- or ten-fold. In this way, adding an appropriate adjuvant can decrease the amount of herbicide applied and lowers the total costs of weed control (Green 2000). The adjuvants, using own lipophilic attribute, cause better penetration the herbicide into leaf tissue (Zabkiewicz 2000). The aim of this study was a quick assay of the biological activity of the herbicide Dicuran 80 WP, applied in a mixture with two typical adjuvants by means of chlorophyll fluorescence in leaves of crop oat and wild oat plants, by analysis of the rate of photosynthetic electron transport. We showed in our earlier papers that the adjuvants have various effects on plants, depending mainly on the kind of herbicide and plant species (Skórska and Swarcewicz 2005, 2006).

MATERIAL AND METHODS

Plants of oat (*Avena sativa* L.) cv. Akt and wild oat (*Avena fatua* L.) were grown in containers with soil in the vegetation hall under natural light of ca. PPFD 500 μmol m^{-2} s^{-1} and at temperature of 22°C/18°C day/night, in June 2006. For analyses, 2 cm tip sections of leaves from the three weeks old plants were placed on solution of Dicuran 80 WP (a.s. chlortoluron, 80%, $C_{10}H_{13}ClN_2O$, 3-(3-chloro-p-tolyl)-1,1-dimethylurea, Syngenta Crop Protection) at a concentration level of 0.5 μmol dm^{-3}, containing 0.5% (v/v) of Adpros 85 SL, Atpolan 80 EC or on the distilled water (0). The samples were kept in light (PPFD 200 μmol m^{-2} s^{-1})

for two hours, then for 15 minutes in darkness, and afterwards the chlorophyll fluorescence was measured with a PAM-210 fluorometer (Walz GmbH, Germany) using the saturating pulses method. Weak red measuring light ML (650 nm, PPFD 0.04 μmol m^{-2} s^{-1}), pulse saturating light SP (665 nm, 3200 μmol m^{-2} s^{-1}) and actinic light AL (665 nm, PPFD 120 μmol m^{-2} s^{-1}) were used. Parameter ETR, determining photosynthetic electron transport rate in photosystem II, exactly described by Skórska and Swarcewicz (2005), was analysed. All measurements were done in 6 biological replications, mean and standard errors were calculated. Treatment effects on chlorophyll fluorescence were determined by one-way analyses of variance (ANOVA) following checks for normality and equal variance distributions. This analysis allowed separating homogeneous groups by means of Newman-Keule test at 0.05 significance level using the Statistica 8.0 software.

RESULTS AND DISCUSSION

Chlortoluron, as an inhibitor of photosynthetic electron transport in photosystem 2, blocks electron transfer between Q_A and Q_B, because this herbicide joins its particle to D1 protein, in which Q_B acceptor is connected, and changes its redox capacity so that it causes slowing of electron transport in PSII (Jansen *et al.* 1993, Retzinger and Mallory-Smith 1997). As a result, a decrease of ETR parameter of chlorophyll fluorescence is observed, what can be measured by means of special pulse fluorometers (Schreiber *et al.* 1994). The Dicuran 80 WP, when applied alone, caused considerable decrease of ETR value in the leaves of *Avena fatua* by 26% compared to the control, i.e. leaves on distilled water, whereas it did not change photosynthetic electron transport in photosystem 2 in *Avena sativa* (Fig. 1). Activity of Dicuran 80 WP in mixtures with Adpros 85 SL or Atpolan 80 EC was significantly increased with respect to wild oat, because the ETR value was much lower, by 85% or 62% of the control, respectively. In the case *Avena sativa* only Adpros 85 SL in mixture with the herbicide caused decrease of ETR was by 42% in comparison with the control. The adjuvants applied alone did not affect the ETR value of leaves of both species.

Van Oorschot and Van Leeuwen (1992) described experiments on detached black-grass leaves subjected to chlortoluron. The inhibition of photosynthesis by chlortoluron was measured using the fluorescence induction. Leaves from resistant plants showed partial to full recovery from the inhibition, while susceptible plants did not. These results for fluorescence induction were compared with the inhibition of photosynthesis rate in intact black-grass plants by chlortoluron in a nutrient solution, and the recovery that occurred when the roots were placed again in herbicide-free nutrient solution. The similarity of the results indicates that the

procedure for fluorescence induction can replace the more labour-intensive and complicated demonstration of inactivation by measuring photosynthesis rate.

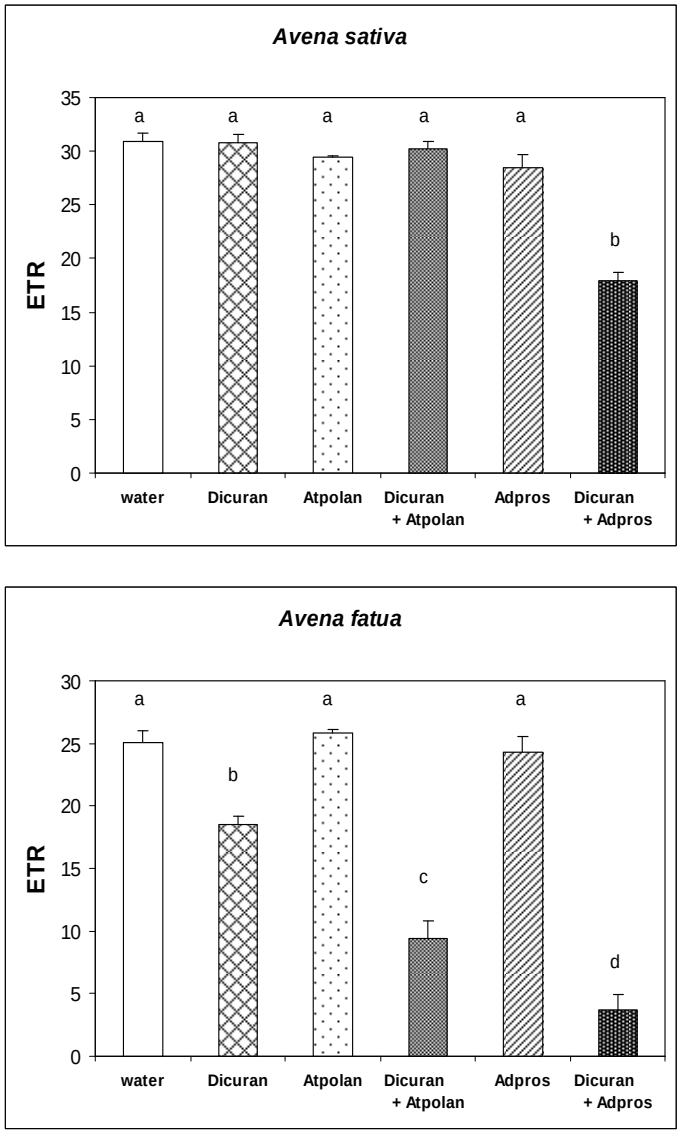


Fig. 1. Values of photosynthetic electron transport rate (ETR) in leaf samples after 2 hours in distilled water, Dicuran 80 WP (0.5 μmol dm^{-3}) alone, adjuvants Atpolan or Adpros alone, and Dicuran 80 WP with those adjuvants treatment in light, PPFD 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$

Korres *et al.* (2003) investigated the influence of chlortoluron on two wheat cultivars, Maris Huntsman and Mercia, which exhibited differential responses to the herbicide. The ratio F_v/F_m (maximal quantum efficiency of photosystem II) and area above the fluorescence induction curve are appropriate chlorophyll fluorescence parameters for detection of differential herbicide response between wheat cultivars.

Yordanova *et al.* (2001) showed that non-transgenic tobacco plants (susceptible to chlortoluron) were more inhibited by this herbicide than transgenic tobacco plants tolerant to chlortoluron, and the quantum yield of photosystem II (Y) was much more decreased than the ratio F_v/F_m. The last ascertainment is consistent with our results of many experiments which showed that the ratio F_v/F_m is an indicator of photoinhibition changes and has secondary quality in stress conditions for plants (Murkowski and Skórska 1997). In the case of herbicide treatment, a primary indicator of inhibition of photosynthetic electron transport is a coefficient of delayed luminescence decay (Devlin *et al.* 1983) or – presented in this work – the ETR value which depends on the quantum yield of photosystem II, but complies also with the light conditions of the studied plants (Schreiber et al. 1994, Skórska and Swarcewicz 2006). It is worth noting that the used ETR value allows for detecting the effects of a herbicide at concentration levels on the order of 0.5 micromoles per dm^3, whereas the traditional method, including the most frequently applied measurement of the F_v/F_m parameter, allows for detecting of concentration levels that are 100 times greater (Van Oorschot and Van Leeuwen 1992, Korres *et al.* 2003, Abbaspoor *et al.* 2006). The results presented in this work indicate a genetic reason of tolerance or susceptibility to chlortoluron in the case *Avena* species, more than the mechanism of metabolism of this herbicide described above.

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PORÓWNANIE WRAŻLIWOŚCI LIŚCI OWSA UPRAWNEGO ODMIANY AKT I OWSA GŁUCHEGO NA DICURAN 80 WP W MIESZANINIE Z DWOMA ADJUWANTAMI MIERZONE ZA POMOCĄ FLUORESCENCJI CHLOROFILU

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S t r e s z c z e n i e . W pracy przedstawiono wyniki pomiarów fluorescencji chlorofilu liści roślin owsa uprawnego odmiany Akt i owsa głuchego poddanych działaniu herbicydu Dicuran 80 WP (s.a. chlorotoluron) z dwoma adiuwantami (Adpros 85 SL, Atpolan 80 EC). Chlorotoluron jako herbicyd mocznikowy jest inhibitorem fotosyntetycznego transportu elektronowego w fotosystemie II, co można mierzyć za pomocą specjalnego fluorymetru impulsowego PAM-210 poprzez parametr ETR (szybkość transportu elektronów). Dicuran 80 WP o stężeniu 0,5 $\mu mol \cdot dm^{-3}$ spowodował zmniejszenie wartości ETR w liściach owsa głuchego, podczas gdy nie wpłynął na zmianę wartości tego parametru w porównaniu do kontroli w liściach owsa uprawnego. Adiuwanty Adpros 85 SL i Atpolan 80 EC dodane do Dicuranu wzmacniały działanie tego herbicydu.

S ł o w a k l u c z o w e : *Avena fatua*, *Avena sativa*, chlorotoluron, fluorescencja chlorofilu, fotosystem II, inhibitor fotosyntezy, owies, owies głuchy, transport elektronów