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Decrease of the herbicide fenoxaprop phytotoxicity in drought conditions: the role of the antioxidant enzymatic system

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Abstract: This study investigated the effects of the herbicide from the graminicide group fenoxaprop, on oat plants under a normal and reduced water supply. The study was done in vegetative experimental conditions. It was established, that plants treated with fenoxaprop after 2 days following a reduction of the water content in soil with 60% to 40% of full field moisture capacity, significantly reduced phytotoxic action. At the decreased level of water supply in the oat plants, activity of antioxidant enzymes – superoxide dismutase, and catalase increased. The fenoxaprop action did not lead to a substantial increase in superoxide anion radical and hydrogen peroxide content, which had been observed under the action of the herbicide when normal levels of water were supplied to the plants. It was concluded, that the phytotoxic action of the herbicides from the graminicide group mediated the formation of reactive oxygen species and reduction of phytotoxicity on the background effect on plants of various stressors. This reaction was due to the increased activity of antioxidant enzymes, associated with nonspecific reaction of plants to these stressors.

Key words: drought, fenoxaprop, herbicide, induced pathogenesis, prooxidant-antioxidant balance, water supply

Introduction

Herbicides that affect the key enzyme of fatty acid synthesis – acetyl-coA-carboxylase (ACC) (EC 6.4.1.2), form a group called graminicides, since they have a phytotoxic effect only on the cereal family (Poaceae/Gramineae) (Harwood 1999). Although graminicides are the most effective class of modern herbicides, their major disadvantage is that the phytotoxic action of these herbicides is dependent on the weather conditions (Collings *et al*. 2005). The effectiveness of graminicides may decline significantly when weeds are under water stress for quite a long period (Boydston 1992; Rossi *et al.* 1993). In field experiments, the effectiveness of controlling the annual grass weed; green foxtail (*Setaria viridis* L.), by the graminicides fluazifop, haloxyfop, fenoxaprop, and sethoxydim was found to decrease if after an herbicide application, the duration of the drought was more than 10 days (Boydston 1991). When the drought lasted less than 2 days before or after graminicide fluazifop was applied, the effectiveness of fluazifop on foxtail did not change (Boydston 1992). There was no significant influence of the plant moisture content to the translocation and metabolism of graminicide fluazifop (Boydston 1992). For this reason, we can assume that the decrease in the effectiveness of graminicide in drought conditions, is due to changes in the passage of induced pathogenesis.

Some data indicate that the development of the phytotoxic action of the graminicides is mediated by the formation of reactive oxygen species (ROS). The antioxidant ionol, when combined with graminicide haloxyfop-R--methyl, was found to slow down the appearance of necrosis in the root meristem of maize seedlings, but hydrogen peroxide did the opposite. At the same time, ionol reduced, and exogenous hydrogen peroxide enhanced the induced increase of the graminicide action of the ROS level in the meristems (Palanytsa *et al*. 2009).

The change of the graminicide phytotoxic action when graminicide is applied with other herbicides is determined by the influence of the latter on the condition of pro-oxidant-antioxidant balance in the cereal family plants (Radchenko *et al*. 2013). In particular, it was shown, that by adding the specific inhibitor of the antioxidant enzyme, superoxide dismutase (SOD), the antagonistic interaction of graminicide fenksaprop-P-ethyl with the herbicide acetolactate synthase inhibitor in the mixture, changed to additive interaction (Radchenko *et al*. 2013a). Recent data are direct evidence that the reduction of graminicide phytotoxic action under stress, may be associated with increased activity of antioxidant enzymes, caused by the influence of the stressor.

In this regard, the objectives of this study was to investigate the influence of moisture conditions on the phytotoxic action of graminicide. We determined the increased level of ROS induced by herbicide action (hydrogen peroxide and superoxide anion radical (SAR)) and change in the activity of antioxidant enzymes: SOD, guaiacol peroxidase and catalase.

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Materials and Methods

The study was about the influence of the moisture level on the graminicide fenoxaprop-P-ethyl (FP) (Bayer CropScience) phytotoxic effects. The experiment was conducted as a growing experiment in greenhouse conditions at average temperatures (day: 24°C; night: 17°C). Oat plants sensitive to FP were used as the object (*Avena sativa* L.). Plants were grown in soil culture conditions in plastic pots. The pots had a capacity of 1 kg (a mixture of soil and sand at a ratio of 3 : 1).

A normal level of moisture was maintained through pot irrigation by weight; 60% of full field moisture capacity (FFMC). Model drought was created by the termination of pot irrigation, bringing the soil moisture content to 40% FFMC. The herbicide treatment of plants was carried out in 3 leaf phases (in the variant with reduced water supply – 2 d after irrigation termination) by immersing the aerial parts of the plants for 5 min in a working solution (contains: 69 g/l FP + 18.75 g/l mefenpyr-diethyl (safener)) with a concentration of FP 5×10^{-4} M. The phytotoxic action of FP was evaluated by the inhibition of the mass-growing of the above-ground plant parts, and reduction of the content of photosynthetic pigments in the leaves. The pigments were extracted from the leaves using dimethylsulfoxide and determined spectrophotometrically using the Welburn method (Welburn 1994). The hydrogen peroxide content was determined using the ferricyanide method of Sagisaka (Sagisaka 1976), SAR by the Shorning method (Shorning *et al*. 2000). The method of Beauchamp and Fridovich (1971) was used to determine the SOD activity, based on the ability of SOD to compete with nitro blue tetrazolium for SAR. To determine the activity of the soluble form of guaiacol peroxidase, the Ridge and Osborne method (Ridge 1970) and the catalase method (Polidoros 1999) were used. The investigations were independently carried out four times; biochemical assays were performed three times.

Results and Discussion

Reducing the moisture content in the pots reduced the growth rate of the oat plants. The phytotoxic action of FP was evaluated after the 11th day following the treatment. The weight of the above-ground oat plant parts in the control group at 40% FFMC, was 1.4 times less than the normal level of moisture. At the same time, the inhibition of the mass-growth of the plant under the effect of FP was significantly higher at a normal level of moisture content than at a reduced level of moisture content (Fig. 1).

A reduced phytotoxic action of FP on oat plants with reduced moisture level was seen through the reduction of the herbicide's impact on the content of photosynthetic pigments in leaves (Table 1).

At the normal level of moisture on the 8th day following the FP treatment, chlorophyll *a* decreased to 26%, chlorophyll *b* – 27%, and carotenoids – 63%, relative to the control at 60% FFMC. Then, the reduced moisture content level of this pigment was thereafter, 55%, 59%, and 68%, relative to the control at 40% FFMC.

On the 5th day after the moisture content had been reduced to 40% FFMC, there was a slight but significant SAR reduction down to 8%. Up to about the same level, there was a decreased content of SAR on the 3rd day after plant treatment by FP under a normal moisture level.

During the plant treatment which involved a reduced level of moisture, significant difference to the corresponding control variant was not observed. Later, the nature of

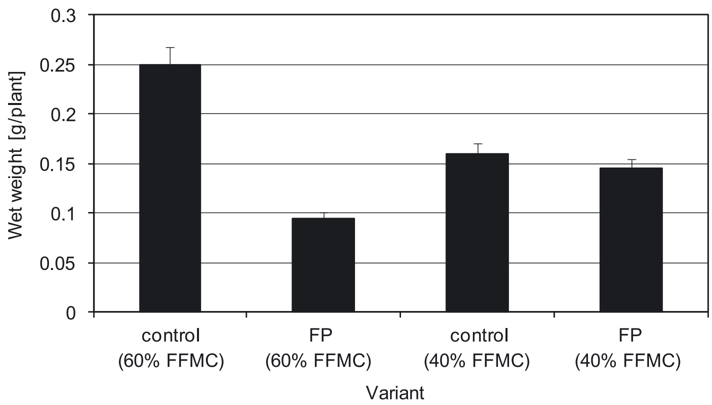

Fig. 1. Wet weight of above-ground plant parts of oats on the 11th days after the treatment of fenoxaprop-P-ethyl (FP)

Table 1. The photosynthetic pigment content in oat leaves after 8 days following plant treatment by fenoxaprop-P-ethyl

Variant	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
	[mg/g of wet matter]		
The control (60% FFMC)	0.65±0.02	0.26±0.03	0.16±0.01
FP (60% FFMC)	0.17±0.01	0.07±0.0	0.10±0.01
The control (40% FFMC)	0.78±0.01	0.27±0.04	0.19±0.02
FP (40% FFMC)	0.43±0.05	0.16±0.02	0.13±0.01

FFMC – full field moisture capacity; FP – fenoxaprop-P-ethyl the impact of stressors on the content of the active oxygen forms (AOF) changed. On the 10th day after the plant treatment by FP at 60% FFMC, the SAR content increased 30%. At the same moment, in the control with 40% FFMC, the SAR level was approximately 17% higher than the normal level of the water supply. In the variant with the treatment of FP under a decreased moisture level, the level of SAR not only increased but also even decreased compared to the control with 60% FFMC. The nature of the changes in the hydrogen peroxide (HP) content was almost identical to modify the content of SAR (Fig. 2).

With the influence of multiple stressors on plants, the following phenomena could be observed: a synergistic effect when action is enhanced or cross-adaptation when adaptation to the action of one factor causes adaptation to the action of another (Kuznetsov 2001). To assess the relative influence of two stressors, the value of the cumulative effect is used. The formula is as follows:

$$K = (F_{(A+B)}/F_B) \times 100,$$

where: K – the value of the cumulative effect of a percentage; $F_{(A+B)}$ – the value of the parameter used to assess the impact of stressors in their joint action, expressed as a percentage of the control; F_B – the value of the same parameter when exposed to a stressor that causes a greater effect. If K is greater than 100, the interaction is characterised as a cross-synergism, and if K is less than 100, the interaction is characterised as cross-adaptation (Alexieva 2003).

Our data indicate that the decline in a water supply prevents an increase in ROS caused by the action of the graminicide. At 10th day after the FP treatment of the oat plants, the cumulative effect for HP was 66%, and for SAR 56%, clearly indicating the presence of cross-adaptation.

The observed cross-adaptation was likely due to the influence of the moisture level on antioxidant enzyme activity. On the 3rd day after the FP treatment of the plants, the SOD activity increased both in the normal and lower moisture levels (Fig. 3). In the variant with a 40% FFMC, an increase in SOD activity, caused by the action of the herbicide, comes amid increasing activity associated with a decrease in the moisture level.

Subsequently, changes in SOD activity associated with the reaction of the plants to FP, depended on the water supply level. On the 15th day after the FP treatment in the variant with 60% FFMC, the SOD activity was approximately twice lower than in the controls. In the variant in which the FP treatment was carried out after a water supply reduction, the SOD activity was also slightly decreased in comparison with the corresponding control, but remained at a level substantially higher than that in the control activity at 60% FFMC – and even more so in a variant in which the plants were treated by FP with a normal water supply.

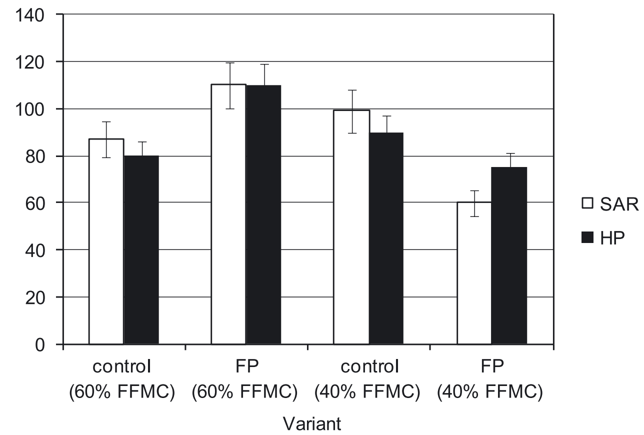

Fig. 2. Superoxide anion radical (SAR) content (conventional units/g dry matter) and hydrogen peroxide (HP) content (mM/g dry matter) on the 10th days after fenoxaprop-P-ethyl (FP) treatment of oat plants

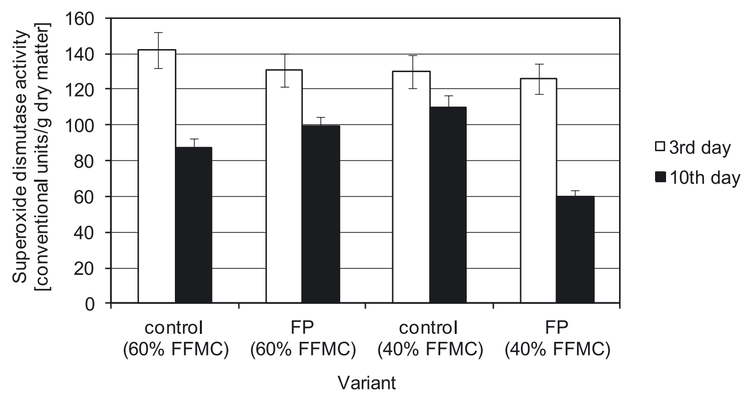

Fig. 3. Superoxide dismutase activity after treatment of oat plants by fenoxaprop-P-ethyl (FP)

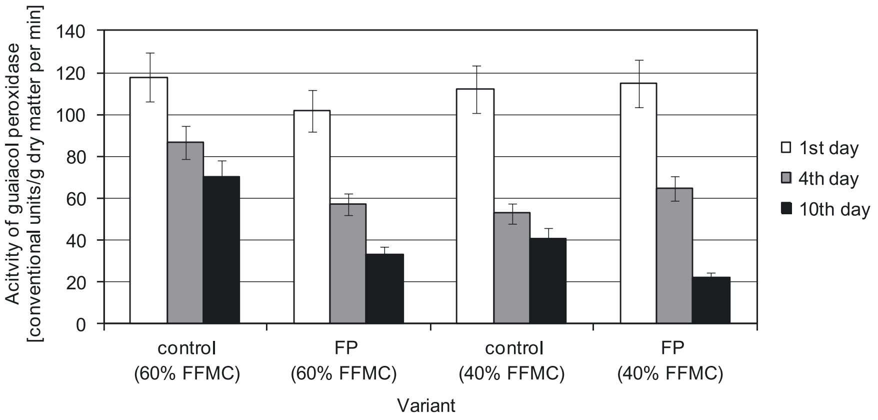

Fig. 4. Activity of the guaiacol peroxidase soluble form after treatment of oat plants by fenoxaprop-P-ethyl (FP)

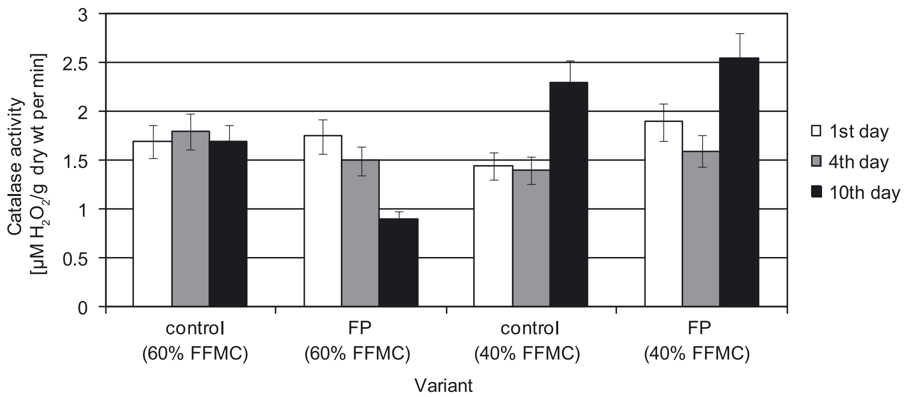

Fig. 5. Catalase activity under the action of fenoxaprop-P-ethyl (FP)

During the observation period, from day 1 to day 10 after the FP plant treatment, the activity of the guaiacol peroxidase soluble form decreased in all variants, including in the controls (Fig. 4). No significant difference in the values of the activity associated with the action of FP or a decrease in the level of the water supply was observed.

Unlike guaiacol peroxidase, the catalase activity in the control at 60% FFMC did not change throughout the observation period (Fig. 5). Reduction of the water supply initially led to some reduction in activity, however, during the last analysis, which corresponded to the 12th day after the irrigation was terminated, there was a sharp increase in the catalase activity. The treatment in which FP was used at different water supply levels led to diametrically opposite changes in activity.

In a variant of 60% FFMC on the 4th day after the FP treatment, there was a tendency towards reduced activity, and on the 10th day, the catalase activity decreased by almost two times compared with that in the controls. At a reduced level of water supply, the treatment using FP did not cause any reduction of the catalase activity. On the 1st day after treatment, the activity even increased slightly compared with that of the control at 40% FFMC.

The obtained data indicated that the reduced water supply level in oat plants increased the antioxidant enzymes SOD and catalase, whereas the FP action does not lead to a significant increase in the ROS content. Thus, on the 10th day after the FP plant treatment, the value of the correlation coefficient between the catalase activity in the plant and the content of hydrogen peroxide in oat plants was –0.83.

According to the obtained results, it can be concluded that the decrease of graminicide phytotoxic action with decreasing levels of a water supply was due to the increased activity of antioxidant enzymes associated with a nonspecific response of the plants to the stressor action.

The presence of a certain lag phase in the dynamics of changes in the activity of antioxidant enzymes, particularly catalase caused by a decrease in the plant water supply, may explain the fact that the reduction of graminicide phytotoxicity was observed only if the duration of the drought before the herbicide treatment exceeded a certain minimum period (Boydston 1992).

Thus, based on the obtained results, it is possible to conclude that the graminicides' phytotoxic action indeed mediated ROS formation. It is also possible to conclude that a decrease in phytotoxicity when graminicides' treatment performed on the background effects on plants having stressors, is due to the increase of antioxidant enzyme activity associated with a non-specific reaction of the plants to these stressors.

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