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Differential Reaction of *Alopecurus myosuroides* Biotypes to ACCase Inhibitors †

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Abstract: Herbicide-resistant blackgrass (*Alopecurus myosuroides* Huds.) populations in winter cereal crops are increasing in Europe, leading to severe restrictions in cereal production. In addition, resistant biotypes often perform better than sensitive biotypes. The aim of the study was to evaluate the response of *A. myosuroides*, potentially resistant to ACCase inhibitors, to different doses of fenoxaprop-P-ethyl and pinoxadene. The blackgrass populations tested showed different responses to the herbicides used. In the case of fenoxaprop -P-ethyl treatment, a reduction in the biomass of the treated plants was only observed for population RI compared to the untreated control. Treatment with pinoxaden resulted in a reduction of biomass accumulation in 3 populations. The obtained results indicate that the studied biotypes are able to induce mechanisms reducing the negative impact of the herbicides used, some of them have a stimulating effect.

Keywords: *Alopecurus myosuroides* Huds., graminicides; biomass; fitness; hormesis

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1. Introduction

Competition between weeds and crops is one of the more serious problems of modern agriculture. [1,2]. In recent years, weed control relies heavily on herbicides targeting specific metabolic pathways, e.g., acetolactate synthase (ALS, also referred to as acetohydroxyacid synthase, AHAS), acetyl-coenzyme A carboxylase. (ACCase) and D1 protein in photosystem II etc. [3]. The widespread use of chemical method of weed control without sufficiently changing mechanisms of action of herbicides leads to the selection of resistant weed biotypes in several species.

Alopecurus myosuroides Huds. is a weed commonly found in winter crops [4,5]. In Poland, it is considered an archaeophyte that was introduced around the 15th century [6]. In recent years, black grass has become one of the most noxious herbicide-resistant weeds in Europe [5,7]. This species produces about 500 viable seeds from one plant. Since only about 20% of seeds germinate in spring, most of them in autumn, winter crops are more at risk [8].

The germination and growth times of black grass seedlings from different populations vary; therefore, the species shows high plasticity and tolerance to changing habitat conditions. Abundant populations of *A. myosuroides* with high adaptability to habitat conditions and the acquisition of resistance have been identified in many European countries, including the UK, France and Germany in winter crops, especially wheat [5,9,10].

There is a prevailing opinion in the literature that mutations conferring resistance to pesticides will generate an adaptation cost in the absence of selection pressure [11]. However, an increased fitness of herbicide-resistant populations is increasingly noted [12,13]. Fitness or success in this field can be defined as the ability to establish itself, survive and reproduce successfully in a given species environment. Changes in fitness can be used to predict population dynamics and establish resistance management strategies [14].

Weeds gaining tolerance to the applied herbicides become more competitive against both undesirable and cultivated vegetation, and attempts of their control contributes to better growth and development of such weeds. This phenomenon is called hormesis and has been defined by Paracelsus (1493–1541) who stated that 'The poison is in the dose' [15]. Researchers noted an increase in the biomass of the sprayed weed from a few, even [16] up to 150% [17] compared to the control, especially when the herbicide was used in incomplete dosing [15].

The aim of the study was to evaluate the response of *A. myosuroides*, potentially resistant to ACCase inhibitors, to different doses of fenoxaprop -P-ethyl and pinoxadene.

2. Materials End Methods

Seven biotypes of *A. myosuroides* were tested in this trial. Field samples of four populations suspected to be resistant to the applied herbicides were collected in 2017 from the districts of Nowodwory (RII), Mallbork (RIII) and Gdańsk (RVII) in Pomerania and from the districts of Lidzbark (RI), Braniewo (RIV) and Kętrzyn (RV, RVI) in Warmian-Masurian. Samples were suspected to be resistant to ACCase inhibitors based on interviews with farmers and chemical advisors working in the crop protection supply chain. Each sample contained more than 100 ears from at least 30 different plants. Individuals from winter wheat fields with very poor herbicide control of *A. myosuroides*, despite being sprayed with full label doses, were harvested and classified for further greenhouse and laboratory tests. In laboratory the seeds of black grass were then threshed, cleaned and stored in a refrigerator for seven days at a temperature of about −4 °C in order to interrupt seed dormancy. The seeds were sown in multiplates with a pot diameter of 5.5 cm, filled with potting mixture. After germination the number of plants was reduced to 5 per pot (3 pots for one herbicide dose). Both herbicides were applied at the 2–3 leaves stage at six doses from 0 to 8× label dose (LD) where a label dose (N1) was 1.2 $L \cdot ha^{-1}$ for fenoxaprop -P-ethyl and 0.9 $L \cdot ha^{-1}$ for pinoxaden, using a laboratory pot sprayer (KAMA, Polanica-Zdrój, Poland) fitted with a boom with one nozzle calibrated to deliver 200 $L \cdot ha^{-1}$ at a spraying pressure of 200 kPa. Three weeks after treatment fresh weight of shoots was recorded. To compare results, a one-way ANOVA was performed using Statgraphics Plus 4.1 (Statpoint Technologies Inc., Warrenton, VA, USA). Differences between means of samples were evaluated by HSD Tukey test at $\alpha = 0.05$

3. Results

Tested populations of blackgrass showed different reactions to applied herbicides (Figure 1). When analysing the biomass of unsprayed controls, it was found that the RI biotype plants were the largest, the RII and RIV biotypes the smallest (2.68 and 1.80 g pot^{-1} respectively) among analysed biotypes.

In the case of fenoxaprop -P-ethyl treatment the reduction of biomass of treated plants was observed only for the RI biotype in comparison to non-treated control. Biomass of the remaining biotypes was increased, especially for doses 0.5 LD–4 LD (label dose) (Figure 2). The biomass of the sprayed plants of the RIV biotype in all treatments was higher than that of the control plants and ranged from 118 to 146% of the controls.

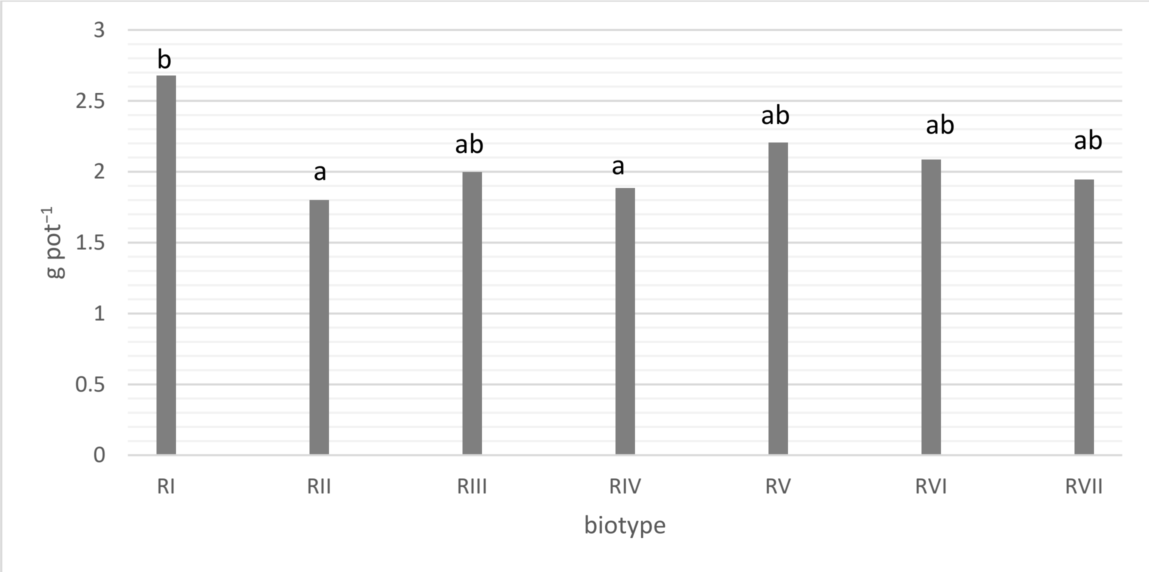

Figure 1. Shoot fresh biomass accumulation in 7 populations of *Alopecurus myosuroides* seedlings non-treated with fenoxaprop -P-ethyl and pinoxaden.

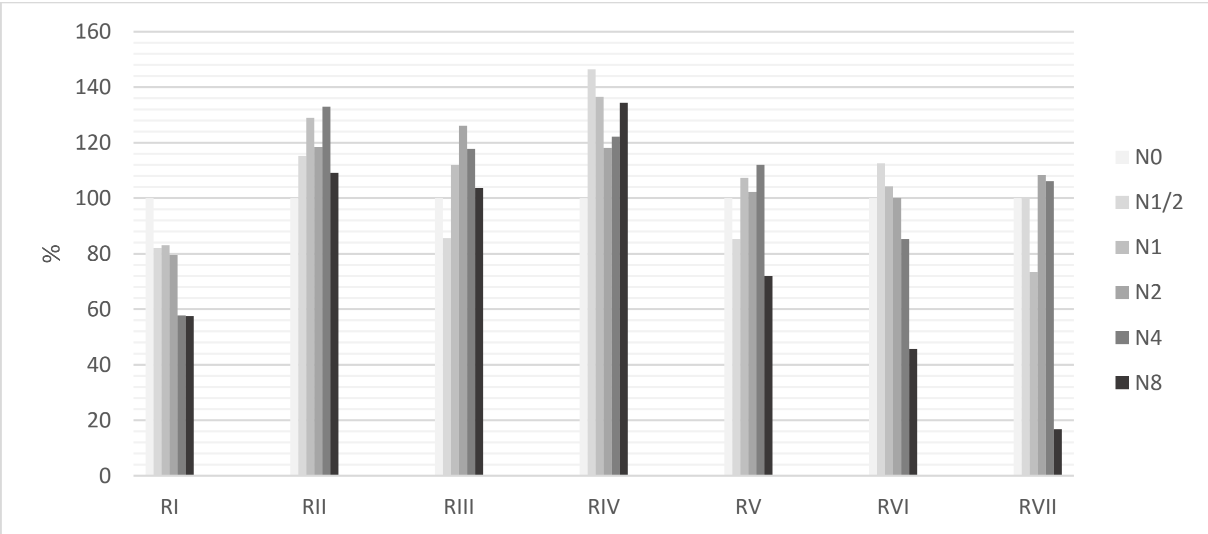

Figure 2. Shoot fresh weight of sprayed and non-sprayed fenoxaprop -P-ethyl plants of *A. mysouroides*, expressed as a percentage of control.

Pinoxaden treatment resulted in biomass accumulation decrease in 3 populations (RI, RVI, RVII). For 4 biotypes (RII-V) increase of biomass accumulation was observed after herbicide treatment in comparison to non-treated control (Figure 3). The highest stimulation effect was observed for the biotype RIV for the label dose and for the biotype RII for the N2 dose. The biomass accumulation of plants treated with N1 dose was 57% higher than in the untreated control. In plants from the biotype RII this parameter was 41% higher than in the control.

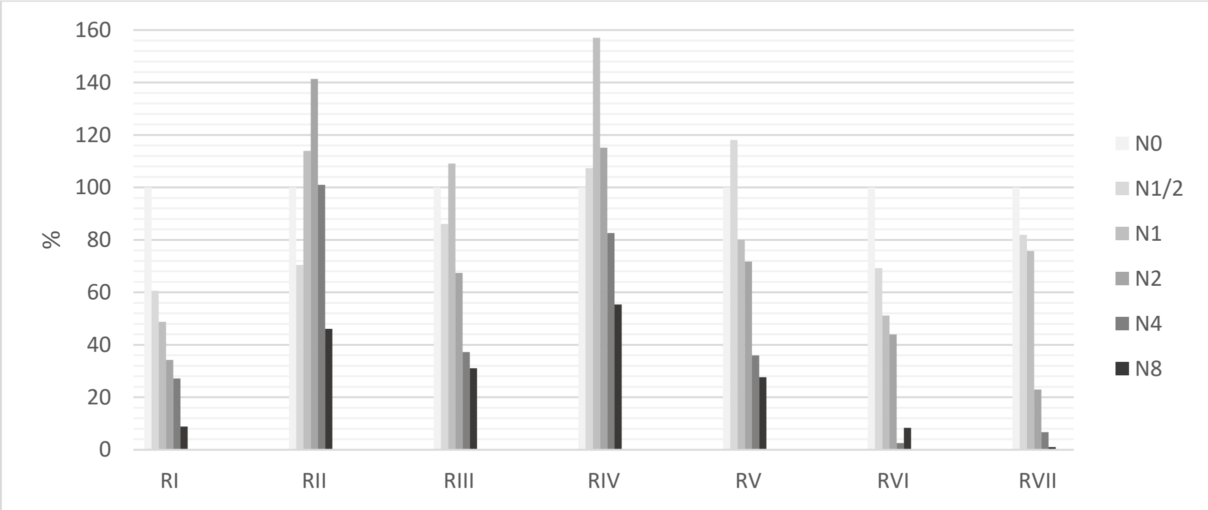

Figure 3. Shoot fresh weight of sprayed and non-sprayed pinoxaden plants *A. mysourides*, expressed as a percentage of the control.

4. Discussion

The obtained results indicate that the studied blackgrass biotypes differ in the level of biomass accumulation and sensitivity to the applied graminicides. It was found that the largest biomass of non-sprayed controls was produced by the RI population, which showed sensitivity to both fenoxaprop -P-ethyl and pinoxaden. Lower biomass accumulation noted in resistant RII and RIV biotypes for untreated plants It confirms the results of other authors who associate the development of herbicide resistance with decreased plant fitness [12,13].

As reviewed by Beltz et al. hormesis is commonly observed at subtoxic doses of herbicides however some weeds that have evolved herbicide resistance may have hormetic responses to recommended herbicide application rates. Especially for biotypes with high resistance factors, the recommended field rate may represent a low dose [15]. Some of blackgrass biotypes observed in our study showed hormesis for doses of fenoxaprop -P-ethyl and pinoxaden less than, equal to or above the label dose. Similar stimulation was described for ACCase-target-site-resistant blackgrass biotypes treated with fenoxaprop -P-ethyl and cycloxydim [18]. This phenomenon is especially dangerous in field conditions because it may indirectly influence the development of resistance by making hormetically enhanced resistant weeds more reproductive, more competitive or more resistant to a second weed control measure.

Institutional Review Board Statement:

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Data Availability Statement:

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