



Susceptibility of *Echinochloa crus-galli* Biotypes from Rice Crop to Profoxydim and Impact of the Weed Growth Stage

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ABSTRACT

Barnyardgrass (*Echinochloa crus-galli*) is one of the most problematic weeds on rice crop. The research was to evaluate the response of several barnyardgrass biotypes from Greece to profoxydim and evaluate the role of different rates and growth stages on the overall effectiveness. Three experiments were conducted in 2018. The response of the several biotypes had high variability (20-100%), with the most susceptible biotypes originating from regions in which crop rotation is a common practice (Fthiotida). Moreover, dose-response experiments showed that the rate of profoxydim required to adequately control the resistant population G17 was 4-times higher than the maximum recommended dose. Concerning the phenology effect on profoxydim effectiveness, This study revealed a higher efficacy at the earlier growth stage BBCH 13 (15-50% higher than growth stages BBCH 22 and 30), probably it was due to the smaller size and the higher growth rate of the younger plants. Consequently, the significant effect of weed growth stage on the effectiveness of profoxydim implies that application of the herbicide at earlier growth stages could optimize herbicide use and result in a better control with the condition of avoidance crop injuries.

INTRODUCTION

Rice is an important crop for more than half of the global population and a plant which has determined the culture and diet of thousands of millions people. In fact, rice as source of food calories is used as to support the half of the world's population (Farooq et al., 2011). Globally, rice crop occupies more than 158 million ha of the arable land (FAO, 2014). In Greece, even if its cultivation is located in specific areas, it is considered one of the most competitive exporting Greek products, with an annual production often higher than 120,000 ton and almost half of which is exported.

Between 40 and 96% of average yield losses in rice is caused by weed competition (Chauhan & Johnson, 2011; Ekeleme et al., 2009; Mahajan, Chauhan, & Johnson, 2009). *Echinochloa* is a cosmopolitan genus, with several weedy species, commonly found in many annual crop fields and perennial orchards. Among the several species, barnyardgrass (*E. crus-galli*) is considered to

be as one of the most competitive, noxious and problematic weeds for many crops including rice with yield losses up to 80% (Smith Jr., 1981). It has a huge adaptability, a wide distribution and has already been classified as a serious weed in 36 crops in 61 countries including Greece (Holm, Plucknett, Pancho, & Herberger, 1977). This C4 species is highly competitive with rice, with its vast seed production and rapid growth rate holding a pivotal role (Marambe & Amarasinghe, 2002).

Globally, *E. crus-galli* var. *crus-galli* has already developed resistance to several herbicides, with 45 individual cases to be already confirmed (Heap, 2019). It is noticeable that in Italy, ALS-inhibiting herbicide (e.g. azimsulfuron, bispyribac-sodium, cyhalofop-butyl, imazamox and penoxsulam) caused resistant of 14 *E. crus-galli* populations, while five of them caused multiple resistant to both the ALS and the ACCase inhibitor profoxydim (Beckie, 2011; Panozzo, Scarabel, Tranel, & Sattin, 2013). In general, controlling problems of this weed

are widespread in countries like Greece, Italy, Spain, Portugal and Turkey, while in general profoxydim was considered to be a good alternative.

Related to the difficulty of *Echinochloa* spp. to be controlled in Greece, this research was conducted to assess the occurrence of profoxydim-resistant biotypes of barnyardgrass from several areas. Specifically, this research was to evaluate the response of several barnyardgrass biotypes to profoxydim and evaluate the role of different rates and growth stages on the overall effectiveness.

MATERIALS AND METHODS

E. crus-galli seeds from 20 fields of rice crop were collected from August to September 2017, in the several prefectures of Greece such as Etoloakarnania (western Greece), Fthiotida (eastern Greece), Thessaloniki and Serres (northern Greece) and were labeled as G1, G2, ..., G20 (Table 1). Poor control of barnyardgrass was occurred in some sites based on farmer complaints registered at local cooperatives. To get representative sample from each site, the seeds were collected from 10 to 20 plants. A biotype barnyardgrass that never been treated with profoxydim was also included and used in this research as the susceptible population.

Table 1. Collected *E. crus-galli* biotypes from the different areas (labels-codes are also shown in parentheses).

Region	Number (and codes) of collected biotypes
Etoloakarnania	6 (G1, G2, G3, G4, G5, G6)
Fthiotida	5 (G7, G8, G9, G10, G11)
Serres	3 (G12, G13, G14)
Thessaloniki	6 (G15, G16, G17, G18, G19, G20)

There were four seeds from each biotype that sown into separated pots (15 cm diameter by 30 cm deep). Media were used an herbicide-free soil combined with a peat substrate (1:1, v/v). During the experiments, water mixed with 70 ml/pot of modified Hoagland's solution (0.25%) every week was applied for each plot (Hogland & Arnon, 1950). A photoperiod of 11-13 hours and air temperatures ranging from 24 to 35°C were kept to maintain the experiment. When seedlings had two tillers (BBCH 22), 0.2 kg profoxydim a.i./ha (1 l/ha of AURA 20 EC, BASF Agro B.V., Arnhem, NL) were sprayed by a custom-built, compressed-air, low pressure flat-fan nozzle experimental sprayer delivering herbicide

in 300 l/ha water at 250 kPa. As recommended rate at 1.0 l/ha, a non-ionic surfactant (Dash HC, BASF, Cheadle, UK) was added. At 14 days after treatment (DAT), fresh weight of aboveground plant biomass was calculated to present a percentage of the untreated control for each biotype. It was conducted twice.

Based on biomass reduction after spraying with profoxydim at 0.2 kg a.i./ha, biotypes were classified as potentially susceptible (higher than 70% reduction) and potentially resistant (less than 30% reduction) (Travlos & Chachalis, 2010; Urbano et al., 2007). Population number of susceptible, resistant and intermediate populations were two, one and two respectively used for dose-responses experiment. Populations were used to determine the herbicide rate needed for a 50% reduction in biomass (GR_{50}). It was conducted from July 6, 2018 to September 23, 2018 under air temperature between 22 to 36°C. Sowing was conducted as same as previously. Plant were treated with profoxydim at 0, 0.05, 0.1, 0.2, 0.4 and 0.8 kg a.i./ha, when reached BBCH 22 and it corresponded to 0, 1/4, 1/2, 1, 2 and 4 times as maximum recommended rate for profoxydim. The application of herbicide were conducted as previous description, and a completely randomized design was adopted in this experiment. Four replication per treatment were used and each pot was a replication. At 14 DAT, relative percentage for each biotype was recorded by the fresh weight of aboveground plant biomass calculation.

Spraying was conducted to evaluate the growth effect on plants at three phenological stages such as 1) seedlings with 3 leaves (BBCH 13), 2) plants with 2 tillers (BBCH 22) and, 3) plants at the beginning of stem elongation (BBCH 30). At the beginning of stem elongation, pseudostem and tillers were erect, first internode began to elongate and the top of inflorescence was at least 1 cm above tillering node. One for each of potential, susceptible and intermediate biotype were chosen based on the previous dose-response experiments. For the specific experiment there were selected Three *E. crus-galli* biotypes used for the specific experiment at similar type and rate of growths. A completely randomized design was adopted for three biotypes and growth stages, and it was replicated five times for each combination. The same equipment was used for all treatments as previous description. At 14 DAT, the reduction of fresh weight of aboveground plant biomass was calculated compared to control.

The data were analyzed by Analysis of Variance (ANOVA). If there were significance, post-hoc test at 5% is conducted to calculated significance distance between each treatment. For the dose-response data, Probit Analysis was adopted (Finney, 1952) to determine the dose resulting in a 50% reduction in plant growth (GR_{50}). Normality test was used to evaluate each data before ANOVA, and further analyses. All analyses were conducted by Statistica 9.0 software package (StatSoft, Inc. 2300 East 14th Street, Tulsa, OK74104, USA).

RESULTS AND DISCUSSION

This study confirmed significant differences in biomass reduction in response to profoxydim at the registered dose, collected from different locations in Greece. In particular, biomass reduction of the several biotypes was ranged from 20 to 100% of the untreated control of each biotype (Fig. 1). The name

of the biotype is adjusted to the name of the region. Fig. 1 describe biomass reduction (compared with the untreated control) for the several biotypes at 14 DAT.

According to the classification proposed by Travlos & Chachalis (2010) and Urbano et al. (2007) and the results of the present study, 12 barnyardgrass biotypes showed mean plant biomass 30% lower than the untreated control biomass and were considered potentially susceptible, whereas 2 biotypes exhibited plant biomass 70% higher than the untreated controls and can be characterized as potentially resistant (Table 2). It has to be noted that the potentially resistant populations were confined to Etoloakarnania prefecture, while all biotypes from Fthiotida region were potentially susceptible, probably due to the crop rotation which is a common practice in this area (Travlos, Apostolidis, Tsekoura, Cheimona, & Antonopoulos, 2019).

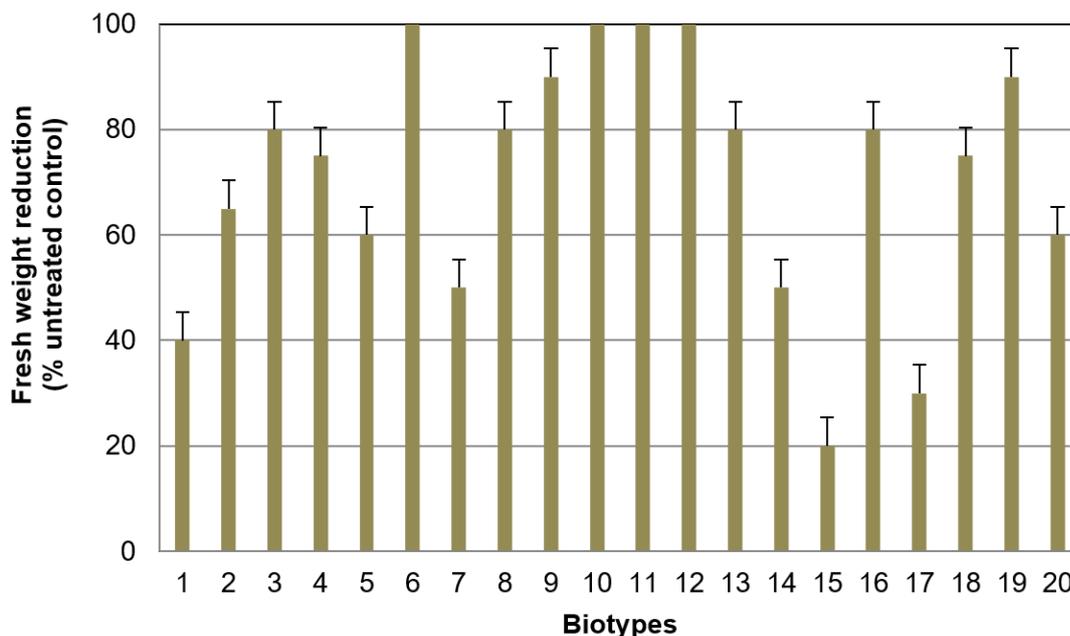


Fig. 1. Biomass reduction (compared to the untreated control) for the several biotypes at 14 DAT.

Table 2. Characterization of *E. crus-galli* biotypes after profoxydim application at a rate of 0.2 kg a.i./ha

Category*	Percentage of studied biotypes
Potentially susceptible	60
Intermediate	30
Potentially resistant	10
Total	100

Remarks: * Potentially susceptible = biomass reduction > 70% relative to untreated control; Intermediate = biomass reduction 30-70%; Potentially resistant = biomass reduction < 30%

In a previous study, Vasilakoglou, Dhima, & Gitsopoulos (2018) found that profoxydim applied at the recommended dose resulted in a very good control (> 90%) of seven biotypes of late watergrass (*Echinochloa phyllopogon*) from northern Greece. Matzenbacher, Kalsing, Menezes, Barcelos, & Merotto Junior (2013) also found that profoxydim effectively controlled resistant barnyardgrass biotypes to other herbicides, while there was a clear evidence of synergism between profoxydim and cyhalofop.

Dose-response experiment revealed significant differences in the response of the five selected biotypes. Particularly, G6 and G9 were susceptible biotypes, G2 and G20 intermediate and G17 resistant to profoxydim. It has to be noted that the rate required to adequately control the resistant population was 4 times higher than the maximum recommended dose (Table 3). Moreover, GR₅₀ value for the resistant biotype (G17) was more than 10 times higher than the corresponding values for the most susceptible biotype (G6).

Table 3. Biomass reduction (% of the untreated control) for one resistant, two susceptible and two intermediate biotypes of *E. crus-galli* in response to increasing profoxydim rate

Profoxydim rate (kg/ha)	<i>E. crus-galli</i> biotypes				
	G2	G6	G9	G17	G20
0	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
0.05	30 ^b	60 ^b	50 ^b	5 ^b	20 ^b
0.1	40 ^b	80 ^{ab}	75 ^c	15 ^c	40 ^c
0.2	65 ^c	100 ^c	90 ^d	30 ^d	60 ^d
0.4	75 ^c	100 ^c	100 ^d	40 ^d	85 ^e
0.8	100 ^d	100 ^c	100 ^d	75 ^e	100 ^f

Remarks: Means followed by different case letters in each column are significantly different according to Fischer's LSD test (P < 0.05)

In regards to the phenology effect of barnyardgrass on profoxydim effectiveness, our study resulted in some very interesting data. In particular, for all three biotypes there was a higher efficacy at the earlier growth stages, probably it was due to the smaller size and the higher growth rate of the younger plants (Table 4). This finding is in agreement with previous studies on *Echinochloa* spp. and other weed species (Kalsing et al., 2017; Travlos & Chachalis, 2010; Urbano et al., 2007). It has

also to be noted that this trend was obvious even for the resistant population (G17), despite the fact that the overall control was significantly lower than that of the susceptible population (G6). Consequently, the present study revealed the importance of early herbicide application as stated in previous studies and several weed species (Travlos et al., 2018).

Table 4. Control of several *E. crus-galli* biotypes (% biomass reduction related to the untreated control) at 14 DAT after profoxydim application at different growth stages: BBCH 13 (3 leaves), BBCH 22 (2 tillers) and BBCH 30 (beginning of stem elongation)

Growth stage	G6	G17	G20
BBCH 13	100 ^a	40 ^c	70 ^e
BBCH 22	100 ^a	30 ^c	60 ^e
BBCH 30	85 ^b	10 ^d	20 ^f

Remarks: Means followed by the same letter within each column are not significantly different according to Fisher's LSD test at P < 0.05

According to Vasilakoglou, Dhima, & Gitsopoulos (2018), the level of resistance of weeds like late watergrass indicated a very high risk of rice monoculture in specific areas of Greece with a shift in weed flora to more resistant biotypes and therefore herbicide and crop rotation are among the practices that should be promoted. This research findings also confirmed significant differences between the several biotypes with an ongoing problem of resistance development. Consequently, the integration of various weed management approaches along with chemical control is strongly recommended towards an effective, sustainable, and long-term weed control (Beckie, 2011; Chahal & Jhala, 2015).

CONCLUSION AND SUGGESTION

The results of the present study suggest that there is an issue of barnyardgrass resistance to the herbicide profoxydim. The response of the several tested biotypes was variable, with the most susceptible biotypes originating from regions in which crop rotation is a common practice. Furthermore, the significant effect of weed growth stage on the effectiveness of profoxydim implies that application of herbicides at earlier growth stages could optimize herbicide use and result in a better control as long as crop injuries are avoided.

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