



Article

Efficacy of Different Herbicides on *Echinochloa* colona (L.) Link Control and the First Case of Its Glyphosate Resistance in Greece

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Abstract: E. colona is a C₄ annual summer grass which is troublesome to major summer annual and perennial crops. Due to recent complaints by the farmers, the objectives of the present study were to evaluate the efficacy of penoxsulam, profoxydim, cycloxydim, cyhalofop-butyl, florpyrauxifen-benzyl and glyphosate against six E. colona accessions, and also to evaluate the response of these accessions to different rates of glyphosate in a dose-response experiment. In the first experiment, herbicides were applied at their maximum recommended label rates, while in the dose-response experiment, glyphosate was applied at six doses corresponding to 0, 1/4X, 1/2X, X, 2X, and 4X of the recommended rate. The dry weight of the biotypes TH8 and TH7 treated with profoxydim was 66% and 68% of the untreated control, respectively. The efficacy of cyhalofop-butyl against three accessions was lower than 30%, while two accessions were susceptible to this herbicide. The efficacy of penoxsulam against the biotypes ET2 and ET4 was lower than 10%, while dry weight of FT5 and TH8 was only reduced by 23%–28% as compared to the control. Cycloxydim application provided control higher than 75% at 21 days after treatment (DAT) of three accessions, while the majority of E. colona accessions was adequately controlled by the application of florpyrauxifen-benzyl. The response of the different accessions to glyphosate varied. The results of the glyphosate dose-response experiment revealed that the GR₅₀ values of the resistant *E. colona* accessions ET2 and ET4 were up to 1098 and 1220 g a.e. ha⁻¹ of glyphosate, respectively, whereas the GR₅₀ value of the susceptible accession (FT5) was only 98 g a.e. ha⁻¹. The resistance indices of ET2 and ET4 were 12.4 and 11.2, respectively, indicating that they have already developed resistance to glyphosate. Three more accessions could be also of developing resistant to glyphosate. This is the first report of glyphosate resistance from E. colona accessions in Greece, with indications of multiple resistance also present. Further research is needed in order to evaluate the efficacy of several herbicides under different soil and climatic conditions, conduct baseline sensitivity studies, reveal the evolvement of resistance patterns to glyphosate from accessions of Echinochloa spp., and search for alternative options of weed management in annual and perennial crops due to the clear indications of multiple resistance situations.

Keywords: *E. colona*; profoxydim; cyhalofop-butyl; penoxsulam; florpyrauxifen-benzyl; glyphosate; dose-response; resistance index

1. 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 [1]. Globally, rice crop occupies more

than 158 million ha of arable land [2]. 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. Weed competition is detrimental to rice production since up to 96% of average yield losses in rice are attributed to weed competition [3-5]. Echinochloa spp. pose a significant threat to rice productivity globally and are usually reported as noxious weeds in several economically important crops around the world [6]. Echinochloa colona (L.) Link (also known as jungle rice, Deccan grass, or awnless barnyard grass) is a C₄ annual summer grass native to Europe and India. It is a problematic weed in more than 60 countries and 35 crops [7]. E. colona has vigorous growth traits and high seed production since each *E. colona* plant is capable of producing up to 42,000 seeds. Seed germination occurs at different ranges of soil temperature and soil moisture conditions [8,9]. It has to be noted that *E. colona* is a troublesome weed species in rice, but also in other summer crops, including maize (Zea mays L.), cotton (Gossypium hirsutum L.), sugarcane (Saccharum officinarum L.), sorghum ((Sorghum bicolor (L.) Moench), and also in several perennial crops [10]. The presence of E. colona has been reported at remarkable densities in 24 countries in dry-seeded rice, in 12 countries in wet-seeded rice, and also in transplanted rice [5,11]. Season-long competition of E. colona caused a 43% yield reduction in transplanted rice [12]. Fischer et al. [13] observed up to 62% average yield loss in rice due to E. colona infestation. E. colona competition effects are more obvious in direct-seeded rice as compared to transplanted rice because of crop-weed interference during the early growth stages rice [14]. Moreover, this weed is common in orchards with many reports of difficulties on its management [9,10].

E. colona control may include several management options, such as mechanical, cultural (utilizing resistant rice phenotypes), allelopathic control etc. However, in many cases its control is mainly dependent on synthetic herbicides, either pre-emergent (including metribuzin, pendimethalin etc.) or post-emergent. At least for Mediterranean countries, farmers mostly use pre-emergent herbicides. For instance, penoxsulam is one of these herbicides, an Acetolactate synthase inhibitor with activity in broadleaf and grass weeds [15]. Cyhalofop-butyl is an aryloxyphenoxypropionate (AOPP) herbicide which controls grasses post-emergence in rice cultivation, especially Echinochloa spp. [16]. Cycloxydim belongs to the cyclohexanedione oxime group for controlling grass weeds of many agricultural and horticultural broad-leaved crops [17]. Profoxydim which belongs to the same group, is applied in rice crop for the post-emergence management of the grass weeds [18]. Florpyrauxifen-benzyl ester is a pyridine-2-carboxylate auxin herbicide with unique mode of action and high efficacy against broadleaves, grasses, and sedges [19]. Glyphosate is one of the most widespread and efficient non-selective herbicides globally, whose application is a common control practice for E. colona [20–22]. However, due to the continuous use of glyphosate, several E. colona accessions have been confirmed as resistant to glyphosate [23–26]. This is true either in genetically modified (GM) annual crops or in perennial crops, whereas the use of glyphosate is very often and extended. Unfortunately, according to Heap (2020), E. colona biotypes from many countries (e.g., Argentina, Australia, USA, Benezuela, and Bolivia) have already developed resistance to several herbicides (including glyphosate, trazine, cyhalofop-butyl, fenoxaprop-P-ethyl, and propanil).

The present study was conducted due to our personal communications and the reports of *E. colona* becoming difficult to control in both annual and perennial crops of Greece. Consequently, the first objective of the present study was to evaluate the efficacy of penoxsulam, profoxydim, cycloxydim, cyhalofop-butyl, florpyrauxifen-benzyl, and glyphosate against six *E. colona* accessions. The second objective was to study the response of these accessions to different application rates of glyphosate in order to clarify whether some accessions have really developed resistance to glyphosate and in what extent.

2. Materials and Methods

2.1. Plant Material Collection and Seed Pretreatment

The prefectures of Etoloakarnania (ET), Thessaloniki (TH), and Fthiotida (FT) were selected for *E. colona* seed collection surveys from 27 August and 30 September 2017 (Table 1). In the prefecture of Thessaloniki (a region of Chalastra), rice is cultivated as a monoculture, whereas crop rotation regimes are often adopted in the prefectures of Etoloakarnania (a region of Katochi) and Fthiotida (a region of Anthili). Etoloakarnania was also selected since there were some reports concerning difficulties with *E. colona* control by means of glyphosate in perennial crops (olives and citrus) near the rice fields. Each surveyed field was walked through by the two diagonals and after personal communication records were kept of the *E. colona* plants present. In each field, panicles and seeds were collected from 20 plants for testing at the Laboratory of Agronomy (Agricultural University of Athens).

Table 1. Prefecture, code and geographical position of *E. colona* accessions included in the present study. Single accessions were collected from each location.

Prefecture	Code	Position
Etoloakarnania	ET2 ET4	38°23′11″ N, 21°09′09″ E 38°22′02″ N, 21°08′43″ E
Chalastra	TH7 TH8	40°37′28″ N, 22°44′49″ E 40°36′21″ N, 22°44′33″ E
Fthiotida	FT3 FT5	38°51′04″ N, 22°31′04″ E 38°50′18″ N, 22°32′02″ E

The seeds were separated, air-dried and stored in paper bags in a non-air conditioned glasshouse. Seeds were chemically scarified in concentrated sulfuric acid followed by rinsing in deionized water. For the two pot experiments, ten of the collected seeds from each accession were sown in separate plastic pots $(12 \times 13 \times 5 \text{ cm}^3)$. Pots were filled with a mix of herbicide-free soil from the experimental field of the Agricultural University of Athens and peat at the ratio of 1:1 (v/v). The pots were watered as needed. During the experiment, the pots were uniformly watered as needed and supplied with 50 mL of modified Hoagland's solution (0.25 strength) pot every 10 days [27]. All pots were placed outdoors, with average air temperatures ranging from 22 to 36 °C. The pot experiments were arranged in a randomized complete block design, with five replicates for each treatment. The pots were randomized every 5 days in order to achieve uniform growth conditions for all plants.

2.2. Efficacy of Herbicides

In Experiment 1, the systemic herbicides profoxydim, cyhalofop-butyl, cycloxydim, penoxsulam, florpyrauxifen-benzyl, and glyphosate were applied at their maximum recommended label field rates at the 3–4 leaf growth stage of *E. colona* (Table 2). The experiment was carried out from 2 May until 12 August 2018. The herbicide application was carried out by using a custom-built sprayer equipped with a flat-fan nozzle, calibrated to deliver 300 L ha $^{-1}$ at 250 kPa. Products were solely used, without any surfactant or other additive in the tank mix. Each accession included 50 plants from which the half were kept untreated. Ten plants were cut at 7 and 14 DAT, above the soil surface, and then dried at 60 °C for 48 h. The dry weight of the samples was measured at 7 and 21 DAT and calculated as a percentage of the untreated control (readability of 0.01 g, PFB 200-3, KERN).

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Chemical Class	Chemical Class Active Ingredient		Trade Name	
Cyclohexadione	Profoxydim	$150 { m g \ a.i. \ ha^{-1}}$	Aura® 20 EC	
Cyclohexadione	Cycloxydim	$200 {\rm g} {\rm a.i ha^{-1}}$	Focus [®] 10 EC	
Aryloxyphenoxypropionate	Cyhalofop-butyl	$300 \mathrm{g} \; \mathrm{a.i.} \; \mathrm{ha}^{-1}$	Clincher™ Neo 200 EC	
Triazolopyrimidine	Penoxsulam	$40.8 \mathrm{g} \; \mathrm{a.i.} \; \mathrm{ha^{-1}}$	VIPER OD	
Arylpicolinate	Florpyrauxifen-benzyl	$300 \text{ g a.i. ha}^{-1}$	Loyant [®] 25 EC	
Phosphanoglycine	Glyphosate	720 g a.e. ha^{-1}	ROUNDUP FLEX	

Table 2. Application rates for the herbicides applied in Experiment 1.

2.3. *Glyphosate Dose-Response Experiment*

Experiment 2 was carried out to study the response of all the six $E.\ colona$ accessions to glyphosate in a dose-response experiment. This experiment was performed to determine the herbicide dose needed for a 50% reduction in dry weight (GR50) and was conducted from 2 May until 12 August 2018. All experiments were repeated, without significant differences between the two runs. Herbicide treatments were applied with the same experimental sprayer which was described above without the addition of any surfactant. Glyphosate was applied at six doses corresponding to 0, 1/4X, 1/2X, X, 2X, and 4X of maximum recommended label field rate (X). At 21 DAT, control of weed accessions was assessed by determining the dry weight of all survived plants in each pot and data were expressed as percentage of the untreated control for each accession.

2.4. Statistical Analysis

Data obtained from the pot experiments were analyzed by ANOVA. There were not significant differences between the two runs of experiments. Fisher's protected LSD test at an = 5% probability level was used to separate means regarding the efficacy of each herbicide applied on all the accessions studied. The weed biomass data are expressed as a percentage of the untreated control. The GR_{50} values were obtained by nonlinear regression using the following log-logistic equation [28]:

$$y = c + \langle (d - c)/1 + \exp\{b [\log(x) - \log(GR50)]\} \rangle$$
 (1)

where y represents dry weight at herbicide dose (X) whereas c and d denote the lower and upper limits, respectively, GR_{50} is the herbicide dose centered between the asymptotic values, and b is the slope of the response curve. The level of resistance was expressed by means of the resistance index (RI), which was calculated as the ratio of the GR_{50} of each potentially resistant (R) accession by the GR_{50} of the most susceptible (S) biotype [29,30].

3. Results

3.1. Efficacy of Several Herbicides on E. Colona Accessions

In Experiment 1, profoxydim reduced dry weight of ET2 and TH8 by 30% as compared to the control. Similar findings were revealed for the dry weight of TH7 and FT5 in the measurement carried out at 7 DAT. Biomass of ET4 was significantly lower (58% of control) as compared to the value recorded for the accessions mentioned above, whereas biomass of FT3 was reduced at approximately 50% of the untreated control. The results of the first measurement showed the slight effects of cyhalofop-butyl spray on three *E. colona* accessions (TH7, TH8, and ET4). Dry weight of ET2 was significantly lower (*p*-value < 0.001) and was recorded at 68% of the untreated control. FT3 biomass was reduced by 56% as compared to the biomass of the untreated plants and the most susceptible accession to cyhalofop-butyl was FT5. Cycloxydim was not effective against TH7 and ET2, whereas TH8 dry weight was recorded at 44% of control. The efficacy of this herbicide was significantly improved on ET4 and FT3. The most susceptible accession was FT5 whose biomass was reduced by 82% in comparison to the value recorded for the untreated plants. The low efficacy of penoxsulam against five out of

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six accessions studied was remarkable since their dry weight was recorded at 74%–94% of control. In contrast, florpyrauxifen-benzyl application resulted in 60%–66% lower biomass than the value recorded for the untreated plants for five accessions as observed from the measurement conducted at seven days after treatment. It was also noticed that FT5 was the most susceptible accession to glyphosate since its dry weight was reduced by 76% as compared to the control at seven days after treatment. Dry weight of FT3 and TH8 was recorded at 36% and 42% of the untreated control, respectively, whereas dry weight of TH7 was significantly higher than the values recorded for the accessions mentioned above (p-value < 0.001). In addition, extremely low efficacy of glyphosate was observed against ET2 and ET4 (Table 3).

Table 3. Efficacy of several herbicides on *E. colona* accessions at 7 days after treatment (7 DAT). Dry weight of each accession was expressed as % of control.

Accession	PXD ²	СВ	CXD	PX	FB	GLY
ET2	70 a ¹	68 b	74 a	94 a	37 b	85 a
ET4	58 b	75 ab	27 c	92 a	35 b	84 a
TH7	65 a	73 ab	78 a	87 a	64 a	54 b
TH8	70 a	76 a	44 b	43 c	35 b	42 c
FT3	49 c	44 c	34 c	79 b	40 b	36 c
FT5	65 a	34 d	18 d	74 b	34 b	24 d
LSD (0.05)	6	7	8	7	7	8
<i>p</i> -Value	**	***	***	***	***	***

 $^{^1}$ Different letters in the same column indicate the significant differences between the means of the accessions for each herbicide at a = 5% significance level. 2 PXD = Profoxydim, CB = Cyhalofop-butyl, CXD = Cycloxydim, PX = Penoxsulam, FB = Florpyrauxifen-benzyl, GLY = Glyphosate. **, *** = significant at 0.01 and 0.001, respectively.

In the measurement carried out at 21 DAT, dry weight of TH8 and TH7 after profoxydim treatment was recorded at 66 and 68% of the untreated control, respectively, while dry weight of ET2 was significantly lower than the values recorded for TH8 and TH7 (p-value < 0.001). Biomass of ET4 and FT3 was reduced at approximately half the value recorded for the untreated plants and FT5 was significantly the most susceptible accession to profoxydim. The results of the second measurement also revealed that TH7 and TH8 were slightly affected by cyhalofop-butyl application. Dry weight of ET2 and ET4 was significantly lower and was recorded at 59% and 66% of control, respectively. FT5 biomass was reduced by 66% as compared to the value recorded for the untreated plants and biomass of FT3 was even lower. Cycloxydim spray was quite ineffective against TH8 whereas its efficacy was greater against ET2 accession. Dry weight of TH7 was reduced by 66% as compared to the control. Moreover, dry weight of FT5 and ET4 was reduced by 76% and 82%, respectively, as compared to the values recorded for the untreated plants and FT3 was the most susceptible accession, out of all, to this herbicide. The efficacy of penoxsulam against ET2 and ET4 was extremely low, whereas dry weight of FT5 and TH8 was reduced only by 23%-28% as compared to the control at the measurement carried out at 14 DAT. Penoxsulam was effective only against TH7 whose dry weight was reduced by 67% as compared to the control due to the application of this herbicide. Florpyrauxifen-benzyl application resulted in 73%-76% lower dry weight for five accessions than the value recorded for the untreated plants, as noticed in the second measurement conducted at 21 DAT. TH8 dry weight was reduced at approximately half of the value recorded for the untreated plants. ET2 and ET4 dry weight was reduced by only 18% and 22%, respectively, as compared to the control when treated with glyphosate. TH7 dry weight was significantly lower than that of ET2 and ET4 (p-value < 0.001), whereas dry weight of TH8 and FT3 was recorded at 27% and 29% of control, respectively. The most susceptible accession to glyphosate was FT5 whose biomass was recorded at 12% of the untreated control at 21 DAT (Table 4).

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Accession	PXD ²	CB	CXD	PX	FB	GLY
ET2	60 b ¹	59 b	64 b	84 b	27 b	82 a
ET4	51 c	66 b	18 d	90 a	25 b	78 a
TH7	68 a	80 a	34 c	33 e	25 b	41 b
TH8	66 a	82 a	80 a	77 c	47 a	27 c
FT3	51 c	24 d	10 e	67 d	25 b	29 c
FT5	43 d	34 c	24 d	72 cd	24 b	12 d

Table 4. Efficacy of several herbicides on *E. colona* accessions at 21 days after treatment (21 DAT). Dry weight of each accession was expressed as % of control.

7

5

6

4

7

5

3.2. *Glyphosate Dose-Response Experiment*

LSD (0.05)

v-Value

In Experiment 2, when treated with glyphosate at 1/4 of the recommended rate the dry weight reduction for TH7, FT3 and TH8 was 30%, 32%, and 37%, respectively, as compared to the untreated control. ET2 and ET4 were almost unaffected whereas the dry weight of the most susceptible accession was reduced by approximately 50% of the value recorded for the untreated individuals. Applying glyphosate at half of the recommended rate resulted in 72% lower dry weight for the susceptible accession whereas dry weight of TH8, FT3, and TH7 was recorded at 47%, 51%, and 59% of the control. In addition, slight reductions were observed in the dry weight of ET2 and ET4. On the contrary, biomass of FT3 and TH8 was by 71%–73% lower as compared to the untreated control, whereas for the susceptible accession, dry weight reduction was up to 88%. Furthermore, TH7 dry weight was reduced by 59% compared to the untreated plants. When treated with double of the recommended rate, dry weight of the susceptible accession (FT5) was almost zero (all plants dead), while FT3 and TH8 biomass was reduced by almost 80% as compared to the control. Biomass of TH8 was recorded at 33% of control whereas even greater values were recorded for ET2 and ET4. Even when treated with glyphosate at 4-fold rate, ET2 and ET4 dry weight reductions were lower than 75% as compared to the untreated control. The reduction observed for TH7 was about 80%. TH7 and FT3 dry weight was recorded at 9%-11% of the control, whereas biomass of the susceptible accession was completely eliminated (Table 5).

Table 5. Dry weight and GR_{50} of *E. colona* accessions after application of glyphosate at different rates. The measurement was carried out at 21 days after treatment (21 DAT). Dry weight of each accession was expressed as % of control.

Accession	GR_{50} (g a. e. ha $^{-1}$)	0	1/4X	1/2X	х	2X	4X
ET2	1220	100	94	87	82	49	30
ET4	1098	100	95	86	78	44	27
TH7	418	100	70	59	41	33	21
TH8	356	100	63	47	27	19	9
FT3	363	100	68	51	29	18	11
S ¹	98	100	47	28	12	3	0

¹ S (FT5) was the most susceptible accession to glyphosate.

According to the GR_{50} values obtained in the present study, RI for glyphosate ranged between 3.7 and 12.4 (Table 6). ET2 was the most resistant *E. colona* accession, since its resistance index was up to 12.4. ET2 was almost three times more resistant than TH7 and its resistance index value was by approximately 70% higher than the corresponding value for TH8 and FT3. ET4 was the second

 $^{^1}$ Different letters in the same column indicate the significant differences between the means of the accessions for each herbicide at a = 5% significance level. 2 PXD = Profoxydim, CB = Cyhalofop-butyl, CXD = Cycloxydim, PX = Penoxsulam, FB = Florpyrauxifen-benzyl, GLY = Glyphosate. **, *** = significant at 0.01 and 0.001, respectively.

most resistant accession to glyphosate, since its resistance index was 11.2. This accession was about three times more resistant to glyphosate than TH8 and FT3 and its resistance index was 62% greater than the corresponding value of the resistance index estimated for TH8. Resistance indices of TH8, FT3 and TH7 were 3.6, 3.7, and 4.3, respectively. The results of the dose-response experiment indicate that ET2 and ET4 accessions are resistant to glyphosate whereas TH7, FT3, and TH8 could be potentially resistant; however, more experiments are required.

Table 6. Resistance index (RI) of *E. colona* accessions according to the dose-response experiment. The data is based on GR_{50} values calculated from dry weight data.

Accession	Resistance Index
ET2	12.4
ET4	11.2
TH7	4.3
TH8	3.6
FT3	3.7
S ¹	1.0

¹ S (FT5) was the most susceptible accession to glyphosate.

4. Discussion

The results of the current study revealed the low efficacy of profoxydim against three *E. colona* accessions. These findings are in line with the ones presented by Kanatas [18], who indicated that dry weight of one *Echinochloa crus-galli* (L.) P. Beauv. accession was reduced by 40% when treated with profoxydim at a rate of 400 g a.i. ha⁻¹ and this accession was characterized as resistant to this herbicide (while other two accessions were characterized as intermediate resistant). In a previous study, profoxydim application at the recommended rate resulted in excellent control of over the level of 90% for seven accessions of *Echinochloa phyllopogon* [Stapf] Stapf ex Kossenko [31]. Profoxydim has been reported to effectively control barnyardgrass biotypes resistant to imidazolinones [32], even if in some cases there was cross-resistance to penoxsulam, bispyribac, imazamox, foramsulfuron, nicosulfuron, and rimsulfuron [33]. There is evidence that the efficacy of profoxydim for the control of *Echinochloa* spp. varied and is dependent-among the others-on the growth stage of the weeds. For instance, applying the herbicide at BBCH 13 and BBCH 22 stages results in 20%–30% and 30–50% increased control, respectively, as compared to the case where the herbicide is applied at BBCH 30 growth stage [18].

In our study, the efficacy of cyhalofop-butyl against TH7 and TH8 was extremely low, whereas two accessions were susceptible to this herbicide. Singal et al. [34] recorded complete mortality for all the Echinochloa spp. accessions treated with cyhalofop-butyl. In the study of Vidotto et al. [35] it was noted that Echinochloa spp. relative growth was reduced by approximately 74% and 83% when treated with half of and the full recommended rate of cyhalofop-butyl, respectively. However, there is evidence from a study conducted in Northern Greece suggesting that some *E. phyllopogon* accessions are less sensitive to cyhalofop-butyl as compared to E. crus-galli populations since stem number reduction for the first species was recorded at 52%-56% of control while for the second species the corresponding value of reduction was recorded at 92%–96% of the control [36]. Similar were the findings of another study carried out in Italy where E. crus-galli populations showed increased sensitivity to this herbicide in comparison to E. phyllopogon populations [35]. Moreover, Kalsing et al. [37] demonstrated that emphasis must be put on the timing of the application since sufficient control can be achieved only if spraying is carried out among the BBCH 12 and BBCH 14 growth stages of *Echinochloa* spp. The importance of applying cyhalofop-butyl up to the phenological stages of three to four leaves in order to assure optimum control of Echinochloa spp. populations has also been highlighted by Damalas et al. [36]. However, in another study, application of cyhalofop-butyl even at four leaf stage

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was quite ineffective against *E. colona*, since seedling survival was up to 62% of the untreated control, whereas a further delay caused a total loss of efficacy [38].

Penoxsulam showed low efficacy against five out of six E. colona accessions and this outcome is in agreement with a previous study where three biotypes of *E. crus-galli* were confirmed as resistant to this herbicide [39]. Resistant indices for one E. colona and one E. crus-galli population were 10.95 and 14.98, respectively, in the dose-response experiment of Sanchotene et al. [40]. In addition, resistance indices to penoxsulam have been reported to reach the values of 28.1 and 13.7 for E. phyllopogon and E. crus-galli, respectively [41]. E. crus-galli populations resistant to penoxsulam were also found in the studies of Yang et al. [42] and Le et al. [43]. In another study, the efficacy of penoxsulam was low against E. crus-galli accessions since it ranged from 25% to 32% for plants derived from seeds collected from plots treated with penoxsulam [44]. Penoxsulam applied at a rate of 40 g a.i. ha⁻¹ to 320 g a.i. ha⁻¹ provided intermediate reduction of shoot number and fresh weight of one E. colona biotype in the dose-response experiment of Vasilakoglou et al. [31]. There is evidence suggesting that the efficacy of penoxsulam for the control of Echinochloa spp. is dependent on the species, as well as on the growth stage of the weeds when herbicide application is carried out. In particular, penoxsulam application at the rate of 25 g a.i. ha⁻¹ has been found to be effective against *E. colona* when the application is carried out at 4-leaf growth stage, whereas efficient control of E. crus-galli can be achieved by the same treatment at 8-leaf growth stage [38].

The results of the current experiments also revealed that cycloxydim application provided sufficient control of three accessions and such findings are in agreement with the ones of other studies. In particular, all Echinochloa spp. accessions were controlled adequately when treated with all the cycloxydim rates below the maximum one as it was observed in the dose-response experiments of Claerhout et al. [45]. Field history has been reported as a factor affecting the efficacy of cycloxydim for the control of Echinochloa spp. Compared to populations from monoculture corn fields, populations of Echinochloa crus-galli originating from organic fields were significantly more sensitive to cycloxydim, whereas populations from the conventional crop rotation system showed intermediate sensitivity levels [46]. Particularly, when treated with cycloxydim, lower biomass was recorded for E. crus-galli populations collected from organic fields where crop rotation systems had been adopted, as compared to the values recorded for populations collected from fields where maize monoculture was the rule [47]. In cycloxydim-tolerant maize crop, applying cycloxydim at a rate of 150 g a.i. ha⁻¹ resulted in 96%–99% less ground coverage for E. crus-galli in comparison to control treatment [48]. Regarding the efficacy of florpyrauxifen-benzyl, it was noticed that the majority of the E. colona accessions was adequately controlled by the application of this herbicide and this finding is in accordance with those of previous studies. Florpyrauxifen-benzyl is well recognized as a new herbicide providing an attractive alternative for Echinochloa spp. control and an effective resistance management tool due to its unique binding site [49]. The dose-response experiment of Duy et al. [50] revealed that the LD₉₀ values of seven E. crus-galli accessions treated with florpyrauxifen-benzyl ranged between 15.1 and 19.3. Miller et al. [51] also found a dry weight reduction of 152 E. crus-galli accessions (including the quinclorac-resistant ones) higher than 90% at 21 days after application of florpyrauxifen-benzyl at 30 g a.i. ha⁻¹. In the study of Miller and Norsworthy [52], E. crus-galli biomass and plant height reduction after florpyrauxifen-benzyl application at a rate of 30 g a.i. ha⁻¹ were up to 84% and 86%, respectively, with values significantly higher than the corresponding values recorded when other herbicides were applied.

The results of the glyphosate dose-response experiment revealed that the GR_{50} values of the resistant *E. colona* accessions, ET2 and ET4, were estimated at 1220 and 1098 g a. e. ha^{-1} of glyphosate, respectively, whereas the GR_{50} value of the susceptible accession was 98 g a. e. ha^{-1} . This finding of the very low glyphosate efficacy against the two accessions from Etoloakarnania is probably related to the extended use of glyphosate in the nearby orchards of Katochi region. This outcome is in line with the results of Goh et al. [20], where the estimated GR_{50} values for the susceptible and the resistant populations were 173 and 1440 g a. e. ha^{-1} , respectively. In addition, Mahajan et al. [53] reported that the GR_{50} values of the resistant *E. colona* accessions ranged between 1086 and 1153 g a.e. ha^{-1} .

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Less than 20% of control has been reported for three E. colona populations following treatments with glyphosate at the rates between 325 and 2600 g a.e. ha^{-1} [53]. It is well established that overdependence on glyphosate for control of E. colona led to the problem of glyphosate-resistant E. colona [54]. All glyphosate-resistant E. colona plants were unaffected when treated with either the recommended or the double rate in the study of Mutti et al. [55]. The development of glyphosate-resistant populations of E. colona has also been mentioned by other scientists [23–26]. Model analysis of Bagavathiannan et al. [56] revealed that the risk of Echinochloa spp. resistance to glyphosate is high in fields characterized by high barnyardgrass seedbank levels, seedling emergence, and seed production per square meter, while the risk is low in fields with high levels of post dispersal seed loss and annual seedbank loss. The current study revealed that the application of the recommended label rate of glyphosate reduced dry weight of the resistant E. colona accessions (ET2 and ET4) only by 18%–22%, as compared to the untreated control. Such findings are in accordance with the corresponding of Mollaee et al. [22] who noticed that the application of the recommended rate of glyphosate reduced the dry weight of a resistant E. colona by 25% under conditions of increased soil moisture, whereas this accession under water stress conditions was completely unaffected. The susceptible accession studied during this experiment was controlled by 72%, 88%, and 97% when treated with 1/2X, X and 2X rates of glyphosate. Dose-response studies under greenhouse conditions showed that a susceptible population was controlled by 91% and 99% with 0.86 and 1.72 kg ha⁻¹ rates, respectively, while a suspected resistant *E. colona* population was controlled by 55% and 84% with glyphosate at 1.92 and 3.85 kg ha⁻¹, respectively [21].

It should be noted that there is evidence that *E. colona* resistance to glyphosate can be influenced by environmental factors [57]. Soil moisture conditions as well as temperature are also key factors affecting the efficacy of glyphosate against Echinochloa spp. In particular, Mollaee et al. [22] noted that when treated with 1440 g a. e. ha⁻¹ of glyphosate, the resistant population survival was recorded at 19% under increased soil moisture conditions, whereas this value was by 43% higher under water-stress conditions. Increased GR₅₀ values, resistance indices, and survival percentage were observed at 30 °C in glyphosate-resistant E. colona populations during both the experiments of Nguyen et al. [58]. In the current study, the application of 1/2X rate of glyphosate resulted in 72 and 13%–14% lower dry weight for the susceptible and the resistant accessions, respectively. These observations are in conformity with the findings of another study where treatment with 1/2X of glyphosate reduced survival rate of the susceptible and the resistant *E. colona* populations by 66% and 12%, respectively [20]. Goh et al. [20] also mentioned that applying glyphosate at the recommended label rate resulted in 88% and 23% lower survival while similar results were obtained during the present study. In our study, resistance indices of ET2 and ET4 were higher than 10, whereas Gaines et al. [59] and Goh et al. [20] estimated that the resistance indices of the moderately resistant and the resistant E. colona populations were 5.6 and 8, respectively. The resistance levels of ET2 and ET4 were also higher than those reported from Han et al. [57] (2–2.5), Alarcón-Reverte et al. [60] (6.6), and Alarcón-Reverte et al. [61] (4–9). In any case, the present study is the first report of glyphosate-resistant E. colona in Greece. It has also to be noted that the low efficacy of the several herbicides against E. colona observed in the present study is clearly not a matter of natural tolerance, since all of them-according to their label-are known to adequately control the specific weed. Nevertheless, a baseline sensitivity study along with shikimic tests and studies on mechanisms involved are necessary for the future studies on E. colona and the accurate quantification of glyphosate resistance. The findings of the present study are in accordance with the ones reported in previous studies, confirming that the efficacy of several herbicides against E. colona is low, while some accessions of this noxious grass weed species have already developed resistance to glyphosate. Our results reveal that there are indications for multiple resistance issues in this species and therefore, alternative control options need to be evaluated for their efficacy in the management of Echinochloa spp. either in annual or in perennial crops.

5. Conclusions

The results of the current study revealed the low efficacy of profoxydim against three *E. colona* accessions. The efficacy of cyhalofop-butyl against two accessions was low, whereas two accessions were susceptible to this herbicide. Penoxsulam showed low efficacy against five out of six *E. colona* accessions. Cycloxydim application provided sufficient control of three accessions. It was also noticed that the majority of the *E. colona* accessions was adequately controlled by the application of florpyrauxifen-benzyl. Our findings also confirmed that ET2 and ET4 have developed resistance to glyphosate, with resistance indices higher than 10. Three more accessions might be resistant as well, with further studies required. This is the first case of resistance to glyphosate from *E. colona* accessions in Greece. Further research is needed in order to evaluate the efficacy of several herbicides under different soil and climatic conditions, conduct a baseline sensitivity study, reveal the evolvement of resistance patterns to glyphosate from accessions of *Echinochloa* spp., and search for alternative options of weed management in annual and perennial crops due to the clear indications of multiple resistance situations.

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