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Herbicide resistance development in winter wild oat (*Avena sterilis* subsp. *ludoviciana*) populations: Field margins vs. within fields

Hamidreza Sasanfar[a,*], Eshagh Keshtkar[b], Eskandar Zand[a], Mohammad Hossein Zamani[b], Behrouz Khalil Tahmasebi[a]

[a] Department of Weed Research, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran. [b] Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

Abstract: **Background:** The resistance of grass weeds to herbicides is expanding in wheat fields. An effective strategy for managing herbicide resistance is to prevent the likelihood of resistance development spreading from field margins to within fields. **Objective:** This study was conducted to evaluate the resistance development in winter wild oat (*Avena sterilis* subsp. *ludoviciana*) populations collected from within fields and field margins of 11 winter wheat fields to the commonly used ACCase and ALS-inhibiting herbicides. **Methods:** Seeds of 22 *A. sterilis* subsp. *ludoviciana* populations were collected, both from field margins and within winter wheat fields. The seeds were grown in greenhouse, and the seedlings at the three- to four-leaf stage were treated with the recommended field rates of the following four herbicides, clodinafop-propargyl (CP), pinoxaden (PN), mesosulfuron+iodosulfuron (MI), and mesosulfuron+iodosulfuron+diflufenican (MD). **Results:** All populations from within fields evolved resistance to CP, but none showed cross-resistance to PN. Importantly, there were notable variations in CP and MI resistance, with less than half of the samples from within fields demonstrating higher resistance, while for the remaining populations no differences were observed between field-margin and within-fields samples. Contradictory trend was observed in response to MD, where around 70% of populations followed the trend for CP and MI, surprisingly, around 30% of populations exhibited opposite results. **Conclusions:** Overall, the incidence of herbicide resistance to CP, MI, and MD was more common in populations from within fields, suggesting stronger selection pressure. However, some field margin populations showed MD resistance, underscoring the need for weed control in field margins as a proactive resistance management strategy.

Keywords: ACCase inhibitor; ALS inhibitor; Herbicide resistance; Winter wild oat

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* Corresponding author:
<sasanfar@iripp.ir>

1. Introduction

The development of resistance to herbicides is a worldwide dilemma. Currently, 523 unique cases of weeds have evolved resistance to 167 different herbicides with 21 known sites of action in 99 crops in 72 countries (Heap, 2023). Herbicide-resistant weed species are difficult to control in the agroecosystems of developed and developing countries, in which herbicides are still the most cost-effective method for weed control (Ofosu et al., 2023; Peterson et al., 2018), especially in broadcast cropping systems such as winter wheat. The majority of cases of weed resistance to herbicides have been reported in cereals, with wheat (*Triticum aestivum* L.) having the most cases, at 359 (Heap, 2023).

Wheat is indeed a major crop grown in almost all provinces of Iran, due to its resilience to the varied climates and the importance of this crop in food security (Ministry of Agriculture Jihad, 2023). More than 400 weed species are found in wheat fields, including winter wild oat (*Avena sterilis* subsp. *ludoviciana* (Durieu) Gillet & Magne), rigid ryegrass (*Lolium rigidum* Gaudin) and little-seed canarygrass (*Phalaris minor* Retz.) which are the most troublesome monocots and result in significant yield loss (Zand et al., 2019). Acetyl coenzyme a carboxylase (ACCase) and acetolactate synthase (ALS) inhibitor herbicides have been widely used in wheat fields of Iran for over the past two decades to control grass weeds, especially *A. sterilis* subsp. *ludoviciana*. Although herbicides are effective tools in weed management, consecutive and inconsiderable application of herbicides with similar modes of action in wheat fields has led to resistance to the two mentioned herbicide groups in weeds (Gherekhloo et al., 2016). These two groups of herbicides, alongside photosystem II (PSII-serine 264 binders) and enolpyruvyl shikimate phosphate synthase (EPSPS) inhibitors, have the highest frequency of resistance cases among all herbicide groups (Heap, 2023). The Poaceae family with 90 resistant species accounts for the most herbicide resistant cases, which wild oat, ryegrass, blackgrass and canary grass are the most prevalent weeds that have developed resistance to herbicides in wheat (Heap, 2023).

Resistance to ACCase- and ALS-inhibiting herbicides in *A. sterilis* subsp. *ludoviciana* populations were first reported in France in 1996 and in Australia in 2005 (Heap, 2023). In Iran, following the use of simple cultivation system and the consecutive use of herbicides with the same mode of action, the first case of herbicide resistance was reported in a *A. sterilis* subsp. *ludoviciana* population collected from wheat fields to fenoxaprop-P-ethyl, an ACCase-inhibiting herbicide (Zand et al., 2004), while the first ALS resistant *A. sterilis* subsp. *ludoviciana* population was detected in 2009 (Heap, 2023). The patterns of cross and multiple resistance to ACCase and ALS inhibitors, as the dominant graminicides, in *A. sterilis* subsp. *ludoviciana* populations have also been well characterized (Hassanpour-bourkheili et al., 2021; Joumi et al., 2022; Sasanfar et al., 2017a). Multiple resistance to the two modes of action was confirmed in both Canadian and Iranian *Avena* spp. populations (Beckie et al., 2020; Heap, 2023). Moreover, the target-site and non-target-site mechanisms conferring multiple resistance to ACCase- and ALS-inhibiting herbicides have been reported in spring and winter wild oat populations (Beckie et al., 2012; Torres-García et al., 2018).

Herbicide resistance management programs must encompass both proactive and reactive strategies. One of the best management practices for proactively combating herbicide resistance is managing field borders to prevent the entry of herbicide-resistant weeds (see BMP 12 in (Norsworthy et al., 2012). Some of these field-margin tactics include implementing haymaking, maintaining dense cover crops, sowing less-weedy species, mowing, and the application of non-selective herbicides in the field margins (Noordijk et al., 2011; Norsworthy et al., 2012). However, farmers often neglect the employment of these field-margin tactics to prevent herbicide-resistant weed entry into their fields, especially in small-scale lands of Iran. This implies that field-margin weeds might rarely be exposed to herbicides compared to those weeds grown within fields. Consequently, it can be expected that the herbicide resistance level in weeds grown on field margins might be lower than those grown within fields. However, it is generally accepted and has been claimed that field margin weeds can act as a pathway not only for the introduction and spread of new weed species but also for herbicide-resistant weed biotypes (Norsworthy et al., 2012). Yet, to the best of our knowledge, the later hypothesis, i.e., field margin weeds as a source of herbicide resistance, has not been tested.

Therefore, the objective of this research was to elucidate whether the occurrence of herbicide resistance in *A. sterilis* subsp. *ludoviciana* populations grown in field margins differs from those grown within fields. In a province-wide study, we sought to answer this question surveying several winter wheat fields and conducting herbicide screenings using four common herbicides including clodinafop-propargyl, pinoxaden, mesosulfuron-methyl+iodosulfuron-methyl-sodium and mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican in Iran. The findings are of high importance and provide insight in management of herbicide-resistant *A. sterilis* subsp. *ludoviciana* populations.

2. Material and Methods

2.1 Plant material

A total of 11 winter wheat fields in Ardabil, one of the main wheat production regions in the northwestern of Iran, were selected to evaluate the patterns of the winter wild oat populations resistance to common herbicides. Seeds from 22 populations of winter wild oat were sampled in June 2021, both from field margins and within 11 winter wheat fields, where the growers were not satisfied with the efficiency of the applied herbicides belonging to ACCase inhibitors (e.g., clodinafop-propargyl) and ALS inhibitors (e.g., mesosulfuron-methyl+iodosulfuron-methyl-sodium). The seeds were kept at room temperature until the start of the experiments.

2.2 Screen experiment

To detect the herbicide resistance patterns between the populations collected inside and outside (i.e., the margins) the fields, whole-plant bioassays in pots were carried out testing the recommended field rates of four commonly used herbicides in wheat (Table) using a completely randomized design with four replicates. The experiments were carried out at the research greenhouse of the Weed Research Department at the Iranian Research Institute of Plant Protection, in winter 2022.

Seed dormancy of collected seeds was eliminated before planting through dehulling and moist chilling methods. The seeds were first dehulled manually and lemma and palea were removed from the seed coats and they were placed in the petri dishes filled with two sheets of filter paper. Then, the seeds were semi-soaked in distilled water and the petri dishes were incubated at 4 °C for 72 hours in continuous

Table 1. Herbicides names, mechanisms of action, formulations and recommended field rates applied in the experiments.

Common Name	Trade Name	Mechanism of action	Formulation	Field rate (g a.i. ha^{-1})	Company Name
Clodinafop-propargyl	Topik®	ACCase inhibitor	EC 8%	80	Syngenta
Pinoxaden	Axial®	ACCase inhibitor	EC 5%	60	Syngenta
Mesosulfuron-methyl+iodosulfuron-methyl-sodium	Atlantis®	ALS inhibitor	OD 1.2 (1+0.2)%	18	Bayer
Mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican	Othello®	ALS inhibitor + PDS inhibitor	OD 6 (0.75+0.25+5)%	96	Bayer

ACCase= acetyl-coenzyme A carboxylase, ALS=acetolactate synthase, PDS=phytoene desaturase darkness. After that, the petri dishes were transferred into a growth chamber at alternating temperatures 20/15 °C for 16/8 h light/dark cycles. Immediately after germination (radicle length Approx. 2 mm), for each population, 10 winter wild oat seeds were transplanted at a depth of 1 cm in 500 ml (10-cm diameter) plastic pots containing a mixture of field soil, sand, clay, organic matter and perlite.

The pots were placed in a greenhouse with a 16/8-hour day/light photoperiod at a temperature of 20/15 °C. Irrigation of plants was done whenever required. As the seedlings reached the one-leaf stage, they were thinned to seven individuals in each pot. The seedlings were sprayed with the recommended field rate of the four commonly used herbicides in wheat including clodinafop-propargyl, pinoxaden, mesosulfuron-methyl+iodosulfuron-methyl-sodium and mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican (Table) at the 3-4 leaf stage using an automatic laboratory cabinet sprayer equipped with an even nozzle calibrated to deliver 150 L/ha at a constant pressure of 280 kPa. Non-herbicide treated pots were also assigned as control treatments for each population.

Four weeks after herbicide treatment, the survived and dead individuals were recorded in each pot. The plants were cut at the soil surface, and then seedling dry weights were measured after being dried at 75°C for 48 h. The experiment was repeated twice in the two greenhouse units at the same time.

2.3 Statistical Analysis

The survival and dry weight of plants in every pot were expressed as a percentage of the untreated controls, serving to characterize herbicide resistance (Adkins et al., 1997). The data was analyzed using ANOVA with a mixed model approach, where the experiment run was considered as a random effect, while herbicide, population, and location of sampling (i.e., within and margin) were considered as fixed effects.

All the data were analyzed using the R statistical software (R Core Team, 2021). The ANOVA assumptions, including the normality of residuals and homogeneity of variance, were assessed using the Shapiro–Wilk and Bartlett's tests, respectively, and they were met. The mixed model was run using *lmer* and *anova* functions through the add-on packages "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017). The denominator degrees of freedom were determined using the Satterthwaite method (ddf = "Satterthwaite"). The *lsmeans* and *cld* approaches were used to compare and classify the mean values of treatments using the "lsmeans" (Lenth, 2016) and the "multcomp" packages (Hothorn et al., 2008), respectively. As a three-way interaction emerged among the three main factors (i.e., herbicide, population, and location of sampling), the slicing approach utilizing the *pairwise* function, available in the "lsmeans" package (Lenth, 2016), was employed to exclude/slice the herbicide factor, as the comparison would otherwise be complicated.

3. Results and Discussion

3.1 Clodinafop-propargyl

The survival and dry weight percentages of the populations collected from both within fields and in field margins were affected by the application of clodinafop-propargyl, the most commonly used graminicide in the studied wheat fields. The vast majority of populations, whether collected from within fields and in field margins, exhibited resistance to this herbicide. Other reports also highlight a widespread resistance to Fops in grass species, especially in *A. sterilis* subsp. *ludoviciana* populations (Sasanfar et al., 2017a; Sasanfar et al., 2017b; Travlos et al., 2011). Regarding survival, the overall trend in the population's response indicated that samples from within fields showed higher survival rates compared to those from the field margins. However, with the exception of samples collected from the field margins in W63 and W69 fields, no significant differences were observed in the remaining populations, which maintained a survival rate of over 85%. While the samples collected from within fields of W63 and W69 exhibited 100% survival rates after the clodinafop-propargyl application, those from the corresponding margins showed survival rates of 41.73% and 2.5%, respectively, indicating a statistically significant difference (Figure 1A). These observed discrepancies between within field and field margins samples collected from the W63 and W69 fields could be possibly attributed to the increased selection pressure exerted by the herbicides applied within the fields.

The dry weights of the majority of samples also mirrored the trend in survival rates, showing a tendency in higher dry weights for samples from within fields. However, no statistically significant difference was observed in the seedling dry weights between the studied samples within fields and field margins, except for the W63 and W69 populations. Although samples collected from the W63 and W69 fields retained 90.65% and 84.19% of their dry weights, respectively, in response to clodinafop-propargyl compared to the untreated control, samples collected from the associated fields, i.e., field margins, had dry weights of 21.47% and 39.69%, demonstrating significant differences (Figure 1B).

According to the results, it can be concluded that resistance to clodinafop-propargyl has developed both within fields and at field margins, except for the W63 and W69 populations. This observation may indicate a historically broader exposure of the populations to the selective pressures exerted by this commonly used herbicide, as well as potentially other herbicides with a similar mechanism of action. However, it is worth noting that the samples from within these fields exhibited higher survival rates and dry weights compared to their counterparts from the field margins. Garibaldi et al.

Figure 1 - Survival (A) and dry weight (B) percentages of winter wild oat populations (*Avena sterilis* subsp. *ludoviciana*) collected from both field margins and within 11 winter wheat fields following clodinafop-propargyl application. Similar letters indicate non-significant differences among mean values

(2023) found that smaller fields with higher edge density had a lower presence of herbicide-resistant weeds, suggesting that weeds grown on field margins may have lower resistance levels. Therefore, it may not be claimed that field margin weeds can be considered a source for the introduction and spread of herbicide-resistant weed populations within the fields. This study rejected this hypothesis in at least two out of 11 cases, while a similar trend was observed in the rest of the population. Accordingly, out-crossing weeds grown in field margins can be considered an herbicide-susceptible gene pool spreading susceptible alleles and mating with herbicide-resistant alleles, which may ultimately lead to a dilution of herbicide-resistant alleles within the field. Storer et al. (2003) mentioned that untreated field margins can provide a pool of susceptible individuals, which may be important in delaying the evolution of resistance.

3.2 Pinoxaden

Contrary to the response to the herbicide clodinafop-propargyl, an ACCase inhibitor herbicide belonging to the aryloxyphenoxypropionate (Fop) chemical family, all samples exhibited entirely different reactions to pinoxaden, another herbicide in the same group but of a different chemical family, i.e., the phenylpyrazoline (Den). It means all samples demonstrated susceptibility to pinoxaden based on the survival rates. Neither among the populations nor the collection sites (i.e., within fields and field margins) was there a statistically significant difference. Except for the sample collected from within W49 field, which exhibited a 10.34% survival rate in response to the application of pinoxaden, none of the individuals in the other samples survived after the herbicide application (Figure 2A).

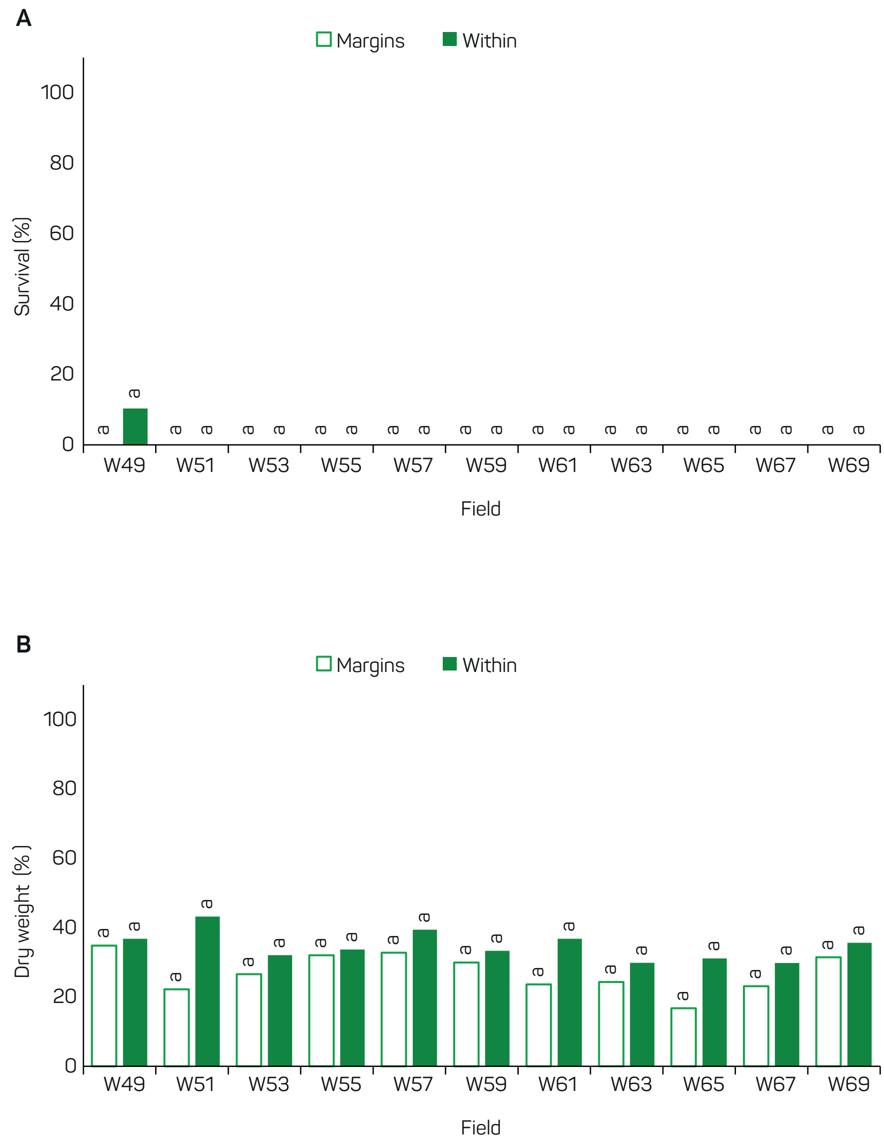

Figure 2 - Survival (A) and dry weight (B) percentages of winter wild oat populations (*Avena sterilis* subsp. *ludoviciana*) collected from both field margins and within 11 winter wheat fields following pinoxaden application. Similar letters indicate non-significant differences among mean values

Across all 11 tested fields, the samples collected from within fields exhibited a tendency in higher dry weight compared to those collected from the field margins. However, no statistically significant difference was observed in terms of dry weight among populations or between the collection sites (i.e., within fields and filed margins), patterning the results of survival rate. It means pinoxaden is still an effective herbicide for controlling *A. sterilis* subsp. *ludoviciana* compared to clodinafop-propargyl. The susceptibility of *A. sterilis* subsp. *ludoviciana* populations to pinoxaden can be attributed to its less frequent historical use in the fields compared to the repeated application of clodinafop-propargyl. In addition, compared to FOP and DIM herbicides, pinoxaden possesses a distinctive chemical structure and a slightly different binding site within the ACCase carboxyl transferase domain (Yu et al., 2010). Hence, reports suggest that this herbicide is more resilient to resistance occurrence in several troublesome grasses to some FOP herbicides (Jang et al., 2013; Sasanfar et al., 2017b). Pinoxaden is considered the ultimate choice among ACCase family herbicides for effectively managing key grass weeds like *Avena* species in specific cereal crops (Kaundun, 2014; Kaundun, 2021).

It is worth noting that, despite susceptibility to pinoxaden, the dry weight of populations collected from both within and margins of the 11 fields ranged from 16.47% (W65, field margins) to 43.18% (W51, within fields) compared to the untreated control (Figure 2B). This not only suggests a potential creeping development of cross-resistance to this herbicide, particularly within fields where weeds are subjected to extensive chemical pressure, but also challenges the general hypothesis that field margins can serve as a pathway for herbicide resistance into fields.

3.3 Mesosulfuron-methyl+iodosulfuron-methyl-sodium

All the 22 *A. sterilis* subsp. *ludoviciana* populations were impacted by the application of mesosulfuron-methyl+iodosulfuron-methyl-sodium, an ALS-inhibiting herbicide. The survival rate results indicated the occurrence or development of resistance to this mechanism of action in most populations. Resistant to mesosulfuron-methyl+iodosulfuron-methyl-sodium, as well as multiple resistance to ACCase and ALS inhibitor herbicides, has been documented in *Avena* spp. populations (Beckie et al., 2012; Joumi et al., 2022). In Iran, ALS-inhibiting herbicides, such as mesosulfuron-methyl+iodosulfuron-methyl-sodium, were registered aiming to manage troublesome grass species resistant to ACCase inhibitors (group 1) in wheat fields. Over the course of several years, ALS inhibitor herbicides have become the predominant and widely adopted option in wheat fields, leading to selected cases of multiple resistance and further complicating management efforts due to their high-risk essence for evolving resistance (Moss et al., 2019).

Importantly, the survival rate was notably higher in samples collected from within fields compared to those gathered from field margins, with statistically significant differences observed between samples collected from within fields and field margins in five fields, including W53, W55, W57, W63, and W69. While the samples collected from within the five fields, including W53, W55, W57, W63, and W69, survived after the application of mesosulfuron-methyl+iodosulfuron-methyl-sodium at rates of 54.23%, 79.38%, 89.58%, 64.58%, and 45.00%, respectively, samples collected from the margins of these five fields exhibited survival rates of 17.99%, 36.82%, 21.37%, 13.77%, and 7.08%, respectively. However, no statistically significant difference was observed in the survival rates between the populations collected from within fields and field margins in the remaining six fields (Figure 3A).

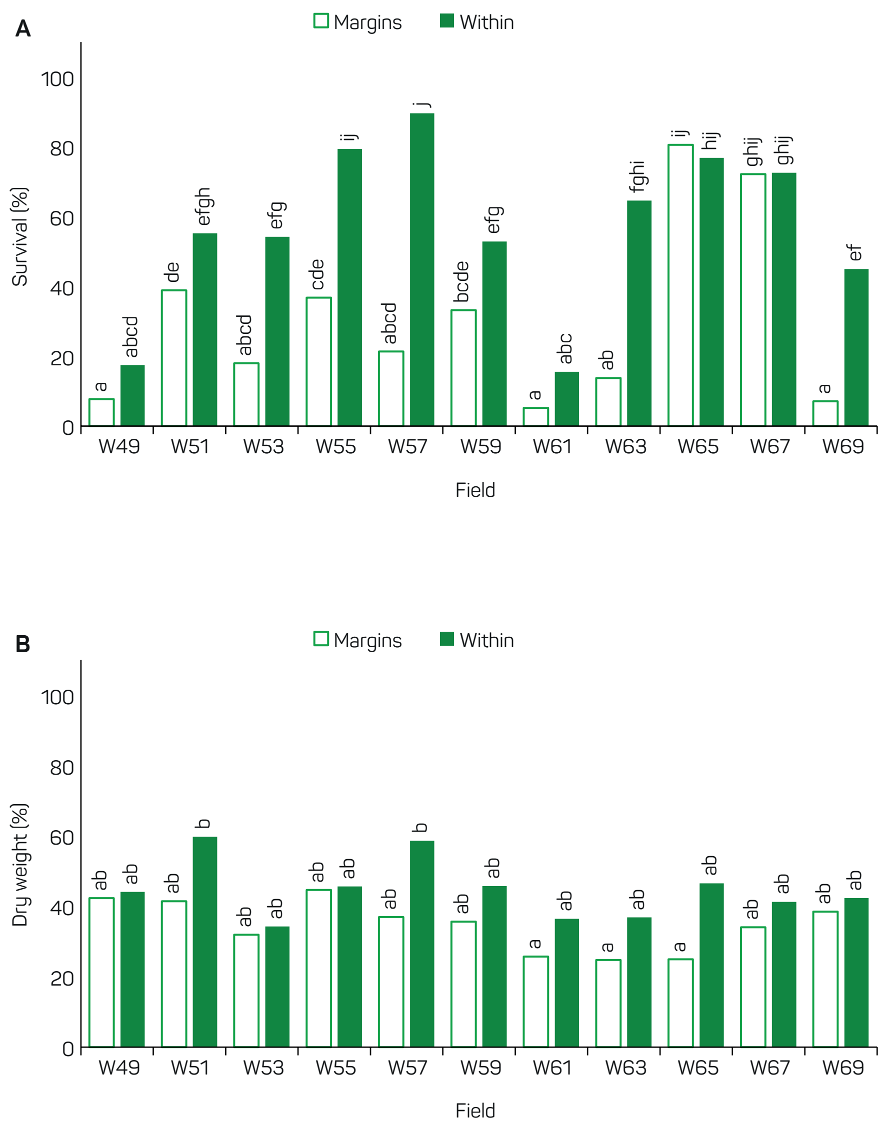

Figure 3 - Survival (A) and dry weight (B) percentages of winter wild oat populations (*Avena sterilis* subsp. *ludoviciana*) collected from both field margins and within 11 winter wheat fields following mesosulfuron-methyl+iodosulfuron-methyl-sodium application. Similar letters indicate non-significant differences among mean values

A tendency of higher dry weight was also observed for the samples collected from within fields compared to those corresponding samples collected in field margins, as aligned with the same general trend observed in the survival rate. On average, the dry weight of samples collected from field margins was 34.65%, whereas the populations collected from within fields exhibited a value of 44.71% in comparison to the untreated control. Nevertheless, among the 22 *A. sterlis* subsp. *ludoviciana* populations, there was no statistically significant difference in the dry weight of the samples collected from within and margins of the 11 fields following the application of mesosulfuron-methyl+iodosulfuron-methyl-sodium (Figure 3B). It is expected that if similar weed management strategies are implemented in these six fields, resistance to mesosulfuron-methyl+iodosulfuron-methyl-sodium might occur, and the difference in herbicide resistance levels to mesosulfuron-methyl+iodosulfuron-methyl-sodium between within fields and field margins will be more pronounced, as observed in the five mentioned fields.

3.4 Mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican

In contrast to the results obtained for clodinafop-propargyl and mesosulfuron-methyl+iodosulfuron-methyl-sodium, inconsistent results were observed for mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican regarding herbicide resistance in samples collected from within field and field margins. In five fields (1, W53, W59, W63, and W69), the survival rates of samples collected from within fields were significantly higher than those of corresponding samples

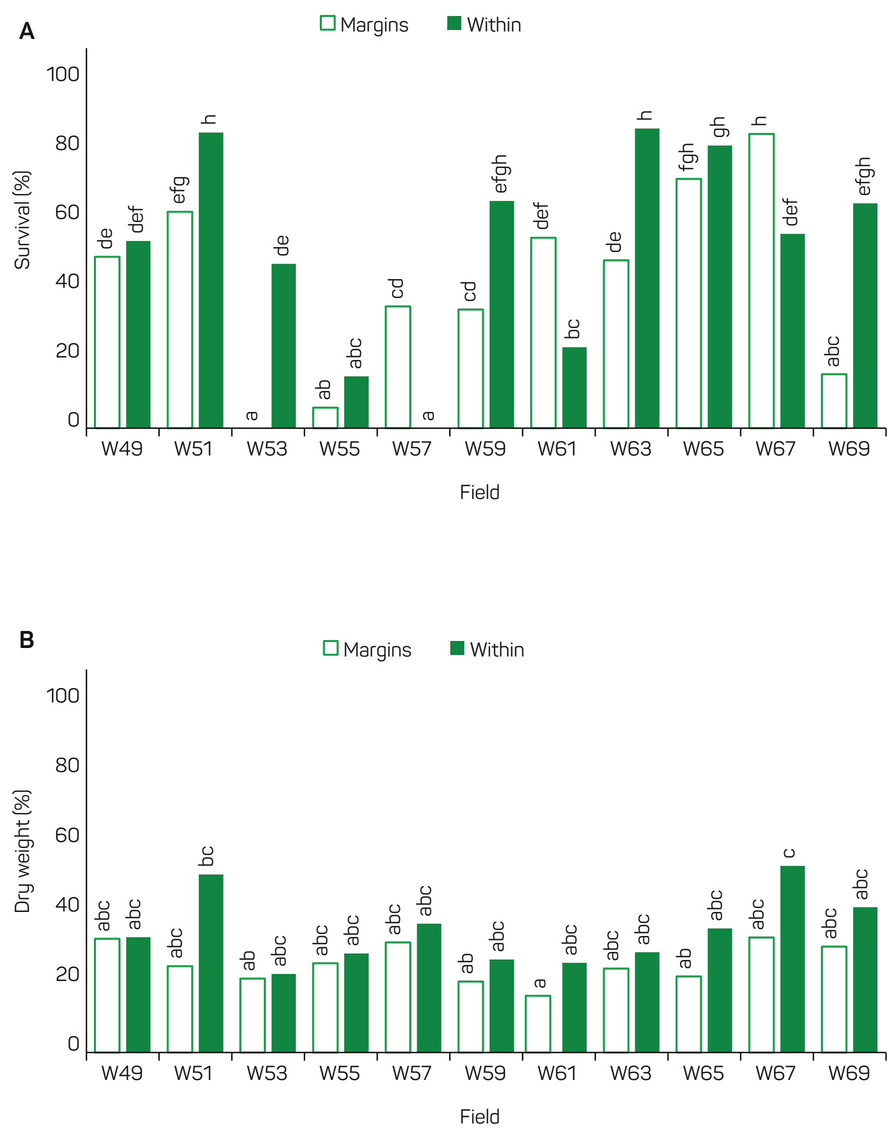

Figure 4 - Survival (A) and dry weight (B) percentages of winter wild oat populations (*Avena sterilis* subsp. *ludoviciana*) collected from both field margins and within 11 winter wheat fields following mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican application. Similar letters indicate non-significant differences among mean values

collected at field margins. Conversely, in three fields (W57, W61, and W67), samples collected from field margins exhibited significantly higher survival rates than those of corresponding samples collected within fields after exposure to mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican. In the remaining three out of the 11 fields (W49, W55, and W65), no statistically significant difference was observed between the samples collected from within fields and field margins. Despite variations in the dry weight of populations in response to mesosulfuron-methyl+iodosulfuron-methyl+diflufenican, no significant difference was observed between the samples collected from within fields and those from field margins, as was also observed for pinoxaden and mesosulfuron-methyl+iodosulfuron-methyl-sodium. However, the general pattern showed a tendency of greater dry weight in all the samples collected from within field than in associated samples from field margins.

Regardless of differences between samples collected from within field and field margins, resistance to the herbicide combination of mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican was observed in seven out of the 11 populations, as they maintained a survival rate and dry weight of over 50% relative to the untreated controls according to the approach suggested by (Adkins et al., 1997) for identifying herbicide resistant populations. Recently, resistance to mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican has been reported in Iranian *A. sterlis* subsp. *ludeovicina* populations (Joumi et al., 2022).

4. Conclusions

This study provides valuable insights into the dynamics of herbicide resistance in *A. sterilis* subsp. *ludoviciana* populations, with a particular focus on patch locations. To the best of our knowledge, this issue was rarely studied. We observed that, although samples collected within fields showed a tendency for higher survival in the presence of clodinafop-propargyl and mesosulfuron-methyl+iodosulfuron-methyl-sodium compared to those from field margins, a significant difference was only noted in a few populations, where fewer than half of the within-field samples displayed increased resistance levels compared to their counterparts at field margins. This suggests a higher selection pressure from recurrent herbicide use within fields and supports the hypothesis that field margin weeds have lower resistance levels. However, pinoxaden showed consistent susceptibility across all samples, making it an effective herbicide for controlling the studied *A. sterilis* subsp. *ludoviciana* populations resistant to the other studied herbicides. Notably, a contradictory trend was observed in response to mesosulfuron-methyl+iodosulfuron-methyl-sodium+diflufenican among samples collected from within fields and field margins, while half of the populations followed the trend for clodinafop-propargyl and mesosulfuron-methyl+iodosulfuron-methyl-sodium, surprisingly, around 30% of populations exhibited opposite results. These contradictions within certain populations suggest the possibility of external sources introducing seeds to these fields, potentially through crop seeds, equipment, grazing, and other means.

Recognizing the importance of field margin populations in introducing and spreading herbicide-resistant biotypes within fields, as suggested by Norsworthy et al. (2012), it is crucial to emphasize preventive strategies in weed management. This includes maintaining crop hygiene and clearing weeds from irrigation canals and field margins. However, in small-scale lands in Iran, farmers often overlooked weed management in these areas. This neglect may result in lower herbicide exposure for field-margin weeds compared to those within fields, leading to delayed herbicide resistance development, as observed in some populations in response to clodinafop-propargyl and mesosulfuron-methyl+iodosulfuron-methyl-sodium. It's worth noting that in self-pollinating species like *Avena* spp., gene transfer and resistance development through gene flow are uncommon due to high selfing rates. Additionally, the hexaploidy of *Avena* spp. can dilute resistant genes, reducing the likelihood of resistance development spreading from field margins to within fields. In contrast, diploid species like *Lolium* spp., with high outcrossing rates, may exhibit a completely reversed trend. It means, field margins outcrossing weeds may serve as a reservoir of susceptible individuals, potentially contributing to the delay in the evolution of herbicide resistance in weeds. However, intriguingly, the confirmation of resistance in the populations collected from field margins underscores the need for vigilant non-chemical management practices.

In conclusion, this study underscores the complexity of herbicide resistance dynamics. While the presence of weeds in field margins can be seen as a double-edged sword, their precise impact on the occurrence or potential contribution to the delay of resistance remains unknown. To our knowledge, weeding field margins, at least for self-pollinating species like *Avena* spp, stands as a foundational principle in proactive weed management, favoring non-chemical methods. It is only through a holistic approach, employing integrated management strategies, that we can safeguard the efficacy of herbicides and ensure the sustainability of agricultural practices in the face of evolving resistance.

Author's contributions

HS, EK, and EZ: conceived and designed the experiments. HS, MHZ, and BKT: conducted the experiments. EK: analyzed the data. HS and EK: wrote the manuscript. HS, EK, EZ, MHZ, and BKT: read and approved the manuscript.

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