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Identification of sterile wild oat (*Avena sterilis* L.) resistance to acetolactate synthase (ALS)-inhibiting herbicides using different assay techniques

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Abstract

Different techniques have been devised to detect herbicide resistance in weeds, and the overall aim from this study was to compare four different assay techniques for evaluating acetolactate synthase (ALS)-inhibiting herbicide resistance in sterile wild oat (*Avena sterilis* L.). A resistant sterile wild oat population (R) was collected from the wheat field in Kozan, Adana province, Turkey. The susceptible (S) population was collected from the border of the same field. Effects of different doses of mesosulfuron-methyl + iodosulfuron-methyl--sodium and pyroxsulam + cloquintocet-mexyl were assessed in agar based (seed and seedling) assay, Petri dish with seeds, and whole plant pot assay. In the agar based assays, the level of resistance was evaluated by measuring coleoptile and hypocotyl lengths, and survival of seedlings. Plant height and shoot dry weight were measured in the Petri dish and whole plant pot assays, respectively. Results from the dose response analyses showed that both the R and S populations were extremely sensitive to mesosulfuron-methyl + iodosulfuron in the seedling bioassay. The resistance indices (*RI*'s) of the R biotype treated with mesosulfuron-methyl + iodosulfuron in the agar based seed, Petri dish, and whole plant assays were 2.29, 2.63 and 4.18, respectively. The resistance indices of the R biotype treated with pyroxsulam + cloquintocet-mexyl was 3.41, 5.05 and 2.82 in the agar based seed, Petri dish, and whole plant pot assays, respectively. The agar based seed assays and Petri dish assay provided feasible, accurate, rapid, and cost effective opportunities to identify resistance in sterile wild oat.

Keywords: acetolactate synthase, agar-based assay, *Avena sterilis*, Petri-dish assay, whole--plant assay

Introduction

Herbicides have been a major tool for weed control in agricultural crops for the past 65 years (Heap 2014) Despite the importance of cultural, physical and mechanical weed control, herbicides remain the most important method for controlling weeds in arable crop production (Peterson *et al.* 2018). Acetolactate synthase (ALS)-inhibiting herbicides, also referred to as acetohydroxyacid synthase (AHAS), were discovered and commercialized in the early 1980's (Yu and Powles 2014). The ALS enzyme is essential in the biosynthesis of branched-chain amino acids valine, leucine and isoleucine (Duggleby *et al.* 2008; Panozzo *et al.* 2017). Moreover, it is the common target site of 56 active ingredients from numerous chemical classes such as sulfonylureas (SUs) and triazolopyrimidines (TPs). ALS-inhibiting herbicides are widely used due to their

broad-spectrum effects in a wide range of crops, and these herbicides have provided effective weed control in fields where resistance to acetyl-CoA carboxylase (ACCase)-inhibiting herbicides has evolved (Bi *et al.* 2013; Layton and Kellogg 2014). Unfortunately, the continuous use of ALS-inhibitors for a long period of time has led to the development of resistance in weed species that were previously controlled with these herbicides (Beckie *et al.* 2000; Preston and Powles 2002). The first case of resistance to an ALS-inhibiting herbicide was reported in rigid ryegrass (*Lolium rigidum* Gaudin) in 1982 (Yu and Powles 2014). Since then, globally 160 weed species (63 grasses and 97 broadleaved species) have developed resistance to ALS herbicides (Heap 2019).

Sterile wild oat (*Avena sterilis* L.) is an annual grass species, and a problematic weed in winter cereals in Mediterranean climate regions (Fernandez-Moreno *et al.* 2016). *Avena sterilis* is a highly competitive weed species which mimics the life cycle of cereal crops. It is self-pollinated and has a high seed production ability that leads to large soil seed banks (Papapanagiotou *et al.* 2019). Currently, 14 cases of herbicide resistance in *A. sterilis* to three different modes of action of herbicides have been documented in nine countries (Heap 2019).

Unsatisfactory control of *A. sterilis* with ALS-inhibiting herbicides is often seen in agricultural fields in Turkey (Uludag *et al.* 2007). Monoculture cropping with no alternation of a herbicide with a different mode of action and repeated applications of ALS herbicides (2–3 times per growing season) has caused Turkish farmers to face these challenges. A large number of resistance cases have been reported worldwide but only a few cases of resistance to ALS herbicides in *A. sterilis* have been reported in Turkey.

Whole-plant pot bioassay is a classical method used to detect herbicide-resistance in weeds; however, it needs resources in the shape of space and time (Tal *et al.* 2000; Uludag *et al.* 2006; Tursun 2012). There is a dire need for simple and rapid assays to detect herbicide-resistant weed populations for fast identification and subsequent management. In previous studies agar-based (seed and seedling) and Petri dish bioassays were used to evaluate resistance in ryegrass *Lolium rigidum* Gaud.) (Kuk *et al.* 2000), Japanese foxtail (*Alopecurus japonicus*) (Yang *et al.* 2007), monochoria [*Monochoria vaginalis* (Burm. f.) Kunth] (Kuk *et al.* 2003) and green foxtail [*Setaria viridis* (L.) Beauv.] (Beckie *et al.* 1990). The objective of this study was to compare different assay techniques for evaluating the level of resistance to mesosulfuron-methyl + iodosulfuron-methyl-sodium and pyroxsulam + cloquintocet-mexyl in *A. sterilis* populations in order to devise reliable, simple and inexpensive assay techniques.

Materials and Methods

Seed collection

Seeds of *A. sterilis* were collected from a wheat field in the province of Kozan (Adana, southeast Turkey [37°28′14.52″ N, 35°45′59.82″ E]) in the summer of 2016 (May–June). Herbicide treatment in the field failed to provide satisfactory control. At maturity, 300–400 possibly resistant plants were collected by two people walking in an inverted design (*W*) across the field. The possibly susceptible (*pS*) sample was collected from the boundaries of the field (>4 m from the field sites). It was assumed that the sample had never been exposed to herbicides. The seeds were cleaned by hand, air-dried and kept in a refrigerator at 4°C for 45–60 days to break dormancy.

Seed germination assay in agar medium

Plant agar (Duchefa) at $10 \mathrm{~g \cdot l^{-1}}$ (1% wt/v) was melted in a microwave and allowed to cool down to 35–40°C before adding herbicide solutions of mesosulfuron-methyl + iodosulfuron-methyl ($30 \mathrm{~g \cdot kg^{-1}}$ a.i.) and pyroxsulam + cloquintocet-mexyl ($75 \mathrm{~g \cdot kg^{-1}}$ a.i.). The stock solution of each herbicide was prepared and diluted to give targeted concentrations. The concentrations of mesosulfuron-methyl + iodosulfuron-methyl were 0, 0.009, 0.018, 0.036, 0.072, 0.144 and $0.288 \mathrm{~mg \cdot l^{-1}}$. The concentrations of pyroxsulam + cloquintocet-mexyl were 0.018, 0.037, 0.075, 0.15, 0.3 and $0.6 \mathrm{~mg \cdot l^{-1}}$. These concentrations were prepared in a conical glass and added to agar medium using a micropipette. A total of 20 ml agar medium was poured into glass Petri dishes (size: 9 cm). Ten seeds were placed in each Petri dish. The dishes were covered with lids and kept in an incubator at 10°C. The assay was repeated twice and arranged in a completely randomized design with six replicates of each concentration. Seed germination as well as coleoptile and hypocotyl lengths were determined 15 days after treatment (DAT).

Seedling assay

Seeds from the resistance (R) and susceptible (S) populations were grown in trays in a greenhouse (60% relative humidity, 12/6°C, 10/14 h day/night). The agar media was prepared as described above in the seed germination assay. The test was carried out with $10 \mathrm{~g \cdot l^{-1}}$ mesosulfuron-methyl + $2 \mathrm{~g \cdot l^{-1}}$ iodosulfuron-methyl-Na + $30 \mathrm{~g \cdot l^{-1}}$ mefenpyr-diethyl at concentrations of 0, 0.3, 0.6, 1.2, 2.4 and $4.8 \mathrm{~mg \cdot l^{-1}}$. One hundred milliliters of agar medium containing herbicide solutions was poured into 12 × 12 cm diameter plastic

Petri dishes (Fisher Scientific). The agar media was allowed to settle at 10°C for 24 h. Seedlings at the three-leaf stage (BBCH 13) were collected from the trays and washed using tap water to remove soil from the roots. The growing points of the seedlings (leaf and root) were excised using scissors. The roots of seedlings were carefully placed in the agar medium using forceps. The dishes were covered with lids and placed in a greenhouse. The experiment was conducted using a completely randomized design with five seedlings in each Petri dish and three replications. Herbicide efficacy was visually assessed for seedling growth and survival of new roots and shoots 15 DAT.

A Petri dish assay using seeds

Ten seeds of each R and S populations were germinated in a growth cabinet in Petri dishes (4.5 cm in diameter) on the top of two layers of filter paper. In order to maintain the humidity 30 g washed fine sand was added to each Petri dish after the seed germinated. At the two-leaf stage, the number of plants per Petri dish was reduced to five plants. The Petri dishes were placed in a greenhouse at 20/8°C, 10/14 h day/night, 30% relative humidity (RH) and a light intensity of 250 μmol quanta $m^{-2} \cdot s^{-1}$ of photon flux. Herbicides were applied outdoors at the recommended growth stage (BBCH 13–14) using a charged backpack sprayer (MATABI) equipped with TeeJet-fan nozzles delivering $250 \, l \cdot ha^{-1}$ at 304 kPa at boom height of 50 cm. Two ALS--inhibiting herbicides were used: 3% mesosulfuron-methyl + 0.6% iodosulfuron-methyl-sodium at the rates of 0, 2.25, 4.5, 9, 18, 36 and 72 $g \cdot ha^{-1}$ a.i.; 7.5% pyroxsulam + 7.5% cloquintocet-mexyl at the rates of 0, 4.68, 9.37, 18.8, 37.5, 75 and 150 $g \cdot ha^{-1}$ a.i. To improve herbicide performance, Bio-power 1,000 ml · ha^{-1} and Dash-oil 26–2N, 500 ml · ha^{-1} was added to mesosulfuron-methyl + iodosulfuron-methyl-sodium and pyroxsulam + cloquintocet-mexyl spray solutions, respectively. The assay was carried out using a completely randomized design with six replications of each herbicide rate and repeated twice. The plant height was recorded a day before treatment and at 1, 3, 5, 7, 14, 21, and 28 DAT.

Greenhouse experiments (whole-plant pot assay)

Five seeds from R and S populations were sown in pots (10 × 15 cm) containing a potting mixture (field soil, sand, and peat) at a 1 : 1 : 1 ratio. Pots were placed in a greenhouse (20/8°C, 14/10 h day/night and 25% RH) and subsequently irrigated. The seedlings were thinned to two plants per pot and left for 1 week before herbicide application. Herbicides were applied at the 2–4 growth stage, and spraying conditions were

the same as mentioned above in the Petri dish assay. After treatment, the pots were placed in separate trays to avoid cross-contamination of different herbicide concentrations. The experiment was conducted using a completely randomized design with six replications. Plants were harvested aboveground 28 DAT, dried in an oven at 65°C for 72 h, and dry weights were recorded. The experiment was repeated twice.

Statistical analysis

The data from two experiments were combined. Data of shoot dry weight, plant height, coleoptile, and hypocotyl lengths were converted to the percentage of the untreated control. Dose-response curves were estimated for each assay separately. Data were subjected to non-linear regression analysis using a three-parameter log-logistic model in the *R* statistical software with add-on package *drc* (Ritz *et al.* 2015).

$$y = \frac{d}{1 + \exp[b(\log(x) - \log(ED_{50}))]} \, ,$$

where: y – the response variable (shoot dry weight, plant height, coleoptile, and hypocotyl lengths), d – the coefficient corresponding to the upper limit, x – herbicide dose, ED_{50} – the dosage causing 50% reduction, b – determines the shape of the curve.

Resistance indices *(RI's)* were calculated as the ED_{50} value of the resistant R population divided by the ED_{50} value of the S population.

Results

Seed germination assays in agar medium

The herbicide effect was observed shortly after germination started, and differences between the R and S populations were observed 7 days after incubation. The percentages of germinated seeds treated with a concentration of 0.036 mg · l^{-1} mesosulfuron-methyl + + iodosulfuron-methyl-sodium was 60 and 80% for S and R, respectively. In contrast, more than 95% of germination was recorded in untreated S and R biotypes. Herbicide-treated seeds had shorter coleoptile and hypocotyl lengths than untreated controls (Fig. 1). Figure 2 shows the response of coleoptile and hypocotyl lengths of R and S populations to the applied doses. Seeds treated with a concentration of 0.075 mg · l^{-1} pyroxsulam + cloquintocet-mexyl germinated with 60 and 70% for S and R populations, respectively. Estimated ED_{50} values of the R population treated with mesosulfuron-methyl + iodosulfuron-methyl were 0.108 and 0.072 mg · l^{-1} for coleoptile and hypocotyl, respectively. The ED_{50} of the R population was
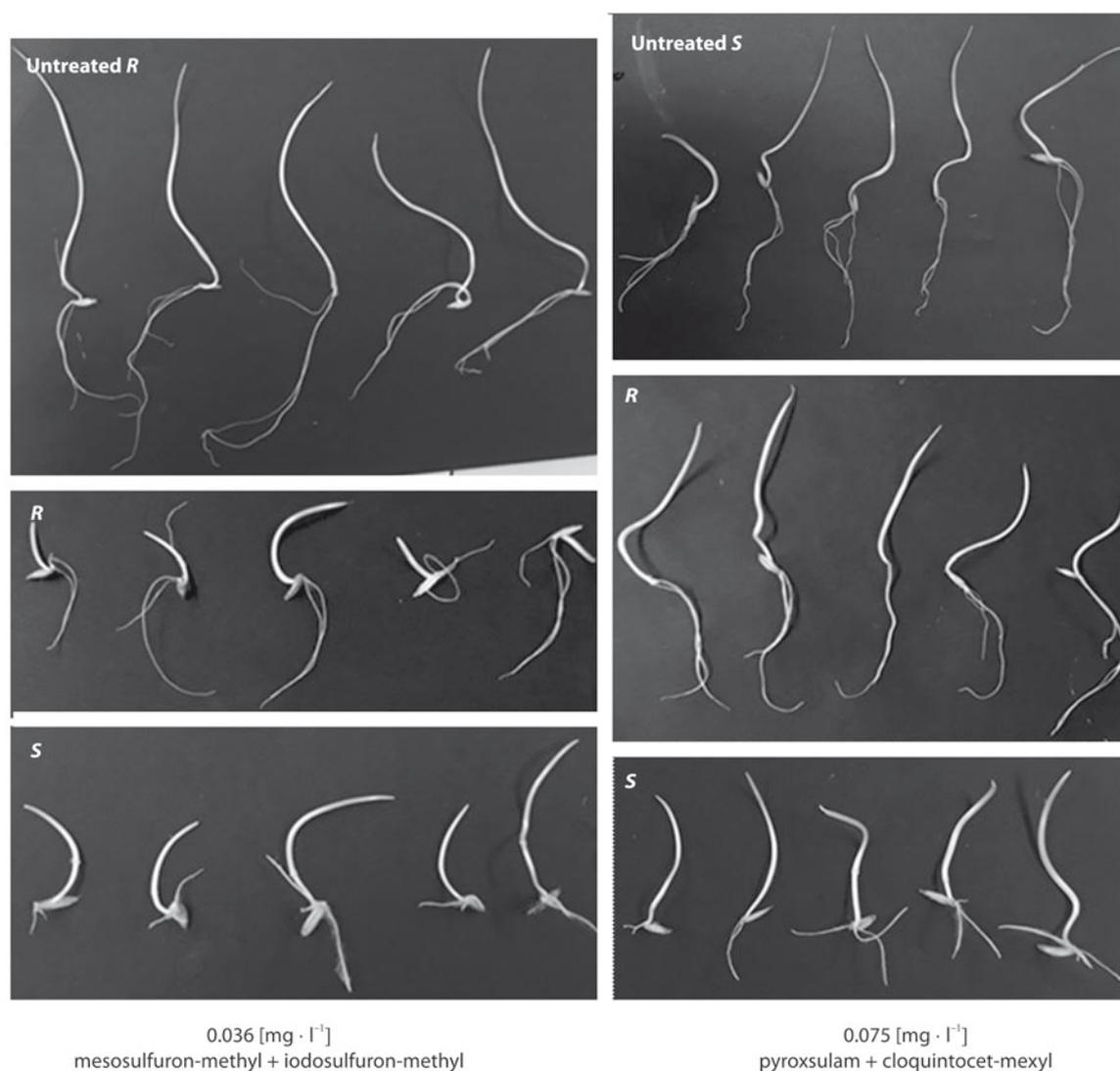
Fig. 1. Effect of mesosulfuron-methyl + iodosulfuron-methyl and pyroxsulam + cloquintocet-mexyl on coleoptile and hypocotyl lengths of resistant (R) and susceptible (S) sterile wild oat populations. Photographs were taken 15 days after treatment (DAT)

Table 1. Estimated parameters of log-logistic curve analysis of mesosulfuron-methyl + iodosulfuron-methyl-sodium doses required to provide 50% reduction (ED$_{50}$) for resistant (R) and susceptible (S) sterile wild oat populations in different assays. Standard errors of means are in parentheses

Method	Population	*b*	*d*	ED$_{50}$	*p*-values	R^2	R/S
Seed assay	S–C	0.78 (0.14)	100.0 (6.73)	0.047 (0.01)	***	0.99	
	R–C	0.85 (0.18)	100.0 (6.57)	0.108 (0.02)	***	0.99	2.3
	S–H	0.60 (0.13)	98.7 (7.33)	0.045 (0.01)	**	0.99	
	R–H	0.70 (0.16)	98.2 (7.37)	0.072 (0.02)	**	0.99	1.6
Seedling assay	S	1.06 (0.22)	100.0 (7.10)	0.31 (0.06)	***	0.99	
	R	0.98 (0.21)	99.9 (7.20)	0.31 (0.07)	***	0.99	1
Petri-dish assay	S	0.77 (0.04)	100.0 (2.21)	8.89 (0.75)	***	0.99	
	R	0.83 (0.05)	100.0 (2.10)	23.36 (1.90)	***	0.99	2.6
Pot assay	S	0.73 (0.19)	100.0 (9.00)	4.38 (1.65)	**	0.97	
	R	0.82 (0.20)	100.0 (8.52)	18.32 (6.01)	**	0.99	4.2

b – determines the shape of the curve; *d* – the coefficient corresponding to the upper limit; S–C – coleoptile of susceptible population; R–C – coleoptile of resistant population; S–H – hypocotyle of susceptible population; R–H – hypocotyle of resistant population; ** ($p \leq 0.01$), *** ($p = 0.001$); R^2 – coefficient of determination; R – resistant sterile wild oat population; S – susceptible population

Table 2. Estimated parameters of log-logistic curve analysis of pyroxsulam + cloquintocet-mexyl doses required to provide 50% reduction (ED_{50}) for resistant (R) and susceptible (S) sterile wild oat populations in different assays. Standard errors of means are in parentheses

Method	Population	b	d	ED_{50}	p-values	R^2	R/S
Seed assay	S–C	0.63 (0.14)	100.0 (7.43)	0.12 (0.04)	**	0.98	
	R–C	0.70 (0.21)	99.6 (7.25)	0.41 (0.14)	**	0.99	3.4
	S–H	0.63 (0.15)	98.4 (7.87)	0.10 (0.03)	**	0.99	
	R–H	0.68 (0.17)	97.6 (7.99)	0.16 (0.05)	**	0.99	1.6
Petri-dish assay	S	1.05 (0.10)	100.0 (3.53)	17.01 (1.80)	***	0.99	
	R	1.02 (0.12)	100.0 (3.02)	85.99 (9.62)	***	0.99	5.1
Pot assay	S	0.79 (0.12)	100.0 (6.34)	12.64 (3.41)	***	0.98	
	R	0.75 (0.13)	100.0 (6.21)	35.74 (9.03)	***	0.98	2.8

b – determines the shape of the curve; d – the coefficient corresponding to the upper limit; S–C – coleoptile of susceptible population; R–C – coleoptile of resistant population; S–H – hypocotyle of susceptible population; R–H – hypocotyle of resistant population; ** ($p \leq 0.01$), *** ($p = 0.001$); R^2 – coefficient of determination; R – resistant sterile wild oat population; S – susceptible population
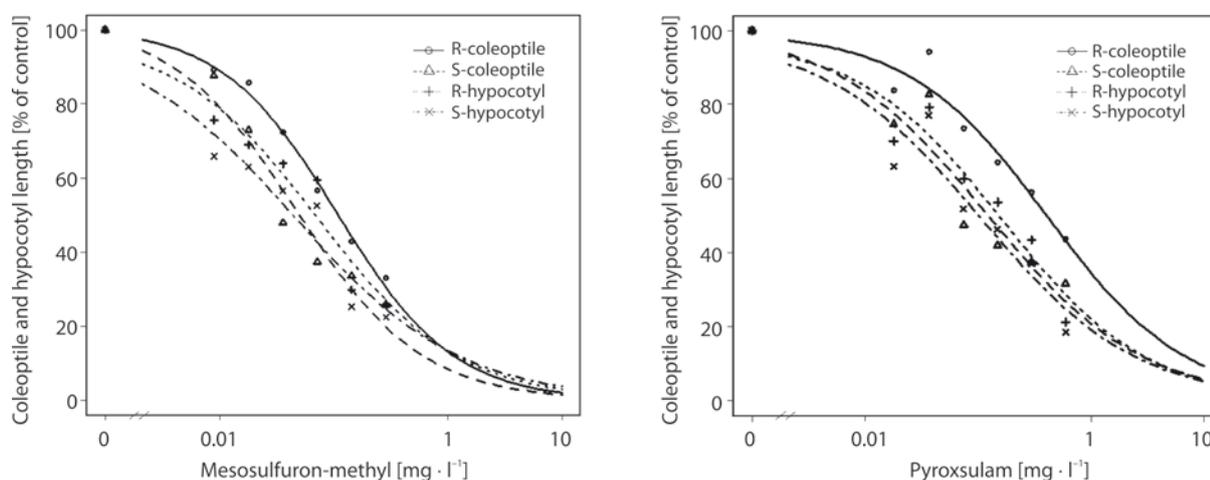
Fig. 2. Effect of mesosulfuron-methyl + iodosulfuron-methyl and pyroxsulam + cloquintocet-mexyl on coleoptile and hypocotyl lengths of resistant (R) and susceptible (S) *Avena sterilis* populations in agar-based seed assay, 15 days after treatment (DAT)

2.29 times higher than the ED_{50} of the S population (Table 1). Calculated ED_{50} values from the R population treated with pyroxsulam + cloquintocet-mexyl were 0.14 and 0.16 mg · l^{-1} for coleoptile and hypocotyl, respectively (Table 2) while the ED_{50} of the S population was 0.108. The results indicated that the R population had a lower level of resistance for both herbicides in this bioassay.

Seedling assay

The agar was a suitable medium to grow *A. sterilis* under greenhouse conditions during the winter. A total of 15 individuals transplanted from each R and S population did not establish new roots or shoots (0%), while in the untreated control all seedling plants established (100%) new healthy roots (Fig. 3). The estimated resistance index (*RI*) exceeded 1, indicating no resistance in this bioassay.

Petri dish assay

Full dose-response curves for R and S populations were obtained based on plant height (Fig. 4). The three-parametric logistic model fit the data well ($R^2 \leq 0.99$) for both herbicides. At 28 DAT, the ED_{50} values of mesosulfuron-methyl + iodosulfuron-methyl for the R and S populations were 23.36 and 8.89 g · ha^{-1} a.i., respectively, with a R/S ratio of 2.62 (Table 1). The ED_{50} values pyroxsulam + cloquintecet-mexyl for the R and S populations were 85.99 and 17.01 g · ha^{-1} a.i., respectively, resulting in a R/S ratio of 5.05 (Table 2). These responses elucidate the need to test large populations rather than testing only a limited number of individuals.

Whole-plant pot assay

The assay was conducted in order to validate the findings from the agar-based seed and seedling assays and the Petri dish assays. Estimated ED_{50} values from the

Fig. 3. Typical resistant (R) and susceptible (S) sterile wild oat populations assayed at the rate of 1.2 mg · l⁻¹ mesosulfuron-methyl + + iodosulfuron-methyl-sodium in the seedling assay, 15 days after treatment (DAT)
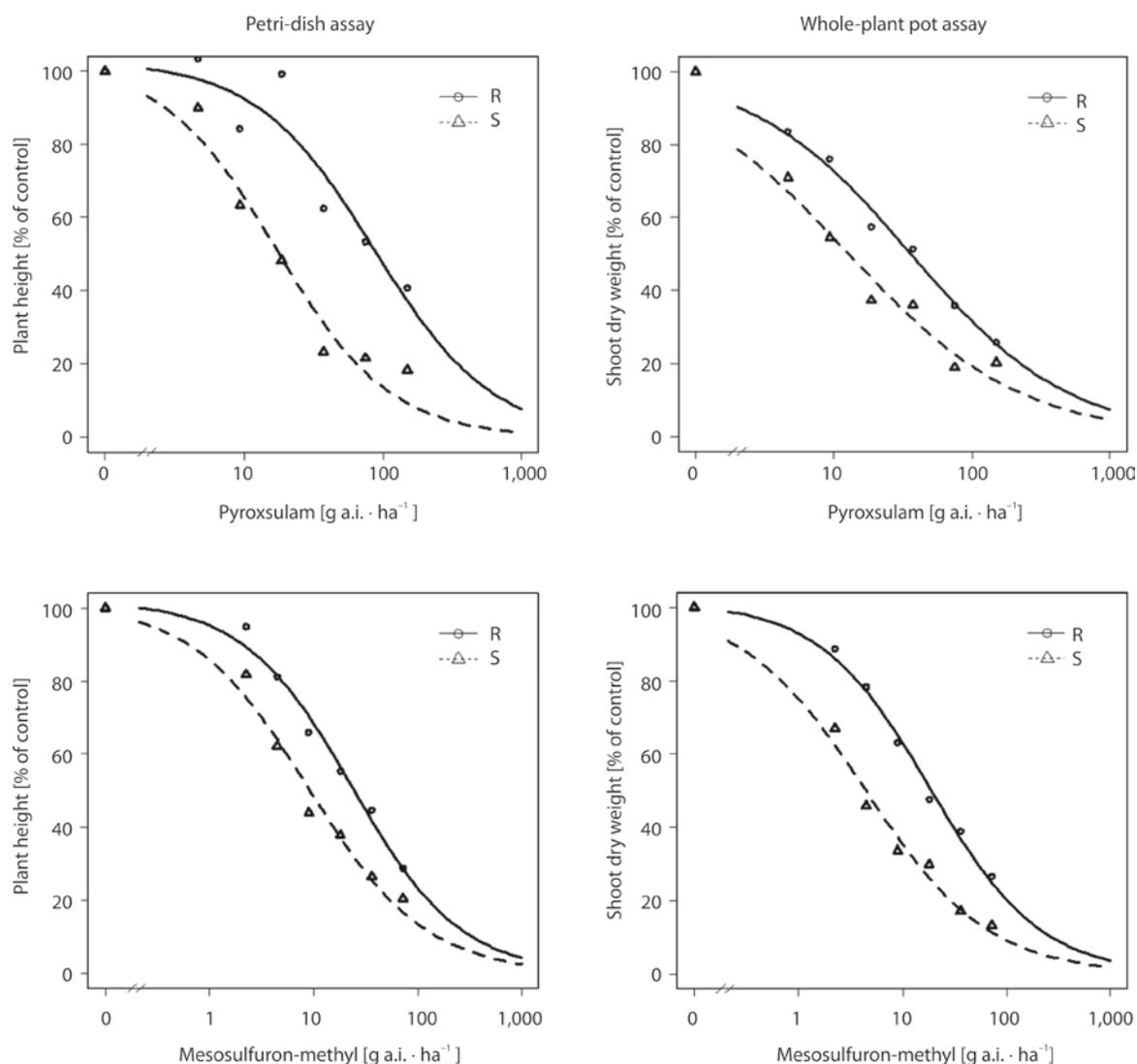
Fig. 4. Percentage of plant height and shoot dry weight for resistant (R) and susceptible (S) sterile wild oat populations in Petri dish and whole-plant pot assays, 28 days after treatment (DAT)

R population treated with mesosulfuron-methyl were 18.32 g · ha⁻¹ a.i., which was 4.18 times higher than the ED_{50} of the S populations. ED_{50} of the R population treated with pyroxsulam was 35.74 g · ha⁻¹ a.i.; which was 2.82 times higher than the S population (Table 1).

It was noted that the *A. sterilis* resistance population had lower resistant levels (*RI* < 5) to two herbicides and cross-resistance patterns to ALS-inhibitors among the population were observed.

Discussion

Cereal crops are infested by numerous grass weed species. The majority of them are sensitive to ALS herbicides. However, target-site resistance can quickly spread and become dominant within a short period of time (Moss *et al.* 2003). The agar media provided an appropriate substrate for the growth of sterile wild oat, although coleoptile and hypocotyl lengths could not be measured (Burke *et al.* 2006). The agar-based seed germination assay demonstrated that the length of coleoptiles was strongly inhibited by increasing the rates of mesosulfuron-methyl + iodosulfuron-methyl-sodium and pyroxsulam + cloquintocet-mexyl. The consistency of the assay seems to be very high as the coleoptile response of the S population was similar to both herbicides. Similar observations have been made in *L. rigidum* treated with sulfometuron and glyphosate (Burnet *et al.* 1994). Ghanizadeh *et al.* (2015) reported a reduction of coleoptile lengths in some broadleaved species e.g, lambsquarters (*Chenopodium album* L.) and wild mustard (*Sinapis arvensis* L.) when treated with dicamba at 0.02 to 0.32 mg a.e. · l^{-1}. In previous studies, coleoptile lengths have been used to show resistance to ALS in flixweed [*Descurainia sophia* (L.) Webb. ex Prantl] (Xu *et al.* 2010), ACCase in Johnsongrass [*Sorghum halepense* (L.) Pers.] (Burke *et al.* 2006), and blackgrass (*Alopecurus myosuroides* Huds.) (Letouze and Gasquez 1999).

In the agar-based seedling assay, the transplanted seedlings of both S and R populations of *A. sterilis* at the 2–3 leaf stage were very sensitive to mesosulfuron-methyl + iodosulfuron-methyl and they did not develop new shoots or roots. This is in contrast to previous studies which showed that when a herbicide is applied at seedling stage it can have a profound impact on the level of resistance in the targeted weed species (Shrestha *et al.* 2007; Kaundun *et al.* 2014). The mortality of seedlings at all concentrations of mesosulfuron-methyl + iodosulfuron-methyl was significantly different from the responses of the whole-plant pot assay. Therefore, the agar-based seed and the seedling assay can easily be used to detect grass weed species such as wild oat (*Avena* spp.), canary grass (*Phalaris prodoxa* L.), blackgrass and ryegrass resistance to ALS synthase inhibitor herbicides within 2 weeks (Boutsalis 2001; Kaundun *et al.* 2011; Brosnan *et al.* 2017), and it can be used to determine resistance of some broadleaved species e.g., horseweed [*Conyza canadensis* (L.) Cronq.] and common waterhemp (*Amaranthus rudis* Sauer) (Kaundun *et al.* 2014). With the agar-based assays, farmers and agronomists will receive quick and accurate results of the level of resistance in their fields. Furthermore, compared to pot assays, these two bioassay techniques were much

faster, required less space, were low-cost, did not need special equipment, did not require watering or any other plant maintenance and the difference between R and S plants was very clear (Yang *et al.* 2007; Sasanfar *et al.* 2017). However, the experiments must be repeated many times (at least two times) (Corbett and Tardif 2006; Burgos 2015). Some disadvantages in agar-based seed assay were noticed. For instance, measuring total coleoptile and hypocotyl lengths for germinated seeds (mm or cm) per dish was time-consuming and higher doses of mesosulfuron-methyl + iodosulfuron-methyl will not prevent seed germination of S *A. sterilis*, although growth was stopped after the cotyledon stage (Cirujeda *et al.* 2001; Abdurruhman *et al.* 2018). Furthermore, seed dormancy issues can affect seed germination in agar medium, as is the case for an assay starting directly after collecting seeds from the field (Moss 1995; Kaundun *et al.* 2014).

Whole-plant pot and Petri dish assays differ from agar-based assays on preliminary materials such as bench-top. It takes more than 4 months from seed collection to evaluate resistance (Burgos 2015). In the Petri dish assay, once the seeds are placed on filter paper, they need water regularly until germination. Fine sand was added to retain plants stand and maintain the moisture. Unexpected challenges of the Petri dish assay were experienced. For example, the plants did not stand upright due to limited space (on the Petri dish). It was also difficult to maintain moisture without access to water Furthermore, there was variation of the temperature range in a phytotron or growth cabinet and the provided light level was inadequate (Cirujeda *et al.* 2001). In other studies on Petri dish assays, the seeds were treated with aqueous herbicides and the shoot length was measured within 7 days of incubation without adding sand to maintain the moisture (Tal *et al.* 2000), In previous papers resistance levels were classified as no resistance ($RI < 2$); low resistance ($RI = 2–5$); moderate resistance ($RI = 6–10$); and high resistance ($RI > 10$) (Beckie and Tardif 2012; Moss *et al.* 2019). With mesosulfuron-methyl + iodosulfuron-methyl resistance levels of 2.63 and 4.18 were obtained in the Petri dish and whole-plant pot assays, respectively, and differed in the population tested with pyroxsulam + cloquintocet-mexyl herbicide with resistant levels of 5.05 and 2.82 for Petri dish and whole-plant pot assays, respectively. This may be due to differences of resistance levels within the individual plant in the assays or it is possible that resistance is still evolving in this population (Uludag *et al.* 2007). Similar variations were observed when laboratory assays were validated with whole-plant pot assay (Kaundun *et al.* 2011). The data indicated significant differences ($p \leq 0.0001$) in the ED$_{50}$ between R and S populations in four assays. This is in accordance with other studies on Flixweed biotypes which had similar ED$_{50}$ when

assessed by the Petri dish bioassay and whole-plant bioassay (Xu *et al.* 2010).

The results from the whole-plant bioassay showed the same tendency as the other bioassays. Xu *et al.* (2010) stated that the whole-plant pot assay was the best technique for defining the levels of resistance since it was more reliable and sensitive to detecting levels of resistance. But it requires a larger space and takes longer (a few months) to complete than other assay methods (Kuk *et al.* 2000). Furthermore, it requires a lot of infrastructure and labor to establish the experiment (Kuk *et al.* 2003). The overall higher or lower levels of plant growth across the different assays may be due to the conditions of the assays (temperature and light). Although direct comparisons are not possible (Tal *et al.* 2000) in general, the results showed similar trends. This is not unexpected, as herbicides are most active on the rapidly-growing plant (Burgos et al. 2013). The level of resistance or cross-resistance patterns to ALS herbicides was found in the R population demonstrating that more than one mechanism of resistance is probably involved in this R population. More studies are required to understand the mechanisms of resistance of ALS-inhibiting herbicides in *A. sterilis* populations. Moreover, our results highlighted the need for other options e.g., herbicide sequence and/or rotation and crop rotation to manage *A. sterilis* earlier before it becomes resistant.

Acknowledgements

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