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Article

Comparing Fitness Cost Associated with Haloxyfop-R Methyl Ester Resistance in Winter Wild Oat Biotypes

Comparação do Custo de Adaptação Associado à Resistência ao Haloxyfop-R Methyl Ester em Biótipos de Aveia Selvagem de Inverno

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ABSTRACT - Consecutive application of herbicides has led to the evolution of herbicide-resistant weeds. This resistance is often associated with a fitness cost. Hence, a completely randomized design experiment with three replications was conducted to evaluate the fitness cost of haloxyfop-R methyl ester resistant winter wild oat biotypes (*Avena ludoviciana* Durieu) possessing Ile-2041-Asn mutation compared to susceptible ones. The pre-germinated F2 generation winter wild oat biotypes were sown in 0.2 m^2 pots containing 50 cm of silty-loam soil outdoors and their growth parameters including tiller number, plant height, leaves per plant, leaf area per plant, chlorophyll content index, leaf dry weight, and plant dry weight were measured 30, 70, 100, 115 and 130 days after planting. Leaf area index, leaf area ratio, specific leaf area, relative growth rate, net assimilation rate, and crop growth rate were also calculated. Seed production, 1000 kernel weight, and flag leaf area were measured at the end of the growth period. According to the results, no fitness cost was observed between susceptible and resistant biotypes, indicating that susceptible biotypes may not overcome resistant ones in the field. Although imposing a new selective pressure via application of an herbicide possessing a different mode of action may control both susceptible and resistant biotypes, herbicide rotation must be adapted to impede the evolution of further resistance. Also, the same non-chemical weed management methods such as careful selection of sowing date can be implemented to ameliorate adverse effects of this weed on crop production.

Keywords: *Avena ludoviciana* Durieu, growth indices, pleiotropic effects, Ile-2041-Asn.

*RESUMO - A aplicação consecutiva de herbicidas levou à evolução de plantas daninhas resistentes a eles. Essa resistência é frequentemente associada a um custo de adaptação. Assim, um delineamento inteiramente casualizado com três repetições foi conduzido para avaliar o custo de adoção dos biótipos de aveia selvagem de inverno resistentes ao haloxyfop-R methyl ester (**Avena ludoviciana** Durieu) com a mutação Ile-2041-Asn, em comparação com os suscetíveis. Os biótipos pré-germinados de aveia selvagem de inverno da geração F2 foram semeados em vasos de 0,2 m^2 contendo 50 cm de solo franco-arenoso ao ar livre, e seus parâmetros de crescimento, incluindo número de perfilhos, altura da planta, folhas por planta, área foliar por planta, índice de clorofila, peso seco da folha e peso seco da planta, foram medidos aos 30, 70, 100, 115 e 130 dias após o plantio. Também foram calculados índice de área foliar, razão de área foliar, área foliar específica, taxa de crescimento relativo, taxa de assimilação líquida e taxa de*

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crescimento de culturas. A produção de sementes, o peso de mil grãos e a área da folha-bandeira foram medidos no final do período de crescimento. De acordo com os resultados, nenhum custo de adoção foi observado entre os biótipos suscetíveis e resistentes, indicando que os primeiros podem não superar os resistentes no campo. Embora a imposição de nova pressão seletiva via aplicação de um herbicida com modo de ação diferente possa controlar biótipos suscetíveis e resistentes, a rotação do herbicida deve ser adaptada a fim de impedir maior evolução da resistência. Além disso, os mesmos métodos não químicos de manejo de plantas daninhas, como seleção cuidadosa da data da semeadura, podem ser implementados para melhorar os efeitos adversos dessa planta daninha na produção agrícola.

Palavras-chave: *Avena ludoviciana* Durieu, índices de crescimento, efeitos pleiotrópicos, Ile-2041-Asn.

INTRODUCTION

Weeds are undesired plants which are considered from the very dawn of agriculture as pests (Powles and Yu, 2010). Introduction of selective herbicides in the 1940s and consecutive development of new herbicides armed the farmers with newfound agrochemicals so they could surpass weed infestation and increase crop production. However, intense reliance on herbicides has led to changes in weed flora as well as the selection of resistant biotypes (Kudsk and Streibig, 2003). Although weed control using chemical methods imposes an acute selective pressure which may result in the elimination of up to 99% of weeds, the few surviving individuals can alter phenotypical and also the genotypical ratio of the population, and biotypes fully resistant to herbicide will emerge in next generations (Maxwell et al., 1990; Gherekhloo et al., 2016). Plants which have evolved resistance show higher fitness compared to susceptible alleles in presence of stress caused by application of that herbicide and thus will prevail. However, these biotypes may exhibit fitness cost if the selective pressure by herbicide is removed from the environment (Delye, 2013; Delye et al., 2013; Vila-Aiub et al., 2011). Thus, the frequency of susceptible individuals in the population will increase compared to that of herbicide-resistant plants (Park and Mallory-Smith, 2005; Tranel and Wright, 2002). Fitness cost is the average success of a phenotype in offspring production compared to another phenotype (Primack and Hyesoon, 1989). Herbicide resistance is expected to be associated with fitness cost for the plant. This fact may be justified by the fewer frequency of herbicide-resistant alleles in the weed population in an herbicide-free environment (Jasieniuk et al., 1996; Preston and Powles, 2002). The reasons behind occurrence of fitness cost resulted by resistant alleles include: 1) mutations in the enzyme-encoding herbicide target leading to resistance may interfere with plant metabolism and function (Vila-Aiub et al., 2009); 2) Herbicide resistant may result in the diversion of resources from growth and propagation to defense (Coley et al., 1985); 3) Pleiotropic effects resulted by resistant alleles might alter ecological relations, e.g. these effects may render the plant less attractive for pollinators (Purrington, 2000; Strauss et al., 2002). It must be noted that resistant inducing mutation does not necessarily impose fitness cost (Vila-Aiub et al., 2005; Menchari et al., 2008). Also, this cost is not inevitably negative and the mutation may even enhance the resistant plants (Wang et al., 2010). Fitness cost plays a vital role in evolution and according to Yanniccari et al. (2016), not only it maintains genetic polymorphism in populations, also prevents adaptive alleles from being fixated.

Study and quantification of fitness cost have been performed by various researchers (Lamego et al., 2011; Westendorff et al., 2013). Keshtkar et al. (2017) studied the fitness of Black grass (*Alopecurus myosuroides* Huds.) biotypes possessing non-target site resistant to ACCase inhibitors grown as a pure stand and in competition with wheat. They reported that susceptible and resistant biotypes did not differ significantly regarding fitness traits such as fecundity, tiller number, and biomass. Black grass biotypes containing Ile-1781-Leu or Ile-2041-Asn mutations in their ACCase encoding enzyme had similar vegetative biomass, height and seed production compared to susceptible ones. However, biotypes with Asp-2078-Gly mutation showed a significant reduction in these traits (Menchari et al., 2008). Quinclorac (Synthetic auxin), penoxsulam and bispyribac-sodium (ALS inhibitors) resistant barnyard grass biotypes (*Echinochloa crus-galli* L.) had lower chlorophyll content compared to susceptible ones (Yang et al., 2017). Iodosulfuron resistant and susceptible radish (*Raphanus sativus* L.) biotypes had similar plant height, shoot dry matter, root dry matter, total dry matter, leaf area, growth rate, relative growth rate, leaf area ratio, number of siliques and seeds produced per plant (Cechin et al., 2017).

Thus, effects of herbicide resistance on fitness-related traits varies depending on weed species, different resistance mechanisms and environmental condition (Goss and Dyer, 2003; Menalled and Smith, 2007; Sibony and Rubin, 2003; Lehnhoff et al., 2013). Therefore, knowledge about biological attributes of herbicide-resistant and susceptible biotypes is very important for the determination of attributes which may contribute to their competitiveness and prove useful in choosing the weed management method to be implemented (Schaedler et al., 2013). The objective of the following study is to evaluate the fitness cost of winter wild oat (*Avena ludoviciana* Durieu.) biotypes resistant to haloxyfop-R methyl ester (EC 10.8%) herbicide compared to susceptible biotype.

MATERIALS AND METHODS

Plant material

The seeds of winter wild oat biotypes (RK5, RK8, RK12, RK14, and RK20) resistant to haloxyfop-R methyl ester were gathered in 2017 from canola fields of Kalaleh Township, Golestan province, Iran. These biotypes had been investigated previously in a molecular assay using allele-specific PCR technique, and their ACCase encoding gene possessed Ile-2041-Asn mutation (Unpublished). Susceptible biotype seeds (S biotype) were gathered from sites which had no history of being sprayed with the mentioned herbicide. The seeds of resistant and susceptible biotypes were propagated for two generations under similar environmental conditions in the field to obtain F2 generation and thus, minimize variance in genetic background.

Pot experiment

The seeds were kept in a refrigerator at 4 ^{o}C for 72 hours for pre-chilling to achieve better germination uniformity and then incubated at 20 ^{o}C for 24 hours. Pre-germinated resistant and susceptible biotype seeds were then sowed on November 22th 2018 in 0.2 m^2 pots containing 50 cm depth of silty loam soil outdoors. Each pot served as one replicate and included 10 rows consisted of 6 individual, resulting in a final plant density of 300 plants per m^2. The pots were irrigated regularly and were maintained weed-free by handweeding during the experiment, except for the winter wild oat plants sown initially. Samplings were done 30, 70, 100, 115 and 130 days after planting (DAP). The two first and last rows in each pot were regarded as margins and thus, were not sampled. In each sampling, plants of one row for all biotypes were first measured vertically to obtain plant height. Chlorophyll content of leaves was measured by a chlorophyll meter (Opti Science USA). Then, the number of tillers for each plant in resistant and susceptible biotypes was recorded. The plant shoots were subsequently separately cut from the soil surface and transferred to the lab. Leaves were detached from the shoots and after being counted, leaf area was measured with leaf area meter apparatus (Delta-T, Burwell, England). Then, shoots and leaves were dried separately in the oven at 75 ^{o}C for 72 hours, and subsequently, their dry weight was recorded. Growth analysis was performed using the formulas presented in Table 1. Seed per plant, 1000 kernel weight, and flag leaf area were measured at the end of the growing period.

Statistical analysis

Changes in plant height, dry weight, leaf area per plant and number of leaves and tiller per plant were described using a three-parameter sigmoidal function (Equation 1) fitted to the data.

$$Y = \frac{a_{max}}{1 + e^{-\left(\frac{d - d_{50}}{b}\right)}} \quad \text{(eq. 1)}$$

In which Y is the extent of changes over time, a_{max} is the maximum value estimated for the trait, d is time, d_{50} is time to reach 50% maximum value of the trait and b is slope at d_{50}.

Table 1 - Formulas used for calculation of growth indices

Index	Formula	Unit
Leaf area index (LAI)	$\frac{Leaf\ area\ of\ the\ plant}{Area\ covered\ by\ the\ plant}$	$(\frac{m^2{}_{leaf}}{m^2{}_{ground}})$
Leaf area ratio (LAR)	$\frac{Leaf\ area\ per\ plant}{Plant\ dry\ weight}$	$(\frac{m^2{}_{leaf}}{g_{plant}})$
Specific leaf area (SLA)	$\frac{Leaf\ area}{Leaf\ weight}$	$(\frac{m^2{}_{leaf}}{g\ _{leaf}})$
Relative growth rate (RGR)	$\frac{ln_{w2} - ln_{w1}}{t_2 - t_1}$	$(\frac{g}{g\ day})$
Net assimilation rate (NAR)	$\frac{(w2 - w1)\ \times (ln_{la2} - ln_{la1})}{(t_2 - t_{1)} {}_{\times} (la_2 - la_1)}$	$(\frac{g}{cm^2\ day})$
Crop growth rate (CGR)	$\frac{w_2 - w_1}{(t_2 - t_1) \times A}$	$(\frac{g}{m^2\ day})$

LA: leaf area; W: plant weight; t: time; A: área.

Growth analysis indices of LAR and LAI were also analyzed the same as traits mentioned above. RGR, CGR, NAR, and chlorophyll content index changes over time were described as a scatter-line graph. Data related to changes in SLA were fitted to a linear function.

Seed production per plant, 1000 kernel weight, and flag leaf area were measured at the end of the experiment period, and their data were analyzed as a completely randomized design (CRD) with three replication using SAS software ver. 9 and the means were compared via the LSD method at $p<0.05$. All figures were prepared using SigmaPlot software ver. 12.5.

RESULTS AND DISCUSSION

According to Figure 1, growth parameters had followed an almost similar trend over time, and the parameters estimated showed that these traits had no significant differences between susceptible and resistant biotypes. Maximum values for plant height, plant dry weight, leaf per plant, leaf area per plant and leaf dry weight of susceptible and resistant biotypes were estimated respectively 107.75-110.87 cm, 7.30-7.85 g, 38.89-44.14 leaf per plant, 235.8-260.69 cm^2 and 1.14-1.25 g. Time to reach 50% of maximum value for these traits were recorded 68.67-71.88 days, 90.33-95.15 g, 71.31- 77.78 days, 68.90-72.98 cm^2 and 75.85-85.53 g, respectively (Table 2). Chlorophyll content index reached its maximum (ranging approximately from 35-38) 100 days after sowing and then declined (Figure 1).

Changes in growth analysis indices were also largely similar between susceptible and resistant biotypes (Figure 2). Specific leaf area for the studied biotypes decreased over time at a rate ranging from 0.32 to 0.34 m^2 leaf. g^{-1} leaf per day. Leaf area ratio and Leaf area index of susceptible and resistant biotypes had peak values of 0.018-0.020 m^2 leaf. g^{-1} plant and 7.004-7.820 m^2 leaf. m^{-2} ground, and time to reach 50% of these values ranged from 82.964-88.242 days, respectively (Table 3). Although the trends of crop growth rate and net assimilation rate of the biotypes slightly differed at the middle stages, this difference was not significant. Changes in the relative growth rate of susceptible and resistant biotypes, however, were more identical and no significant differences were observed as well (Figure 2).

Since d_{50} of resistant and susceptible biotypes for all studied traits including the ones demonstrating degrees of variations between the biotypes was similar and it was not possible to distinguish the differences using this parameter, analysis of variance based on completely randomized design with three replications was performed for the data points at which the differences between biotypes were more pronounced. This selected point for leaf area index, leaf dry weight and leaf area per plant was 100 days after sowing (the point at which the differences between biotypes commenced to be more obvious), whereas dry weight was chosen to be analyzed

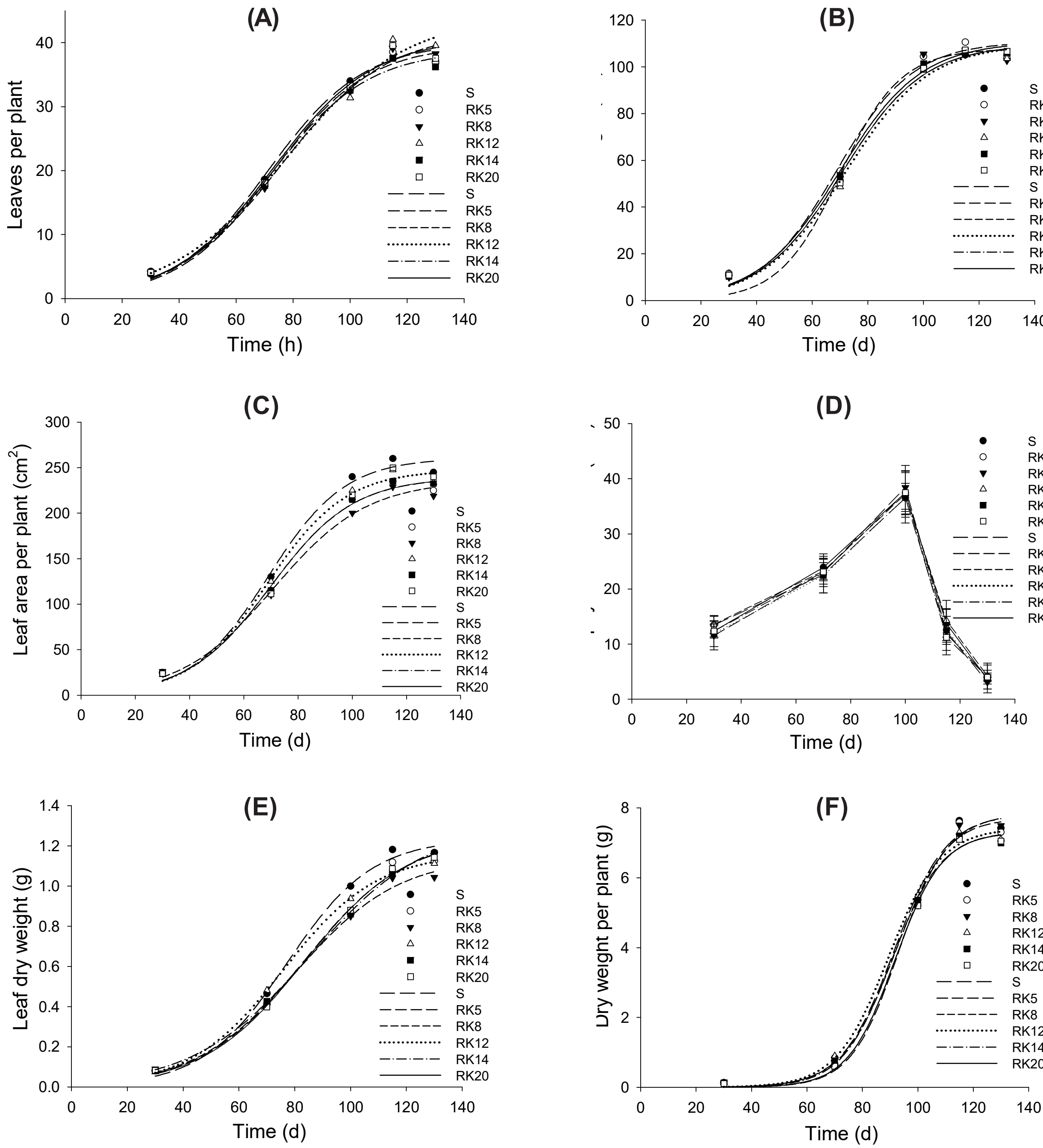

Figure 1 - Changes in (A) leaf per plant, (B) plant height, (C) leaf area per plant, (D) chlorophyll content index, (E) single-leaf dry weight and (F) dry weight per plant of susceptible and resistant winter wild oat biotypes over time.

at the end of the experiment (130 days after sowing). According to results, the values associated with these points were also not significantly different between biotypes (Table 4).

Seed per plant of susceptible and resistant biotypes ranged from 74.55 to 77.00, and 1000 grains of these biotypes weighed 12.99-14.50 grams. Also, the area of the flag leaf varied from 57.99 to 59.45 cm^2. None of these traits showed significant difference between susceptible and resistant biotypes (Table 5).

According to the results, it may be deducted that Ile-2041-Asn mutation leading to haloxyfop R-methyl resistance had no fitness cost on winter wild oat biotypes. Therefore, in absence of herbicide selective pressure, these resistant biotypes may not be outdone by the susceptible ones in the field. Travlos (2013) also investigated ACCase resistant winter wild oat under competitive and non-competitive conditions and no fitness cost was observed between resistant

Table 2 - Parameter estimates for plant height, dry weight per plant, leaf per plant, leaf area per plant and single-leaf dry weight of susceptible and resistant winter wild oat biotypes

Biotype	Plant height (cm)			Plant dry weight (g)			Leaf per plant			Leaf area per plant (cm^2)			Leaf dry weight (g)		
	Max	Slope	d_{50} (d)	Max	Slope	d_{50} (d)	Max	Slope	d_{50} (d)	Max	Slope	d_{50} (d)	Max	Slope	d_{50} (d)
S	109.62 (4.9)	14.62 (3.1)	70.71 (2.8)	7.82 (0.4)	9.43 (1.9)	91.49 (2.6)	40.10 (2.7)	16.77 (2.2)	71.31 (4.4)	260.69 (13.5)	14.21 (3.8)	69.00 (3.8)	1.23 (0.1)	15.27 (2.5)	77.30 (2.8)
RK5	110.87 (6.7)	13.81 (4.6)	68.67 (3.9)	7.70 (0.4)	8.49 (2.2)	92.9 (2.7)	39.67 (2.4)	16.75 (3.7)	72.84 (4.0)	239.33 (12.6)	15.24 (3.6)	69.94 (3.4)	1.25 (0.1)	18.53 (3.7)	81.23 (5.0)
RK8	107.75 (6.0)	10.90 (5.1)	69.98 (3.0)	7.85 (0.3)	9.58 (1.6)	95.15 (3.0)	41.67 (2.8)	18.50 (3.7)	75.74 (4.3)	235.8 (14.2)	17.20 (3.7)	71.09 (4.0)	1.14 (0.1)	18.41 (3.3)	80.44 (4.32)
RK12	109.71 (6.5)	14.88 (3.9)	71.88 (3.7)	7.42 (0.3)	9.38 (1.5)	90.33 (2.8)	44.14 (4.6)	20.70 (5.2)	77.78 (6.8)	247.71 (11.6)	14.40 (3.4)	68.90 (3.0)	1.16 (0.1)	16.75 (1.8)	75.85 (4.8)
RK14	109.52 (4.8)	14.65 (3.0)	70.80 (2.7)	7.32 (0.3)	9.01 (2.6)	90.69 (2.3)	38.89 (2.2)	17.12 (3.5)	72.12 (3.7)	240.13 (12.8)	14.24 (2.9)	70.15 (3.2)	1.30 (0.1)	20.89 (3.0)	85.53 (5.8)
RK20	110.68 (4.2)	14.64 (2.6)	69.90 (2.5)	7.30 (0.3)	8.50 (1.2)	91.79 (1.8)	40.71 (2.7)	17.45 (4.2)	72.98 (4.6)	255.91 (14.6)	15.42 (3.6)	72.98 (3.6)	1.25 (0.1)	18.33 (2.8)	83.58 (3.4)

Values in parentheses are standard errors.

and susceptible biotypes. On contrary, wild oat biotypes resistant to difenzoquat, imazamethabenz, flucarbazone, and tralkoxydim produced respectively 67% and 43% less tillers and seeds compared to susceptible biotypes (Lehnoff et al., 2013). In contrast to the results obtained in this study, Papapanagiotou et al. (2015) reported that winter wild oat plants containing Ile-2041-Asn mutation in their ACCase exhibit lower fresh weight and panicle number in comparison with susceptible biotype, but also stated that their results for various populations studied was inconsistent due to the selection of non-resistance associated alleles.

No fitness cost implies that the same non-chemical weed management practices can be applied for both resistant and susceptible biotypes. Application of the herbicide mentioned above at recommended rate will wipe out susceptible individuals but will fail to suppress resistant plants and thus, will lead to an increase in the relative frequency of resistant alleles in the population. Other herbicides having different modes of action to which winter wild oat has not yet developed resistance may be implemented to eradicate both susceptible and resistant biotypes, but on the other hand, it will serve as a new selective pressure. The continuity of the pressure will gradually increase the relative frequency of alleles resistant to this herbicide in the population (Gherekhloo et al., 2012). To avoid serious consequences of the evolution of multiple resistant plants, it may be wise to advocate non-chemical weed management methods and try to weaken the weed in competition.

According to Leverett (2017) germination phenology plays an important role in the competition of a species due to the influence it has on plant establishment in the environment. Winter wild oat seeds are attributed with thermo-dormancy (Whittington et al., 1970), and noting the higher optimum temperatures of germination for canola)Lakzaei et al., 2017; Khalaj et al., 2012) compared to winter wild oat (Forozesh et al., 2018), early sowing of canola or increasing soil temperature using plant residues may be considered as proper management strategies. Wheat and canola rotation is very common in the studied region, and since the presence of these plant residues can increase winter wild oat biomass by up to 10 times compared to residue-free conditions (Purvis et al., 1985), early sowing seems to be a more proper method. Canola cultivation in the region is usually performed from late October to November. Hence considering the difference between maximum temperatures of October and November, early sowing of canola in October may be a feasible approach to attain faster emergence and establishment of the crop. Canola stand, therefore, can close the canopy before the occurrence of winter wild oat plants and have the edge in the competition. However, supplementary irrigations may be required, especially in dry seasons. Careful selection of sowing date is also applicable to other areas all over the world infested with either wild-type or Ile-2041-Asn winter wild oat biotypes, provided that required meteorological information is available.

Removing selective pressure of herbicide form the environment is also an option. Winter wild oat is a self-pollinating species, but it shows some degree of cross-pollination (Cavan et al., 1998). Since no fitness cost was observed as a result of resistance evolution, the presence of

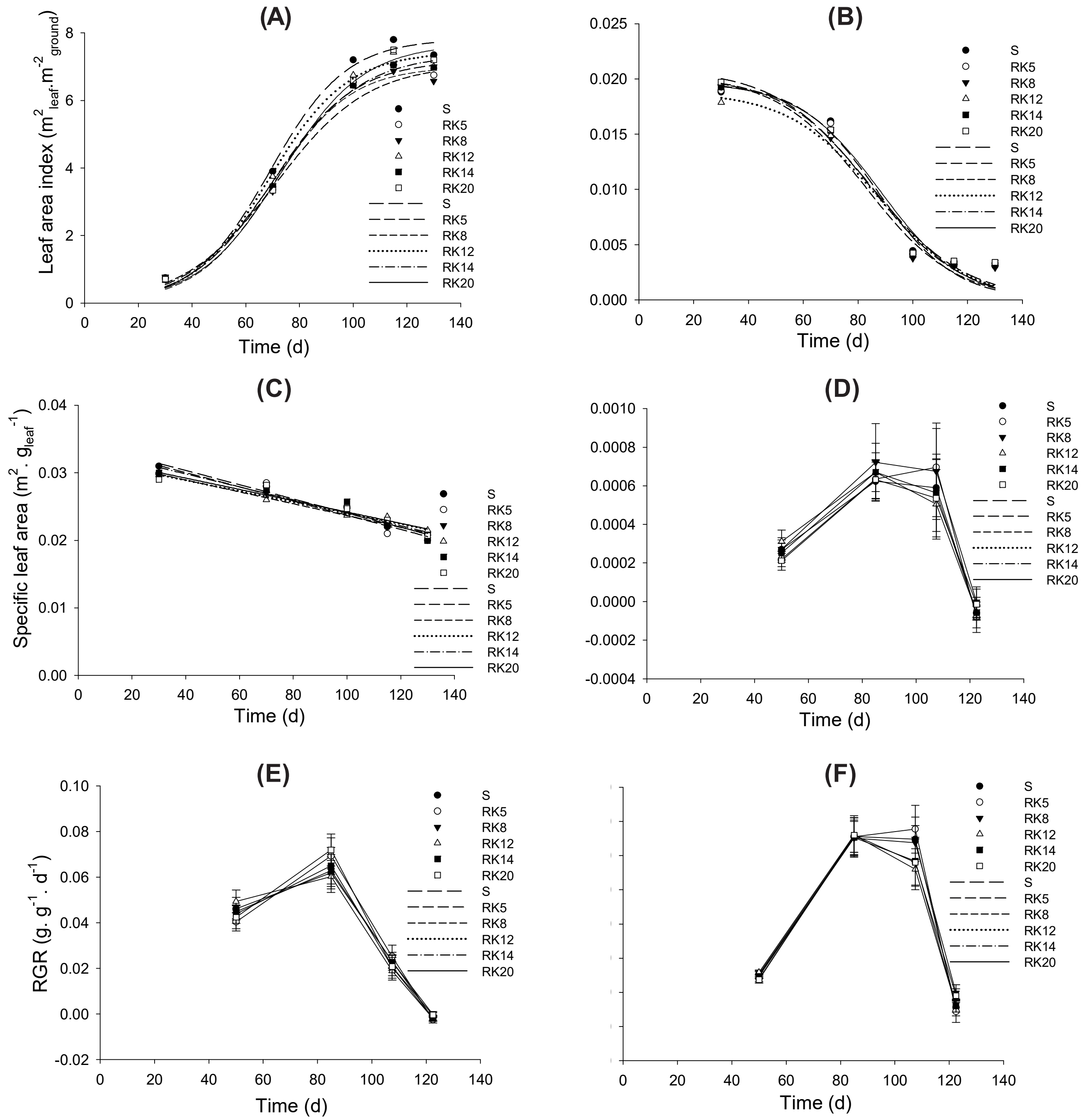

Figure 2 - Changes in (A) leaf area index, (B) leaf area ratio, (C) specific leaf area, (D) net assimilation rate, (E) relative growth rate and (F) crop growth rate of susceptible and resistant winter wild oat biotypes over time.

susceptible individuals between resistant biotypes will lead to cross-pollination between them. Thus, the population will contain both susceptible and resistant alleles and consequently, the relative frequency of resistant alleles in the population will decrease over time. Removal of selective pressure along with integrated weed management methods may be also regarded as a stratagem to battle this weed.

It must be noted that the present study has not taken into account other genetic variations non-associated with herbicide-resistant which would impose a further fitness cost on winter wild oat biotypes, so probably this cost may have been underestimated (Vila-Aiub et al., 2011). Anyway, from an ecological point of view, if resistance endowing genetic mutations impose insignificant fitness cost or even higher fitness compared to wild-type individuals, herbicide resistance in the population will develop more rapidly (Vila-Aiub et al., 2015). This fact clearly expresses the necessity of serious measures to suppress herbicide resistant winter wild oat weed and adopting management strategies to preserve crop production.

Table 3 - Parameter estimates for specific leaf area, leaf area ratio and leaf area index of susceptible and resistant winter wild oat biotypes

Biotype	SLA (m^2 $_{leaf}$. g^{-1} $_{leaf}$)			LAR (m^2 $_{leaf}$. g^{-1} $_{plant}$)			LAI (m^2 $_{leaf}$.m^{-2} $_{ground}$)		
	a	*b*	R^2	Max	Slope	d_{50} (d)	Max	Slope	d_{50} (d)
S	-1.05E-4 (7.32E-6)	0.034 (0.02)	0.99	0.019 (0.002)	-14.885 (5.353)	88.242 (8.115)	7.820 (0.407)	14.209 (3.839)	69.008 (3.384)
RK5	-1.05E-4 (1.91E-5)	0.034 (0.02)	0.96	0.019 (0.002)	-14.168 (4.795)	87.104 (7.439)	7.004 (0.453)	14.394 (4.615)	70.055 (4.139)
RK8	-8.76E-5 (-8.71E-5)	0.032 (0.02)	0.98	0.020 (0.002)	-16.214 (4.884)	82.964 (7.521)	7.073 (0.426)	17.200 (3.565)	71.097 (4.026)
RK12	-8.08E-5 (-6.08E-6)	0.032 (0.02)	0.98	0.018 (0.002)	-16.301 (6.017)	86.907 (9.098)	7.431 (0.349)	14.441 (3.431)	68.904 (3.082)
RK14	-9.59E-5 (-1.58E-5	0.033 (0.02)	0.96	0.020 (0.002)	-16.260 (5.521)	84.913 (8.460)	7.364 (0.312)	16.229 (2.754)	70.886 (2.791)
RK20	-8.36E-5 (1.55E-5)	0.032 (0.02)	0.95	0.020 (0.002)	-17.302 (5.806)	84.222 (8.895)	7.677 (0.440)	15.542 (3.601)	72.987 (3.595)

Values in parentheses are standard errors.

Table 4 - Mean comparison for leaf area per plant, leaf dry weight, leaf area index and dry weight of susceptible and resistant winter wild oat biotypes

Biotype	Leaf area per plant (cm^2)	Leaf dry weight (g)	Leaf area index (m^2 $_{leaf.}$ m^{-2} $_{ground}$)	Dry weight (g)
S	240.25	1.01	7.22	7.42
RK5	215.54	0.86	6.45	7.30
RK8	200.50	0.85	6.50	7.52
RK12	225.77	0.93	6.75	7.12
RK14	215.35	0.86	6.45	7.00
RK20	220.00	0.88	6.66	7.05
LSD	22.55	0.12	0.47	0.30

Table 5 - Mean comparison for seed per plant, 1000 seed weight and flag leaf area of susceptible and resistant winter wild oat biotypes

Biotype	Seed per plant	1000 kernel weight (g)	Flag leaf area (cm^2)
S	77.00	14.50	58.55
RK5	76.66	13.85	59.45
RK8	74.55	14.00	57.99
RK12	74.66	12.99	58.00
RK14	75.75	13.55	58.25
RK20	75.33	14.28	59.05
LSD	4.36	1.75	2.12

Lack of fitness cost indicates that susceptible winter wild oat biotypes may not be able to outdone resistant ones in the field. Imposing a new selective pressure via herbicide rotation may make the evolution of further resistance slower. Also, non-chemical weed management methods such as careful selection of sowing date may prove useful to attenuate the adverse effects of this weed on crop production.

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