Romanian Biotechnological Letters *Vol. 22, No. 2, 2017*

ORIGINAL PAPER

Fitness studies on invasive weedy sunflower populations from Serbia

Received for publication, February, 02, 2015
Accepted, September, 09, 2015

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Abstract

Weedy sunflower has become a problem worldwide, giving significant yield losses in sunflower and soybean fields even at low densities, decreasing their oil quality and allowing gene flow among crops and weeds. Its evolution differs among countries due to availability of wild forms. The problem is not only weedeness but also herbicide resistance in the Republic of Serbia. Three weedy sunflower populations from Serbia were studied: RWS1 and RWS2, which were presumably ALS herbicide resistant and SWS which is susceptible. Plant height, fresh weight, leaf area, relative chlorophyll content, fecundity and percentage of germination, length and weight of seedlings with and without nicosulfuron application were recorded. The most frequently RWS1 and RWS2 populations were of better ecological fitness than the SWS population under the conditions with and without nicosulfuron application. The number of seed produced was higher in RWS1 without herbicide application and RWS2 with nicosulfuron application. It was concluded that the differences in the level of herbicide-resistance could result in different fitness level of weedy sunflower populations which could promote the invasiveness of these populations in landscape.

Keywords: fitness, *Helianthus annuus*, herbicide resistance, invasiveness, nicosulfuron, weedy sunflower

Abbreviations: DAT – days after treatment; IMI-R – imidazolinone resistant; IMI-S – imidazolinone susceptible; RCC – relative chlorophyll content; SWS – susceptible weedy sunflower; RWS1– presumably resistant weedy sunflower population1; RWS2 – presumably resistant weedy sunflower population 2

1. Introduction

Weedy sunflower (*Helianthus annuus*) has become a problem not only in the North America where is the native area [1] but also in the other parts of the world. Its evolution might differ from place to place [2], which is suggested that is originated from wild introductions in Argentina while from pollen contamination of commercial seed with wild plants or crop-wild hybrids in Spain in a comparative study [3]. Sunflower might be introduced to Europe in early sixteenth centuary through Spain as ornamental and spread throughout Europe during the centuary [4] and multiple introductions into Europe were occured [5]. Sunflower as a crop has increased since 1970s in Europe [6] and the records of weedy sunflower in Europe date back to 1980s [7]. In all evolving conditions, after weedy sunflower is established, hybridization between crop and weedy/wild/feral populations is inevitable [2, 6, 8, 9, 10, 11, 12, 13, 14]. Volunteer populations are also subject to hybridization with crop and feral populations [10].

Weedy sunflower is an issue worldwide, which causes decline in yield over 50% under more than 4 plants m^{-2} in sunflower crop, 97-95% under 4.6 plants m^{-2} in soybean and lesser amount in wheat [7, 15, 16, 17]. In addition, weedy sunflower affects quality and quantity of oil due to lower oil content and different fatty acid composition [17]. It can become troublesome for cultivated sunflower given their genetic similarity that allows gene flow: weedy-to-crop and crop-to-weedy [18]. Especially use of genetically or biologicaly engineered varieties require more attention on weedy sunflowers [19, 20, 21, 22]. Weedy sunflower is not only difficult to control weed but also its spread accelerates after its population reaches certain level [23]. Weedy sunflower is also considered an issue of major concern in the sunflower growing areas of the Balkan Peninsula such as Hungary, Croatia and Romania. Weedy sunflower populations in arable and non-arable lands have been observed in these neighboring countries where traditionally sunflower hybrids been cultivatied. Given that there is a realistic possibility for interspecies and intraspecies crossing in *H. annuus* and its close relatives [9, 24] and resulting in that spreading resistant weedy sunflower can be a big problem. The biggest populations are in southern Srem and southern Banat (north part of Serbia) [25]. Although no studies carried out in Serbia, it might be more detrimental for small holding farmers of Serbia because of inadequate agricultural practices and poor weed management. Herbicide resistant common sunflower populations have been reported in Serbia and the other places [26, 27, 28]. The wide-spread adoption of herbicide-resistant crops (sunflower hybrids resistant to herbicide ALS inhibitors) has exposed herbicides such as nicosulfuron the weedy population to the high risk of crop-to-weedy gene flow. Resistance to ALS herbicides occurs rapidly and spreads because of lack of fitness cost [29]. On the other hand, fitness cost can provide some advantage to population to cause delay in germination [30, 31]. Fitness of herbicide resistant populations is a measure of adaptation of populations and gives estimation of their possible extension and management ways if fitness estimations can be done accurately [32]. Fitness of herbicide resistant weed populations under competitive and non competitive conditions without herbicide applications show differences although herbicide resistant weed populations under herbicide application conditions have fitness advantage [33, 34, 35, 36]. In addition, gene flow herbicide resistant crop to its weedy populations can result in higher fitness advantage of these weeds as it has been shown in weedy rice [37]. Increasing feral populations and their damages on crops, expanding use of engineered crops, developing herbicide resistance and rising environmental concerns require understanding ecology and biology of weedy sunflower populations. The aim of the current study was reveal fitness of herbicide resistant and susceptible weedy sunflower populations with and without herbicide applications, which might end up with a strategy proposal for weedy sunflower control based on evaluated fitness and invasiveness of recorded populations.

2. Materials and methods

Weedy sunflower seeds for the field experiment were collected in 2006 from three sites: Surcin 1 (SWR1) and Surcin 2 (SWR2) where ALS inhibitor herbicides were applied successively six and three years, respectively and Padinska Skela (SWS) where herbicides had never been applied. Field experiments were conducted at the Institute PKB Agroekonomik, Belgrade in 2008 and 2009. The soil type was alluvial black marsh with 2.6% of organic matter and pH 7.8. The experimental area was managed according to the conventional agronomic practices and irrigated as needed. Meteorological data (Table 1) in 2008 was characterized by lower rainfall during the first part of the season, accumulating 89.8 mm between 1 April to 30 June, and higher rainfall in the following months (157 mm from 1 July to 31 August). The 2009 growing season had a relatively high rainfall during the first part of the growing season (146.8 mm accumulated until 1 July) and low rainfall in the later months of the season (90.6 mm from 1 July to 31 August). The daily mean temperatures were similar for both years. Between 1 April and 1 September, 1396.7 d °C (d °C = Σ [(T_{max} + T_{min}) / 2-T_{base}]; T_{base} = 10°C) were accumulated in 2009 compared to 1388.6 d °C in 2008. The experimental model was a randomized complete block design with four replications. The size of each plot was 5.0 × 4.2 m. Plants which were grown in pots were transplanted (108 individuals per plot) in the field 10 days after emergence. Plants were treated with nicosulfuron (40 g a.i. ha^{-1}, BASF) at the 2–4 true-leaf growth stage (17 to 19 days after transplanting) using a knapsack sprayer Neptune 15, Kwazar® equipped with a TeeJet 1004 nozzle delivering 300 L ha^{-1} at 200 kPa pressure on 17 May in both years. All plots were maintained weed-free for the other weeds by hoeing. Plant height, fresh weight, leaf area and relative chlorophyll content (RCC) were recorded five times (0, 15, 30, 45 and 60 DAT). Fresh weight was taken immediately after cutting plants from the soil level. Leaf area was measured using a Delta-T leaf area meter. Changes in RCC of the same leaves were measured between the midrib and margin using a Minolta SPAD 502 chlorophyll meter [38]; two measurements were taken per leaf and averaged. After the completion of SPAD reading, leaves were taken from 10 plants and stored at –20°C until total chlorophyll content was recorded. A standard curve was constructed on the basis of SPAD readings and total chlorophyll content from laboratory analysis. Based on standard curves and „n" SPAD readings, RCC was determined. Fecundity (number of heads $plant^{-1}$, head diameter, number of seeds $plant^{-1}$) measurements, from two central rows of each plot, were made at maturity. After harvesting the seed samples were collected from each treatment and stored in the dark at room temperature until use. Thirty seeds (10 seeds × 3 replications) of each treatment were placed in petri dishes and 5 ml of distilled water was added and were placed in growth chamber at 25°C. The percentage of germination, length and weight of seedlings were recorded after 7 days. Each experiment was conducted twice. Statistical procedures were carried out using STATISTICA 5.0 software. Having in mind that there were no significant differences between years, then combined data were subjected to one-way ANOVA (F-values) to evaluate main effects of nicosulfuron application on germination, vegetative productivity, relative chlorophyll content and fecundity of weedy sunflower populations.

Results

Fitness of the different weedy sunflower populations was evaluated based on plant height, fresh weight and leaf area under conditions with and without herbicide applications. The effect of year as a factor had no significant influence on the most of the fitness parameters examined. Therefore data from both years were combined. Plant height, fresh weight and leaf area was higher in plants not sprayed comparing to survived plants after spraying (Figure 1). At the late season, plant height reached to 241, 238, and 220 cm for not sprayed populations for RWS1, SWS and RWS2, respectively. Generally, under the conditions without herbicide application WSR1 plants were the tallest during the season although significant differences in plant height between weedy sunflower populations at the evaluation time throughout the season were not always found (Figure 1a). Nicosulfuron application caused height inhibition among survived plants: the lowest plant height inhibition has been noted for RWS1 (23-44%), followed by RWS2 (28-50%) and SWS plants (49-52%). Differences in plant height between weedy sunflower populations after nicosulfuron application were significant ($p<0.01$) except between RWS1 and RWS2 at the second and between RWS2 and SWS at the last evaluation time.

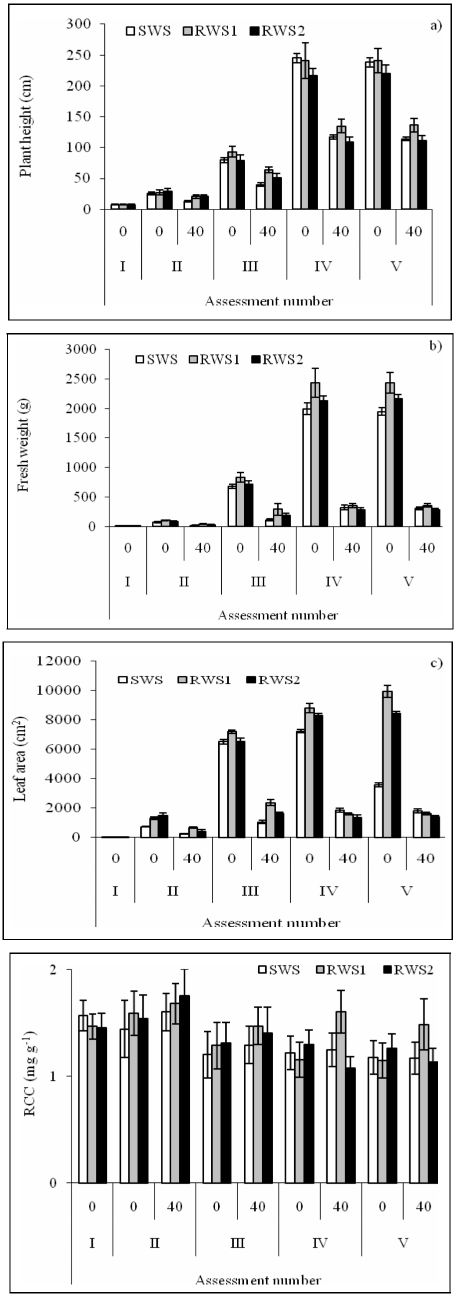

Figure 1. Plant height (**a**), fresh weight (**b**), leaf area (**c**) of weedy sunflower populations, which nicosulfuron was applied (40 g a.i. ha^{-1}) or not applied (0 g a.i. ha^{-1}).

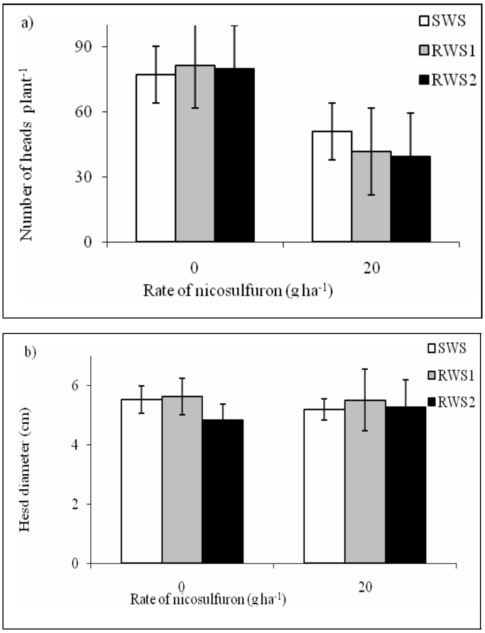

Figure 2. Relative chlorophyll content (RCC) of weedy sunflower populations under nicosulfuron (40 g a.i. ha^{-1}) applied or not applied.

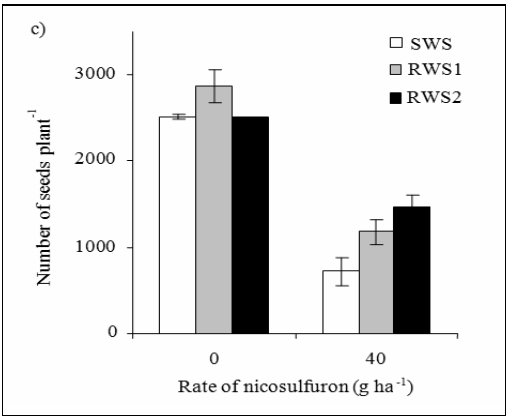

Figure 3. Number of heads $plant^{-1}$ (**a**), head diameter (**b**) and number of seeds $plant^{-1}$ (**c**) with and without nicosulfuron (40 g a.i. ha^{-1}) application.

Table 1. Rainfall and GDD in 2008 and 2009 at the experimental field

Month	Rainfall (mm)		GDD (^{o}C)	
	2008	2009	2008	2009
April	1.2	9.4	129.8	139.1
May	49.0	39.2	218.6	229.8
June	39.6	98.2	320.1	273,1
July	82.0	35.8	333.7	366.3
August	75.0	54.8	386.4	388.4
Total	**246.8**	**237.4**	**1388.6**	**1396.7**

Table 2. Seed germination, seedling length and weight of weedy sunflower populations with and without nicosulfuron application

Parameter	Rate of nicosulfuron (g a.i.ha^{-1})	SWS	RWS1	RWS2
		(mean value± SD)		
Seed germination (%)	0	5.75 ± 0.85	6.50 ± 1.75	8.75 ± 2.30
	40	5.65 ± 1.46	3.75 ± 1.94	8.75 ± 1.88
Seedling length (cm)	0	1.42 ± 0.52	2.50 ± 1.73	7.45 ± 1.66
	40	3.13 ± 0.70	3.12 ± 2.48	6.81 ± 1.20
Seedling weight (g)	0	0.22 ± 0.03	0.23 ± 0.02	0.27 ± 0.11
	40	0.23 ± 0.06	0.21 ± 0.02	0.29 ± 0.06

Only the inhibitory effect of herbicide to RCC was confirmed for RWS2 at the last two evaluations. Nicosulfuron applied plants produced significantly less heads and seeds than no herbicide applied ones; but diameter of heads were similar (Figure 3). When the herbicide was not applied plants of WSR1 population produced significantly more seeds (2865 seeds plant^{-1}) compared to the other two populations (SWS:2510 and RWS2: 2499 seeds plant^{-1}). Regarding to the number of head plant^{-1}, RWS1 population averaged the same as the SWS (53), but was lower compared to RWS2 population (55). Populations with the lower number of heads plant^{-1} contained heads with a larger diameter (RWS1:5.6 cm, SWS:5.5 cm, RWS2:4.8 cm) and had a greater seed production. Under the conditions with nicosulfuron application, the number of seeds plant^{-1} were RWS2:1473, RWS1:1182 and SWS:718; number of heads plant^{-1} was RWS2:30, RWS1:24 and SWS:14; otherwise head diameter were RWS2:5.3, RWS1:5.5 and SWS:5.2. Generally, weedy sunflower seeds had very poor germination, almost 4 to 9%. Germination of the seeds (% germination, seedling length and weight) that originated from the plants that developed without nicosulfuron application or from the plants survived the herbicide application the previous year, were the best for RWS2 plants (Table 2). Differences in percentage of seed germination, seedling length and weight (data not shown) were insignificant ($p>0.05$) and therefore the application of nicosulfuron the previous year had no influence on seed germination of weedy sunflower populations.

3. Discussions and Conclusions

There is no evidence for a fitness cost of herbicide resistance [39, 40] or for an invasiveness of weedy sunflower populations. As in our study, the previous studies demonstrated that environmental stresses can alter fitness or productivity of plants [41]. This hypothesizes an interaction of hybridization and growing conditions. Variation in environmental conditions and genetic on fitness is likely to play an important role in invasion of weedy sunflower plants. Alternatively, similar environmental conditions may differently affect the survival and reproduction of different populations of weedy sunflower. In this study, three weedy sunflower populations were grown under the same conditions, suggesting that the source of the populations may have an important influence on fitness. We found considerable variation in many measured parameters (plant height, fresh weight, leaf area, relative chlorophyll content, seed germination and fecundity) between the tested populations. With respect to almost all of the measured parameters, ecological fitness of weedy sunflower populations was higher under the condition without herbicide application. The exception to this was RCC value in which case very often chlorophyll content in treated plants was higher compared to the untreated plants for all three weedy sunflower populations. On one hand this shows that weedy sunflower populations battled herbicide stress by increasing chlorophyll production [42], and on the other to be the result of the fact that the synthesis of plant pigments is not the primary target of nicosulfuron [43]. The most frequently RWS1 and RWS2 populations were of better ecological fitness than the WSS population under the conditions with and without nicosulfuron application. However, sometimes there were no differences in growth parameters between SWS and the other two resistant populations of weedy sunflower, especially in plant height and rarely in leaf area under the condition without herbicide application. This result is partially in agreement with an earlier publication, which reported no differences in growth parameters between IMI-R (imidazolinone resistant) and IMI-S (imidazolinone susceptible) common sunflower, except in early growth stages when high growth rate in resistant plants was found [44].

In our study the leaf area in RWS1 and RWS2 plants was significantly greater than in SWS, except in the late growth period when a high leaf area in SWS plants was found under conditions with nicosulfuron application. Also it was described similar changes of the leaf area between IMI-R and IMI-S common sunflower during the growth period, while in the case of IMI-R and IMI-S prairie sunflower hybrids the opposite was true [45]. Moreover, in all vegetative parameters of fitness (plant height, fresh weight, leaf area) RWS1 was better than RWS2 population under the condition with as well as without nicosulfuron treatment, that point out a higher level of resistance to nicosulfuron than RWS2 plants. Concerning generative parameters of fitness SWS plants had considerably lower fecundity (number of heads plant^{-1}, number of seeds plant^{-1}, and head diameter) than the other two populations, particularly under conditions with nicosulfuron application where seed production was reduced by 39% and 51% with regard to RWS1 and RWS2 plants. Similarly to our findings, it was showed that R plants of *Lolium rigidum* resistant to glyphosate produced more seeds than the susceptible plants [46]. Resistant weedy sunflower plants showed superiority with regard to different parameters, e.g. RWS1 showed better fitness with respect to vegetative parameters [44] and probably a greater capacity for invasiveness. The superiority of resistant plants was also shown regarding seed germination, e.g RWS2 showed greater fecundity and seed germination after application of nicosulfuron. In a recent study, it was confirmed different ALS activity in vitro between resistant and susceptible weedy sunflower plants ($IR_{WS\text{-}R1}$:6.13, $IR_{WS\text{-}R2}$:3.80), which was corresponding to fitness parameters of these populations that were grown under the conditions with and without nicosulfuron application [26]. Consequently, our results suggest that RWS1 and RWS2 plants possess a certain superiority compared to SWS plants, which can impose fitness costs. Those fitness differences could result from different resistance alleles themselves [44, 47] or their genetic polymorphism [41].

Finally, it suggests that the relative fitness of resistant plants compared with susceptible plants can promote the spreading and invasiveness of weedy sunflower. Similarly to this it was found that fitness differences between resistant and susceptible plants are usually inferred from comparison of relative plant vigor, productivity, or competitiveness, as measured using specific traits including: seed dormancy, flowering date, seed production, aboveground biomass and fecundity of the species[48]. In addition, it was found that susceptible plants of *Kochia scoparia* germinated more rapidly than the susceptible plants in the absence of herbicide [49]. Moreover, it was conferred that resistant plants of *K. scoparia* and *Lactuca serriola* germinated faster than susceptible plants [50]. Differences between R and S plants were suggested to be due to genetic polymorphism and not because of the resistance traits. As well, it was reported that resistant and susceptible sunflower plants displayed a high degree of dormancy [51], which was most probably the case in our study as all three WS populations showed poor seed germination. We have found that the distinct level of herbicide-resistance could result in different fitness of weedy sunflower populations which could promote the invasiveness of these populations in landscape. A major finding of this study was that resistant weedy sunflower populations are pre-eminent in comparison to susceptible populations and possess ecological fitness as a promoter of their invasiveness. Fitness measures describe the potential evolutionary success of a population based on survival, competitive ability and reproductive success and promote dispersal and invasiveness. Finally, weedy sunflower as invasive plants could become troublesome for sunflower crops, given their genetic similarity which allows gene flow in both directions (weedy sunflower to crop and crop to weedy sunflower), especially in the regions of traditional sunflower planting.

4. Acknowledgments

We thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for supporting this investigation Project III 46008 and EU-FP7-REGPOT-AREA Project No 316004.

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