Effect of nicosulfuron on the populations of invasive weedy sunflower

Die Auswirkung von Nicosulfuron auf invasive Unkraut-Sonnenblumenbestände

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Abstract

Weedy sunflower *Helianthus annuus* L. (WS) can become troublesome for cultivated sunflower given their genetic similarity which allows gene flow from weed-to-crop and from crop-to-weed. Also, it causes yield losses in different spring-sown crops. Despite this, there is still no data about its response to herbicides.

Field experiments were conducted to quantify the effects of nicosulfuron on WS populations. Plants of three populations (R1-WS and R2-WS = presumably resistant and S-WS = susceptible) were treated with 40 g nicosulfuron ha-1 at the 2–4 true-leaf growth stage. Vegetative parameters (plant height, fresh weight, leaf area) and relative chlorophyll content (RCC) were measured 30 days after herbicide application. Generative parameters (number of heads plant-1, head diameter, number of seeds plant-1) were measured at maturity. After harvesting, seed germination of collected seeds was studied in Petri dishes at 25°C.

Nicosulfuron was found to have a significant effect on vegetative and generative production of WS plants. The effect on RCC and postharvest seed germination was not so prominent. All this effects depended on the population, which is probably a result of different history of herbicide application and possible development of resistance in the populations R1-WS and R2-WS.

Key words: Generative parameter, *Helianthus annuus*, resistance, vegetative parameters, weedy sunflower

Zusammenfassung

In Anbetracht der genetischen Ähnlichkeit zwischen der Kulturpflanze und seinem zugehörigen Unkraut, die einen Genfluss in beiden Richtungen ermöglicht, ist es zu erwarten, dass auch die Unkraut-Sonnenblume, *Helianthus annuus* (WS) eine bedeutende Entwicklungsstörung bei der Ackersonnenblume hervorrufen und genauso Ertragsverluste bei anderen Sommerungen verursachen kann.

Angesichts der fehlenden Daten bezüglich der Herbizidreaktion des obengenannten Unkrauts wurden entsprechende Felduntersuchungen durchgeführt, mit dem Ziel die Auswirkungen von Nicosulfuron auf WS-Populationen quantitativ zu bestimmen. Drei unterschiedliche Pflanzenbestände (R1-WS und R2-WS = vermutlich resistent, und S-WS = anfällig) wurden mit 40 g Nicosulfuron/ha, jeweils auf das zweite Blattpaare behandelt. Vegetative Parameter (Pflanzengröße, Frischmasse, Blattfläche) und relativer Chlorophyllgehalt (RCC) wurden 30 Tage nach dem Herbizideinsatz gemessen. Generative Parameter (Anzahl der Pflanzenköpfe, Kopfdurchmesser, Anzahl der Pflanzensamen) wurden im reifen Zustand gemessen. Nach der Ernte, wurde noch die Keimung der gesammelten Samen in Petrischalen mit einer Temperatur von 25 °C genauer beobachtet.

Die Ergebnisse deuteten darauf hin, dass Nicosulfuron eine erhebliche Auswirkung auf vegetative so wie auch auf generative Produktion von WS-Pflanzen hat. Die Auswirkung auf RCC und Keimung durch Ernte gewonnener Samen zeigte sich jedoch nicht in dem gleichen Maße auffällig. Alle Effekte standen im Zusammenhang mit den Pflanzenpopulationen, was wahrscheinlich eine Folge vom andersartigen Verlauf der früheren Herbizidanwendungen, und vermutlicher Resistenzentwicklung in R1-WS und R2-WS Populationen ist.

Stichwörter: Generative Parameter, *Helianthus annuus*, Resistenzentwicklung, Vegetative Parameter, Unkraut-Sonnenblumenbestände

Introduction

Harvesting *Helianthus annuus* or other crops inevitably results in some unintentional loss of seeds. In subsequent years when these seeds germinate they are called „volunteer plants“. If volunteer seeds become incorporated into the soil seed bank and subsequently germinate and flower, newly-germinated populations may continue to be exposed to gene flow even in the absence of nearby related crops (REAGON and SNOW, 2006). Hybridization between cultivated and their volunteer or wild/weedy sunflower (WS) is inevitable in regions of traditional sunflower crop production (HVARLEVA et al., 2009). Weedy forms of sunflower are morphologically clearly different from the volunteers originating (MULLER et al., 2009). The F-1 hybrids between the cultivated sunflower and WS are fertile and may hybridize more easily with wild relatives than cultivated sunflowers (SERIEYS and CHRISTOV, 2005). Weedy sunflower populations are characterized by a high morphological diversity, with plants combining, in different proportions, the traits of cultivated and wild sunflower (POVERENE et al., 2009; MULLER et al., 2009). Those plants have adapted to different environments in an undesired way, becoming harmful and invasive weeds (ARNOLD, 2004; MULLER et al., 2009). The main traits distinguishing WS are strong branching, without apical dominance, production of many seed heads, reduced size of the seed head and achene in comparison with the cultivated sunflower, seed dormancy and shattering (BURKE et al., 2002). In the past decade weedy sunflower was frequently reported as an invasive species in both arable and non-arable lands (MARSHALL et al., 2001; BENÉCSNÉ BÁRDI et al., 2005; POVERENE et al., 2009; POVERENE and CANTAMUTTO, 2010). It can become troublesome for the cultivated sunflower, due to their genetic similarity, which allows gene flow: weed-to-crop and crop-to-weed (URETA et al., 2008). In France, MULLER et al. (2009) reported that WS caused yield losses of the cultivated sunflower in more than 50% of the heavily infested patches. According to our experience in spring sown crops, yield losses can be more than 70% in the case of inadequate agricultural practices and poor weed management. Weedy sunflower is also a big problem for the entire area of the Balkan Peninsula, where sunflower is planted, e.g. in Hungary, where it is the 18^{th} most harmful weed species (BENÉCSNÉ BÁRDI et al., 2005), Croatia, Romania, etc. In Spain, the WS were found mainly in crop fields, where the largest populations affected about 1500 m^2 and were composed of no more than 200 plants (POVERENE and CANTAMUTTO, 2010). In some parts of central Italy, WS is spreading from the marginal areas into cultivated fields, where sunflower has not been cultivated for more than 5 years (VISCHI et al., 2006).

In Serbian maize fields, *Sorghum halepense* L. (Pers.) and several broad-leaved species including WS are common dominant weeds. Although there are several ALS inhibiting herbicides which effectively control weeds in corn, farmers often choose nicosulfuron due to its high efficacy for *S. halepense* (BAGHESTANI et al., 2007; NOSRATTI et al., 2007), even at low rates of application (ROSALES-ROBLES et al., 2001). Nicosulfuron is a POST-applied sulfonylurea herbicide, whose mode of action is inhibition the ALS (acetolactate synthase) enzyme, which is essential for the production of amino acids leucine, isoleucine and valine. Intensive application of nicosulfuron has caused a development of resistant populations in several grass and broad-leaved weed species (*Setaria viridis* (L.) Beauv., *Setaria faberi* Herrm., *Conyza albida* Willd., *Amaranthus retroflexus* L.) (VOLENBERG et al., 2001; OSUNA and DE PRADO, 2003; SCARABEL et al., 2007; LAPLANTE et al., 2009). As nicosulfuron is a widely used herbicide for weed control in maize and WS is one of the most widespread weeds in this crop in some parts of Serbia, the aim of this study was to check effects of nicosulfuron on WS and conclude if this herbicide is an adequate for WS population control in maize fields.

Materials and Methods

Seeds of three weedy sunflower populations with different herbicide application history were collected: R1-WS and R2-WS, from the fields where ALS inhibitor herbicides were applied successively for six and three years, respectively, and S-WS seeds, which were collected from the field where any herbicides had never been applied. Field experiments were conducted in two consecutive years. The experimental area was managed according to the conventional agronomic practices, including deep autumn plowing and seedbed preparation before setting up an experiment. The soil was alluvial black marsh with 2.6% of organic matter and pH 7.8. Precipitation and growing degree days (GDD, d °C = Σ [$(T_{max} + T_{min})$ / 2-T_{base}]; T_{base} = 10 °C) are summarized in Table 1.

Tab. 1 Rainfall and GDD in 1st and 2nd year.

Tab. 1 *Niederschlagsmenge und Wachstumsgradtage im 1. und 2. Jahr.*

Month	**Rainfall (mm)**		**GDD (°C)**	
	1st	**2nd**	**1st**	**2nd**
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**

The experimental model was a fully randomized block design with four replicates and size of each plot was 5 × 4.2m. Plants were treated with the recommended rate of nicosulfuron (40 g a.i.ha^{-1}, BASF) at the 2–4 true-leaf growth stage, using a knapsack sprayer Neptune 15, Kwazar®, equipped with a TeeJet 1004 nozzles. The sprayer was calibrated to deliver 300 L ha^{-1} at 200 kPa pressure. Control plots (without herbicide treatment) and plots in which weeds survived the herbicide treatment were maintained weed-free from other weeds by hoeing.

The measurements of plant height, fresh weight, leaf area and relative chlorophyll content (RCC) were made 30 days after herbicide application (DAHA). Leaf area was measured using a Delta-T leaf area meter. RCC was calculated based on SPAD-readings measured using a Minolta SPAD 502 chlorophyll meter. A standard curve for RCC calculation was constructed based on SPAD readings and total chlorophyll content was determined in laboratory after the extraction with acetone. The measurements of the number of heads plant^{-1}, head diameter and number of seeds plant^{-1} were done at maturity of plants. After harvesting, seed germination of collected seeds (10 seeds × 3 replications per treatment) was studied in Petri dishes. Distilled water (5 ml) was added and Petri dishes 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.

All data was processed by one-way ANOVA (F-values) using software STATISICA 5.0. Data for both years were analyzed as one set because effect of nicosulfuron on WS did not differ significantly between years.

Results

Despite the differences in the distribution of rainfall during the vegetation season (Tab. 1), the experimental year as a factor had no significant influence on the differences between most of the parameters examined. Therefore, all the results reported represent average values of the two year testing (not shown).

Generally, the three WS populations, with different herbicide application history (S-WS, R1-WS, R2-WS), differed in their vegetative parameters (plant height, fresh weight, leaf area) in response to the recommended rate of nicosulfuron applied (40 g a.i.ha^{-1}). The recommended rate of this herbicide was not enough to cause mortality of WS populations, but results presented in Table 2 indicate that nicosulfuron significantly ($P<0.01$) reduced the plant height, fresh weight and leaf area in all three populations. Namely, 30 days after the application of nicosulfuron the lowest inhibition of plant height had been registered for R1-WS (31.48%), followed by R2-WS (35.76%) and the highest reduction in growth was registered for S-WS plants (49.97%). With regard to fresh weight, nicosulfuron caused the lowest inhibition in R1-WS plants (64.83%), followed by R2-WS (73.30%), while the strongest effects were found in S-WS plants (83.50%). The response of leaf area to nicosulfuron showed the same trend like plant height and fresh weight. Furthermore, the lowest inhibition was detected for R1-WS plants (67.06%), followed by R2-WS (75.02%) and S-WS (83.68%).

Tab. 2 Plant height, fresh weight and leaf area (mean ± SD) of WS populations 30 DAHA.

Tab. 2 *Pflanzenhöhe, Frischmasse und Blattfläche der WS-Pflanzenbeständen 30 Tage nach Applikation.*

Population	Rate of nicosulfuron (g a.i. ha^{-1})	Plant height (cm)	Fresh weight (g)	Leaf area (cm^2)
S-WS	0	79.71±3.97	679.21±37.12	6505.74±178.20
	40	39.88±2.63	112.04±15.63	1061.51±97.97
R1-WS	0	92.92±8.76	830.79±80.89	7195.21±108.29
	40	63.67±2.63	92.21±101.86	2369.51±201.83
R2-WS	0	78.88±9.28	719.83±56.58	6528.47±208.82
	40	50.67±7.11	192.17±38.14	1631.21±92.37

In general, the RCC (Tab. 3) did not change significantly (P>0.05) in plants treated with nicosulfuron, in comparison with the untreated plants, but in all populations these values were slightly higher, when compared to the RCC values of the untreated plants.

Tab. 3 Relative chlorophyll content (RCC) of weedy sunflower populations.

Tab. 3 *Relativer Chlorophyllgehalt (RCC) von Ausfallsonnenblumen.*

Population	Rate of nicosulfuron (g a.i. ha^{-1})	RCC (mg g^{-1})
S-WS	0	1.20±0.22
	40	1.29±0.18
R1-WS	0	1.29±0.22
	40	1.47±0.17
R2-WS	0	1.31±0.20
	40	1.40±0.24

Generative production of WS populations (Tab. 4) was different in treated and untreated plants. Actually, values of generative parameters in untreated plants were higher than the same parameters in treated plants. Untreated plants of R1-WS, R2-WS and S-WS population produced significantly (P<0.01) more seeds (2865 seeds plant^{-1}, 2499 seeds plant^{-1} and 2510 seeds plant^{-1}) when compared with the treated plants (1182 seeds plant^{-1}, 1473 seeds plant^{-1} and 718), respectively. With regard to the number of heads plant^{-1}, the production of untreated plants (R1-WS: 53, R2-WS: 55 and S-WS: 53) was significantly (P<0.01) higher than the production of treated plants (R1-WS: 24, R2-WS: 30 and S-WS: 14). Populations with the lower number of heads plant^{-1} contained heads with a larger diameter (R1-WS = 5.6 cm, WSR2 = 4.8 cm, S-WS = 5.5 cm), in plants without herbicide application, while with plants with herbicide application that relationship was not observed (R1-WS = 5.5cm, R2-WS = 5.3 cm, S-WS = 5.2cm).

Tab. 4 The number of heads plant^{-1}, head diameter and number of seeds plant^{-1} (mean ± SD) at maturity.

Tab. 4 *Anzahl der Pflanzenköpfe, Kopfdurchmesser, Anzahl der Pflanzensamen im reifen Zustand.*

Population	Rate of nicosulfuron (g a.i. ha^{-1})	Number of heads plant^{-1}	Head diameter (cm)	Number of seeds plant^{-1}
S-WS	0	53±2.12	5.5±0.12	2510 ±24.71
	40	14± 0.58	5.2±0.23	718±52.19
R1-WS	0	53± 4.11	5.6±0.31	2865±34.18
	40	24±2.22	5.5±0.24	1182±9.26
R2-WS	0	55± 3.12	4.8±0.18	2499±99.28
	40	30±1.56	5.3 ±0.25	1473±11.42

Generally, WS seeds germinate very poorly (4 to 9%). The germination of the seeds (% germination) which originated from the plants that developed without the application of nicosulfuron, or from the plants which survived the herbicide application in the previous year, were the best for WSR2 plants (Tab. 5). Differences in seed germination were insignificant (P>0.05), and therefore the application of nicosulfuron during the previous year had no influence on seed germination of WS populations. Seedling length differed between treated and untreated plants in all three populations, but in populations S-WS and R1-WS it was longer in that case when herbicide was applied in the previous year, while in the population R2-WS, it was shorter. Contrary to that, nicosulfuron did not affect the seedling weight.

Tab. 5 Seed germination (%), seedling length (cm) and seedling weight (g) (mean ± SD) of WS populations.

Tab. 5 *Samenkeimung (%), Sprosslänge (cm) und Sämlingsgewicht der WS-Pflanzenbestände.*

Population	Rate of nicosulfuron (g a.i. ha^{-1})	Seed germination (%)	Seedling length (cm)	Seedling weight (g)
S-WS	0	5.75 ± 0.85	1.42 ± 0.52	0.22 ± 0.03
	40	5.65 ± 1.46	3.13 ± 0.70	0.23 ± 0.06
R1-WS	0	6.50 ± 1.75	2.50 ± 1.73	0.23 ± 0.02
	40	3.75 ± 1.94	3.12 ± 2.48	0.21 ± 0.02
R2-WS	0	8.75 ± 2.30	7.45 ± 1.66	0.27 ± 0.11
	40	8.75 ± 1.88	6.81 ± 1.20	0.29 ± 0.06

Discussion

Weedy sunflower populations are present in many European countries including France, Spain, Hungary, Croatia, Romania, Serbia, etc. (MULLER et al., 2009; POVERENE and CANTAMUTTO, 2010; BENÉCSNÉ BÁRDI et al. 2005; SAULIC et al., 2013), where they cause yield losses in cultivated plants. Despite this, there is no data about the response of weedy sunflower to herbicides. Although nicosulfuron is a herbicide commonly used for weed control in corn, where weedy sunflower is one of the dominant weeds, according to our knowledge, the response of this species to nicosulfuron has not been previously studied. BRIGHENTI et al. (2011) found that nicosulfuron did not have a phytotoxic effect on the sunflower crop plants, while STREIT (2012) found that nicosulfuron applied at rate 30 g a.i.ha^{-1} has a low phytotoxicity to sunflower crop varieties/hybrids. But, it may not be possible to extrapolate the results of these studies to weedy sunflower populations due to high morphological and genetically variability of different sunflower forms.

Herbicidal effects of nicosulfuron on WS populations were examined using the manufacturer's recommended rate (40 g a.i. ha^{-1}). The used rate was not enough to cause mortality of WS population. But, populations with history of herbicide application (R1-WS, R2-WS) were less susceptible to nicosulfuron than population S-WS which have never been treated by any herbicide, based on all of their vegetative parameters. Reductions due to the application of nicosulfuron in the plant height, fresh weight and leaf area were 31-50%, 65-84% and 68-84%, respectively, depending on the population. Plant height was the parameter least sensitive to nicosulfuron, while the sensitivity of fresh weight and leaf area was similar. These results are in accordance with the previous findings of BOZIC et al. (2013), who studied the effects of nicosulfuron on *Xanthium strumarium,* and discovered that the plant height is the least sensitive to nicosulfuron, in comparison with the fresh weight and leaf area index (LAI).

Although most post-emergence herbicides (including nicosulfuron) are applied at the seedling stage and, thus, do not directly affect the plant's reproduction, some of them also affect the seed production. In our study, nicosulfuron reduced the number of heads (45-74%) and seeds (41-71%) $plant^{-1}$, while there was no effect on the head diameter. Similarly to our findings, ZHANG et al. (1994) showed that bentazone significantly reduced the weight of burs of *X. strumarium.* Also, BOZIC et al. (2013) confirmed the reduction of burs production (weight $plant^{-1}$ or number $plant^{-1}$) in *X. strumarium* treated with nicosulfuron, but its reduction was not as strong as the reduction of vegetative parameters.

Contrary to other parameters (vegetative and generative), the RCC values in treated plants were higher, when compared to the untreated, for all of the three weedy sunflower populations studied. On the one hand, this shows that weedy sunflower populations battled herbicide stress by increasing chlorophyll production (RIETHMULLER-HAAGE et al., 2006), and on the other to be the result of the fact that the synthesis of plant pigments is not the primary target of nicosulfuron, (MEKKI and LEROUX, 1994).

It is well known that the conditions in which plants are grown affect the germination of produced seeds. In our study, the application of nicosulfuron on WS populations had no significant effect on the germination of seeds produced by the survived plants and the seedlings' weight, while the seedling length differed between treated and untreated plants. But, it cannot be attributed to the nicosulfuron, due to the fact that the seeds from treated plants of S-WS and R1-WS germinate better than the seeds from untreated plants, while in the R2-WS population that was contrary.

In conclusion, nicosulfuron was found to have a significant impact on vegetative and generative production of WS plants, while its effect on RCC and postharvest seed germination was not so prominent. Due to that, nicosulfuron application leads to a lowered competitive ability of WS plants in the field and therefore to a lower seed bank accumulation in the soil. Also, we conclude that the effect of nicosulfuron on populations with different history of herbicide (acetolactate synthase (ALS)- inhibitors) application were different. Additional future studies should be done to check does the history of herbicide application have an impact on WS response to nicosulfuron.

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