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Article

Chemical Options for the Control of Silverleaf Nightshade (*Solanum elaeagnifolium*)

*Opções para Controle Químico de Melãozinho-do-Campo (**Solanum elaeagnifolium**)*

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ABSTRACT - Silverleaf nightshade is a difficult-to-control perennial weed. Field experiments were conducted in northern Greece to evaluate the control of silverleaf nightshade with POST applications of glufosinate (1,500 g a.i. ha^{-1}), glyphosate (3,600 g a.i. ha^{-1}), tembotrione (148.5 g a.i. ha^{-1}), and a mixture of tembotrione plus bentazon (148.5 plus 1,440 g a.i. ha^{-1}) at an early vegetative stage (plant height 10-15 cm) and at the beginning of flowering (plant height 30-50 cm). Glufosinate provided > 95% control of silverleaf nightshade from 7 to 39 days after treatment (DAT), regardless of the vegetative stage at herbicide application. Similarly, glyphosate provided up to 90% control around 39 DAT at either growth stage applied, exhibiting gradually increasing efficacy. Tembotrione alone controlled silverleaf nightshade 85% when applied at the early vegetative stage and 48% when applied at the beginning of flowering. The mixture of tembotrione plus bentazon applied at the beginning of flowering exhibited 74% control at 21 DAT; however, the control was decreased to 41% at 35 DAT. When the mixture was applied at the early vegetative stage, *S. elaeagnifolium* control was 61% at 23 DAT, which was decreased to 27% at 39 DAT. Glufosinate and glyphosate were found to be reliable options for control of silverleaf nightshade when applied at either weed growth stage; tembotrione could be also another reliable option, however, when applied only at an early vegetative stage. The results have significant implications for developing appropriate management practices for silverleaf nightshade, taking into account chemical options for preventing the evolution of herbicide resistance.

Keywords: application stage, herbicide efficacy, invasive weed.

RESUMO - Melãozinho-do-campo é uma planta daninha perene de difícil controle. A fim de avaliar o controle dessa planta, ensaios de campo foram realizados no norte da Grécia, com aplicações em pós-emergência de glufosinate (1.500 g i.a. ha^{-1}), glyphosate (3.600 g i.a. ha^{-1}), tembotrione (148,5 g i.a. ha^{-1}) e uma mistura de tembotrione mais bentazon (148,5 mais 1.440 g i.a. ha^{-1}), numa fase vegetativa (altura da planta de 10-15 cm) e no início da floração (altura da planta de 30-50 cm). O glufosinate proporcionou controle de melãozinho-do-campo acima de 95% de 7 a 39 dias após o tratamento (DAT), independentemente do momento da aplicação. Da mesma forma, o glyphosate proporcionou controle de 90% em aproximadamente 39 DAT, em cada fase de crescimento, exibindo eficácia gradualmente aumentada. O tembotrione sozinho controlou 85% do melãozinho-do-campo na fase vegetativa e 48% no início da floração. A mistura de tembotrione e bentazon aplicada no início da floração controlou 74% aos 21 DAT; no entanto, o controle foi reduzido para 41% aos 35 DAT. Quando a mistura foi aplicada no início da fase vegetativa, o controle de ***S. elaeagnifolium***

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foi de 61% aos 23 DAT, que foi reduzido para 27% aos 39 DAT. Glufosinate e glyphosate demonstraram ser opções confiáveis para o controle de melãozinho-do-campo; o tembotrione poderia ser também uma outra opção confiável, mas apenas quando aplicado no início da fase vegetativa. Os resultados têm implicações importantes para o desenvolvimento de práticas de gestão adequadas para melãozinho-do-campo, tendo em vista as opções de químicos para prevenir a evolução da resistência aos herbicidas.

Palavras-chave: estádio de aplicação, eficácia de herbicidas, planta daninha invasora.

INTRODUCTION

Silverleaf nightshade (*Solanum elaeagnifolium*), a perennial broadleaf weed, is considered one of the most invasive plants worldwide (EPPO, 2007; Brunel, 2011). It has been reported as a weed in several annual and perennial crops, such as corn (*Zea mays*), wheat (*Triticum aestivum*), grain sorghum (*Sorghum bicolor*), cotton (*Gossypium* spp.), tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), alfalfa (*Medicago sativa*), grapes (*Vitis vinifera*), olive (*Olea europaea*), and peaches (*Prunus persica*) (EPPO, 2007; Brunel, 2011). This weed species can be found in Morocco (Taleb and Bouhache, 2006), Tunisia (Mekki, 2006), USA, Australia, Egypt, Greece, India, Israel, Zimbabwe, Sicily, South Africa, Northwest Africa, and Spain (Sforza and Jones, 2007). Silverleaf nightshade is a very adaptable plant that can tolerate relatively high summer temperatures (20-34 ^{o}C), low annual rainfall (250-600 mm), drought and saline soil conditions. It primarily reproduces from buds present on underground root fragments and also from seeds. Its seeds are able to remain viable for years (at least 10 years) (Brunel, 2011).

Silverleaf nightshade is a hard to control weed with the current cultural, mechanical, chemical, and biological control means (EPPO, 2007). In particular, mechanical control (soil tillage) is not considered the best control practice, since it breaks roots into fragments aiding in the spread of the weed, besides being expensive, and may damage soil structure (Ensbey, 2011). Considering chemical control, silverleaf nightshade is not easily controlled with herbicides due to its deep root system that is capable of developing new plants from the root buds; thus, infestations of this weed cannot be successfully controlled with a single herbicide application. The systemic herbicides might be able to provide some level of control of this weed (Eleftherohorinos et al., 1993).

Glyphosate is among the recommended herbicides for silverleaf nightshade control for non-selective treatments (Eleftherohorinos et al., 1993; Baye and Bouhache, 2007; Ensbey, 2011). Glyphosate is a systemic herbicide that controls a wide range of annual and perennial broadleaf weeds, grasses, and sedges. It inhibits the aromatic amino acid biosynthesis by blocking the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway. It can be used in fruit orchards, vineyards, and other row-seeded crops with directed-spray applications. It has been reported to efficiently control silverleaf nightshade in glyphosate-resistant cotton (Choudhary and Bordovsky, 2006; Joy et al., 2008).

Glufosinate, a non-selective contact herbicide, could be used for weed control in both non-agricultural and agricultural areas, such as in minimum tillage row crop production systems, fruit orchards, and vineyards, with directed-spray applications to control a broad range of annual and perennial weeds. Glufosinate inhibits glutamine synthetase in plants and blocks the synthesis of glutamine from glutamate and ammonia, resulting in rapid accumulation of toxic levels of ammonia within the cells (Mersey et al., 1990). It has been reported to control silverleaf nightshade effectively in glufosinate-resistant cotton (McCormick, 2005). However, in Europe glufosinate is not labeled for the control of this weed species.

Tembotrione belongs to the triketone class of herbicides and it is registered for selective POST control of a wide range of broadleaf weeds and some annual grasses in field corn grown for grain or silage, sweet corn, popcorn, and corn for seed production (Santel, 2009). It is mobile both in the plant symplast (phloem) and in the apoplast (xylem) (Schulte and Körcher, 2009). Tembotrione acts by inhibiting the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPD) in numerous weed species, which results in disruption of carotenoid formation that consequently leads to chlorophyll oxidation and inhibition of photosynthesis (Santel, 2009). As a result, the newly developing plant tissues in sensitive species appear white (bleached) (Hawkes, 2007). Tembotrione has been recently reported to inhibit growth of silverleaf nightshade plants causing 74 and 80% reduction in fresh weight of the surviving plants when applied at an early vegetative stage (Gitsopoulos et al., 2014).

Combination of herbicides with different mode of action is a component of integrated weed management practice for widening the weed control spectrum and decreasing the possibility of evolution of herbicide resistance. Bentazon belongs to inhibitors of photosystem II (PSII) and is used for postemergence control of broadleaf weeds and sedges. It has been used in tank mixtures with HPPD herbicides to increase weed control (Armel et al., 2008; Walsh et al., 2012). Both PSII and HPPD inhibitors cause inhibition of electron transfer between the plant photosystems, resulting in oxidative stress and reactive oxygen radicals that damage cellular constituents (Choe et al., 2014). Previous research reported synergism between PSII and HPPD inhibitors when applied POST (Bollman et al., 2008). Mesotrione, an HPPD-inhibitor, plus bentazon in mixture improved the control of yellow nutsedge over either herbicide applied alone; however, the control achieved was not considered commercially acceptable (Armel et al., 2008). Mesotrione has shown better control of purple (*Cyperus rotundus*) and yellow nutsedge (*C. esculentus*) when applied in combination with bentazon than with atrazine (both PSII inhibitors) (Armel et al., 2008). In another study, the tank mixture of atrazine plus tembotrione in sweet corn showed higher and more consistent weed control than tembotrione applied alone (Williams et al., 2011).

In the current study, tembotrione was tank-mixed with bentazon to determine any increase or decrease in silverleaf nightshade control. Concerning the appropriate growth stage for maximum control of silverleaf nightshade with the application of herbicides, there is conflicting information (Choudhary and Bordovsky, 2006). For silverleaf nightshade control, it is important to target rootbank with systemic herbicides, but when the seeds are the start of new infestations it is essential to control seedbank as well. The fruits and seeds of silverleaf nightshade are consumed and spread by birds and livestock and can be transported as a contaminant of hay and fodder products or spread via floodwaters and sheep can carry the seeds in their digestive tract for several weeks without affecting the seed germination capability (Anonymous, 2010). The berries are also toxic to livestock and may reduce the value of agricultural land (Sforza and Jones, 2007). In addition, early control is essential for a “clean” fruit orchard field and limiting weed competition. There are limited herbicides available for the control of silverleaf nightshade. The objectives of this study were to evaluate the efficacy of three herbicides (glufosinate, glyphosate, tembotrione) and a tank-mixture (tembotrione plus bentazon) applied POST at two growth stages of silverleaf nightshade.

MATERIALS AND METHODS

Two separate field experiments (herbicide treatments at the beginning of flowering and at an early vegetative stage) were conducted in summer 2015 to study the weed control with: glufosinate (Basta® 15 SL, glufosinate-ammonium 15% w/v, Bayer Hellas S.A., Greece), glyphosate (Meteor® 36 SL, glyphosate acid 36% w/v, isopropylamine salt of glyphosate 48.6% w/v, Alfa Agricultural Supplies S.A., Greece), tembotrione (Laudis® 66 OD, tembotrione 4.4% w/v plus isoxadifen-ethyl 2.2% w/v, Bayer Hellas S.A., Greece), and tembotrione plus bentazon (Basagran® 48 SL, bentazon 48%, sodium salt of bentazon 49.8% w/v, BASF Hellas S.A., Greece). The experiments were repeated (second run) again in the same year (2015). Initially, silverleaf nightshade plants were treated at the beginning of flowering (plant height 30-50 cm) on 15 July 2015. In early August, the half of both fields (runs) were ploughed letting the weed to re-emerge and on 20 August 2015 the emerged silverleaf nightshade plants were treated at early vegetative stage (plant height 10-15 cm). The mean temperature 7 days before up to 10 days after herbicide applications was similar for both experiments (Table 1). The two mentioned field runs of both experiments were conducted in two naturally infested fields with dense populations (12-18 plants m^{-2}) of silverleaf nightshade at a distance of 200 m to obtain similar weed density. The experiments were conducted at the experimental unit of Institute of Plant Breeding and Phytogenetic Resources in Thermi, northern Greece. Herbicide treatments included glufosinate at 1,500 g a.i. ha^{-1}, glyphosate at 3,600 g a.i. ha^{-1}, tembotrione at 148.5 g a.i. ha^{-1}, and a mixture of tembotrione plus bentazon (at 148.5 plus 1,440 g a.i. ha^{-1}). All herbicides were applied at label

Table 1 - Basic weather conditions (mean temperature and rainfall) before, after and on the day of herbicide applications

Days from applications	Beginning of flowering[1]		Early vegetative stage[2]	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
-7	29.0	0.0	29.0	0.0
-6	28.2	0.0	28.3	0.8
-5	27.3	0.4	28.5	0.0
-4	26.5	0.0	29.4	0.0
-3	25.8	0.0	28.9	0.0
-2	26.0	0.0	28.4	0.0
-1	28.0	0.0	28.2	0.0
0	28.3	0.0	28.1	0.0
+1	28.5	0.0	25.6	4.2
+2	28.4	0.0	23.0	4.6
+3	28.5	0.0	24.5	0.0
+4	29.4	0.0	25.5	0.0
+5	29.4	0.0	26.2	0.0
+6	30.8	0.0	26.4	0.0
+7	30.5	0.0	26.5	0.0
+8	29.0	0.0	26.1	0.0
+9	28.3	0.0	26.7	0.0
+10	27.8	0.0	27.5	0.0

(1) <http://penteli.meteo.gr/meteosearch/data/thessaloniki/2015-07.txt>. (2) <http://penteli.meteo.gr/meteosearch/data/thessaloniki/2015-08.txt>.

rates, except from tembotrione for which an increased rate (1.5X of the label rate for weed control in corn) was used. Non-treated plots were used as controls. Experiments were arranged in a randomized complete block design with three replications. Plot size was 4 m long and 3 m wide.

Herbicides were applied with an AZO hand-held boom sprayer equipped with six twin flat spray nozzles (TeeJet® TTJ60-11002 Twin Flat Spray Tips, Spraying systems Co., Illinois) at 207 kPa pressure. The water volume was 600 L ha^{-1} for glufosinate and 400 L ha^{-1} for the other herbicide treatments according to the label of each product for the control of perennial weeds.

Visual control estimates of silverleaf nightshade were taken at 7, 21, and 35 days after treatment (DAT) (herbicide application at the beginning of flowering) and 5, 12, 23, and 39 DAT (herbicide application at the early vegetative stage). The 6 week period was considered adequate time for estimating herbicide efficacy on plant growth and seed-set. Weed control was rated on a scale of 0 to 100%, where: 0% is no visual injury symptom and 100% refers to plant death, based on the extent of chlorosis, necrosis, and stunting of plants compared to the non-treated control. At the end of each experiment, ten randomly selected plants were cut at the ground level from the center of each plot and the fresh weight was measured after removing the necrotic plant parts. Fresh weight data were expressed as percentage of control based on fresh weight reduction compared to the non-treated control.

All data were subjected to ANOVA separately for each growth stage due to different sampling number and time after spraying. Before the analysis, all percentages data were arcsine transformed to normalize variance. Transformation did not alter mean ranking and interpretation; therefore, the actual mean values are presented. Means were compared with Fischer's protected LSD test at $P<0.05$. Mean daily temperature and rainfall before, after, and on day of herbicide applications are summarized in Table 1.

RESULTS AND DISCUSSION

Since there was no interaction between herbicide treatments and experimental runs, data were pooled over the two runs of each experiment. When applied at the beginning of flowering, glufosinate resulted in 95-97% control of silverleaf nightshade at 7 to 35 DAT (Table 2). The symptoms of glufosinate activity consisted of total discoloration (deep brown color of the foliage) resembling burned plants. Glyphosate treatments revealed a gradually increasing high efficacy level, reaching 88% at 35 DAT (Table 2). The symptoms differed to those caused by glufosinate and consisted of wilting and chlorosis of leaves in the majority of the plants. Tembotrione alone resulted in 48% control of silverleaf nightshade at 35 DAT (Table 2). Although the silverleaf nightshade plants exhibited the typical bleaching symptoms, they finally did not get necrotic, but survived with an evident retardation in their growth rate. Although the mixture of tembotrione plus bentazon showed 74% control at 21 DAT, the control declined to 41% at 35 DAT (Table 2) due to regrowth of the treated plants. The fresh weight reduction at 35 DAT was 99% in plants treated with glufosinate, 87% in plants treated with glyphosate and around 61% in plants treated either with tembotrione or with tembotrione plus bentazon (Table 2).

Table 2 - Visual control and fresh weight reduction of silverleaf nightshade plants treated at the beginning of flowering (plant height 30-50 cm)

Treatment	Rate (g a.i. ha^{-1})	Visual control(1)(2)			Fresh wt reduction(1)(2)
		7 DAT	21 DAT	35 DAT	
Non-treated control(3)	-	0	0	0	0
Glufosinate	1,500	95 a	99 a	97 a	99 a
Glyphosate	3,600	63 b	84 b	88 b	87 b
Tembotrione	148.5	7 d	40 c	48 c	61 c
Tembotrione + bentazon	148.5 + 1,440	42 c	74 b	41 c	62 c

(1) Means are averaged over two field runs. (2) Means within each column followed by the same letter(s) are not significantly different according to Fisher's protected LSD test ($P < 0.05$). (3) Data of the non-treated control were not included in the analysis.

At early vegetative stage, glufosinate resulted in 96% control of silverleaf nightshade at 39 DAT (Table 3). Glyphosate provided 90% control at 39 DAT and tembotrione alone showed 85% control at 39 DAT (Table 3). In particular, there was a gradual increase in tembotrione efficacy with time. The sprayed plants developed extensive bleaching symptoms and most of them did not manage to survive. These symptoms appeared in shorter time after treatment and they were more pronounced compared to tembotrione application at the beginning of flowering. The mixture of tembotrione plus bentazon caused initial symptoms of herbicide activity which provided 77% control at 12 DAT (Table 3); however, these symptoms gradually disappeared, the plants started to regain their green color and finally survived. The fresh weight reduction at 39 DAT was high with glufosinate, glyphosate, and tembotrione (99, 88, and 85%, respectively), suggesting that these herbicides provide good to excellent control of silverleaf nightshade. On the contrary, the fresh weight reduction caused by tembotrione plus bentazon mixture was low (44%) (Table 3).

Glufosinate and glyphosate are both non-selective herbicides and they can be used for the control of silverleaf nightshade in fruit orchards, fallow fields and in non-transgenic row crops, such as cotton, with directed-spray application. The higher efficacy of glufosinate and glyphosate compared to the other treatments makes them suitable for the control of silverleaf nightshade. For silverleaf nightshade, both seedbank and rootbank control are essential. Applying glyphosate at the end of summer can enhance rootbank control, because during this period the carbohydrates and the nutrients move into the root system and this contributes to increased movement of the herbicide towards roots (Kidston et al., 2007). However, Choudhary and Bordovsky (2006) found that applying glyphosate late in the season was not effective on silverleaf nightshade. Previous research has provided variable results related to the most appropriate growth stage of silverleaf nightshade to apply glyphosate (Feuerherdt, 2010). It was reported that the plants are more susceptible at the berry stage (Stubblefield and Sosebee, 1986). In Morocco, however, silverleaf nightshade was found more susceptible at full bloom than at the green berry stage (Bouhache et al., 1996), whereas in Greece, no differences in control between applications at full bloom or the berry stage were found (Eleftherohorinos et al., 1993). In a more recent study (Stanton et al., 2010), an autumn application of herbicides for optimum silverleaf nightshade rootbank control was suggested in contrast to recommendations for summer applications at the flowering or the early berry stage. In our study, glyphosate provided around 90% visual control when applied either at early vegetative stage or at the beginning of flowering and prevented weed growth.

Table 3 - Visual control and fresh weight reduction of silverleaf nightshade plants treated at early vegetative stage (plant height 10-15 cm)

Treatment	Rate (g a.i. ha^{-1})	Visual control(1)(2)				Fresh wt reduction(1)(2)
		5 DAT	12 DAT	23 DAT	39 DAT	
Non-treated control(3)	-	0	0	0	0	0
Glufosinate	1,500	98 a	99 a	98 a	96 a	99 a
Glyphosate	3,600	69 b	82 b	83 b	90 b	88 b
Tembotrione	148.5	20 d	42 c	75 bc	85 b	85 b
Tembotrione + bentazon	148.5 + 1,440	33 c	77 b	61 c	27 c	44 c

(1) Means are averaged over two field runs. (2) Means within each column followed by the same letter(s) are not significantly different according to Fisher's protected LSD test ($P<0.05$). (3) Data of the non-treated control were not included in the analysis.

To achieve an effective and consistent control of silverleaf nightshade without enhancing the possibility of herbicide resistance evolution, herbicides with different mode of action should be applied along with other integrated weed management. More specifically for the former, it is well known that mixtures of herbicides with different site of action reduce the herbicide selection pressure and delay the evolution of herbicide resistance (Beckie, 2011; Norsworthy et al., 2012; Chahal and Jhala 2015). In particular, in new infestations of silverleaf nightshade beginning by seed, glufosinate could be an alternative for effective control. Glufosinate with glyphosate could be used for long-term management by applying the former at the time of the first appearance of the weed in late spring, continuing with later applications as needed during summer, while application of glyphosate in autumn can take place for high effect on the underground reproductive organs, as stated above. Tembotrione could be another alternative herbicide for silverleaf nightshade control. However, the present study showed that tembotrione was not effective when applied at the flowering stage and this was the reason for repeating the herbicide applications later in summer at an early growth stage. In contrast, an acceptable level of control (85%) was observed with tembotrione when applied at an early vegetative stage. These results are in agreement with the results of our preliminary experiments which showed 74 to 80% fresh weight reduction in silverleaf nightshade at rates varying from 100 to 150 g a.i. ha^{-1} when applied at plants 15 cm tall (Gitsopoulos et al., 2014). Tembotrione is an important herbicide for weed control in corn at 100 g a.i. ha^{-1} (Santel, 2009). Since silverleaf nightshade has been reviewed as a weed in corn (EPPO, 2007; Brunel, 2011) tembotrione applied at an early vegetative stage could be an alternative for silverleaf nightshade control in corn. The mixture of tembotrione plus bentazon, although at the beginning exhibited higher efficacy level compared to that of tembotrione applied alone regardless the time of application, the weed control was drastically decreased over time. As mentioned above, there have been several reports showing increased efficacy either of bentazon in mixture with other HPPD inhibitors or tembotrione with other PSII inhibitors. However, this has not been always observed, which comes in agreement with our study; for example tembotrione efficacy was not improved for the control of volunteer potato when applied in mixture with bentazon (Koepke-Hill et al., 2010). It should be mentioned that altering the ratio of the mixture might provide different efficacy results; however, this was beyond the aim of the present study.

Glufosinate, glyphosate, and tembotrione can be significant tools for silverleaf nightshade control. These herbicides can be used according to the type of field infested (e.g., fruit orchard, row crop, fallow fields) in weed control programs with herbicide alternation to avoid herbicide resistance. Future research should focus on the rates of these herbicides (glufosinate, glyphosate, and tembotrione) required for effective control of silverleaf nightshade and other factors, including adjuvants, influencing their efficacy.

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