

# Effects of application date and rate of foliar-applied glyphosate on pine seedlings in Turkey

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**Abstract** Glyphosate is the herbicide most extensively used for site preparation and conifer release. It is a broad-spectrum herbicide and therefore crop safety is a critical issue. This study assessed the early effects of 14 different treatments, including no weed control, manual weed control, and 12 foliar-applied herbicide treatments at low, intermediate, high, and highest application rates and application timing on glyphosate phytotoxicity of containerized seedlings of Austrian pine (*Pinus nigra* J.F. Arnold.), Scots pine (*P. sylvestris* L.) and maritime pine (*P. pinaster* Aiton), conifer species widely used for afforestation and supplementary plantings in Turkish forestry. In general, Scots pine seedlings were tolerant to glyphosate compared to the other species. Glyphosate phytotoxicity varied significantly according to the time and rate of application. Seedlings were relatively tolerant to glyphosate in April whereas they were intolerant in May. The highest herbicide rate (1.2% v:v) was consistently phytotoxic to all species. Moreover, the effect of herbicide rate on seedling survival and growth varied significantly according to application date (i.e., application rate × date interaction). Seedlings appeared tolerant to glyphosate at

low and intermediate rates (0.2, 0.4% v:v) between mid-spring and mid-summer, whereas they demonstrated significant sensitivity to the highest rate across all time periods. Glyphosate at the high rate (0.8% v:v) was particularly more phytotoxic when applied in May. Application of glyphosate at rates up to 0.8% could be recommended for weed control without significant pine damage in mid-spring when the needles presumably have a dense leaf epicuticular wax layer limiting herbicide penetration. Applications of 0.8 and 1.2% v:v are not recommended during May–June.

**Keywords** Application date and rate · Glyphosate screening · Pines · Seedling tolerance

## Introduction

Herbaceous weeds compete with young tree seedlings for light, soil, water, nutrients and space and significantly reduce survival, growth and quality. Therefore, they are a major impediment to forest regeneration and in nursery production (McCarthy and O'Reilly 2001). By carrying out adequate weed control on sites where conifers grow, diameter and volume are significantly increased (Wagner et al. 2004).

Weeds are controlled almost exclusively by manual or mechanical methods in Turkey (Saatçioğlu 1957; Eşen and Yıldız 2000; Yıldız and Eşen 2006; Yıldız et al. 2007; Boydak and Çalışkan 2014). However, the temporary effectiveness on weeds and relatively high costs associated with manual and mechanical controls increasingly limit their use (Eşen and Yıldız 2000; Eşen and Zedaker 2004). As a result, herbicides are widely used for site preparation and conifer release in forest areas and for vegetation

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control in forest nurseries (McCarthy and O'Reilly 2001; Radosevich et al. 2007).

Crop safety is a critical issue with chemical weed control (Ross and Lembi 1989; Radosevich et al. 2007). Application rates and time are vital factors for herbicide phytotoxicity in pine seedlings (Newton and Knight 1981; Radosevich et al. 2007). Currently, screening tests are being employed extensively in Europe and North America in order to rapidly assess herbicide phytotoxicity and species tolerance of many tree species (Bunn et al. 1995; Ezel and Nelson 2001; Willoughby et al. 2003; Woeste et al. 2005; Mitchell et al. 2006; Stanley et al. 2014). Although a few screening tests for herbicide phytotoxicity in some hardwood species have been carried out in Turkey (Eşen et al. 2006, 2012), no study has to date been conducted on screening for herbicide phytotoxicity and tolerance for the major conifer species of Turkey.

Glyphosate is the most widely used herbicide in forestry and in forest nurseries worldwide for vegetation management (Duryea and Landis 1984; Tanjung 2001; Thompson and Pitt 2003; Wagner et al. 2004). It is a broad-spectrum, symplastically translocated herbicide to control many herbaceous and perennial weeds (Ross and Lembi 1989; Thompson and Pitt 2003; Duryea and Landis 1984). It is often used to release conifers from unwanted vegetation, including jack pine, (*Pinus banksiana* Lamb), black spruce (*Picea mariana* Mill.), and white spruce (*P. glauca* Moench.) (Thompson and Pitt 2003). Research indicates that the glyphosate tolerance of conifer seedlings varies by species and application times and rates (Prasad 1989; Tanjung 2001). There is, however, no information on the phytotoxicity of glyphosate on the major conifer species used in Turkish forestry, particularly taking application dates and rates into consideration.

This study aimed to determine the early effects of application dates and rates on the phytotoxicity of glyphosate to young containerized seedlings of Austrian pine (*Pinus nigra* J.F.Arnold.), Scots pine (*P. sylvestris* L.) and maritime pine (*P. pinaster* Aiton), the conifer species commonly used for afforestation and supplementary plantings in Turkish forestry (Boydak and Çalıřkan 2014; Anonymous 2014), and to help foresters to achieve effective, cost-efficient weed control in forested and nurseries areas.

## Materials and methods

One-, two-, and three-year-old containerized seedlings of maritime pine (Izmit–Kefken origin), Austrian pine (Bolu–Sarpuncuk origin), and Scots pine (Bolu origin) were supplied by the Pınar Forest Nursery of the Düzce Directorate of Forestry in the Western Black Sea Region of

Turkey. The maritime and Austrian pine seedlings were grown in 2.1 L polyethylene nursery bags, whereas the Scots pine seedlings were grown in 2.8 L bags. The growing medium consisted of 60% mineral soil, 30% peat moss and 10% perlite. Seedlings received supplemental watering as needed during the study. The study employed 14 different treatments, including no weed control (NWC), manual weed control (MWC), and 12 herbicide weed control (HWC) treatments with different application rates and dates (Table 1). For the MWC treatment, herbaceous weeds growing in the containers were pulled out by hand in May 2014. Over-the-top application of glyphosate [N-(Phosphonomethyl)glycine, Roundup® Star SL (441 g l<sup>-1</sup>)] herbicide was applied to the pine seedlings to the point of run-off on three different dates (early April, May, and June 2014) using a 18-L CO<sub>2</sub> polyethylene plastic knapsack electric battery sprayer (Gardenjoy®, China) (Table 1).

Ground line diameter (GLD) and height of all seedlings were measured prior to treatments and at the end of the growing season, and seedling vigor (height-to-GLD ratio) was determined for each seedling. In addition, the effects of the treatments on the seedlings were also rated ocularly using a 1–6 point visual health index based on the degree of injury symptoms (i.e., epinasty, chlorosis and necrosis) in the foliage prior to treatments and at the end of the growing season. Visual 1 designated dead seedlings with 100% crown necrosis, while Visual 6 described healthy seedlings with no visible morphological effects. The percent of seedling survival was calculated for each treatment for each species at the end of the growing season.

A randomized complete block design with four replications was used for the study. Each experimental unit contained 20 seedlings, and 1120 seedlings in total were used. Two sets of analyses were carried out; in the first set, data from all treatments, including NWC, MWC, and HWC applications, were analyzed using one-way ANOVA. In the second set, only HWC data were assessed using two-way ANOVA with application rate (AR) and application date (AD) as the main effects along with the AR × AD interaction effect (Ott 1993). In addition, the correlation of AR, including the control treatment (0% v:v) with the dependent variables (height, GLD, vigor (H/D), visual health index, and survival) was assessed in the third analysis across the ADs with three separate correlation analyses.

Means were separated using the Duncan mean separation test (MST) at  $\alpha = 0.05$ . Statistical analyses were carried out according to SAS (2008) and data were checked for violation of the ANOVA assumptions.

**Table 1** Effects of no weed control (NWC), manual weed control (MWC) and 12 foliar-applied glyphosate treatments (HWC) with various application dates (AD) and rates (AR) on mean height, ground

No.	Treatment <sup>1</sup>	AD	AR		Height (cm)	GLD (mm)	Visual	Survival <sup>3</sup> (%)
			(%, v:v)	(g a.i. ha <sup>-1</sup> )				
1	NWC	–	–	–	21.53 ± 1.3ab <sup>2</sup>	6.67 ± 0.5a	4.03 ± 0.4ab	91.3 ± 4.3b
2	MWC	June	–	–	23.09 ± 0.6ab	6.98 ± 0.2a	4.75 ± 0.1a	98.8 ± 1.3ab
3	G-A-0.2	April	0.2	172	20.54 ± 0.9ab	6.78 ± 0.3a	4.10 ± 0.2ab	97.5 ± 1.4ab
4	G-M-0.2	May	0.2	172	23.50 ± 1.3a	7.02 ± 0.1a	4.51 ± 0.2a	98.8 ± 1.3ab
5	G-J-0.2	June	0.2	172	22.69 ± 1.8ab	7.14 ± 0.3a	4.35 ± 0.4a	96.3 ± 2.4ab
6	G-A-0.4	April	0.4	344	23.86 ± 1.6a	7.01 ± 0.1a	4.59 ± 0.3a	100.0 ± 0a
7	G-M-0.4	May	0.4	344	23.76 ± 0.5a	7.06 ± 0.1a	4.33 ± 0.1a	98.8 ± 1.3ab
8	G-J-0.4	June	0.4	344	22.91 ± 3.2ab	6.66 ± 0.3a	4.13 ± 0.2ab	97.5 ± 1.4ab
9	G-A-0.8	April	0.8	689	19.93 ± 0.3b	6.33 ± 0.1a	3.53 ± 0.1bc	98.8 ± 1.3ab
10	G-M-0.8	May	0.8	689	21.22 ± 0.6ab	6.82 ± 0.3a	3.14 ± 0.3c	75.0 ± 8.7c
11	G-J-0.8	June	0.8	689	22.12 ± 1.8ab	6.29 ± 0.2a	2.80 ± 0.2cd	70.0 ± 6.1c
12	G-A-1.2	April	1.2	1032	19.78 ± 0.7b	6.74 ± 0.4a	2.71 ± 0.3cd	71.3 ± 5.5c
13	G-M-1.2	May	1.2	1032	24.05 ± 0.4a	7.05 ± 0.4a	2.25 ± 0.3d	47.5 ± 9.2d
14	G-J-1.2	June	1.2	1032	21.54 ± 1.3ab	6.24 ± 0.5a	2.94 ± 0.3cd	76.3 ± 8.0c

Nontransformed values were used for actual means

<sup>1</sup>Treatment main effect was significant ( $p \leq 0.05$ )

<sup>2</sup>Means within the same column with different letters are significantly different ( $p \leq 0.05$ )

<sup>3</sup>Arcsin (square root) transformed values were employed for separation of the mean for this variable

## Results and discussion

There were no significant pre-treatment differences for any variables for any species. Mean pretreatment GLD and height were 3.06 mm and 11.33 cm for 1-year-old maritime pine seedlings, 5.74 mm and 16.23 cm for 2-year-old Austrian pine seedlings, and 16.7 mm and 37.85 cm for 3-year-old Scots pine seedlings.

### Austrian pine

In the first set of analyses, significant differences were found for all dependent variables except for seedling vigor (H/D) (Table 1). Survival and the visual health index were generally high across treatments except for May/June applications at the high rate (0.8%) and the April, May, and June applications at the highest rate (1.2%). The April 0.4% application rate caused no seedling mortality, while the May application at the highest rate (1.2%) resulted in the lowest seedling survival. The highest glyphosate application applied in May significantly reduced the mean survival rate by more than 30, 47 and 52% when compared to the June-0.8%, NWC, and April-0.4% treatments, respectively (Table 1).

In the second set of analyses, the AD was found to have had a significant effect on mean height and vigor index (H/

D) (Table 2). The seedlings sprayed in May averaged a significantly higher mean height (10%) than those sprayed in April, which averaged a significantly lower vigor index when compared to those sprayed in May and June. In addition, the main effect of AR was significant for mean

**Table 2** *Pinus nigra* seedlings 1 year after treatment: (Upper section) effects of application date (AD) on mean height and vigor index (H/D); (Lower section) effects of application rate (AR) on mean height and visual health index (Visual)

AD <sup>1</sup>	Height (cm)	H/D
April	21.03 ± 0.6b <sup>3</sup>	31.59 ± 0.7b
May	23.04 ± 0.5a	33.75 ± 0.9a
June	22.32 ± 0.7ab	34.40 ± 0.8a
AR <sup>2</sup>	Height (cm)	Visual
0.2	22.24 ± 0.8ab <sup>3</sup>	4.32 ± 0.1a
0.4	23.51 ± 0.6a	4.35 ± 0.1a
0.8	20.97 ± 0.6b	3.15 ± 0.1b
1.2	21.79 ± 0.7ab	2.63 ± 0.2c

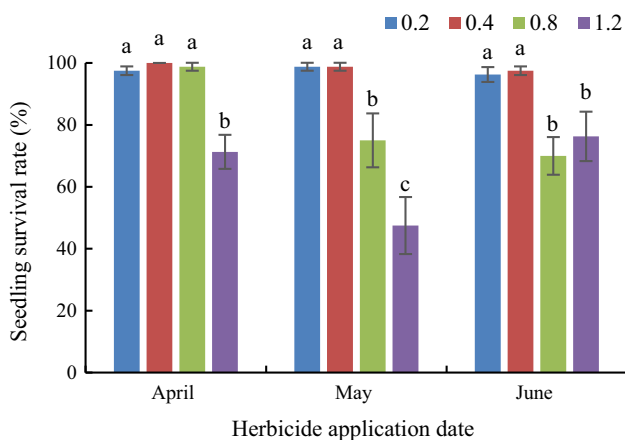
<sup>1,2</sup>Herbicide application date and rate effects were significant, respectively ( $p \leq 0.05$ )

<sup>3</sup>Means within the same column within the same table with different letters are significantly different ( $p \leq 0.05$ )

seedling height and visual health index (Table 2). Increasing the rate inflicted greater damage on the seedlings. At the high (0.8%) and highest (1.2%) rates, the progressive deterioration of seedling health was significant (20 and 85%, respectively).

In addition, the effect of application rate on seedling survival varied substantially depending on AD, i.e., there was a significant AD  $\times$  AR interaction (Fig. 1). Mean seedling survival at the low (0.2%) and intermediate (0.4%) rates was consistently high in April, May, and June, but there were significant differences when compared to the high (0.8%) and the highest (1.2%) rates. Seedlings showed substantially different responses to the high and highest rates depending on the date. Although the high rate caused almost no seedling mortality in April, it considerably reduced survival rate in May and June. Seedling survival at the highest rate averaged significantly lower than the other rates for April and May. The seedlings seemed less sensitive to the highest rate in June when compared to the May application (Fig. 1).

The third correlation results were also in line with the results of the previous analysis. The application rate did not appear to be significantly correlated with seedling height, GLD, and H/D across all three application dates (Table 3). However, application rate consistently had a significant, negative correlation with seedling visual health index and survival; increasing rate significantly decreased seedling visual health index and survival in April, May, and June. The negative effects of AR on these variables appeared to be pronounced in the May application (Table 3).



**Fig. 1** Effects of herbicide application rates by application dates on mean survival rates (%) of *Pinus nigra* seedlings 1 year after treatment, based on application rate  $\times$  date interaction effect ( $p \leq 0.05$ ). Arcsine transformed values were used for the mean separation test for seedling survival, whereas nontransformed values were used for the actual means. Means within the same date with different letters are significantly different ( $p \leq 0.05$ )

## Maritime pine

In the first analysis, seedling survival was high for the first nine treatments (Table 4). The May and June applications at 0.8% and the April and June applications at 1.2%, however, caused a significantly lower survival when compared to the first group. Seedling mortality decreased more with the May application at the highest rate. Only slightly more than a quarter of the maritime pine seedlings survived with this treatment. The index assessing the overall health of the seedlings demonstrated a similar pattern to that of the survival data. The NWC and the April application of the 0.4 and 0.8% treatments showed the greatest significantly different vigor index (H/D). Similarly to the survival rate, treatments 10–14 resulted in the poorest vigor indices of all treatments. The GLD and height data produced results that were generally in line with the previous variables (Table 4).

When the data were reanalyzed without the data from the nonchemical treatments, a significant effect of application rate was found for the GLD of maritime pine seedlings (Fig. 2). Seedling diameter was the poorest at the highest rate (1.2%) of glyphosate. The low and intermediate (0.2 and 0.4%, respectively) rates, however, showed the greatest mean seedling diameter. The effect of the high rate (0.8%) application was not significantly different from the other rates (Fig. 2).

A significant AR  $\times$  AD interaction effect was found for seedling height, vigor and visual health indices, and survival rate for this species (Table 5). The low and intermediate rates (0.2 and 0.4%, respectively) consistently showed high seedling survival, whereas the highest AR (1.2%) reduced the survival rate the most across all application dates. The highest rate was the most harmful to pine seedlings in May. Although the high rate (0.8%) was not phytotoxic when applied in April, it inflicted damage on seedlings in later applications. The height, vigor and visual health indices generally demonstrated results similar to the survival rate (Table 5).

The correlation analysis demonstrated that AR had a consistent, significant, negative correlation with height, seedling visual health index and survival of maritime pine in all of the three ADs. Increasing the application rate appeared to significantly reduce the vigor index (H/D) in the May application. The negative effects of AR on seedling variables were greater with the May application (Table 3).

## Scots pine

Although significant treatment differences were found for height, GLD, vigor and visual health indices, and survival rates for Scots pine seedlings, this species did not

**Table 3** Correlation of application rate (AR) with mean height, ground line diameter (GLD), vigor index (H/D), visual health index (Visual), and survival rate of *Pinus nigra*, *P. pinaster*, and *P. sylvestris* 1 year after treatment on three application dates

	Height	GLD	H/D	Visual	Survival
<i>Pinus nigra</i>					
AR (April)	- 0.2492	- 0.0823	- 0.3085	- 0.5954*	- 0.4873*
AR (May)	0.1952	0.1380	0.1810	- 0.7266*	- 0.7135*
AR (June)	- 0.0164	- 0.3185	0.3611	- 0.6214*	- 0.5423*
<i>Pinus pinaster</i>					
AR (April)	- 0.4760*	- 0.4319	- 0.4415	- 0.6815*	- 0.6293*
AR (May)	- 0.6799*	- 0.3567	- 0.7427*	- 0.8760*	- 0.8446*
AR (June)	- 0.5435*	- 0.2469	- 0.6370*	- 0.8081*	- 0.6742*
<i>Pinus sylvestris</i>					
AR (April)	- 0.2403	- 0.3395	- 0.0552	- 0.4970*	- 0.4615*
AR (May)	- 0.5104*	- 0.3035	- 0.4903*	- 0.8102*	- 0.6503*
AR (June)	- 0.4018	0.0981	0.1556	- 0.7192*	- 0.5511*

\*  $p \leq 0.05$ **Table 4** Effects of weed control treatments on mean height (H), ground line diameter (GLD), vigor index (H/D) and visual health index at the end of the first growing season (Visual), and survival rate of seedlings of *Pinus pinaster* 1 year after treatment

No.	Treatment <sup>1</sup>	Height (cm)	GLD (mm)	H/D	Visual	Survival <sup>3</sup> (%)
1	NWC	21.95 ± 2.3ab <sup>2</sup>	4.67 ± 0.5ab	49.22 ± 3.4a	5.11 ± 0.3ab	97.5 ± 1.4a
2	MWC	21.32 ± 1.4ab	4.61 ± 0.2ab	48.33 ± 1.8ab	5.35 ± 0.1a	97.5 ± 1.4a
3	G-A-0.2	19.21 ± 0.8bc	4.12 ± 0.2abc	46.80 ± 3.0ab	4.76 ± 0.1ab	98.8 ± 1.3a
4	G-M-0.2	21.74 ± 2.3ab	4.93 ± 0.3a	45.16 ± 2.7abc	5.34 ± 0.1a	100.0 ± 0a
5	G-J-0.2	19.70 ± 0.5bc	4.55 ± 0.3ab	44.92 ± 2.6abc	4.69 ± 0.3ab	97.5 ± 1.4a
6	G-A-0.4	25.05 ± 0.5a	4.87 ± 0.2ab	52.42 ± 1.2a	5.44 ± 0.1a	100.0 ± 0a
7	G-M-0.4	19.86 ± 2.0bc	4.43 ± 0.4ab	46.78 ± 3.3ab	4.63 ± 0.5ab	97.5 ± 1.3a
8	G-J-0.4	17.41 ± 1.3bcd	4.37 ± 0.3ab	40.88 ± 1.7bcd	4.46 ± 0.1b	97.5 ± 2.5a
9	G-A-0.8	21.56 ± 1.1ab	4.54 ± 0.2ab	49.39 ± 2.5a	4.80 ± 0.1ab	98.8 ± 1.3a
10	G-M-0.8	14.12 ± 0.8cd	3.88 ± 0.4bc	36.93 ± 2.4de	2.44 ± 0.4c	57.5 ± 10.5b
11	G-J-0.8	17.30 ± 1.8bcd	4.65 ± 0.4ab	38.08 ± 2.4cde	3.20 ± 0.5c	68.8 ± 11.4b
12	G-A-1.2	12.74 ± 0.9d	3.39 ± 0.2c	37.72 ± 2.2cde	2.60 ± 0.2c	67.5 ± 7.8b
13	G-M-1.2	13.97 ± 0.7d	4.32 ± 0.2abc	32.45 ± 1.5e	1.59 ± 0.1d	25.5 ± 5.4c
14	G-J-1.2	15.19 ± 1.1cd	4.01 ± 0.4abc	38.90 ± 2.7cde	2.53 ± 0.4c	62.5 ± 11.3b

Nontransformed values were used for actual means

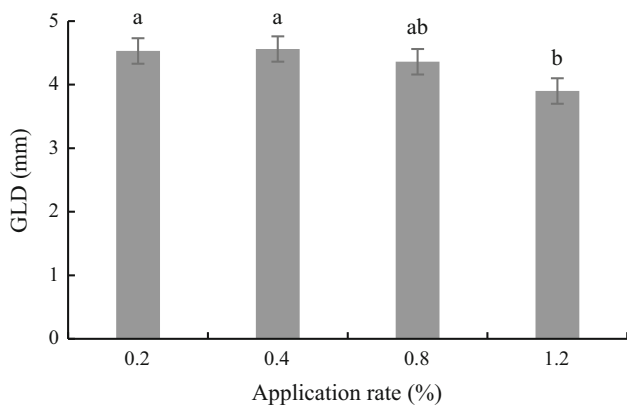
<sup>1</sup>Treatment effect was significant ( $p \leq 0.05$ )<sup>2</sup>Means within the same column within the same year with different letters are significantly different ( $p \leq 0.05$ )<sup>3</sup>Arcsine transformed values were employed for the mean separation test for this variable

demonstrate as significant a response to different weed control treatments as the other pine species (Table 6). Seedling survival was consistently high (> 92%) across the 14 treatments. Only the May application at the highest glyphosate rate (1.2%) demonstrated a significantly lower—yet still relatively high—survival rate compared to the other treatments. However, the visual health index showed more differentiation. The May application at the high herbicide rate (0.8%) and the May and June applications at the highest rate significantly lowered (> 18%) the

mean visual health index of the seedlings compared to other treatments (Table 6).

Differences in GLD amongst treatments were small. However, NWC and MWC treatments showed significantly different mean diameters from the May and June applications at the intermediate herbicide rate (0.4%). The former two treatments also showed significantly higher height than the April application at the low (0.2%) and high (0.8%) ARs and the May application at the highest (1.2%) rate (Table 6).





**Fig. 2** Effects of herbicide application rate on ground line diameter (GLD) of *Pinus pinaster* seedlings 1 year after treatment. Application rates showed significant effect ( $p \leq 0.05$ ). Means with different letters are significantly different ( $p \leq 0.05$ )

When only herbicide treatments were analyzed, a significant AR effect was found for seedling survival (Fig. 3). Mean seedling survival was considerably high for the low, intermediate, and high rates of glyphosate with no significant differences. Scots pine seedling mortality, however, significantly increased with the highest herbicide rate (Fig. 3).

In addition, a significant AR  $\times$  AD interaction was found for the visual health index of the seedlings (Fig. 4). Seedlings sprayed with the highest herbicide rate demonstrated the poorest health across all application dates. The rest of the ARs did not appear to inflict significant damage on the seedlings except for the May treatments. With the May application at the high rate, the mean visual health

index of the seedlings decreased significantly more than with the low- and intermediate-rate applications and was not significantly different from the health index at the highest AR (Fig. 4). No significant or interaction effect was found for the other seedling variables analyzed.

The correlation analysis demonstrated that application rate had a consistent, negative correlation with seedling visual health index and survival on the three application dates. In addition, increasing the application rate significantly reduced seedling height and vigor index (H/D) in the May application (Table 3).

Conifers are reported to be tolerant to herbicides, including glyphosate at low rates (0.11% and 0.33%); however, this tolerance disappears at high rates (1%) (Tanjung 2001). This was shown in all the analyses in this study by consistent pine tolerance at the low and intermediate application rates (0.2 and 0.4%) and increasing phytotoxicity at higher rates, especially the highest rate (1.2%).

The varying sensitivity of young Austrian, maritime and, to a lesser extent, Scots pine seedlings to glyphosate application rates at various application dates in the present study, as indicated by consistently significant AR  $\times$  AD interactions, underlines the point that applying the treatment at the proper rate and time is key to controlling weeds effectively while maintaining crop safety (Newton and Knight 1981; Radosevich et al. 2007). The increased herbicide phytotoxicity in the May application at the 0.8 and 1.2% rates in the present study confirms relevant past research indicating increased sensitivity of young conifer seedlings to foliar-applied glyphosate in the middle of the growing season when seedlings are physiologically the

**Table 5** Effects of herbicide application rate (AR) on mean height, vigor index (H/D), visual index at the end of the first growing season (Visual), and survival rate of *Pinus pinaster* seedlings by application dates 1 year after treatment

AD <sup>1</sup>	AR <sup>1</sup> (% v:v)	Height (cm)	H/D	Visual	Survival <sup>3</sup> (%)
April	0.2	19.21 $\pm$ 0.8b <sup>2</sup>	46.80 $\pm$ 3.0a	4.76 $\pm$ 0.1b	98.8 $\pm$ 1.3a
	0.4	25.06 $\pm$ 0.5a	52.42 $\pm$ 1.2a	5.44 $\pm$ 0.1a	100 $\pm$ 0.0a
	0.8	21.56 $\pm$ 1.1b	49.39 $\pm$ 2.5a	4.80 $\pm$ 0.1b	98.8 $\pm$ 1.3a
	1.2	12.76 $\pm$ 0.9c	37.72 $\pm$ 2.2b	2.60 $\pm$ 0.2c	67.5 $\pm$ 7.8b
May	0.2	21.74 $\pm$ 2.3a <sup>2</sup>	45.16 $\pm$ 2.7ab	5.34 $\pm$ 0.1a	100 $\pm$ 0.0a
	0.4	19.86 $\pm$ 2.0a	46.78 $\pm$ 3.3a	4.63 $\pm$ 0.4a	98.8 $\pm$ 1.3a
	0.8	14.12 $\pm$ 0.8b	39.93 $\pm$ 2.4bc	2.44 $\pm$ 0.4b	57.5 $\pm$ 1.5b
	1.2	13.97 $\pm$ 0.7b	32.45 $\pm$ 1.5c	1.59 $\pm$ 0.1b	25.0 $\pm$ 5.4c
June	0.2	19.7 $\pm$ 2.3a	44.92 $\pm$ 2.6a	4.69 $\pm$ 0.3a	97.5 $\pm$ 1.4a
	0.4	17.4 $\pm$ 1.2a	40.88 $\pm$ 1.7a	4.46 $\pm$ 0.1a	97.5 $\pm$ 2.5a
	0.8	17.3 $\pm$ 1.8a	38.08 $\pm$ 2.4a	3.20 $\pm$ 0.5b	68.8 $\pm$ 1.4b
	1.2	15.2 $\pm$ 1.1a	39.90 $\pm$ 2.7a	2.53 $\pm$ 0.4b	62.5 $\pm$ 1.3b

Nontransformed values were used for actual means

<sup>1</sup>Herbicide application rate  $\times$  date interaction was significant ( $p \leq 0.05$ )

<sup>2</sup>Means within the same column within the same year with different letters are significantly different ( $p \leq 0.05$ )

<sup>3</sup>Arcsin (square root) transformed values were employed for separation of the mean for this variable

**Table 6** Effects of various weed control treatments on mean height (H), ground line diameter (GLD), vigor index (H/D) and visual index at the end of the first growing season (Visual), and survival rate of *Pinus sylvestris* seedlings 1 year after treatment

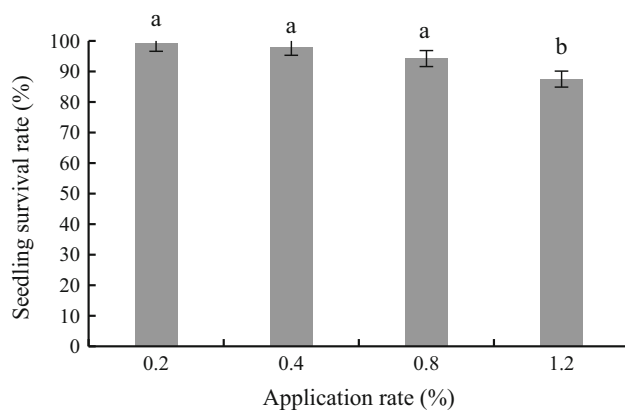
No.	Treatment <sup>1</sup>	Height (cm)	GLD (mm)	H/D	Visual	Survival <sup>3</sup> (%)
1	NWC	49.19 ± 1.4a <sup>2</sup>	10.39 ± 0.2a	48.13 ± 0.4abc	5.9 ± 0.0a	100.0 ± 0.0a
2	MWC	47.90 ± 2.1ab	10.42 ± 0.4a	46.71 ± 0.8abcd	5.9 ± 0.1a	100.0 ± 0.0a
3	G-A-0.2	40.74 ± 0.7d	10.04 ± 0.1ab	41.50 ± 0.2d	6.0 ± 0.0a	100.0 ± 0.0a
4	G-M-0.2	44.99 ± 1.7abcd	9.90 ± 0.3ab	50.46 ± 1.2a	5.9 ± 0.1a	100.0 ± 0.0a
5	G-J-0.2	45.85 ± 1.1abcd	10.04 ± 0.3ab	46.56 ± 1.2abcd	5.8 ± 0.1a	100.0 ± 0.0a
6	G-A-0.4	42.89 ± 1.6bcd	10.12 ± 0.2ab	43.16 ± 1.2cd	5.9 ± 0.1a	100.0 ± 0.0a
7	G-M-0.4	42.84 ± 2.0bcd	9.52 ± 0.4b	46.30 ± 3.3abcd	5.8 ± 0.1a	96.3 ± 2.4ab
8	G-J-0.4	46.10 ± 0.7abc	9.50 ± 0.1b	49.55 ± 1.0ab	5.5 ± 0.1ab	100.0 ± 0.0a
9	G-A-0.8	42.10 ± 2.1cd	9.85 ± 0.3ab	43.73 ± 2.4bcd	6.0 ± 0.0a	100.0 ± 0.0a
10	G-M-0.8	45.78 ± 1.4abcd	10.04 ± 2.5ab	45.85 ± 0.8abcd	4.5 ± 0.2c	92.5 ± 3.8abc
11	G-J-0.8	46.85 ± 1.2abc	9.97 ± 0.1ab	48.18 ± 1.5abc	5.1 ± 0.2bc	100.0 ± 0.0a
12	G-A-1.2	44.31 ± 2.3abcd	9.81 ± 0.3ab	45.83 ± 1.3abcd	5.5 ± 0.3ab	98.8 ± 1.3abc
13	G-M-1.2	42.74 ± 1.1cd	9.86 ± 0.1ab	44.14 ± 1.3bcd	4.6 ± 0.3c	93.8 ± 6.3bc
14	G-J-1.2	45.52 ± 1.2abcd	9.88 ± 0.1ab	46.13 ± 1.3abcd	4.9 ± 0.4c	100.0 ± 0.0a

Nontransformed values were used for actual means

<sup>1</sup>Treatment effect was significant ( $p \leq 0.05$ )

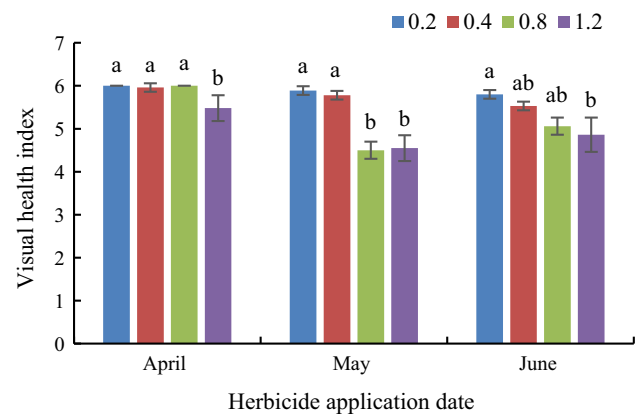
<sup>2</sup>Means within the same column within the same year with different letters are significantly different ( $p \leq 0.05$ )

<sup>3</sup>Arcsin transformed values were employed for separation of the mean for this variable



**Fig. 3** Effects of herbicide application on mean survival of *Pinus sylvestris* seedlings 1 year after treatment. A significant herbicide application rate effect was found ( $p \leq 0.05$ ). Means within the same column within the same year with different letters are significantly different ( $p \leq 0.05$ )

most active (Lund-Hoie 1975; Tanjung 2001; Kelpsas et al. 2015). In another example, a Canadian study reported that glyphosate caused more injury to young seedlings of lodgepole pine (*Pinus contorta* Douglas ex Loudon), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) when applied in May, compared to September application at the end of the growing season (Tanjung 2001). Moreover, Yeiser and Ezell (2010) reported that glyphosate



**Fig. 4** Effects of application rate by application dates on visual health index of *Pinus sylvestris* seedlings 1 year after treatment, based on the significant application rate × date interaction effect ( $p \leq 0.05$ ). Means within the same date with different letters are significantly different ( $p \leq 0.05$ )

substantially injured young loblolly pine (*Pinus taeda* L.) seedlings in a plantation in the southern USA in mid-May, and recommended earlier applications of this herbicide for optimum weed control and crop safety.

The epicuticular wax layer on leaf surfaces is the major barrier to many abiotic stresses for plant species, including high temperatures and irradiance, drought, altitude, cold and frost (Shepherd and Griffiths 2006). The seasonal variation in the content and composition of the epicuticular

wax layer (i.e., the “leaf wettability”) of conifer needles during the year also determines the seasonal differences in their tolerance to herbicides (Holloway 1970; Shepherd and Griffiths 2006; Pallardy 2008; Wang et al. 2015) by influencing herbicide uptake, translocation, and efficacy (Kirkwood 1999; Hess and Foy 2000). Wang et al. (2013) used the contact angle on the adaxial surface of cedar needles as a surrogate for leaf wettability and found that the contact angle at the end of May was lower (i.e., the wettability and in turn, herbicide sensitivity was increased) than that at the end of April. Anfodillo et al. (2002) reported that the contact angle of the adaxial surface of recent and 1-year-old needles of *Pinus cembra* L. in Italy significantly decreased from February to August. In the present study, the seasonal variation in leaf wettability probably explains the variation in herbicide tolerance of the seedlings and their greatest sensitivity in May.

Drought, low relative humidity, altitude, pollution and high solar radiation generally result in an increase in leaf wax content and inhibited herbicide uptake by plants (Cape 1983; Shepherd and Griffiths 2006; Pallardy 2008). The pine species of the present study typically grow on sites where high irradiance and summer temperatures and low soil water availability are major issues (Genç 2012). Lower leaf wettability due to a probable intrinsic increase in the leaf wax content in the seedlings in response to these abiotic stresses (Shepherd and Griffiths 2006) might explain the slight increase in herbicide tolerance observed from May to June.

The relatively greater glyphosate tolerance of 3-year-old Scots pine seedlings when compared to 1- and 2-year-old Austrian and maritime pine seedlings, respectively, confirms previous reports that pine sensitivity to glyphosate is species- and age-specific (Prasad 1989; Tanjung 2001). Pine seedlings generally acquire more tolerance to glyphosate 1 year after establishment (Osiecka and Minogue 2014). Moreover, the greater epicuticular wax density in pine needles confers tolerance to abiotic stresses such as altitude, cold, frost, high irradiance and drought (Shepherd and Griffiths 2006; Vilčinskas and Kupčinskienė 2012). Scots pine generally grows on south-facing slopes at higher elevations (Genç 2012). In addition to higher age (Osiecka and Minogue 2014), the greater needle cuticle content of Scots pine as a possible adaption to frost and drought damage at high elevations (Shepherd and Griffiths 2006; Vilčinskas and Kupčinskienė 2012) might be responsible for its greater herbicide tolerance compared to the other pine species.

## Conclusions

The tolerance of young Austrian, maritime and Scots pine seedlings to foliar-applied glyphosate depended on the date and rate of application. In addition, the effects of application rates on seedlings varied according to application dates, i.e., there was a significant application rate  $\times$  date interaction. Seedlings were tolerant to glyphosate at low and intermediate rates (0.2–0.4% v:v or 172–344 g a.i. ha<sup>-1</sup>), whereas they were extremely sensitive to glyphosate at the highest rate (1.2% v:v or 1032 g a.i. ha<sup>-1</sup>), resulting in substantial mortality and injury. Glyphosate tolerance at the rate of 0.8% v:v (689 g a.i. ha<sup>-1</sup>) varied according to application date. This rate is recommended only in the early mid-spring period when needles presumably have a dense leaf epicuticular wax layer providing limited herbicide penetration. Three-year-old Scots pine seedlings appear to have relatively greater glyphosate tolerance than Austrian and maritime pines.

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