Screening for Metsulfuron Methyl Phytotoxicity in Seeds of Various Pine Species

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Abstract

In forestry, herbicide screening of woody plant seeds has been successfully used for the selection of safe and effective herbicides for tree species. Consequently, research time and costs can be reduced when compared to the respective traditional field experiments. Metsulfuron methyl is a systemic sulfonylurea herbicide used to selectively control herbaceous and woody weeds in conifer plantations. In the present study, the cumulative germination rate and germination speed of Austrian pine (*Pinus nigra* J.F.Arnold), Scots pine (*Pinus sylvestris* L.), and maritime pine (*Pinus pinaster* Aiton) seed presoaked in sulfometuron methyl solutions of 15 different concentrations (0-5% v: v), including a control, were evaluated in a rapid seed-screening trial in order to predict early field phytotoxicity of the herbicide on these pine species. At the end of the 28-day germination trial in the laboratory, the cumulative rate and speed of germination of the three pine species seed varied across different concentrations of sulfometuron. Sulfometuron methyl was not significantly phytotoxic to pine seed germination at low and intermediate concentrations, while seed germination was depressed at high concentrations. The herbicide appeared to affect germination speed more than cumulative germination. The use of this herbicide at low to intermediate concentrations in nursery beds and forest areas could afford young seedlings a rapid establishment and growth without significant phytotoxicity to seed germination. These results could be used to predict early efficacy of sulfometuron on young pine seedlings in the field along with field confirmation of the results. Selecting a herbicide concentration that is both efficacious on weeds and safe for crop trees is vital for the successful establishment and growth of pine seedlings on nursery and plantation sites.

Keywords: Metsulfuron methyl, Phytotoxicity, Pine, Seed germination

Çeşitli Çam Türlerinin Tohum Çimlenmesinde Metsulfuron Metil Fitotoksisitesi

Özet

Odunsu bitki herbisit tarama yöntemi ormancılıkta kullanılacak etkili ve güvenilir herbisitlerin seçiminde başarıyla kullanılmaktadır. Bu yöntem, muadili geleneksel saha denemelerine kıyasla araştırma süresi ve maliyetini oldukça düşürmektedir. Metsülfuron metil sülfonil üre sınıfında sistemik seçici bir herbisit olup ibreli plantasyonlarında yapılan otsu ve odunsu zararlı bitki mücadelesinde kullanılmaktadır. Bu çalışmada kullanılan hızlı tohum herbisit tarama yöntemiyle, sulfomethuron metil herbisitinin kontrol dozu da dahil olmak üzere 15 farklı doz solüsyonu (%0-5, hacim bazında) ile ön işleme tabii tutulan Anadolu karaçamı (*Pinus nigra* J.F. Arnold), sarıçam (*Pinus sylvestris* L.) ve sahilçamı (*Pinus pinaster* Aiton) tohumlarının birikimli çimlenme yüzdesi ve hızı incelenerek herbisitin çam türleri üzerindeki ilk dönem saha etkisinin öngörülmesi amaçlanmıştır. Laboratuarda yapılan 28 günlük çimlenme testi sonunda, çam tohum çimlenmesinin farklı herbisit dozlarında farklı tepki verdiği tespit edilmiştir. Sülfometuron metil, düşük ve orta seviyeli dozlarda çimlenmeye önemli bir zarar vermemiş ancak yüksek dozlarda çimlenmeyi oldukça düşürmüştür. Herbisit uygulamasının birikimli çimlenme yüzdesinden ziyade çimlenme hızı üzerinde etkili olduğu görülmüştür. Fidanlık yastıklarında ve orman sahalarında düşük ve orta dozlarda kullanılacak sulfomethuron metil asli türlere önemli bir zarar vermeden hızlı bir fidan gelişiminin elde edilmesine yardımcı olabilecektir. Elde edilen bu sonuçlar saha teyidinin yapılmasından sonra sulfomethuron metilin genç karaçam, sarıçam ve sahilçamı fidanları üzerindeki saha fitotoksisitesinin tahmini için kullanılabilir. Zararlı bitkiler üzerinde etkili fakat asli türler için güvenilir herbisit seçimi fidanlık ve dikim sahalarında fidan tutma başarısı ve gelişimi için büyük önem arz etmektedir.

Anahtar Kelimeler: Methsulfuron metil, Fitotoksisite, Çam, Tohum çimlenmesi

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Introduction

Seed sowing is commonly used as a major or supplemental method for tree establishment in natural forests and nurseries in many parts of the world including Turkey (Boydak and Çalışkan, 2014) and the UK (Willoughby et al., 2003). Weeds pose significant vegetation management problems in forest regeneration areas and nurseries. The impact of herbaceous competition on young tree seedlings is particularly pronounced in forest areas established by sowing (Willoughby et al., 2003) and in nurseries, due to intensive practices such as irrigation, fertilization and soil tillage (Owston and Abrahamson, 1984). In the absence of effective weed control, young conifer seedlings suffer greatly from competing weeds (Owston and Abrahamson, 1984).

Herbicides are widely used in forest and nursery management around the world, with great control effectiveness and cost-efficiency (Radosevich et al., 2007). Chemical weed control is the most appropriate technique for the removal of competing vegetation among densely and, in most cases, irregularly spaced newly germinated tree seedlings on forest and nursery sites (Willoughby et al., 2003). Due to the lack of research on chemical weed control and the skill and knowledge necessary for its use, forest and nursery managers in Turkey generally prefer to employ high-cost manual weed control, which is only partially effective and requires using a labor force, a resource which is increasingly becoming less available (Eşen and Yıldız, 2000; Eşen et al., 2005; Eşen et al. 2006; Radosevich et al., 2007; Boydak and Çalışkan, 2014).

Rapid woody plant herbicide screening is a technique successfully used to screen the efficacy and safety of forestry herbicides on tree species. This method reduces the one-year duration normally required for the research period to less than six months. Herbicide volume can be reduced from kilograms to milligrams, and the cost is less when compared to the respective time-consuming, traditional field experiments (Zedaker and Seiler, 1988; Bunn et al., 1995; Blair et al., 2006; Stanley et al., 2014). In particular, the rapid herbicide seed screening technique (i.e., soaking seeds in herbicide solutions prior to germination tests)can successfully predict herbicide phytotoxicity in tree seedlings in a much shorter period (1 month or less),compared with extended (e.g., 10 months) conventional field experiments. Seed screening trials have effectively predicted the field performance of clopyralid, triclopyr, imazapyr, and several developmental herbicides for phytotoxicity in young loblolly pine seedlings (Bunn et al. 1995, Blair et al. 2006, Stanley et al. 2014).A high correlation was found between the efficacies of triclopyr and imazaypr on the seed germination of loblolly pine and on the height growth of the species in the field one year after treatment (Blair et al., 2006).

Metsulfuron methyl (Escort®,(methyl 2-[[[[(4-methyoxy-6-methyl-1,3,5-triazin-2-yl)amino]-carbanyl]amino]sulfonyl]benzoic acid] is a systemic sulfonylurea herbicide used to selectively control herbaceous and woody weeds in pastures and conifer plantations (Bowes and Spurr 1996, Monaco et al. 2002) including those of the southern pines (*Pinus teada* L., *Pinus palustris* Mill., *Pinus echinata* Mill., and *Pinus elliottii* Engelm.) and Douglasfir (*Pseudotsuga menziesii* (Mirb.) Franco) in the US (Minogue et al., 1991; Lefebvre, 2013; Osiecka and Minogue, 2014), and radiata pine (*Pinus radiata* D.Don) in South Africa (Gous, 1996) and New Zealand (Tran et al., 2015). The 15th-day cumulative germination rate of the seeds pre-treated with triclopyr solutions successfully predicted, in terms of height and injury rates, the field efficacy of the herbicide on one-year-old loblolly pine seedlings32 weeks after treatment (Blair et al., 2006).

Austrian pine (*Pinus nigra* J.F. Arnold), Scots pine (*Pinus sylvestris* L.), and maritime pine (*Pinus pinaster* Aiton) are ecologically and economically significant conifer species in Turkey (Genç, 2012). The former two pine species are native to Turkey while maritime pine is considered a naturalized tree species in Turkey. Forest nurseries have large seedling

production programs for these conifers to support progressively increasing afforestation and artificial regeneration activities in the country (Genç, 2012; Boydak and Çalışkan, 2014).

This study evaluated the cumulative germination and speed rates of the seeds of Austrian, Scots, and maritime pine soaked in solutions of various concentrations of metsulfuron methyl. The outcome of the present study will aid in the designing of an effective weed control program in forest nurseries that is acceptable for crop safety. In tandem with confirmation of the results in the field, this study will also shed some light on the phytotoxicity of the herbicide on young seedlings of the conifer species in forest and nursery areas.

Material and Methods

The seeds of Austrian, pine (*Pinus nigra* J.F. Arnold), Scots pine (*Pinussylvestris* L.), and maritime pine (*Pinus pinaster* Aiton) supplied from the seed collection of Bolu Forest Nursery of the Bolu Regional Forest Directorate, Turkish Ministry of Forest and Water Affairs in the fall of 2015. Austrian pine seeds were collected from a seed stand located in Mengen-Daren (Bolu) between December 2014 and February 2015. Scots pine seeds were collected from a seed orchard of Aladağ (Bolu) origin, established in Mengen (Bolu) between October 2014 and February 2015. Maritime pine seeds were collected from Kefken Research Forest (İzmit) between February-March of 2015 (Cantürk, 2016, pers. comm.). Seeds had been collected from a varying number of individuals with the desired phenotypes (e.g. straight bole, free of bole and crown defects, good natural pruning, large growth increment) growing on the same aspect in the selected altitudinal zone. Seeds were subsequently stored in air-tight containers and refrigerated at 4-6^{o}C in the seed lab of the Düzce University Forestry Faculty until the experiment. According to K. Cantürk, Manager of the Bolu Forest Nursery, Bolu Regional Directorate of Forestry, all pine species seeds show abundant germination, and therefore, no stratification is used in forest nurseries in Turkey for any of the pine species prior to treatments (personal communication, 2015).

Fourteen different solutions of sulfometuron methyl herbicide, in concentrations varying between 0.2-5.0% (v:v) in deionized water, were prepared in separate plastic containers (Table 1). A control solution with no herbicide (i.e., 0% herbicide) was also prepared. For each pine species, 250 seeds were separately placed in each of the 15 containers containing 100 ml of the respective herbicide solutions and soaked for 24 h in the lab using a procedure similar to Blair et al (2006). The seeds were then drained and placed on dry filter paper in petri dishes. For each pine species, 60 dishes (4 replications × 15 treatments), each containing 50 seeds, were employed. The dishes were later placed in a dark growth chamber at 20 °C for 28 days and checked every other day for germination. Seeds with a 5 mm-long radicle were considered germinated. The dishes were also regularly checked for moisture, and dampened with1-2 ml of deionized water as needed (Blair et al., 2006).

The mean cumulative seed germination rate (GR) was calculated for day 7,14, and 28 (GR7, GR14, and GR28, respectively) for each pine species. In addition, the speed of germination was graphically demonstrated. A completely randomized design was used for the study, with four replications. One-way ANOVA (analysis of variance) was employed for statistical test. The treatment means were separated using the Duncan's Mean Separation Test ($p \leq 0.05$). The Statistical Analysis System (SAS) was employed for analysis (SAS 1996).

Results

Austrian pine seed

Within the first and second week of the germination trial,44 and 93% of mean GR28 occurred for the control seeds, indicating a high speed of germination (Table 1, Figure 1). The mean GR7, GR14, and GR28 significantly varied across herbicide concentrations (Table 1). At the end of the trial, herbicide concentration was shown to affect germination speed (GS) more than GR. At low concentrations (0.2-0.6%), sulfometuron methyl did not significantly impair seed germination when compared to the control. The low-concentration herbicide even appeared to increase seed germination in the second and last phases of the trial when compared to the control, although the differences were not significant. Although mean GR28 started to decrease at intermediate herbicide concentrations (≥0.8%), significant reductions occurred only at high concentration levels (≥2.0%). The mean GR28 was 27, 46, and 74% lower at 2, 2.5, and 5% concentrations, respectively, when compared to the control treatment.

Table 1. Effects of sulfometuron methyl on mean cumulative seed germination rate of Austrian pine

Herbicide Percentage (%, v:v)[1]	Cumulative Seed Germination Rate (%)		
	7th day[3]	14th day	28th day[4]
0	35.5 ±3.9 a[2]	74.0 ±1.6 a	80.0 ±2.0 a
0.2	19.5 ±3.3 ab	80.5 ±3.2 a	83.5 ±2.9 a
0.4	11.0 ±1.3 bc	77.5 ±4.4 a	82.0 ±5.0 a
0.6	14.0 ±2.6 b	75.5 ±2.8 a	81.0 ±3.9 a
0.8	3.0 ±1.0 def	56.5 ±2.5 bc	68.0 ±3.6 ab
1.0	5.0 ±1.0 cd	59.5 ±3.9 b	70.5 ±3.0 ab
1.2	4.0 ± 1.4 de	52.0 ±4.2 bcd	64.0 ±4.7 ab
1.4	0.5 ±0.5 g	55.0 ±2.6 bc	71.5 ±2.6 ab
1.6	1.5 ±0.3 efg	47.0 ±3.5 cd	63.0 ±3.8 ab
1.8	1.5 ±1.0 fg	46.0 ±2.8 cd	62.5 ±4.2 ab
2.0	1.5 ±1.0 fg	43.0 ±1.3 d	58.5 ±2.2 b
2.5	1.5 ±0.5 efg	31.5 ±4.8 e	43.5 ±6.4 c
3.0	1.0 ±1.0 g	28.5 ±4.7 e	37.0 ±5.5 c
4.0	0.0 ± 0.0 g	16.5 ±2.9 f	27.5 ±5.0 d
5.0	0.0 ± 0.0 g	10.7 ±1.8 f	20.6 ±1.3 d

[1]Herbicide concentration effect was significant ($p < 0.0001$); [2]Means within the same column with different letters are significantly different; [3,4] Log and arcsine, respectively, transformed values were employed for separation of the means.

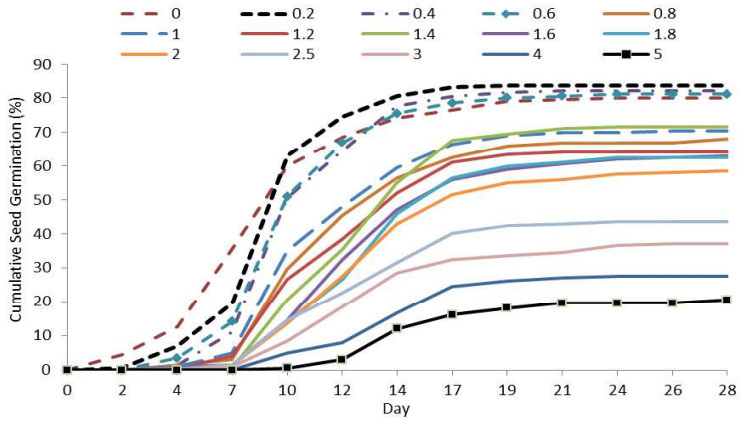

Figure 1. Mean cumulative germination speed of Austrian pine seed pretreated with various sulfometuron methyl percentages

Scots pine seed

For the control seeds,21 and 90% of the mean GR28 occurred in the first and second week of the trial, respectively (Table 2, Figure 2), suggesting a lower GS when compared to Austrian pine.

Similar to Austrian pine, herbicide solutions at 0.8% and higher substantially reduced the mean GR in the early and then in the intermediate phases of the germination trial. The mean cumulative GR7 was 59, 91, and 100% (total mortality) lower at 0.8, 1.4, and 2.5% concentration levels, respectively, when compared to the control. However, as in Austrian pine, the negative effects of the herbicide solutions diminished as the germination trial proceeded to the second week, and then continued until the end of the experiment. The herbicide solutions did not differ significantly from the control treatment for mean GR28, except for the highest-concentration treatment. The latter treatment reduced mean GR28 by 29% when compared to the control treatment (Table 2).

Maritime pine seed

In general, the GR was lower than in the Austrian and Scots pines. The seeds of the maritime pine also began to germinate later than the seeds of the other two pine species, with no germination within the first week of the trial (Table 3, Figure 3).

Table 2. Effects of sulfometuron methyl on mean cumulative seed germination rate of Scots pine

Herbicide Percentage (%, v:v)[1]	Seed Germination (%)		
	7th day[3]	14th day	28th day
0	16.0 ±2.2 ab[2]	69.0 ±2.6 ab	76.5 ±2.0 a
0.2	20.0 ±2.2 a	74.5 ±2.0 a	83.5 ±2.6 a
0.4	11.5 ±2.8 ab	69.5 ±1.8 ab	76.5±2.0 a
0.6	6.5 ±1.0 abc	57.5 ±1.6 bcd	69.0 ±3.2 ab
0.8	3.0 ±1.8 cde	63.5 ±1.8 abc	74.5 ±0.6 a
1.0	5.0 ±1.4 bcd	63.5 ±0.6 abc	74.5 ±1.8 a
1.2	3.5 ±2.0 cde	61.0 ±2.6 bc	74.5 ±3.6 a
1.4	1.5 ±1.0 cde	54.0 ±4.8 cde	75.3 ±3.2 a
1.6	1.0 ±0.6 cde	47.0 ±2.0 def	75.0 ±3.6 a
1.8	1.5 ±1.0 cde	52.0 ±2.6 cde	68.5 ±2.8 ab
2.0	0.5 ±0.5 de	43.0 ±2.4 efg	74.0 ±4.2 a
2.5	0.0 ±0.0 e	32.0 ±2.2 gh	69.0 ±4.2 ab
3.0	0.5 ±0.5 de	37.5 ±2.2 fg	74.0 ±2.8 a
4.0	0.0 ±0.0 e	24.0 ±1.4 hi	69.5 ±3.0 ab
5.0	0.0 ±0.0 e	13.5 ±2.6 i	54.0 ±4.2 b

[1]Herbicide concentration effect was significant ($p < 0.0003$); [2]Means within the same column with different letters are significantly different; [3] Log transformed values were employed for separation of the means and nontransformed values were used for actual means

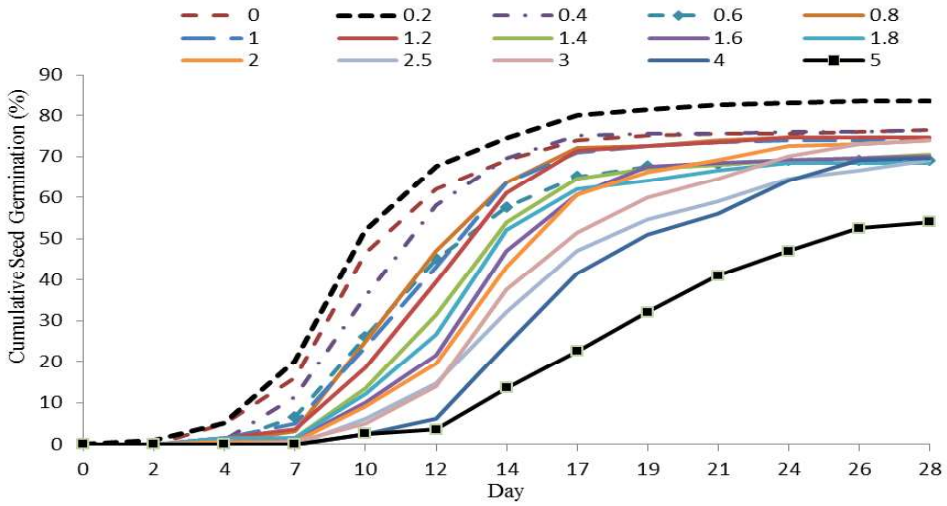

Figure 2. Mean cumulative germination speed of Scots pine seed pretreated with various sulfometuron methyl percentages

Table 3. Effects of sulfometuron methyl on mean cumulative seed germination rate of maritime pine

Herbicide percentage (%, v:v)[1]	Seed Germination (%)	
	14th day	28th day
0	27.5 ±3.1 abc	57.0 ±2.9 abc
0.2	34.0 ±3.3 a	67.0 ±4.0 ab
0.4	32.0 ±0.8 abcd	64.5 ±6.7 ab
0.6	25.0 ±3.9 abcd	71.5 ±5.0 a
0.8	23.5 ±1.3 abcde	67.5 ±6.1 ab
1	21.5 ±3.8 abcde	56.5 ±3.4 abc
1.2	22.0 ±4.1 abcde	63.5 ±5.7 ab
1.4	19.0 ±1.3 bcde	61.0 ±2.1 abc
1.6	16.5 ±1.9 cdef	49.5 ±1.3 abc
1.8	18.5 ±3.4 cde	60.0 ±2.6 abc
2	16.0 ±1.4 cdef	55.5 ±6.0 abc
2.5	14.0 ±1.6 def	57.0 ±4.4 abc
3	11.5 ±1.9 ef	46.0 ±2.4 bc
4	10.5 ±1.9 ef	48.0 ±5.9 bc
5	3.5 ±1.7 f	39.5 ±3.9 c

[1]Herbicide concentration effect was significant ($p < 0.0003$); [2]Means within the same column with different letters are significantly different

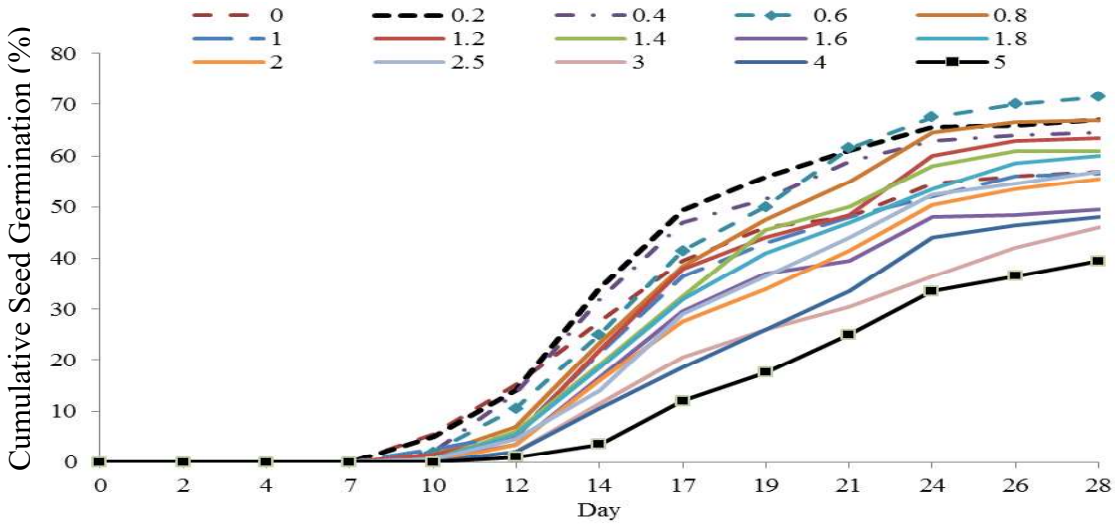

Figure 3. Mean cumulative germination speed of maritime pine seed pretreated with various sulfometuron methyl concentrations

The germination of the maritime pine seeds appeared to be less sensitive to sulfometuron methyl when compared to the germination of the other two pine seeds (Tables 1, 2and 3). The GR14 significantly differed across herbicide concentrations. The mean GR14 was the greatest at 0.2%, which was at least two-fold greater than the mean G14 at 1.6% and higher. However, these differences among treatments disappeared by the end of the trial (Table 3). The treatment demonstrated a lower level of statistical differentiation for GR28.The greatest mean GR28 occurred at the 0.6% concentration level (Tables 3). At high concentration levels (≥3%), the sulfometuron methyl started to be phytotoxic for the maritime pine seeds. At the highest level, the mean GR was reduced by at least 30% when compared to low concentrations. Germination was further reduced with herbicide levels higher than 2.5%. At the highest herbicide concentration, the mean GR decreased to one-tenth of the highest mean GR observed (at 0.6% concentration) in the first two weeks of the trial (Table 3).

Discussion

Concentration-specific and species-specific herbicide phytotoxicity to seed germination has been previously reported for other herbicides and tree species (Willoughby et al., 2003; Blair et al., 2006; Stanley et al., 2014) and plants (Baskin and Baskin, 2014). For example, Willoughby et al. (2003) used nine different herbicides on pots seeded with various broadleaved (oak, cherry, sycamore,and beech) and conifer (Norway pine) tree species. They found that herbicide phytotoxicity varied by species and application level. Pendimethalin and nanpropamide were seen as promising for the majority of the tree species studied, and oak (*Quercus robur* L.) seed germination appeared to tolerate most herbicides at most rates (Willoughby et al.,2003).

The effects of sulfometuronon seed germination of the pine species tested in the present study were also species- and application-level specific. Scots pine and maritime pine germination were fairly tolerant of sulfometuron methyl except for the highest concentration level, whereas Austrian pine seed germination started to display some phytotoxicity effects at intermediate concentration levels which became significant at high levels (≥2%). Baskin and Baskin (2014) reported concentration-dependent herbicide phytotoxicity effects ranging from stimulating, to having no effect, to damaging on the germination of plant species.

Although sulfometuron methyl was in general not considerably phytotoxic to pine seeds at the end of the 28-day trial, in concentrations greater than 0.6%it drastically delayed germination speed. Germination speed is considered a more meaningful variable than

cumulative germination rate for successful sowing (Boydak and Çalışkan, 2014;Çetin,2010). Saatçioğlu (1971) reported that when compared to the cumulativegermination rate, germination speed in the lab is a better representative of the seed germination rate in the field for Austrian pine. Earlier seed germination produces more vigorous and competitive seedlings in the field than later seed germination (Boydak and Çalışkan, 2014). Seedlings under rapid seed germination also show greater adaptation to site conditions, especially for summer drought (Dunlap and Barnett, 1984). Although in the present study sulfometuron methyl was more effective on the seed germination speed than the cumulative germination rate, these negative effects would probably be offset by the positive effects of eliminating competing vegetation (Willoughby et al., 2003).

In the present study, inconsistent cases where higher herbicide concentrations resulted in greater and/or lower germination rates than the control have also been reported by other researchers (Willoughby et al., 2003, Baskin and Baskin 2014). Willough et al. (2003) attributed thesediscrepancies to high data variation which could be alleviated by conducting a greater number of replications in future studies (Willoughby et al. 2003). On the other hand, Baskin and Baskin (2014) attributed these inconsistencies to the stimulation effect of low-concentration level herbicides. Herbicide seed screening can successfully predict the field efficacy of various herbicides on tree seedlings, thus substantially reducing the assessment period and cost (Bunn et al., 1995; Blair et al., 2006; Stanley et al., 2014).

Conclusions

Seed screening can enable the prediction of the early efficacy of herbicides for tree seedlings in the field. The cumulative rate and speed of germination of Austrian, Scots, and maritime pine seed varied according to the different concentrations of sulfometuron. Seed germinations of the studied pine species were not significantly impaired by different levels of sulfometuron methyl, while they were depressed at high levels of herbicide concentration. The herbicide affected germination speed more than cumulative germination rate. The use of this herbicide at low to intermediate concentration levels in nursery beds and seeded forest areas could provide rapid growth of seedlings without substantially depressing the germination rate. Applying sulfometuron methyl at concentrations of≥ 2% is not recommended for Austrian pine, whereas higher concentrations ofup to 4% could be tolerated by Scots and maritime pine seed. In addition, field trials are needed to confirm the results of this rapid seed screening.

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