

PHYTOTOXICITY OF CLOPYRALID AT HIGH RATES ON SEED GERMINATION OF MEDITERRANEAN PINES

FITOTOKSIČNOST KLOPIRALIDA VEZANO UZ KLIJANJE SJEMENA MEDITERANSKIH BOROVA

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SUMMARY

Black pine (*Pinus nigra* J. F. Arnold), maritime pine (*P. pinaster* Aiton), Scots pine (*P. sylvestris* L.), and Turkish red pine (TRP, *P. brutia* Ten.) are ecologically and economically important conifers of the Mediterranean Basin, and in particular for Turkish forestry. They are commonly used for the regeneration and restoration of degraded ecosystems in Turkey. Weeds compete with tree seedlings for vital site elements such as soil moisture and nutrients and solar radiation, substantially reducing tree establishment and growth. Herbicides can offer the practitioner efficient and cost-effective weed control compared to other methods. Rapid herbicide seed screening allows testing of crop-safe herbicides and application rates at much lower costs in a very short time-frame when compared to lengthy field trials. Clopyralid is a systemic herbicide used to rid pine seedlings of competing vegetation. The present study examined the effect of clopyralid phytotoxicity on these pine species using a rapid herbicide seed screening test in order to compare different application rates on seed germination and to identify crop-safe rates. Clopyralid was not phytotoxic to any of the pine species at low rates (i.e., <2%, v:v); however, pine sensitivity to the herbicide increased with increasing rates, especially for rates higher than 3%. Moreover, at high rates, clopyralid reduced the germination speed. This herbicide can be used at low rates for degraded areas and nursery sites in which sowing is used as the main regeneration or restoration method. Field confirmation of the obtained results is also recommended.

KEY WORDS: Forestry, forest nursery, herbicide sensitivity, *Pinus*, seed screening, weed control

INTRODUCTION UVOD

Pines make up a significant share (47%) of Turkish forests (22.7 million ha) (OGM 2019). Turkish red pine (*Pinus brutia* Ten.), black pine (*P. nigra* Arnold), and Scots pine (*P. sylvestris* L.) are important natural Turkish forest pine species, with distributions of 5.7, 4.4, and 1.5 million ha, respectively (OGM, 2019). Maritime pine (*P. pinaster* Aiton) in Turkey is distributed over almost 58,000 ha, mostly in plantations (Güner et al. 2019). These pines benefit the

society greatly with their significant economic (i.e., wood and, specifically for Turkish red pine, honey production) and ecological (i.e., biodiversity, erosion control, and land restoration) contributions and are widely used for natural regeneration and afforestation by the Turkish General Directorate of Forestry (Genç 2012; Boydak and Çalışkan 2014; Dağlar et al. 2016; Bakan et al. 2018; Bakan and Eşen 2018; Cap and Eşen 2018; Türedi et al. 2018; Bakan 2019; Güner et al. 2019). Most of these pines are distributed on degraded land that is under a high level of environmental

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(e.g., drought, erosion) and anthropogenic pressure (Genç 2012; Boydak and Çalıřkan 2014; Yıldız et al. 2018).

Weeds compete with tree seedlings for vital site elements such as soil moisture and nutrients and solar radiation, substantially reducing tree establishment and growth (Wagner et al. 2004; Radosevich et al. 2007). Consequently, effective weed control on natural forest regeneration and afforestation sites becomes essential (Radosevich et al. 2007). Herbicides can offer the practitioner efficient and cost-effective weed control compared to other methods (Eřen and Yıldız 2000; Wagner et al. 2004; Eřen et al. 2005, 2006; Radosevich et al. 2007).

“Rapid herbicide seed screening” is a method wherein tree seeds are pretreated with herbicide formulations before germination assessments. This method allows screening for crop-safe herbicides and application-rates at much lower costs within a very short time frame (i.e., 30 days or less) compared to the expensive and lengthier tests in the field (i.e., 10 months) (Zedaker and Seiler 1988; Bunn et al. 1995; Blair et al. 2006; Stanley et al. 2014; Dađlar et al. 2016, Bakan et al. 2018; Türedi et al. 2018; Bakan 2019). Recently, this method was employed in black pine, Scots pine, and maritime pine to assess the phytotoxicity of glyphosate (Türedi et al. 2018) and methsulfuron methyl (Dađlar et al. 2016) on the seed germination of the species. Herbicide sensitivity of these pines to these herbicides was found to vary substantially according to species and herbicide application rates (Dađlar et al. 2016; Türedi et al. 2018). Additionally, the rapid herbicide seed screening test using black, Scots, and maritime pine seeds pretreated with glyphosate (Türedi et al. 2018) successfully predicted the results of a longer field trial using 1-3-year-old seedlings of the same pine species in terms of glyphosate sensitivity (Cap and Eřen 2018), thus confirming previous reports of the high correlation between short herbicide seed screening and longer field screening trials (Zedaker and Seiler 1988; Bunn et al. 1995; Blair et al. 2006; Stanley et al. 2014; Dađlar et al. 2016; Türedi et al. 2018).

Clopyralid is a growth-regulating, post-emergence herbicide belonging to the pyridine carboxylic acid group (Monaco et al. 2002; Dixon et al. 2005). It is mainly applied to plant foliage and then roots and is easily translocated to the meristematic sites. Clopyralid effectively controls many broadleaf herbaceous and some woody weeds (Monaco et al. 2002; Dixon et al. 2005) and is reported to be safe for young seedlings of many pine species including longleaf pine (*P. palustris* Mill.), slash pine (*P. elliotii* Engelm. var. *elliotii*), and loblolly pine (*P. taeda* L.) (South 2000) as well as Scots pine (*P. sylvestris* L.) (Vea and Palmer 2009).

Clopyralid phytotoxicity on black pine, maritime pine, Scots pine, and Turkish red pine (TRP) at low rates was studied in a recent, preliminary rapid seed screening test in

Turkey (Bakan et al. 2018; Bakan 2019). In this preliminary test, clopyralid demonstrated no significant phytotoxic effects on the listed pine species at low rates of 0-1% (v:v). Further testing of the phytotoxicity of this herbicide at higher rates on these pine species was recommended (Bakan et al. 2018; Bakan 2019).

Consequently, as a continuation of the study of Bakan et al. (2018) and Bakan (2019), the present study, in a separate rapid seed germination test, examined black pine, maritime pine, Scots pine, and TRP for clopyralid phytotoxicity at higher rates. The study aimed to determine the clopyralid sensitivity of the pine species at high rates and to help develop a tree-safe pre-emergence herbicide weed control treatment for pine regeneration and/or restoration sites and forest nurseries where sowing is the principal basis of propagation. Considering the high correlation between the results of the rapid seed screening test and those of the field herbicide screening trial, the findings of the present study could also be used for the post-emergence control of herbaceous weeds in the field.

MATERIAL AND METHODS

METODE I MATERIJALI

Prior to the experiment, pine seeds were obtained from the Bolu Forest Nursery situated in Bolu, Turkey, in the autumn of 2015 and stored in air-tight plastic bags in the refrigerator (4-6 °C) at the Duzce University Forestry Faculty (Dađlar et al. 2016; Bakan et al. 2018; Bakan 2019). The seeds of black, Scots, and maritime pine were collected from their native distribution areas in Bolu-Mengen, Bolu-Aladađ, Izmit-Kefke, and Sakarya-Geyve-Taraklı in the Western Black Sea Region of Turkey in 2014, 2016, 2016, and 2011, respectively. For more detailed information on seed collection and storage, please refer to Bakan (2019). The seeds of these pine did not require pretreatment (i.e., stratification) before germination (Genç 2012).

The present study followed a procedure similar to that of Blair et al. (2006), and more specifically to Dađlar et al. (2016), Bakan et al. (2018), Türedi et al. (2018), and Bakan (2019). Seeds from each of the four pine species were independently presoaked in 100-mL clopyralid (Phaeton 100^o, 100 g L⁻¹ a.i., HEKTAŞ T.A.Ş., Gebze Organize San. Bölgesi, İhsan Dede Cd. 700, Sk. 41480, Gebze-Kocaeli, Turkey) solutions at differing rates of 0-5% (v:v) in deionized water in separate plastic containers for 24 h (Table 1) (Bakan 2019). A 0.4% (v:v) Izolcaptan[®] 50 WP (Metropol Kimya Sanayi ve Ticaret Ltd. Şti., Istanbul, Turkey) was added to the solutions of each rate to prevent the fungus infestations that had occurred in the preliminary test with the lower rates of clopyralid (Bakan and Eřen 2018; Bakan et al. 2018; Bakan 2019). The seeds were then taken from the containers and placed on dry filter paper in 9-cm Petri dishes. For each

pine species, 50 seeds were placed in each dish for each clopyralid rate. Treatments were replicated four times for the test. Thus, the experiment included 128 dishes (eight solutions \times four pine species \times four replications). The dishes containing the seeds were placed in a growth chamber (MMM Aqualytic AL655, Germany) at 20 ± 0.5 °C for 28 days between 07/09/2018 and 05/10/2018. During the germination test, the dishes were monitored every other day. Seeds having a 5 mm-long radicle during the test period were evaluated as germinated (ISTA, 1985). The dishes were conditioned with 1-2 mL of deionized water as needed (Blair et al. 2006; Dađlar et al. 2016; Bakan et al. 2018; Türedi et al. 2018; Bakan 2019).

The mean percentages of cumulative seed germination (CSG) on Days 7, 14, and 28 were discretely analyzed for each conifer species via a completely randomized design (CRD). A one-way analysis of variance (ANOVA) was employed for the statistical test. To separate treatment means, Tukey's MST was employed ($p \leq 0.05$). Results were analyzed using the Statistical Analysis System (SAS) (SAS 1996). The data were transformed following a violation of the assumption of normality.

RESULTS REZULTATI

Black pine – Crni bor

Germination started on Day 4, and more than half of the control seeds had germinated by the second week, showing rapid germination (Table 1, Fig. 1). As in the preliminary test of Bakan et al. (2018), the control (0%) and 1%-rate demonstrated high CSG when compared to the rest of the treatments and did not significantly differ from each other at the end of the test (Table 1). Moreover, no significant phytotoxic effect was observed in the seeds that were pre-treated with 1.5% clopyralid. The herbicide sensitivity of black pine seeds began with the herbicide rates of 2.0% and higher. At the end of the test, the CSG had progressively and significantly decreased by 14, 21, 39, and 54% for the seeds that were presoaked with the 2, 3, 4, and 5% solutions, respectively (Table 1).

Maritime pine – Primorski bor

At the end of the first week, no seed germination had occurred for the maritime pine regardless of treatments (Table 1). Maritime pine displayed the lowest seed germination performance among all of the pine species used in the present study. The majority of seeds had germinated by the end of the second week. Clopyralid affected maritime pine seed germination in a similar manner as with black and Scots pine seeds. Significant herbicide phytotoxicity began with clopyralid rates of $\geq 2\%$ and progressively intensified with

Table 1. Mean cumulative seed germination of black pine (*Pinus nigra*), maritime pine (*Pinus pinaster*), Scots pine (*Pinus sylvestris*), and Turkish red pine (*Pinus brutia*) depending on clopyralid rate after 7, 14, and 28 days of the germination test.

Tablica 1. Srednja vrijednost klijanja sjemena crnog bora (*Pinus nigra*), primorskog bora (*Pinus pinaster*), bijelog bora (*Pinus sylvestris*), i brucijskog bora (*Pinus brutia*) prema Postotku (volumnom) klopivalida nakon 7, 14, i 28 dana unutar testa klijanja.

Herbicide Rate Postotak herbicida (%, v:v) ¹	Mean Cumulative Seed Germination (%) Središnja vrijednost klijanja sjemena (%)		
	Day 7	Day 14	Day 28 ³
Black pine Crni bor			
0	14.8 \pm 1.6 ab ²	55.2 \pm 3.8 a	67.2 \pm 2.9 a ³
1.0	16.0 \pm 1.4 a	48.0 \pm 3.2 a	64.4 \pm 1.7 a
1.5	10.4 \pm 2.3 bc	38.8 \pm 1.5 b	60.8 \pm 2.2 a
2.0	8.0 \pm 1.1 c	39.6 \pm 3.2 b	52.8 \pm 3.4 b
2.5	8.8 \pm 2.1 c	38.8 \pm 2.7 b	48.0 \pm 2.2 bc
3.0	6.8 \pm 1.6 c	31.6 \pm 1.2 bc	41.2 \pm 2.2 c
4.0	8.4 \pm 1.2 c	26.4 \pm 2.3 c	31.2 \pm 1.9 d
5.0	5.6 \pm 1.6 c	15.2 \pm 2.4 d	19.2 \pm 2.7 e
Maritime pine Primorski bor			
0	-	32.0 \pm 2.3 a ²	43.2 \pm 2.4 a
1.0	-	28.4 \pm 2.4 ab	40.8 \pm 2.6 ab
1.5	-	26.8 \pm 3.8 ab	38.0 \pm 3.9 ab
2.0	-	24.4 \pm 1.7 b	33.6 \pm 2.7 bc
2.5	-	24.4 \pm 1.7 b	30.0 \pm 1.8 cd
3.0	-	16.8 \pm 1.5 c	24.0 \pm 2.0 de
4.0	-	14.4 \pm 2.7 c	20.8 \pm 3.1 ef
5.0	-	11.2 \pm 1.2 c	15.6 \pm 1.7 f
Scots pine Bijeli bor			
0	34.8 \pm 1.4 a	68.8 \pm 3.5 a ²	80.8 \pm 2.7 a
1.0	29.2 \pm 1.4 b	66.4 \pm 2.7 a	77.6 \pm 4.4 a
1.5	29.2 \pm 2.4 b	60.8 \pm 4.1 ab	72.2 \pm 3.2 ab
2.0	28.0 \pm 2.6 b	56.4 \pm 3.0 c	63.6 \pm 2.6 bc
2.5	22.0 \pm 1.4 c	47.6 \pm 2.3 c	56.0 \pm 2.6 cd
3.0	22.0 \pm 2.3 c	45.6 \pm 4.1 c	49.6 \pm 5.0 de
4.0	15.6 \pm 0.7 d	38.8 \pm 1.9 c	41.2 \pm 2.4 e
5.0	12.8 \pm 0.8 d	28.8 \pm 1.0 d	29.6 \pm 1.0 f
Turkish red pine Brucijski bor			
0	-	70.4 \pm 2.3 ab ²	90.4 \pm 1.3 a ³
1.0	-	72.8 \pm 3.8 a	87.2 \pm 2.2 ab
1.5	-	65.2 \pm 2.3 bc	84.8 \pm 2.1 bc
2.0	-	61.2 \pm 3.2 c	79.6 \pm 2.5 cd
2.5	-	53.2 \pm 1.9 d	73.2 \pm 3.1 d
3.0	-	46.0 \pm 1.8 e	60.8 \pm 1.6 e
4.0	-	38.8 \pm 2.4 f	48.4 \pm 1.7 f
5.0	-	31.6 \pm 1.2 g	38.8 \pm 1.5 g

¹Herbicide rate effect was significant ($p < 0.0001$); ²Means within the same column with different letters are significantly different ($p \leq 0.05$); ^{3,4}Arctsin transformed values were employed for separation of the means and non-transformed values were used for actual means.

¹Efekat herbicida je bio značajan ($p < 0.0001$); ²Središnje vrijednosti unutar istog stupca s različitim slovima se značajno razlikuju ($p \leq 0.05$); ^{3,4}Korištene su specifične arkus sinus vrijednosti za odvajanje središnjih vrijednosti, a ne-transformirane vrijednosti su korištene za izračunavanje stvarnih središnjih vrijednosti.

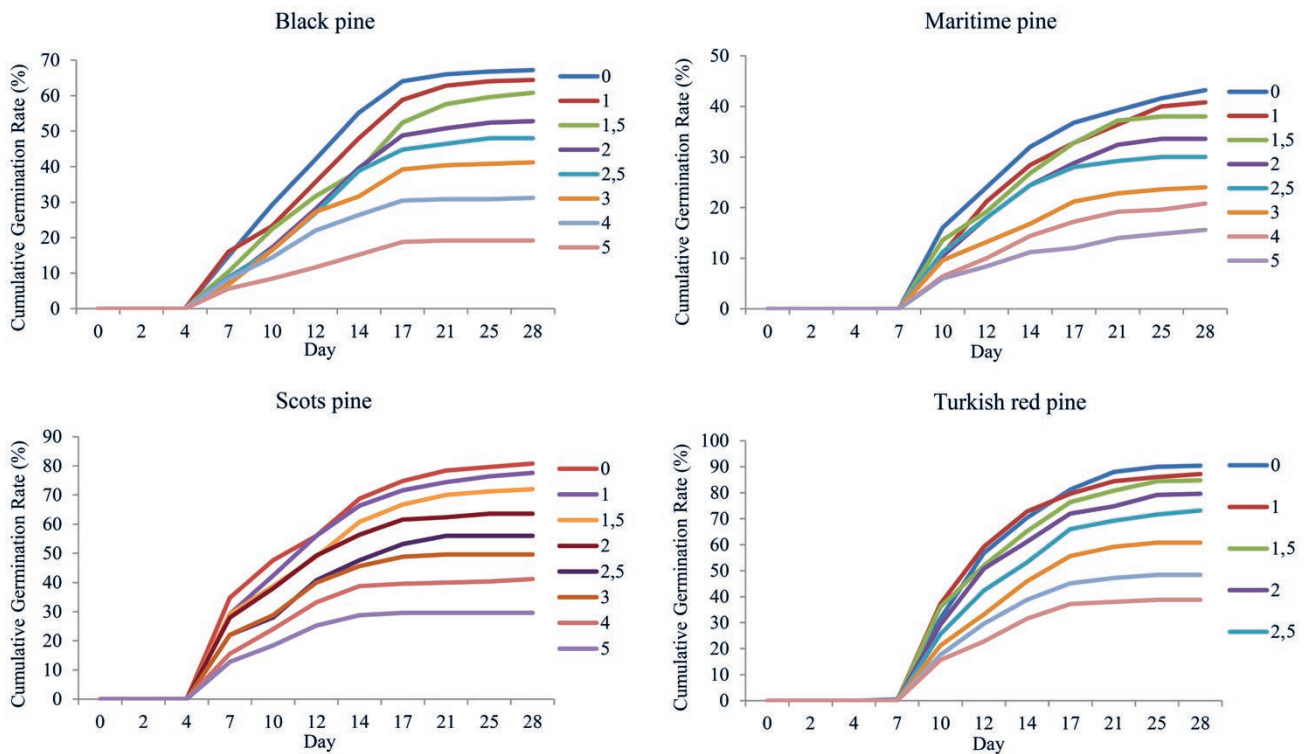


Figure 1. Cumulative germination rate of black pine (*Pinus nigra*), maritime pine (*Pinus pinaster*), Scots pine (*Pinus sylvestris*), and Turkish red pine (*Pinus brutia*) seeds pretreated with varying rates of clopyralid for the germination test.

Slika 1. Brzina klijanja sjemena crnog bora (*Pinus nigra*), primorskog bora (*Pinus pinaster*), bijelog bora (*Pinus sylvestris*) i brucijskog bora (*Pinus brutia*) prethodno tretirana s različitim volumnim postocima klopivalida tijekom testa klijanja.

increasing rates. The mean CSG with the 2, 3, and 5% herbicide rates was 22, 44, and 64%, respectively, compared to that of the control seeds and was lower by the end of the experiment (Table 1). The herbicide rate also had a similar effect on the germination speed (Fig. 1).

Scots pine – Bijeli bor

Germination started on Day 4, and more than two-thirds of the control seeds had germinated by the second week, demonstrating a higher speed of germination than black pine (Table 1, Fig. 1). Similar to black pine, Scots pine seeds were not significantly sensitive to clopyralid at rates of $\leq 1.5\%$; however, herbicide phytotoxicity increasingly intensified with higher rates. Increasing the herbicide rate to 2, 3, and 5% significantly brought about 21, 39, and 63% decreases, respectively, in the mean CSG when compared to the control seeds. Germination speed progressively decreased in parallel with the increasing herbicide rate (Fig. 1).

Turkish red pine – Brucijski bor (TRP)

Similar to maritime pine, TRP exhibited no seed germination by the second week of the germination test, whereas at the end of the second week of the experiment demonstrated the greatest CSG performance among all of the pine species including the control seeds (Table 1). Unlike the rest of the analyzed pine species, TRP began to demonstrate substantial

clopyralid sensitivity at a lower rate (1.5%), and this sensitivity increased more at higher herbicide rates. The mean CSG at 2, 3, and 5% herbicide rates decreased by 12, 32, and 57%, respectively, when compared to that of the control seeds. The effect of clopyralid on the germination speed of TRP was comparable to that seen in the other pine species (Fig. 1).

DISCUSSION RASPRAVA

Most of the distribution area of black, maritime, Scots, and TRP is subject to site degradation with the existence of a high level of environmental (e.g., drought, erosion) and anthropogenic pressure (Genç 2012; Boydak and Çalışkan 2014; Yildiz et al. 2018). Because they provide more favorable site conditions, “microsites” that are dispersed on degraded areas can offer stressed vegetation essential resources and can substantially enhance plant establishment. By supplementing natural seed dispersal and uniting seeds via these microsites, direct seeding can dramatically improve tree seedling survival and growth on such stressed areas (Dixon et al. 2005; Doust et al. 2006; Jinks et al. 2006; Greipsson 2012; Löf et al. 2016; Grossnickle and Ivetić, 2017). On the other hand, weeds that usurp essential site resources (i.e., water and nutrients in the soil) can pose a great threat to the establishment of crops in these already

resource-scarce environments, therefore warranting efficient weed control (Wagner et al. 2004; Radosevich et al. 2007; Galatowitsch 2012, Türedi et al. 2018). Chemical weed control is an effective tool for eliminating unwanted vegetation and enhancing tree establishment and growth in forestry situations (Eşen and Yıldız 2000; Wagner et al. 2004; Eşen et al. 2005, 2006; Radosevich et al. 2007). In this sense, rapid herbicide seed screening tests can significantly help to quickly devise cost-effective, and crop-safe weed control prescriptions (Zedaker and Seiler 1988; Bunn et al. 1995; Blair et al. 2006; Stanley et al. 2014; Dağlar et al. 2016; Bakan et al. 2018; Türedi et al. 2018; Bakan 2019).

The sensitivity of plant seeds to herbicides varies depending on the herbicide, species, and application rate (Blair et al. 2006; Willoughby et al. 2006; Stanley et al. 2014; Bakan et al. 2018; Türedi et al. 2018; Bakan 2019). Black pine, maritime pine, Scots pine, and TRP exhibited similar responses to clopyralid in terms of mean cumulative seed germination and speed of germination. Clopyralid is reported to be comparatively safe in various pine species at relatively low rates. It was not phytotoxic to young seedlings of longleaf, loblolly, and slash pine at rates of 0.2-0.8 kg a.e. ha⁻¹ (South 2000). Moreover, foliar-applied clopyralid effectively controlled Scotch broom (*Cytisus scoparius* L.) populations in young radiata pine (*Pinus radiata* D. Don) plantations at a slightly higher rate (i.e., 1.125 kg a.e. ha⁻¹) without significant phytotoxicity to the pine seedlings one and two years after planting (Watt and Rolando 2014; Rolando et al. 2017). Additionally, clopyralid at low rates was found to be non-phytotoxic to several Mediterranean pines including black pine, Scots pine, maritime pine, and TRP in both the preliminary trial of the present study (Bakan et al. 2018; Bakan 2019) and the present study (Table 1), confirming the results of earlier studies on clopyralid. This herbicide was also found to be non-phytotoxic to Scots pine at low rates in another study where clopyralid was foliar-applied to young pine seedlings at rates of 0.14-0.56 kg a.i. ha⁻¹ (Vea and Palmer 2009). However, this systematic herbicide became increasingly phytotoxic at rates of ≥2.0% for the pine species used in the present study (Table 1). As with the mean cumulative seed germination, clopyralid similarly reduced the germination speed for the pine species. This was particularly apparent after rates higher than 3% (Fig. 1). Consequently, clopyralid at low rates can help the practitioner eliminate critical weed competition on regeneration or propagation sites for these important pine species without significant crop damage, although it can be critically damaging to pine species at higher rates. Using high rates of clopyralid may further hurt seedling survival by decreasing the speed of seed germination because accelerated germination enables newly emerged seedlings to reach site resources earlier than other seedlings having slower germination speeds (Swanton 2003; Radosevich et al. 2007; Türedi et al. 2018).

CONCLUSION ZAKLJUČAK

Black pine, maritime pine, Scots pine, and TRP exhibited similar responses to clopyralid in mean cumulative seed germination and speed of germination. Clopyralid was not phytotoxic to the pine species at low rates (i.e., <2%, v.v); however, pine sensitivity to the herbicide significantly increased with increasing rates, especially for rates higher than 3%. This herbicide can be used at low rates for degraded areas and nursery sites in which sowing is used as the main regeneration or restoration method. Field studies are recommended for confirmation of the obtained results.

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SAŽETAK

Crni bor (*Pinus nigra* J. F. Arnold), primorski bor (*Pinus pinaster* Aiton), obični bor (*Pinus sylvestris* L.), i brucijski bor (TRP, *Pinus brutia* Ten.) su ekološki i ekonomski važne četinjače u mediteranskom području, a posebno za tursko šumarstvo. Obično se koriste za regeneraciju i sanaciju degradiranih eko sustava u Turskoj. Korovske biljke su u kompeticiji sa sadnicama drveća jer im oduzimaju vlagu, hranjive tvari i sunčevu svjetlost, što može značajno smanjiti rast i razvoj sadnica. Herbicidi se u praksi

koriste za učinkovitu i isplativu kontrolu korova jer smanjuju troškove u usporedbi s ostalim metodama njihovog suzbijanja. Brzo skeniranje herbicida za sjeme omogućuje skeniranje herbicida sigurnih za uzgoj uz znatno niže troškove i u vrlo kratkom vremenu u usporedbi s dugotrajnim pokusima na terenu. Klopivalid je sustavni herbicid koji se koristi za zaštitu sadnica istraživanih borova od konkurentne vegetacije. U ovom istraživanju, fitotoksičnost klopivalida je istražena uz pomoć testa za brzo skeniranje herbicida za sjeme kako bi se usporedila njegova primjena kod klijanja sjemena te radi utvrđivanja sigurne doze. Klopivalid nije bio fitotoksičan niti za jednu vrstu bora pri niskim dozama (npr., <2%, v:v), ali se osjetljivost borova na herbicid povećala s većom dozom, posebno ako su doze bile veće od 3 %. Klopivalid je smanjio brzinu klijanja u visokim dozama. Ovaj herbicid se može koristiti u manjim dozama za degradirana i rasadnička mjesta na kojima se koristi sjetva sjemena kao glavna metoda sanacije i regeneracije sastojina. Preporučena je također i potvrda rezultata s terena.

KLJUČNE RIJEČI: šuma, šumski rasadnik, osjetljivost na herbicide, pregled sjemena, kontrola korova