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# THE EFFECT OF DESICCATION STRESS AND HYDROGEL USE ON SEEDLING PHYSIOLOGY AND GROWTH IN *Juniperus foetidissima*

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## ABSTRACT

One of the aims of the present study was to determine the effect of desiccation treatment on physiological quality and growth of 1+0 year-old bare-root *Juniperus foetidissima* Willd. seedlings. The second aim of the study was to evaluate the effect of hydrogel use to protect the roots against desiccation on the same properties. After the seedlings were lifted, they were divided into two groups (without hydrogel and with hydrogel). The seedlings with and without hydrogel were subjected to desiccation for different times (0, 1, 3 and 5 hours) under controlled conditions before planting. There were significant differences in the length of desiccation time and decreasing stem and root moisture content, stem water potential ( $\psi$ ) and root growth potential (RGP). It was found that these properties were higher in the seedlings with hydrogel than without hydrogel. In both treatments, root electrolyte leakage (REL) significantly affected desiccation and REL value increased with the duration of desiccation treatments. In addition, REL values of the seedlings with hydrogel were lower than of the seedlings without hydrogel. Seedling height, stem diameter, stem and root dry weight values of seedlings with and without hydrogel decreased with the length of desiccation time. In all desiccation times, diameter increase and survival of the seedlings treated with hydrogel were higher than of the seedlings not treated with hydrogel. For this reason, immersing the roots of the seedlings into hydrogel after lifting might protect the seedlings from potential desiccation stress and might improve planting success.

**KEYWORDS:** Desiccation, Electrolyte leakage, *Juniperus foetidissima*, Root growth potential, Survival, Water potential

## 1. INTRODUCTION

In recent years, *Juniperus foetidissima* Willd. is one of the preferred species in afforestation activities in ex-

treme growing environments [1]. However, in afforestation activities carried out in extreme dry areas, planting shock is often encountered and planting activities become unsuccessful. One of the reasons of this failure is the use of low-quality seedlings.

Seedlings might be exposed to sun, wind, high and low temperatures lifting and planting process. These environmental factors cause physiological damage in the seedlings, particularly in their root systems [2]. Drought is one of the most damaging climate-related hazards [3]. One of the main reasons for planting shock is water deficit [4, 5]. The roots which are exposed to ambient conditions during lifting and planting lose water and particularly the fine roots dry out. Due to the damages caused by desiccation, new root development is delayed in the new planting environment and water intake of the seedlings is inhibited. As a result, the desiccation of the roots directly affects the survival of the plant [6, 2]. The hydrogels which are termed as hydrophilic polymer, the substances like agricol, clay and water mixture, sphagnum algae were used to increase planting success of bare-root seedlings by directly applying on the root system of the seedling or in planting holes [7-11]. Polymers are the substances which absorb water that is 400-500 times of their own weight according to the purity of water. For this reason, it was reported that polymers might be effective in reducing water stress of the seedlings [8]. It was reported that hydrogels were used in afforestation activities in semi-arid areas [10, 12].

This study investigated the effects of desiccation on stem and root moisture content, water potential, root electrolyte leakage, root growth potential and growth of seedling and the effectiveness of hydrogel use in the desiccation of root system.

## 2. MATERIAL AND METHODS

### 2.1. Plant material and experiment set-up

Çamdağ-origin 1+0 year old bare-root *Juniperus foetidissima* seedlings were grown applying standard nursery procedures in Isparta-Eğirdir Forest Nursery (37° 53' N; 30° 52' E, 926 m altitude). On 31 March 2009, the seed-

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lings were lifted from nursery bed. Root systems of the seedlings were immersed in water and the soil residues around the roots were carefully cleaned to provide homogenous desiccation. To determine morphological properties of the seedlings which would be used in the experiment, root collar diameter, height and dry weight measurements were made on randomly selected 15 seedlings (Table 1). The remaining seedlings were kept in the cooler at +4 °C until the experiment starts.

**TABLE 1 - Morphological characteristic of *Juniperus foetidissima* seedlings (n=15)**

Characteristic	Mean±SE
Root collar diameter (mm)	2.47 ± 0.10
Seedling height (cm)	12.57 ± 0.49
Stem dry weight (g)	1.08 ± 0.11
Root dry weight (g)	0.63 ± 0.06
Stem/Root dry weight (g)	1.81 ± 0.15

A total of 840 seedlings which were kept at cool storage for 2 days were divided into two equal groups: the seedlings without hydrogel (the roots which were not immersed in hydrogel) and the seedlings with hydrogel (the roots which were immersed in hydrogel). A total of 420 randomly selected seedlings were divided in four different desiccation groups (desiccation for 0, 1, 3 and 5 hours) for the treatment of the seedlings without hydrogel. Except the control seedlings (0 h), all the seedlings (root and shoot) were laid on a wire shelf in a controlled environment chamber and were left to desiccation for 1, 3 and 5 h under 20 °C temperature, 40% moisture and photosynthesis photon flux density 242  $\mu\text{mol m}^{-2}\text{s}^{-1}$  conditions. Control (0 h) seedlings were kept in cooler at +4°C during this procedure. Immediately after the desiccation treatments were completed, the physiological condition of the seedling was evaluated using stem water potential ( $\psi$ ), root electrolyte leakage (REL), root growth potential (RGP), stem moisture content (SMC) and root moisture content (RMC). The seedlings which would be used in planting experiment were placed in polyethylene bags immediately after the experiment and were kept at +4°C until the time of planting.

After completing laboratory stage of the treatments without hydrogel, the roots of second group seedlings (420 seedlings) were kept in hydrogel called Scotkosorb for 10 minutes. To prepare Scotkosorb gel, 7.5 g granulated Scotkosorb 500 micro was mixed into 1 liter of water. In one hour, the granules completely absorbed water and took the form of gel. Then the roots of the seedlings were immersed in this gel. The seedlings which were kept at hydrogel for 10 minutes were subjected to desiccation treatment under the same conditions and using the same procedures which were applied on the seedlings without hydrogel. After the treatments,  $\psi$ , REL, SMC, RMC and RGP measurements were made. The seedlings which would be used in planting experiment were placed in polyethylene bags and were kept at +4°C until the time of planting.

Immediately after completing the laboratory stage, the seedlings in both groups (with and without hydrogel)

were planted on 4 April 2009. A total of 50 seedlings from each treatment were planted in 11x 25 cm polyethylene tubes containing forest soil and were left to grow in open nursery conditions in Eğırdir Forest Nursery. The seedlings were regularly watered according to the routine watering program of nursery.

## 2.2. Measurements

For the two treatment groups (with and without hydrogel), a total of 5 randomly selected seedlings from each desiccation treatment were used for  $\psi$  measurements. The seedlings were smoothly cut in root collar with a slight curve using a sharp knife. In this cut surface, approximately 3 cm section was peeled and were placed in the device for water potential measurement.  $\Psi$  was measured in excised stem with a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) [13].

REL values were identified on a total of 10 seedlings which were randomly selected from each desiccation treatments for both treatment groups (with and without hydrogel). Root system of each seedling were washed in tap water to remove soil and then rinsed in deionized water to remove surface ions. Fine roots (<2 mm) from in the middle section of the root systems was removed. Root samples of each seedlings were put in 28 ml capped glass tubes containing 16 ml distilled water whose conductivity are known ( $C_0$ ). The tubes which were closed were shaken well and were then kept at room temperature for 24 h (20-22°C). At the end of 24 h, glass tubes were shaken again. Using an electrical conductivity meter, the conductivity of the solution ( $C_1$ ) was measured. All samples were then autoclaved at 110°C for 10 min to kill the cells. After the samples had cooled to room temperature, a second recording of the conductivity ( $C_2$ ) was made. REL was calculated with the below formula for each seedling [2, 14, 15]:

$$REL = \frac{C_1 - C_0}{C_2 - C_0} \times 100$$

SMC and RMC were determined by using 15 seedlings from each desiccation times for both treatments (with and without hydrogel). After determining fresh weight (FW) and dry weight (after drying to 24 h at 105 °C) (DW) of each seedling, moisture contents (MC) were calculated:

$$MC = (FW - DW) / DW \times 100$$

RPG was determined using 20 seedlings from each desiccation times for both treatments (with and without hydrogel). Prior to planting, newly-formed white roots on the root system of each seedling were removed. Each seedling was planted in polyethylene tubes containing humus and perlite mixture (3:1 by volume) and after watering at field capacity. They were kept under controlled conditions (at 21 °C; 16 h photoperiod) for 40 days. At the end of this time, each seedling was carefully lifted and their root systems were washed. All new white roots ( $\geq 1$  cm) were counted for each seedling [16, 17].

To determine survival and growth, initial seedling height and stem diameter measurements were made 10 days

after the planting. Seedling height was measured from soil surface to the tip of shoots; stem diameter was measured from soil surface. At the end of the first growing season (25 December 2009) survival, height, stem diameter, dry weights were determined and height and diameter increments of seedling were calculated:

$$\text{Diameter increment}(\%) = \frac{\text{autumndiameter} - \text{springdiameter}}{\text{springdiameter}} \times 100$$

$$\text{Height increment}(\%) = \frac{\text{autumnheight} - \text{springheight}}{\text{springheight}} \times 100$$

### 2.3. Statistical analysis

One-way variance analysis was made to determine the effect of desiccation treatments on SMC, RMC,  $\psi$ , REL, RGP and planting performance. If analyses of variance indicated significant differences ( $p < 0.05$ ), the treatment means were separated by Duncan's New Multiple Range test ( $p < 0.05$ ). Prior to the variance analysis, square-root transformation was used for numerical data but, percentage data were subjected to arcsine transformation. All the statistical analyses of the data were done using SPSS version 15.0 software.

## 3. RESULTS AND DISCUSSION

### 3.1. The effect of desiccation and hydrogel use on seedling physiology

Different desiccation times had significant effects on SMC ( $p < 0.001$ ), RMC ( $p < 0.001$ ),  $\psi$  ( $p < 0.001$ ), REL ( $p < 0.001$ ) and RGP ( $p < 0.01$ ) of the seedlings with and without hydrogel (Figs. 1-4).

SMC and RMC of seedlings without hydrogel were lower than of seedlings treated with hydrogel in different desiccation times (Figure 1a,b). While SMC of the seedlings without hydrogel were 158% at the beginning, it decreased to 140% after 1 h, to 112% after 3 h and to 101% after 5 h. SMC of the seedlings treated with hydrogel decreased to 159% after 1 h, to 146% after 3 h and to 111% after 5 h. 1 h after the desiccation treatment, RMC of the seedlings treated with hydrogel was 76% higher than of the seedlings without hydrogel. However, after 5 h, RMC of the seedlings treated with hydrogel decreased and became only 28% higher than the RMC of the seedlings without hydrogel (Figure 1a,b).

A similar situation was observed in  $\psi$ .  $\psi$  of the seedlings treated with hydrogel were found to be significantly higher than of the seedlings without hydrogel. While the  $\psi$  of the seedlings treated with hydrogel was higher than -0.5 MPa 1 and 3 h after exposure to desiccation, in the seedlings without hydrogel,  $\psi$  decreased to -0.93 MPa after 1 h and to -2.55 MPa after 3 h. At the end of 5 h,  $\psi$  was found to be -3.36 MPa in the seedlings without hydrogel and it was -2.26 MPa in the seedlings with hydrogel (Figure 2). A similar result was reported in the study of McKay

and White [18]. Root moisture content and needle water potential of 2+0 years old bare-root seedlings of *Pseudotsuga menziesii* (Mirb.) Franco and *Picea sitchensis* (Bong.) Carr. which were exposed to desiccation for 0.5, 1, 1.5 and 3 h decreased due to the length of desiccation [18]. Brønnum [19], reported that moisture contents generally decreased with longer exposure times in the whole plants, needles and roots in *Abies procera* species.

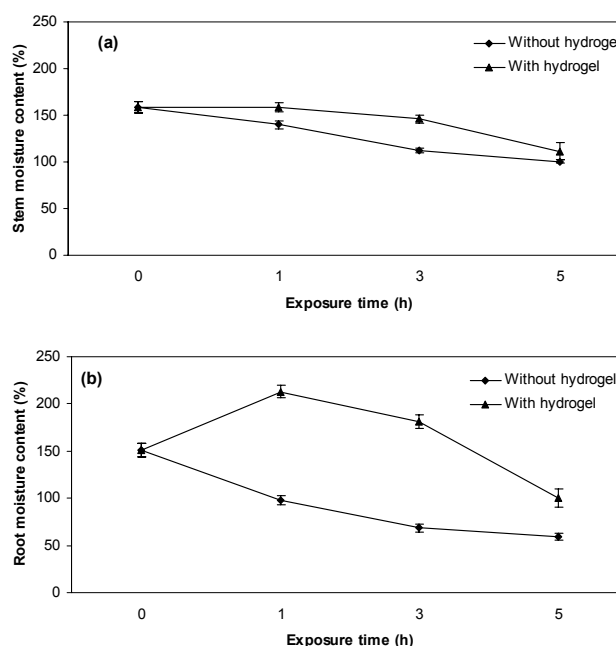


FIGURE 1 - Stem (a) and root (b) moisture contents of the seedlings with and without hydrogel after exposure to desiccation under controlled conditions. Each data point represents mean ( $n=15$ )  $\pm$  SE.

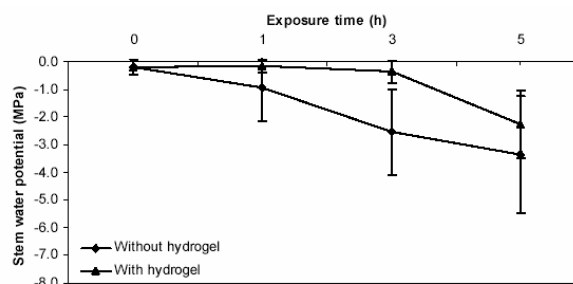


FIGURE 2 - Stem water potential ( $\psi$ ) values of the seedlings with and without hydrogel after exposure to desiccation. Each data point represents mean ( $n=5$ )  $\pm$  SE.

Claery and Zaerr [20] reported that it was necessary to high keep water potential between lifting and planting for seedling quality. Genç [21] suggested that seedling roots should be watered immediately after lifting against drying stress in *Picea orientalis* (L.) Link. Deligöz [22] suggested the same procedure for *Pinus nigra* Arn. subsp. *pallasiana* species. In our study, hydrogel was used to protect the root systems of *Juniperus foetidissima* seedlings against desiccation and the use of hydrogel decreased water stress against desiccation stress.

To determine the physiological damage caused by drying, generally electrolyte leakage measurements are made in many studies [2, 19, 23]. REL significantly increased in the seedlings without hydrogel starting from 1 h desiccation treatment and reached 92% at the end of 5 h. On the other hand, in the seedlings treated with hydrogel, there was no significant difference between 1 and 3 h. Only after 5 h desiccation treatment, REL values reached 80% (Figure 3). It was reported that hydrogel decrease the damage occurring in the roots which were subjected to desiccation prior to planting [24]. In our study, immersing the roots of the seedlings to hydrogel for 10 minutes decreased root damage in desiccation for 1 and 3 h and thus REL values were found to be lower than of the seedling without hydrogel.

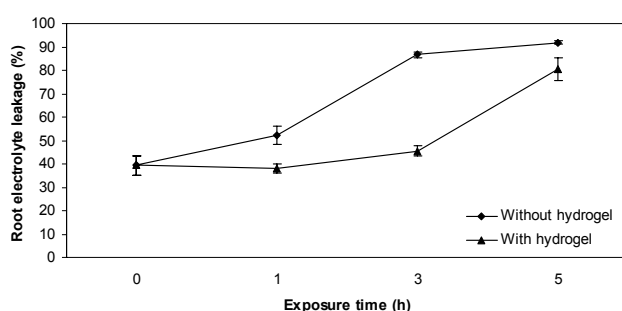


FIGURE 3 - Root electrolyte leakage values of the seedlings with and without hydrogel after exposure to desiccation. Each data point represents mean ( $n=10$ )  $\pm$ SE.

RGP was negatively affected by desiccation treatments. New root growth significantly decreased in the seedlings with and without hydrogel depending on the length of desiccation. Particularly in the seedlings without hydrogel, no new root growth was observed in desiccation procedure for 5 h (Figure 4). Brønnum [19], reported that RGP decreased rapidly with exposure time and was almost zero after only 1.5 h of desiccation. However, field survival after this treatment was still around 60%, meaning that new root growth actually occurred in the field [19].

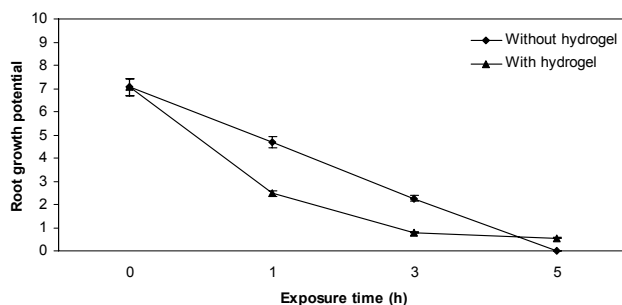


FIGURE 4 - Root growth potential of the seedlings with and without hydrogel at different desiccation times. Each data point represents mean ( $n=20$ )  $\pm$ SE.

### 3.2. The effect of desiccation and hydrogel use on survival and growth

Survival in the treatment without hydrogel decreased with longer desiccation times. While survival was 66% at

the end of 3 h in the treatment without hydrogel, it decreased to 42% at the end of 5 h. The main reason for this decrease is that both SMC and RMC and  $\psi$  significantly decrease depending of the length of desiccation procedure. It was observed that when RMC decreased below 98%, survival significantly decreased. It was reported that after lifting, before and immediately after planting, when water potential of the seedlings decreased below -0.10 MPa, the risk of death started and this risk rapidly increased towards -1.8 MPa [25]. This information is consistent with our findings in the present study. The decrease of  $\psi$  below -0.90 MPa increased death ratio. Dirik [26] found that survival of the Anatolian Black Pine seedlings whose  $\psi$  were -0.52, -1.14, -1.48 and -2.13 MPa were 92.5%, 70%, 57.5% and 32.5% respectively in descending order.

In the treatment with hydrogel, survival was found to be significantly high in all desiccation times and survival was higher than 80% even at the end of 5 h (Figure 5). Survival of the seedlings treated with hydrogel was found to be significantly higher than those of the seedlings without hydrogel. The reason for this is that the use of hydrogel significantly decreased water loss and root damage of the seedlings. Likewise, seedling moisture was defined as an important factor that affects survival percentage of newly planted seedlings [27]. In a study carried out by Hermann in Douglas, it was found that when the seedlings which were lifted in January were kept in open air at 32 °C for 15 minutes, they gave 100% survival percentage; however when the seedlings were kept at open air for 30, 60 and 120 minutes, survival decreased to 90%, 80% and 50% respectively [16].

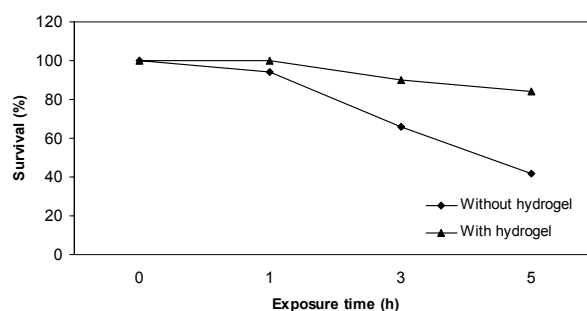


FIGURE 5 - Survival of the treatments with and without hydrogel at the end of the first growing season.

There was a statistically significant difference ( $p \leq 0.05$ ) between the desiccation treatments in terms of all measured morphological properties of the seedlings without hydrogel. However, in seedlings with hydrogel, there was statistically significant difference between desiccation treatments in terms of the morphological properties apart from seedling height and dry root weight (Table 3). It was observed that seedling height, stem diameter, stem and dry root weight values of the seedlings without hydrogel decreased with longer desiccation time (Table 3). For both treatments the lowest values were obtained from 5-hour desiccation treatment in terms of morphological properties.

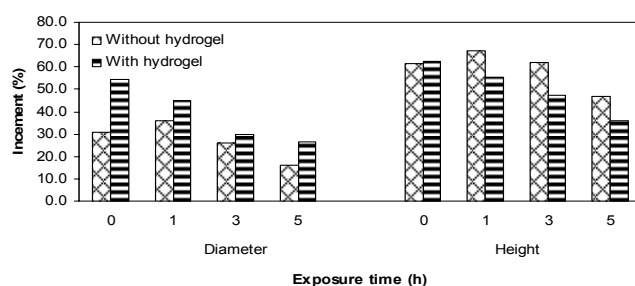


**TABLE 3 - Some morphological properties of the seedlings with and without hydrogel at the beginning and end of the first growing season. Values are means±SE. For each row, means with different letters indicate significant differences ( $p \leq 0.05$ ).**

Treatments		Time (h)			
		0	1	3	5
Without hydrogel	Initial stem diameter(mm)	3.30±.09a	3.30±.10a	3.01±.09b	2.82±.06b
	Initial height (cm)	10.82±.31a	10.37±.32ab	9.53±.27ab	10.13±.29a
	Stem diameter (mm)	4.32±0.20a	4.48±0.21a	3.79±0.13b	3.28±0.15c
	Seedling height (cm)	17.46±0.85a	17.36±0.55a	15.42±0.52b	14.86±0.47b
	Stem dry weight (g)	4.24±0.43a	4.01±0.41a	2.70±0.26b	1.71±0.15c
	Root dry weight (g)	2.79±0.24a	2.54±0.25a	1.82±0.17b	1.13±0.11c
With hydrogel	Initial stem diameter(mm)	3.03±.06a	2.95±.08ab	3.25±.10ab	3.16±.08a
	Initial height (cm)	9.86±.23a	9.87±.26ab	10.57±.24b	10.90±.25b
	Stem diameter (mm)	4.68±0.15a	4.27±0.17ab	4.22±0.17ab	4.00±0.19b
	Seedling height (cm)	16.01±0.40a	15.33±0.54a	15.57±0.54a	14.84±0.61a
	Stem dry weight (g)	3.87±0.36a	3.65±0.30a	3.10±0.25ab	2.70±0.21b
	Root dry weight (g)	2.87±0.29a	2.42±0.22a	2.45±0.25a	2.21±0.18a

Likewise, exposure to desiccation reduced seedling development and survival in *Abies procera* Rehd., *Picea sitchensis* (Bong.) Carr. and *Picea abies* (L.) Karst. species [10, 19, 28, 29]. Sijacic-Nikolic et al [30] found that the polymers had a positive on development of *Pinus sylvestris* L. and *Pinus nigra* Arn. seedling.

Diameter increases of the seedlings treated with hydrogel were found to be higher. This increase decreased with longer desiccation times. Likewise Brønnum [19] found that the effect of desiccation on diameter growth was larger and significant. On the contrary, it was found that the seedlings without hydrogel had higher height increase in 1, 3 and 5 h desiccation treatments (Figure 6). Sarvaš [10] found that diameter and height increase of *Picea abies* (L.) Karst.) seedlings with hydrogel which were exposed to desiccation were lower than of the seedlings without hydrogel. Low REL values give high survival. REL values were higher in the seedlings without hydrogel. Thus, survival and diameter increases of the seedlings without hydrogel were lower than of hydrogel-treated seedlings.



**FIGURE 6 – Seedling height and stem diameter increments of *Juniperus foetidissima* at the end of the first growing season.**

#### 4. CONCLUSIONS

According to the findings of the study, in the bare-root *Juniperus foetidissima* seedlings, SMC, RMC,  $\psi$ , REL and RGP were negatively affected by desiccation treatments. In our study it was found that the use of hy-

drogel significantly decreased water loss and root damage in the of seedlings full exposure. Survival and diameter increment of the seedlings treated with hydrogel was found to be significantly higher than of the seedlings without hydrogel. In conclusion, immersing seedling roots into hydrogel for 10 minutes after lifting will protect them from desiccation stress and might improve planting performance. However, the benefits of using the hydrogel should be tested with field trials

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Received: August 26, 2010

Accepted: November 04, 2010

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