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Research Reports

Pre-germination Seed Treatments and Stratification Affect Germination of Montezuma Cypress¹

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Abstract -

Taxodium distichum (L.) Richard var. *mexicanum* Gordon (syn. *T. mucronatum*), Montezuma cypress, is a valuable ornamental tree species tolerant of a wide range of cultural conditions. However, little is know about the propagation requirements of this species. The objectives of this study were 1) to determine the effect of previously recommended seed treatments for baldcypress (*T. distichum* (L.) Richard var. *distichum*) or pondcypress (*T. distichum* (L.) Richard var. *imbricarium* (Nutt.) Croom; syn. *T. ascendens, T.d.* var. *nutans*) on Montezuma cypress seeds, and 2) to determine the effects of stratification in combination with pre-germination treatments on germination of Montezuma cypress seeds. Open pollinated seeds were collected from a single tree in Southmost, TX (25° 52.576' N, 97° 27.083' W, elevation 4.5 m, USDA Plant Hardiness Zone 9b). Seven pre-germination treatments and three stratification periods were applied to the seeds. If immediate germination of ripe seed is desired, then the best treatments are the citric acid soak and the hot water baths, however, if seeds can be stratified, then no pre-germination rates and cumulative mean germination percentages. Stratification for 45 d appears to be sufficient, although for the best pre-germination treatments stratification requirements were less pronounced.

Index words: seed propagation, germination, scarification.

Species used in this study: Taxodium distichum var. mexicanum, Taxodium mucronatum.

Significance to the Nursery Industry

Taxodium distichum var. *mexicanum* (syn. *T. mucronatum*), Montezuma cypress, is an adaptable tree species that is fast growing and has potential for large urban sites (1, 5). The cultural requirements for the other varieties in this species

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cultural requirements of this taxon of increasing importance in the landscape and nursery trade.

Introduction

Taxodium distichum var. mexicanum, has a distribution from the lower Rio Grande Valley of Texas south into southern Mexico (1, 2, 6). It is a medium to large tree, usually less than 15.3 m (50 ft) tall in Texas, but the famous large tree of Santa Maria de Tule in Oaxaca, Mexico, is estimated to be 38.1 m (125 ft) tall with a circumference of 50 m (162 ft) (1, 2). Montezuma cypress is typically a seed propagated species, but the germination requirements are not well known (5). St. Hilaire has conducted some studies to illuminate these requirements and found that mechanical scarification enhanced germination rates (5). Germination of both baldcypress and pondcypress have been studied and the requirements have been established (3, 4, 5). Murphy and Stanley (3) recommend a 4 hr soak in sulfuric acid to hasten germination of baldcypress and pondcypress. They found that warm-water soaks and cold stratification were no more effective than the untreated control, but had the advantage of producing a more uniform population of seedlings. They reported no embryo dormancy in baldcypress or pondcypress and low germination rates to be the result of a hard, impermeable seed coat. A five minute soak in ethanol followed by 90 d cold stratification or soaking seeds in 3.3C (38F) water for 90 d have also been reported as appropriate treatments to increase germination of baldcypress (4). A 24 to 48 hr soak in 100 mg/liter (100 ppm) citric acid followed by 60 to 90 d cold stratification was reported to enhance pondcypress germination (4).

The objectives of this study were 1) to determine the effect of previously recommended seed treatments for baldcypress or pondcypress on Montezuma cypress seed germination, and 2) to determine the effects of stratification in combination with the above seed treatments on germination of Montezuma cypress seeds.

Materials and Methods

Seeds of *T. distichum* var. *mexicanum* were collected from a single tree on September 19, 2003, in Southmost, TX (25°

52.576' N, 97° 27.083' W, elevation 4.5 m, USDA Plant Hardiness Zone 9b). Cones were slightly immature and were allowed to dry at room temperature (22C, 71.6F) until treatments were applied. Seeds were separated from cone fragments by hand. Seven pre-germination treatments and three stratification periods were applied to the seeds starting on December 4, 2003. Stratification took place in moist peat moss at 2C (35.5F) for periods of 0, 45 and 90 d. Seeds were treated with a 5 min ethanol soak (99.5% solution, Sigma-Aldrich, Inc., St. Louis, MO), a 5 min ethyl ether soak (99.5% solution, Sigma-Aldrich, Inc., St. Louis, MO), 5 hot water baths (42C, 107.6F) allowing the water to cool to room temperature between baths, a 48 hr soak in 100 mg/liter (100 ppm) citric acid (EM Science, Gibbstown, NJ), by nicking the testa with a razor, stratification in water, and a non-treated control. For the 0 d stratification period the water stratification was carried out by placing the seeds in 500 ml of 22C (71.6F) water and then removing them immediately, for the 45 d and 90 d stratification period seeds were left in 22C (71.6F) water for their respective period. Germination was carried out in petri dishes (diameter = 9 cm (3.5 in)) with a double layer of moist filter paper in a growth chamber with 12 hr day/night photoperiods and at a constant 25C (77F). Each dish contained 30 seeds. Three dishes per treatment combination randomly arranged within the growth chamber were evaluated (30 seeds per petri dish per pre-germination treatment per stratification time). Water (5 ml (0.17 oz)) was added to the petri dishes as needed to keep the filter paper wet. The number of seeds germinated was counted daily for the first 14 d and then at 21 d. A seed was counted as germinated when the radicle protruded at least 2 mm (0.08 in).

Data were analyzed using univariate analysis (Table 1) in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, IL) and means were separated using Duncan's Mean Separation ($P \le 0.05$).

Results and Discussion

Pre-germination treatment and stratification had significant effects on the germination rate of *Taxodium distichum* var. *mexicanum* (Table 2). Overall germination was low, with a maximum mean final germination percentage of only 32%

Table 1.	ANOVA tests of between-su	bjects effects for seed ge	ermination of Montezuma cyp	ress, Taxodium distichum var.	mexicanum.

Source	Sum of squares	df	Mean square	F value	Significance (P≤)
Corrected model ^z	10,094.726	314	32.149	7.331	0.000
Intercept	12,146.607	1	12,146.607	2,769.919	0.000
Pre-germination treatment (PT) ^y	636.815	6	106.136	24.203	0.000
Stratification (S) ^x	439.501	2	219.750	50.112	0.000
Day (D) ^w	7,271.202	14	519.372	118.438	0.000
$PT \times S$	362.277	12	30.190	6.884	0.000
$PT \times D$	375.598	84	4.471	1.020	0.436
$S \times D$	673.928	28	24.069	5.489	0.000
$PT \times S \times D$	335.405	168	1.996	0.455	1.000
Error	2,762.667	630	4.385		
Total	25,004.000	945			
Corrected Total	12,857.393	944			

 ${}^{2}R^{2} = 0.785$ (Adjusted $R^{2} = 0.678$; Adjusted R^{2} is an R^{2} adjusted for the number of terms in a model and only increases if the terms added to the model improves it more than would be expected by chance. Adjusted R^{2} can be negative and will always be lower than R^{2} . Adjusted $R^{2} = 1 - [(SS(error) / DF(error)) / (SS(total) / DF(total))].)$

^yThe seven pre-germination treatments included ethanol, ethyl ether, hot water, water, citric acid, mechanical, and control.

*Stratification at 2C (35.5F) in moist peat moss for 0, 45, and 90 d.

"Germination percentages determined at 7, 14, and 21 days.

Table 2.	Mean daily germination percentages for pre-germination
	treatments of Montezuma cypress, Taxodium distichum var.
	mexicanum.

		Stratification period, in days			
		0 ^z	45	90	
Day	Seed treatment	Percent germination			
7 ^y	Ethanol	0.0Bb ^x	4.4Ba	17.8Aa	
	Ethyl Ether	0.0Bb	1.1Ba	18.9Aa	
	Hot Water	12.2Aa	2.2Ba	14.4Aa	
	Water	0.0Ab	5.6Aa	11.1Aa	
	Citric Acid	15.6Ba	11.1Ba	28.9Aa	
	Mechanical	0.0Ab	8.9Aa	14.4Aa	
	Control	0.0Bb	2.2Ba	20.0Aa	
14	Ethanol	18.9Aa	26.7Aa	23.3Aa	
	Ethyl Ether	14.4Aa	24.4Aa	21.1Aa	
	Hot Water	27.8Aa	25.6Aa	17.8Aa	
	Water	23.3Aa	24.4Aa	18.9Aa	
	Citric Acid	28.9Aa	31.1Aa	32.2Aa	
	Mechanical	18.9Aa	21.1Aa	16.7Aa	
	Control	16.7Aa	32.2Aa	24.4Aa	
21	Ethanol	30.0Aa	27.8Aa	23.3Aa	
	Ethyl Ether	20.0Aa	26.7Aa	21.1Aa	
	Hot Water	27.8Aa	27.8Aa	17.8Aa	
	Water	24.4Aa	24.4Aa	18.9Aa	
	Citric Acid	28.9Aa	31.1Aa	32.2Aa	
	Mechanical	21.1Aa	21.1Aa	16.7Aa	
	Control	24.4Aa	32.2Aa	24.4Aa	

^zStratification at 2C (35.5F) in moist peat moss for 0, 45, and 90 d. ^yGermination percentages determined at 7, 14, and 21 days.

^xUpper case letters denote differences in rows and lower case letters indicate differences within columns and days post-stratification. Means followed by the same letter are not significantly different at $P \le 0.05$ using Duncan's Mean Separation (n = 3).

(Table 2). This is consistent with Murphy and Stanley's (3) reports on baldcypress and pondcypress germination.

Stratification and pre-germination treatment effects were confined to early stages of germination. All statistical differences ($P \le 0.05$) among stratification and pre-germination treatments were manifested by seven days of germination time (Table 2), but were not apparent with longer (14 d or 21 d) germination times. Stratification treatments hastened germination, as expressed in increased mean germination percentages for 45 d and 90 d treatments germinating at 7 d (Table 2). The 90 d stratification treatments with ethanol, ethyl ether, citric acid and the control pre-germination treatments typically achieved their final germination rates sooner, than with 45 d or 0 d stratification treatments (Table 2). Stratification for 45 d usually produced the greatest mean final

Pre-germination treatment also had an effect on germination rate, but only during the first seven days of germination. During the first 7 days of germination, citric acid treatment produced the most uniform germination and highest mean germination percentages, but not thereafter (Table 2). Aesthetically, the citric acid treatment produced the most robust seedlings compared to other pre-germination treatments. Seedlings produced appeared to be larger with darker green color compared to other treatments.

A 48 hr soak in 100 mg/liter (100 ppm) citric acid appears to be the best pre-germination treatment studied, if no stratification is applied. Stratification tends to hasten germination. Stratification for 45 d is sufficient, with no improvement in cumulative germination at 90 d stratification. Although stratification hastened germination, this may not have been due to a physiological dormancy (endodormancy), but rather a physical one (ectodormancy). The stratification under moist conditions may simply have allowed the seed to imbibe sufficiently and thereby hasten germination when favorable growing conditions occurred. This would be in agreement with St. Hilaire (5) and Murphy and Stanley (3) who both suggest that there are no physiological dormancy requirements for germination. St. Hilaire (5) found that removing the seed coats had a similar effect to stratification, hastening germination and improving germination uniformity. If immediate germination of ripe seed is desired, then the best treatments are the citric acid soak and the hot water baths, however, if seeds can be stratified, then no pre-germination seed treatment is needed.

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