Journal of Animal & Plant Sciences (J.Anim.Plant Sci. ISSN 2071-7024)
Vol.67(2) : 13264 -13281 https://doi.org/10.35759/JAnmPlSci.v67.2.6

Comparative Analysis on Seed and Cutting propagation in *Cupressus arizonica, Cupressus sempervirens,* and *Thuya orientalis* : influence of seasonal climatic effects conditions

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Keywords: conifers, seedling**, c**utting, season impact.

Submitted 08/01/2026, Published online on 31st March 2026 in the *Journal of Animal and Plant Sciences (J. Anim. Plant Sci.) ISSN 2071 – 7024*

1 ABSTRACT

Various experiments on propagation through seeding and different types of cuttings, on different substrates, were conducted for three species of conifers: *Thuya orientalis (Chinese thuja*), *Cupressus sempervirens (Common Cypress*) and *Cupressus arizonica* (*Arizona Cypress*) . The results demonstrate variability among the different species studied: the ex-situ germination rate ranges from 4% to 50%. Regarding in-situ germination, the study shows that germination of all species is higher in peat. Regarding cuttings performed on different substrate types and during two different periods (winter and spring), the results vary among the different species studied, with low rooting percentages, especially in *Cupressus arizonica*, which showed better rooting during the spring period

2 INTRODUCTION

Conifers, such as pines, firs and spruces, play a crucial role globally: They often occupy a dominant position in forest ecosystems in many regions, especially in boreal and mountainous areas, providing vital habitat for numerous plant and animal species, thus contributing to global biodiversity conservation. Additionally, conifers make an undeniable contribution to landscaping by promoting soil stability, regulating water, providing protection against wind, and adding attractive aesthetics and vertical structure. Unfortunately, one-third of the world's conifers are currently threatened with extinction (IUCN, 2013). Some conifers are widely distributed in Tunisia and hold significant economic value. Besides their economic value, *Cupressus sempervirens* is widely used as an ornamental tree appreciated for its slender shape and as windbreak. *Thuya orientalis* is another popular ornamental conifer in Tunisia, recognizable by its dense foliage and fan-shaped branches, making it a preferred choice for hedges and decorative gardens. In contrast, *Cupressus arizonica*, a cypress species native to the western United States and Mexico, is less common in Tunisia. But recently this species started to gain popularity in landscaping due to its attractive appearance and distinctive blue-green foliage. This comprehensive study focuses on different propagation methods of three conifer species: *Thuya orientalis, Cupressus arizonica, and Cupressus sempervirens*. The main objective is to evaluate and compare the most commonly used propagation methods for these species, emphasizing their effectiveness and impact on the quality of the produced seedlings. The

methods studied are seedling and cutting. These experiments were conducted in two seasons to detect any potential climatic effects on cutting success.

3 MATERIALS AND METHODS

3.1 Study site: The trials are conducted in the nursery of the Higher Agronomic Institute of Chott Mariem, a delegation of the city of Sousse in Tunisia. The Sousse region is characterized by a hot and dry semi-arid climate. The average annual temperature is around 19°C, and the average annual rainfall is approximately 359 mm. Thus, the average annual relative humidity of the air is 68% (National Institute of Meteorology (INM), 2a.

Thuya orientalis *Cupressus sempervirens* *Cupressus arizonica*

Photo 1. Studied species (IA simulation)

3.2 Seeding: Seed sowing was performed in the ISA-CM nursery in February 2023, using two types of substrates: peat and a peat and sand mixture.

3.3 Cutting: Cutting trials were conducted at two different times: in winter (February) and in spring (May). Three types of cuttings were made: top cuttings, semi-hardwood cuttings with and without a heel, and hardwood cuttings. Before planting, cuttings were dipped in a rooting hormone (IBA). Statistical analysis was completed using SPSS software.

4 RESULTS

4.1 Seedling: Seeds of *Cupressus arizonica* were able to germinate successfully only in a peat. The germination curve exhibits a sigmoidal shape, delineating three distinct phases (Figure 1A): a 30-day dormancy period, a 35-day germination period, and a maximum germination rate reaching 3%. The germination of *Cupressus sempervirens* seeds on the peat and sand mixture exhibits a maximum germination rate reaching only 10.88%. In contrast, the germination curve observed on the substrate composed exclusively of peat shows two well-defined plateaus. The first plateau begins in the fifth week, followed by a second plateau after four weeks. For *Thuya orientalis*, the latency periods are approximately 30 and 42 days, respectively, for seeds sown in peat and those in the peat-sand mixture. The germination rate reaches 29.38% for seeds germinated in peat, while it is only 6.67% for those cultivated in the mixture.

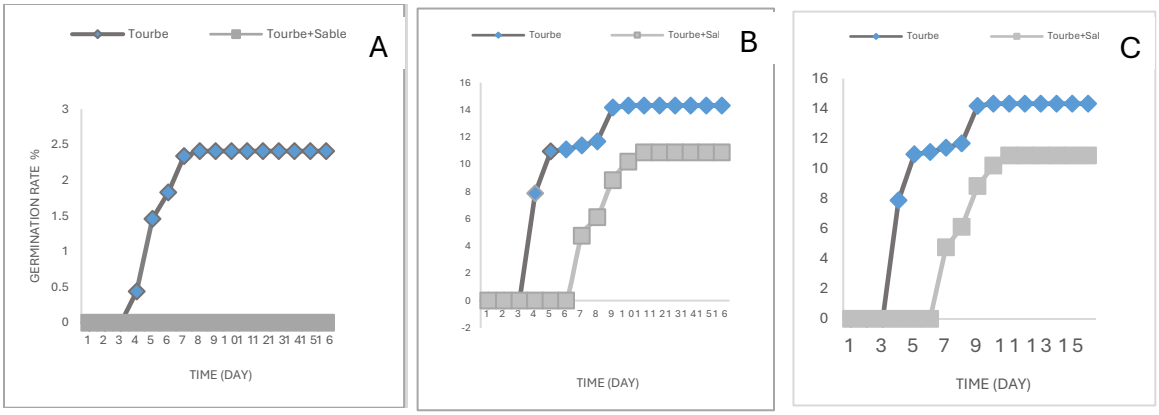

Figure 1. Germination kinetics in *Cupressus arizonica* (A), *Cupressus sempervirens* (B), and *Thuya orientalis* (C) sown on different substrates.

4.2 Cutting: Several studies have demonstrated that the timing of branch collection can have an effect on the success of cutting. To evaluate this effect, two cutting trials were conducted during two different periods. The first trial began in winter, in mid-February, while the second trial was conducted in spring, in late April to early May, during the tree's active period.

4.2.1 Winter Trial: During this period, three types of cuttings were taken:

- hardwood cuttings:
- head cuttings: These cuttings are taken from young, still flexible branches.
- Heel cuttings: This method involves taking cuttings with a substantial section of bark and cambium, to stimulate root production (Bixio and Ysabeau, 1844; Poirier, 2006).

Rooting Substrate: The rooting substrate used is a mixture of sand and peat in respective proportions of 1/3 and 2/3.

4.2.1.1 Winter Trial Results: After four months of cutting of studied species during the winter season, relatively low rooting rates were observed (Figure 2). For *Cupressus arizonica*, only 4 out of 132 softwood cuttings developed root. In contrast, semi-hardwood cuttings and heel cuttings showed no signs of rooting, and drying of the cuttings was observed one month after cutting (Figure 2). Conversely, *Cupressus sempervirens* and *Thuya orientalis* species exhibited a higher number of cuttings developing roots. Out of 160 softwood cuttings of *Cupressus sempervirens*, 54 developed roots (Figure 2). Similarly, *Thuya orientalis* showed that out of 39 softwood cuttings and 5 heel cuttings were developed a roots. Analysis of variance (Table 1) exposed a significant effect of cutting type on rooting ($P < 0.05$). A previous study by Stubbs *et al.* (1997) also highlighted the significant impact of cutting type on rooting percentage. Softwood cuttings, including an apical bud, showed the best results in terms of callus and root formation. Additionally, Hartmann *et al.* (1990) suggested that cutting rooting is associated with shoot tips, probably due to a higher concentration of endogenous root promoters produced in the terminal bud.

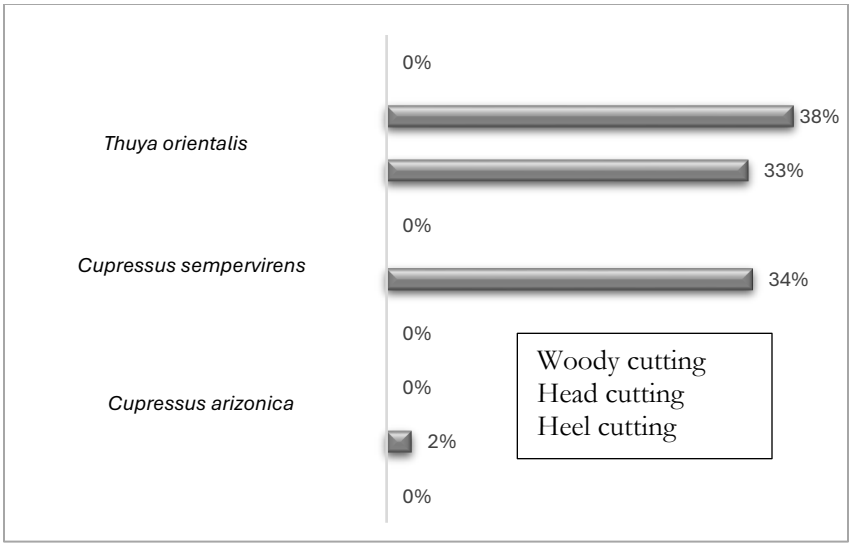

Figure 2: Rate of Cuttings rooting from the winter Trial for the Studied Species

Table 1: Analysis of Variance of the WinterTrial

Source	Sum of type III squares	Df	Mean squares	F	P
Species	1.875	2	.938	6.904	.001
Cutting type	3.220	2	1.610	11.854	.000
species* cutting type	2.176	4	.544	4.006	.003

4.2.3 Spring trial results: After six weeks of experimentation, measurements were taken to evaluate the number of cuttings that developed roots. The interaction between the different species and the type of cutting applied showed a significant effect on the rooting rate of the cuttings ($P < 0.05$) (Table 2). For *Cupressus arizonica*, 11 head cuttings out of a total of 104 cuttings produced a root (Figure 4). Similarly, for *Thuya orientalis*, 12 cuttings produced a roots, while 7 heel cuttings developed a roots (Figure 4). In contrast, no rooting was observed for either type of *Cupressus sempervirens* cuttings.

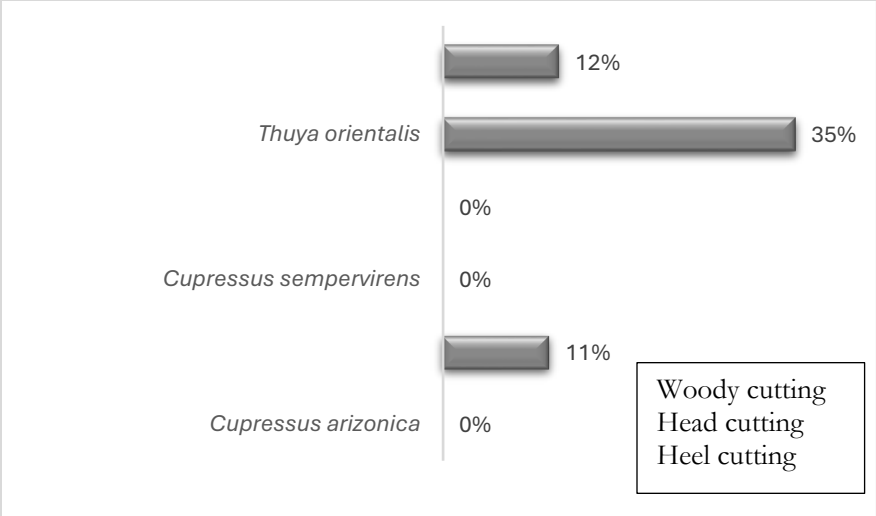

Figure 3. Rate of Cuttings with Calluses and Roots from the Spring Trial for the Studied Species.

Table 2: Analysis of Variance of the Spring Trial Cutting Results

Source	Sum of Type III Squares	df	Mean square	F	P
Species	1.997	2	.998	14.614	.000
Cutting type	.093	1	.093	1.359	.244
Species* cutting type	1.018	2	.509	7.452	.001

***4 3 Evaluation of the Effect of Cutting Collection Season on Propagation Success*:** The success of conifer propagation depends on several factors, among which the season of cutting collection plays a crucial role. This study demonstrated that the timing of cutting collection has a significant effect on the rooting rate of cuttings ($P < 0.05$) (Table 2). The rooting formation rates for the different species studied, obtained during the winter and spring trials, are presented in Figure 4. A significant interaction between the season and species is revealed ($P<0.05$) (Table 3). *Cupressus sempervirens* exhibited a rooting rate of about 17% during the winter trial, but no rooting was observed during the spring trial (Figure 6). These results are consistent with those of the study by Stankova and Panetsos (1997), which showed that winter cuttings of *Cupressus sempervirens* led to higher survival and rooting rates than those obtained in spring. Similarly, *Thuya orientalis* exhibited a higher rooting rate (35%) during the spring trial compared to the winter trial. This result can be explained by the characteristics of the new shoots, which have low carbohydrate storage and high nitrogen content, thus limiting rooting, as suggested by Hartmann and Kester (1983). Therefore, they recommend collecting cuttings between late autumn and late winter to promote rooting in narrow-leaved conifers. Browse (1985) cited by Stankova *et. al* (1997) also supports this period of the year, particularly for the genus *Cupressus*. Additionally, Lamb *et al.* (1985) indicate that February is considered the best period for cypress cutting propagation. However, our study revealed that *Cupressus arizonica* exhibited a higher callus formation rate when cuttings were collected during the spring period.

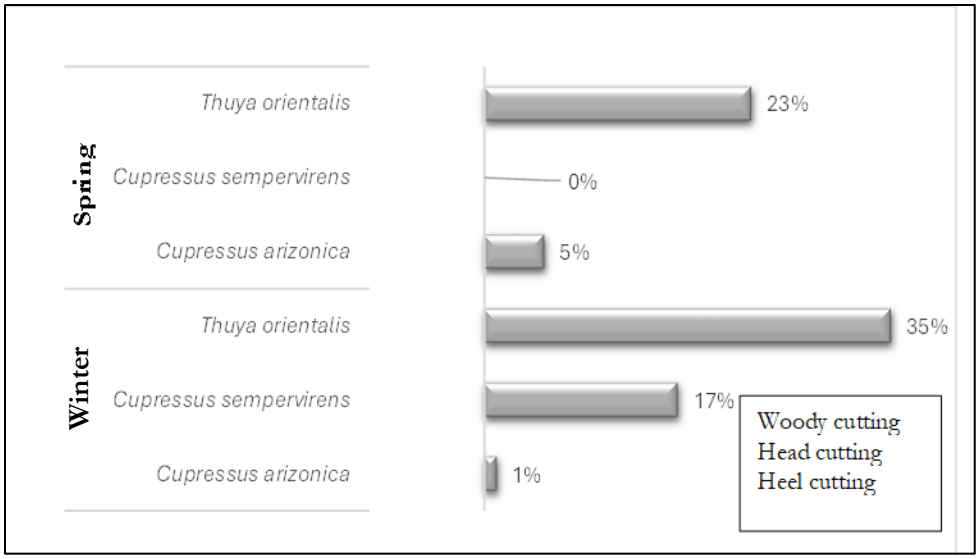

Figure 4. Rate of Rooted Cuttings During the Two Trials (Winter and Spring)

Tableau 3. Analysis of Variance of Cutting Results for the Two Trials (Winter and Spring).

Source	Sum of type III squares	df	Mean squares	F	P
Season	.616	1	.616	5.381	0.21
Species	4.526	2	2.263	19.780	.000
Cutting Type	.186	1	.186	1.623	.203
Season* species	.727	2	.364	3.178	.042
Season* cutting type	.701	1	.701	6.126	.014
species * cutting type	1.062	2	.531	4.643	.010
Season*species*cutting	.770	2	.385	3.366	.035

5 CONCLUSION

This study examined the propagation of three conifer species, *Thuya orientalis, Cupressus sempervirens,* and *Cupressus arizonica*, through both seed and cutting methods, varying substrates, cutting seasons, and types of cuttings. The results highlighted significant variations in ex-situ germination rates, in-situ callus formation and rooting, and the overall success of cuttings depending on the season of collection. Overall, it became evident that the success of cuttings is closely dependent on several factors, including the season of collection. Trials conducted during the winter season often showed higher rooting rates compared to those carried out in the spring. This observation is consistent with previous studies that have emphasized the importance of the winter period for the cutting propagation of narrow-leaved conifers. Additionally, the results also demonstrated differences among the studied species. *Thuya orientalis* showed overall better performance in terms of germination and rooting rates compared to the two *Cupressus* species, with notable variations depending on the seasons and types of cuttings. This study offers valuable insights into best practices for the vegetative propagation of the conifer species examined. It highlights the critical role of the timing of cutting collection, as well as species-specific characteristics, in optimizing the success rate of cuttings.

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