Research Article

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MEDITERRANEAN AGRICULTURAL SCIENCES
(2026) 39: 1
DOI: 10.29136/mediterranean.1742174

Influence of pot design and microbial bioinoculants on early growth of pecan (*Carya illinoinensis*) seedlings

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ARTICLE INFO

Received: July 14, 2025
Received in revised form: September 1, 2025
Accepted: September 5, 2025

***Keywords*:**

Biostimulants
Airpruned
Mycorrhiza
Seedling vigor
Seedling production

ABSTRACT

The process of growth from seed to grafting takes a long time in traditional seedling production in pecan. In addition, pecan orchards are currently established with bare roots, which reduces the planting interval while establishing an orchard. The objective of this study was to determine the effects of microbial fertilizers (Vitormone and Megaflu), mycorrhiza (truffle) alone or in combination with two different pot types (standard and air pruned) on the development of seedlings in pecan cultivar 'Wichita'. A mixture of peat, perlite and vermiculite was used as a substrate. After the transfer of the seedlings to the pot, microbial fertilizers and mycorrhiza were applied once a week through a drip irrigation system for 5 weeks. Stem diameter, stem length, number of leaves, plant internode distance, fresh and dry weights of above and below ground parts were measured during the seedling growing stage. The results showed that the use of Vitormone + truffle combination significantly enhanced growth, producing the highest stem diameter (5.46 mm), stem length (37.16 cm), internode distance (2.41 cm), and leaf number (15.57), compared to the control (4.43 mm, 22.75 cm, 1.80 cm, and 12.54, respectively. Plants grown in standard pots exhibited greater stem diameter (4.90 mm), longer stem length (28.51 cm), and higher root dry weight (3.71 g) compared to those grown in air-pruned pots (4.71 mm, 26.67 cm, and 2.89 g, respectively) At the end of the experiment, the combination of Vitormone with mycorrhiza at a rate of 10% in standard pots was recommended for pecan seedling production.

1. Introduction

Recently, pecan production and consumption have steadily increased worldwide (FAOSTAT 2020; Conner 2011). Pecan nut is native to North America. It is used in various industries for different purposes in many countries (Conner 2011; Casales et al. 2018; Wood et al. 1994). The most important feature of pecans is that they can be easily broken because of their thin shell and their high kernel yield (Sparks 1992; Casales et al. 2018).

Globally, bare rooted pecan nursery plants are usually used to establish pecan groves. The process from seed to grafting takes about 3 years (Zhang et al. 2015). To shorten this process, several germination studies have been conducted on pecan seeds using different methods. However, no successful results have been obtained (Pokomy and Sparks 1967). The beginning of the germination stage to grafting, takes 2 years in pecan. The number of public or private institutions are limited due to the lengthy process of seedling production in Türkiye. A few seedling producers have worked on potted cultivation; however, they did not achieve success due to the deep-root development habit of the pecan. Researchers have also been struggling to find new solutions to produce grafted pecan seedlings globally. Gilman and Beeson (1996) reported that growing seedlings in a pot can negatively affect plant growth. On the contrary, Wood (1996) compared bare-rooted and potted plants in the pecan orchard and observed the shoot development for 6 years. This research reported no differences in terms of growth between the two different planting materials.

Previous studies have showed that potted seedlings have more advantageous than bare- rooted produced ones (Mathers et al. 2007). Additionally, potted production method can shorten the duration. However, the most important problem in the production of potted seedlings is that the pecan has a taproot structure (Ferrini et al. 2000).

Pecan forms a dominant taproot immediately after seed germination, which represents nearly 80% of the root system and plays a critical role in early seedling establishment (Conner 2007; Silva et al. 2023a). The taproot constitutes approximately 80% of the pecan root system and is known to serve as the main root that feeds the plant (Casales et al. 2018). After the taproot, small feeder roots grow parallel to the soil surface. It has been suggested to use pots to change the root system in pecan by air pruning. However, it was determined that the most important problem encountered in the cultivation of plant species with taproots, such as pecan, curled root at the bottom of the pot after reaching the end of the pot. The roots that curl at the bottom of the pot emerge out of the holes at the bottom of the pot in search oxygen and water and encounter the soil (Woodroof 1934). Also, according to the study, it showed that truffle, especially black truffle, has a positive impact on root morphogenesis at an early stage of

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interaction. In this study, white and black truffles (*T. borchii* and *T. melanopsorum*) were used for increasing ethylene and other hormonal activities which induced primary root shortening, root hair stimulation and branching (Splivallo et al. 2009).

The number of pecan nurseries is insufficient in Türkiye. The main reason for this may be that the rootstock needs an average of 2-3 years of growth to reach the graftable stem diameter. Unlike previous studies that mainly focused on germination or general pot cultivation challenges, our research introduces the combined use of microbial bioinoculants and truffle mycorrhiza in two different pot systems (standard and air-pruned), assessing their impact on multiple growth parameters under controlled greenhouse conditions. This approach provides a novel contribution by identifying specific treatment–pot type interactions.

The objective of the study was to determine the effects of microbial fertilizers, truffle solely or in combination with seedling development in standard and air pruned pot types.

2. Materials and Methods

2.1. Materials

This study was carried out between 2020-2021 in a greenhouse at the Faculty of Agriculture and Post-harvest Physiology Laboratory of the Department of Horticulture in Akdeniz University. Seeds of the 'Wichita' cultivar were obtained from a private organization. Two different types of pots, standard and air pruned with a volume of 7 L, were tested. In the research, Vitormone and Megaflu were used as microbial fertilizers with truffles. The *T. melanosporum* species, belonging to the genus *Tuber* were used as mycorrhiza (Table 1). A diluted series was prepared for the truffle concentration. The recommended $2x10^{-4}$ spores mL^{-1} concentration was used.

Table 1. Composition of treatments applied to pecan seedlings (ml)

Treatment	Pure Water (ml)	Fertilizer (ml)	Truffle Concentration (ml)
Control	1000	-	-
Vitormone	630	70	-
Megaflu	630	70	-
Truffle	800	-	200
Vitormone + Truffle	700	100	200

All seeds received identical pre-germination treatments, including 30-day cold stratification at +4 °C in a sand:perlite (2:1) mixture and standardized soaking, germinated under uniform conditions, and were arbitrarily randomly assigned to treatments in a completely randomized design with sufficient replication to account for genetic variation.

Subsequently, the seeds were kept for 72 hours in the solutions prepared with four treatments namely, Control (1000 ml pure water), Vitormone (630 ml pure water + 70 ml commercial fertilizer), Megaflu (630 ml pure water + 70 ml commercial fertilizer), truffle (800 ml pure water + 200 ml concentration) and Vitormone + truffle: 700 ml pure water + 200 ml concentration + 100 ml commercial fertilizer. The seeds were sown in April 2020 according to the treatments given above. A mixture of peat + perlite at a ratio of 2:1 was used as a medium in the vials. To maintain the humidity balance in the environment, the vials were covered with cocopeat medium. Irrigation was carried out regularly with a sprinkler system. Seedlings developed from germinated seeds were then transferred to pots. During the transfer process, 2/3 of the roots of the seedlings were pruned. Peat, perlite and vermiculite in the ratio of 2:1:1 was used in the pots. The pots were placed on benches higher than the ground in the greenhouse. A spaghetti irrigation system was used in the pots, for irrigation.

The above-mentioned treatments started 15 days after the pot transfer at the seedling stage. Each treatment was repeated once a week. In total, five treatments were carried out. In the study, 100 ml of commercial fertilizer was mixed into 1 L of water for each treatment. For Vitormone + truffle concentration, 50 ml of each commercial fertilizer was added to 1 L of water (Furlani et al. 2009).

2.2. Methods

2.2.1. Investigation criteria

Measurements were started 2 months after the seedlings were transferred to the pots (June 2020), repeated once a month and continued for three months. The measurements started 15 days later. Climatic data were measured regularly every month with the HOBO U12-012 data logger device placed in the greenhouse and automatically set to measure every hour. Temperature values were recorded as minimum (22°C), average (33°C) and maximum (42°C), and humidity values were recorded as minimum (23%), average (55%) and maximum (78%).

Stem diameter (mm), stem length (cm), number of leaves, plant internode distance (cm), and fresh and dry weights of aboveground and belowground organs were recorded during the seedling growth stage. Stem diameter and length were measured 5 cm above the pot using a digital caliper (Zhang et al. 2015). Leaf number was determined by counting all fully expanded leaves per plant, and internode length (cm) was calculated as the mean distance between consecutive nodes following Cigsar and Yılmaz (2000). Seedlings were separated into shoots, leaves, and roots, and fresh mass was immediately recorded using an analytical balance (±0.001 g) (Levy et al. 1983). Samples were then oven-dried at 65°C until constant weight (24 h) to determine dry mass according to established protocols (Ferrara et al. 2023; Bertora et al. 2019; Wang et al. 2022).

The experiment was conducted using a completly randomized design with two pot types, five treatments, and three replicates per treatment, each containing five plants. Treatment means were analyzed using XLSTAT software, and differences among means were compared using LSD at $P \leq 0.05$.

3. Results

3.1.Stem diameter (mm)

The effects of different treatments on stem diameter in different pot types are given in Table 2. Among the pot types, the stem diameter value in the air pruned pot type was the lowest (4.71 mm). Among the treatments, the lowest stem diameter value was found in the control treatment with 4.43 mm and the highest mean value was 5.46 mm with Vitormone + truffle combination.

3.2.*Stem length (cm)*

The effects of the different treatments on stem length in different pot types are shown in Table 3. The effects of pot types on stem length showed that the standard pot type gave better results than the air pruned pot type (26.67 cm) with a stem length

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value of 28.51 cm. Among the treatment means, the highest mean value was 37.16 cm with Vitormone + truffle combination while the lowest value was in Vitormone (23.36 cm) and Control (22.75 cm) treatments.

3.3. Number of leaves

The effects of the different treatments on leaf numbers in different pot types are recorded in Table 4. The effect of pot type ×treatment interaction on the number of leaves was found to be statistically significant. However, the effects of pot types on the number of leaves were found to be insignificant. The number of leaves was highest in Vitormone treatment (15.83) and Vitormone + truffle treatment (15.57) with both treatments in the same statistical group.

3.4. Plant internode distance (cm)

The effects of the different treatments on plant internode distance in different pot types were found to be insignificant as can be seen in Table 5. The effects of the treatments on plant internode distance showed that the highest plant internode distance of 2.41 cm was found in Vitormone + truffle treatment.

3.5. Fresh and dry weights of above ground organs

3.5.1. Fresh weights of above ground organs (g)

The effects of the different treatments in different pot types on the fresh weight of above ground organs are shown in Table 6. pot types and treatments on fresh weight of above ground organs had significant had positive effect. Standard pot type gave better results than the air pruned pot type in terms of the fresh weight of above ground organs while truffle gave the lowest value with 2.87 g.

3.5.2. Dry weights of above ground organs (g)

As shown in Table 7, Pot types and treatments on dry weights of above ground organs had significant effect. The interaction analysis showed that the truffle treatment of the standard pot type gave the lowest value (0.57 g), while the Control treatment of the same pot type gave the highest value with 4.87 g. The standard pot type gave better results than the air pruned ones. Depending on the treatments, dry weight values varied between 1.20 and 3.32 g with the highest value found in the Vitormone treatment.

Table 2. Effects of different treatments on stem diameter of pecan grown in different pot types (mm)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	4.35 cd	4.52 cd	4.43 D
Vitormone	3.88 e	5.24 b	4.56 CD
Megaflu	5.28 b	4.45 cd	4.87 B
Truffle	5.04 b	4.37 cd	4.71 BC
Vitormone + Truffle	5.95 a	4.98 b	5.46 A
Pot type (Means)	4.90 A	4.71 B	
LSD	*Pot type x Treatment: 0.355**	*Pot type: 0.159**	*Treatment: 0.251**

**P<0.05*

Table 3. Effects of different treatments on stem length of pecan grown in different pot types (cm)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	22.30 de	23.20 d	22.75 D
Vitormone	17.36 f	29.35 bc	23.36 D
Megaflu	28.62 c	28.62 c	28.62 B
Truffle	30.62 b	21.53 e	26.07 C
Vitormone + Truffle	43.67 a	30.65 b	37.16 A
Pot type (Means)	28.51 A	26.67 B	
LSD	*Pot type x Treatment: 1.408**	*Pot type: 0.63**	*Treatment: 0.996**

**P<0.05*

Table 4. Effects of treatments on the number of leaves of pecan grown in different pot types

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	12.00 g	13.08 efg	12.54 C
Vitormone	13.23 efg	18.43 a	15.83 A
Megaflu	14.75 cd	13.62 def	14.18 B
Truffle	15.48 c	12.37 fg	13.92 B
Vitormone + Truffle	17.13 v	14.00 de	15.57 A
Pot type (Means)	14.52	14.30	
LSD	*Pot type x Treatment: 1.314**	*Pot type: ---^{NS}*	*Treatment: 0.929**

**P<0.05*, ^{NS}: Nonsignificant

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3.6. Fresh and dry weights of the subsoil organs

3.6.1. Fresh weights of subsoil organs (g)

The effects of the different pot types and treatments on the fresh weights of subsoil organs can be seen in Table 8. The lowest subsoil fresh weight value of 3.53 g was recorded in the Control treatment of air pruned pot type. The effects of pot types on the fresh weight of subsoil organs in Table 8 showed that the standard pot type gave better results than the air pruned pot type. Among the treatments, the lowest fresh weight value of 8.37 g was determined in the truffle treatment.

3.6.2. Dry weights of subsoil organs (g)

The effects of the different pot types and treatments on the dry weight of subsoil organs are shown in Table 9. The effects of pot type and treatments on subsoil dry weight were statistically significant. The effects of pot types on subsoil dry weight showed that two statistically different groups were formed. Subsoil dry weight was 3.71 g in the standard pot type and 2.89 g in the air pruned pot type. The effects of treatments on subsoil dry weight exhibited that the highest dry weight value 4.59 g was found in Megaflu treatment followed by Control (3.59 g), Vitormone (3.00 g), Vitormone + truffle (2.69 g) and truffle (2.63 g) treatments, respectively.

Table 5. Effects of treatments on plant internode distance of pecan grown in different pot types (cm)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	1.83 ef	1.76 f	1.80 C
Vitormone	1.60 g	1.34 h	1.47 D
Megaflu	1.92 de	2.07 c	1.95 B
Truffle	1.96 d	1.83 ef	1.89 B
Vitormone + Truffle	2.53 a	2.30 b	2.41 A
Pot type (Means)	1.92	1.91	
LSD	*Pot type x Treatment: 0.083**	*Pot type: ---^{NS}*	*Treatment: 0.059**

**P<0.05, ^{NS}*: Nonsignificant

Table 6. Effects of treatments on fresh weight of above ground organs of pecan in pot types (g)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	12.72 a	2.38 f	7.55 B
Vitormone	6.50 d	11.27 b	8.88 A
Megaflu	7.35 c	6.67 d	7.01 C
Truffle	1.16 g	4.58 e	2.87 E
Vitormone + Truffle	6.77 cd	2.11 f	4.44 D
Pot type (Means)	6.90 A	5.40 B	
LSD	*Pot type x Treatment: 0.411**	*Pot type: 0.184**	*Treatment: 0.291**

**P<0.05*

Table 7. Effects of treatments on dry weight of above ground organs of pecan in pot types (g)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	4.87 a	0.89 eg	2.88 B
Vitormone	2.46 e	4.19 b	3.32 A
Megaflu	2.77 cd	2.85 c	2.81 C
Truffle	0.57 fh	1.83 df	1.20 E
Vitormone + Truffle	2.37 c	0.95 e	1.66 D
Pot type (Means)	2.61 A	2.14 B	
LSD	*Pot type x Treatment: 0.22**	*Pot type: 0.098**	*Treatment: 0.155**

**P<0.05*

Table 8. Effects of treatments on the fresh weight of subsoil organs of pecan in pot types (g)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	36.21 a	3.53 h	19.87 A
Vitormone	11.24 f	13.25 d	12.25 C
Megaflu	14.18 c	16.50 b	15.34 B
Truffle	4.40 g	12.34 e	8.37 E
Vitormone + Truffle	18.74 b	4.10 e	11.42 D
Pot type (Means)	16.95 A	9.94 B	
LSD	*Pot type x Treatment: 0.523**	*Pot type:0.234**	*Treatment: 0.37**

**P<0.05*

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Table 9. Effects of treatments on the dry weight of subsoil organs of pecan in pot types (g)

Treatments	Standard pot	Air pruned pot	Treatment (Means)
Control	6.19 a	0.99 h	3.59 B
Vitormone	2.85 f	3.16 d	3.00 C
Megaflu	3.04 e	6.13 a	4.59 A
Truffle	1.82 g	3.43 c	2.63 E
Vitormone + Truffle	4.65 b	0.74 h	2.69 D
Pot type (Means)	3.71 A	2.89 B	
LSD	*Pot type x Treatment: 0.207**	*Pot type:0.093**	*Treatment: 0.147**

**P<0.05*

4. Discussion

The effects of Vitormone, Megaflu, truffle, and the Vitormone + truffle combination on pecan seedling growth and development were investigated. Seedlings were obtained from seeds, which were genetically heterogeneous. To minimize variability, all seeds were germinated under uniform conditions, seedlings were then randomly assigned to treatments in a completely randomized design, and sufficient replication and statistical analyses were applied. This was in order to minimize genetic differences. Across all parameters, the combined Vitormone + truffle treatment consistently produced the most favorable outcomes. Similar positive effects of microbial inoculants and biostimulants have been reported in other species, including pistachio, citrus, and tobacco (Ortakçı 1999; Üstüner 2001; Zhang et al. 2004; Akgün 2003). Stem diameter and length are critical for grafting, with shorter internodes contributing to compact seedlings with improved physiological efficiency (Hu et al. 2023). In this study, pecan seedlings reached grafting suitability within 6–7 months, with root system architecture playing a key role in post-transplant performance through taproot elongation and lateral root proliferation.

The Vitormone + truffle treatment showed pronounced positive effects on aboveground growth, consistent with previous studies reporting enhanced stem and leaf development via mycorrhizal inoculation (Levy et al. 1983; Ucgun et al. 2009) and increased shoot elongation and leaf number through microbial fertilizers (Ortaş et al. 1998; Matsubara et al. 1994). These effects are often mediated by the stimulation of lateral shoot formation, resulting in a denser and more photosynthetically efficient canopy, while improved root performance enhances water and nutrient uptake (Chen et al. 1996; Reddy et al. 1996; Pulatkan and Var 2010).Interestingly, this treatment was associated with reduced taproot elongation, likely due to a trade-off with lateral root proliferation, a phenomenon supported by studies showing microbial inoculants influence carbon partitioning and root morphology (Silva et al. 2023b; Ferrara et al. 2023). Mycorrhizal associations modulate hormonal signals, promoting lateral root development and nutrient uptake (Gutjahr and Paszkowski 2013; Elsysy et al. 2022), while enhanced phosphorus availability and microbial fertilizers further improve root branching and biomass allocation (Smith and Read 2008; Ozkan et al. 2004; Benucci et al. 2012). This trade-off enhances water and nutrient acquisition, ultimately supporting overall seedling establishment.

No statistically significant differences were observed between standard and air pruned pots over the two-month period. Although air pruned pots can prevent root circling and stimulate lateral roots, the short duration likely limited these effects, suggesting longer trials are needed to fully assess potential advantages in root aeration, nutrient uptake, and stress resilience.

5. Conclusion

In conclusion, although the standard pot type and the Vitormone + truffle treatment at 10% appeared to enhance pecan seedling growth, the short two-month duration of this trial limits the ability to draw definitive recommendations. Therefore, these results should be regarded as preliminary evidence, and future studies on air-pruned pots should extend beyond this period to provide more robust and reliable insights into their effects on seedling development.

Acknowledgement

This project was supported by the Akdeniz University Scientific Research Project Units (Project no: FYL-2021-5648). The authors would like to thank Akdeniz University for contributing to the project support.

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