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INFLUENCE OF TEMPERATURE DURING THE SCIONS GROWTH ON THE PERCENTAGE OF GRAFT TAKE SUCCESS IN WALNUT PROPAGATION

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Abstract

The experiment was conducted at the Fruit Growing Institute-Plovdiv during the period 2022-2025. The local walnut cultivar Izvor 10 was used, grafted onto a rootstock (Juglans regia L.). Scions were collected during winter dormancy, immediately before grafting. The percentage of graft take success was evaluated under two propagation methods: Variant I - plants propagated in a stratification room and Variant II - plants propagated in a heated tunnel with a water-based nutritional solution. Average monthly and maximal outdoor temperatures for the experimental region were recorded for the entire observation period. Statistical analysis revealed a clear tendency toward decreasing graft take success in winter grafting of walnut. The study examined the potential relationship between temperature conditions in the preceding year and the percentage of graft take success under controlled callus-formation conditions (air temperature 26°C and relative humidity 80-90%). The results suggest that elevated summer temperatures may negatively affect scion quality and subsequent grafting success.

Key words*: walnut, propagation, temperature, scions, influence.*

INTRODUCTION

Walnut (*Juglans regia* L.) is considered one of the most difficult fruit species to propagate by grafting, and achieving high graft take success remains a major challenge for nurseries worldwide. Increasing the efficiency of propagation and reducing production costs are therefore of key importance for industrial walnut nurseries (Hassankhah et al., 2017). Successful grafting depends strongly on environmental conditions, particularly temperature, which plays a critical role in callus formation and graft union development. According to Hartmann et al. (1997), optimal callusing temperatures vary among species, but maintaining stable conditions is essential for proper tissue differentiation. For walnut, maintaining a temperature of approximately 26°C and relative humidity of 80-95% for 3-4 weeks is considered optimal for callus formation (Sadeghi-Majd et al., 2022).

A wide range of grafting techniques and propagation systems have been developed for walnut. Classical studies by Lagerstedt (1979, 1982) established the principles of hot-callus grafting and emphasized the importance of performing grafting during winter dormancy. In Bulgaria, extensive work has been conducted at the Fruit Growing Institute - Plovdiv, where various propagation approaches, including hot-callus and epicotyl grafting, have been tested and optimized (Gandev, 2007, 2014, 2016). The use of stratification rooms for indoor walnut propagation has long been practiced in the region (Nedev et al., 1983). Under controlled callusing conditions, high grafting success rates ranging from 65% to 90% have been reported (Paunović et al., 2012).

The quality and physiological state of the scions are among the most important factors determining grafting success. Scions are typically collected during winter dormancy and stored at 1-4°C until grafting (Achim & Botu, 2001; Lagerstedt, 1982; Soleimani et al., 2010; Paunović et al., 2011; Raufi et al., 2017). Other authors recommend collecting scions immediately before grafting (Kasmi et al., 2013; Mostakhdemi et al., 2022). Scion age and thickness also influence grafting success; for example, Sharma et al. (2022) reported that

pencil-thick scions aged 6-12 months are most suitable.

Temperature plays a central role in walnut phenology. Buds require sufficient chilling during winter dormancy to break rest, followed by adequate heat accumulation to initiate budburst (Charrier et al., 2011). Dehghan et al. (2010) demonstrated that heat accumulation may be more influential than chilling in determining the timing of budburst. High summer temperatures can accelerate phenological development, potentially leading to insufficient chilling fulfillment and reduced bud quality (Hassankhah et al., 2017). Since walnut bud differentiation occurs over a long period - from late spring through the following winter - temperature anomalies during the growing season may negatively affect the physiological condition of the buds that later serve as scions.

Given the increasing frequency of temperature extremes in many regions, understanding how climatic conditions influence scion quality and grafting success has become increasingly important. The present study examines the hypothesis that elevated summer temperatures negatively affect the vegetative growth and physiological quality of shoots used as scions for winter grafting. The aim of the study was to establish the relationship between temperature conditions in the preceding year and the percentage of graft take in walnut propagated under controlled callus - formation conditions.

MATERIALS AND METHODS

The study was conducted at the Fruit Growing Institute - Plovdiv during the period 2022-2025. The local walnut cultivar Izvor 10 grafted onto seedling rootstocks of *Juglans regia* L. was used. Two propagation methods were evaluated:

- Variant I: Plants propagated in a stratification chamber;
- Variant II: Plants propagated in a heated tunnel with a water-based nutritional solution.

Grafting was performed in March of each experimental year. During the callus formation period, the grafted plants were maintained for 30 days at a temperature of 26°C and relative humidity of 80-90%, conditions considered optimal for walnut callusing.

Propagation in a Stratification Chamber (Variant I)

Grafted plants were placed in wooden box pallets measuring 75 × 40 × 40 cm. The root systems were covered with moist sand, and the pallets were wrapped with polyethylene film to maintain humidity. The boxes were then placed in a controlled-environment room with stable temperature and humidity suitable for callus formation.

Propagation in a Heated Tunnel with Nutrient Solution (Variant II)

Plants were grafted on a bench using the epicotyl grafting method. After grafting, they were planted in polystyrene trays containing eight cavities, each with a volume of 3 L. A substrate mixture of peat and perlite (2:1, v/v) was used. The trays were placed in a heated tunnel equipped with a water-based nutritional solution maintained at the same temperature and humidity as in Variant I. A combined fertilizer (N:P:K 20:5:10, MgO 2%, SO_3 25%, plus trace elements: Fe 0.07%, Mn 0.04%, Mo 0.004%, Zn 0.025%, B 0.025%, Cu 0.01%) was applied at a concentration of 1 g L^{-1}. For each propagation method, 100 grafted plants were used, each representing a separate replication.

Vegetative Growth Assessment (2023-2024)

The vegetative performance of successfully grafted plants was evaluated during 2023 and 2024. Plants were grown in containers filled with a peat-perlite mixture under 50% shading. Three fertilization treatments were applied:

- Control: No fertilization
- Variant I: N 1.3 – P 0.3 – K 0.7 – Mg 0.1
- Variant II: N 2.7 – P 0.7 – K 1.3 – Mg 0.3

Each treatment included 10 plants; each considered an independent replication. The following parameters were recorded:

- plant height (cm);
- annual shoot thickness (mm);
- root system volume (cm^3);
- number of compound leaves.

Temperature Data

Average monthly and maximal outdoor temperatures for the experimental region were recorded for the entire study period (2021-2025) to assess potential relationships between climatic conditions and graft-take success.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the significance of differences among treatments and years. When ANOVA indicated significant effects ($p \leq 0.05$), the Duncan Multiple Range Test (DMRT) was applied to separate the means (Steele & Torrie, 1980). Statistical analyses were conducted using RStudio (R Core Team, 2020) with the packages **agricolae** (Mendiburu, 2021), **readxl** (Wickham, 2019), **Rcpp** (Eddelbuettel, 2018), and **rstatix** (Kassambara, 2021).

RESULTS AND DISCUSSIONS

Graft Take Success (2022-2025)

The percentage of graft take showed a clear decreasing trend during the four-year study period (Table 1). In plants propagated in a stratification chamber, graft take ranged from 84% in 2022 to 20% in 2025, while in the heated tunnel with a water-based nutritional solution, the values decreased from 60% to 15.6%. Although the absolute percentages differed between the two propagation methods, ANOVA revealed no statistically significant effect of the propagation method, indicating that the observed variation is primarily determined by year-to-year environmental conditions rather than by the technology used.

Table 1. Graft take rate for different walnut propagation methods and statistically significant differences between groups (Duncan Multiple Range Test)

Method	Percentage of graft take, %	
Year	Stratification chamber	Heated tunnel with water based nutritional solution
2022	84% a	60% a
2023	80% a	47% b
2024	50% b	34.4% c
2025	20% c	15.6% d
Average value	58.5 % a	39.25 % a



Picture 1. Walnut plants propagated by different methods

The Duncan Multiple Range Test confirmed that 2025 formed a statistically distinct group, significantly different from 2022, 2023 and 2024. The latter three years did not differ significantly from each other. This pattern demonstrates that the sharp decline in graft take is concentrated in the final year of the study, suggesting the influence of an external factor that changed markedly in 2025.

Temperature Dynamics and Their Relationship to Graft Take

To explore whether temperature conditions in the preceding growing season may explain the observed decline in grafting success, average monthly and maximal temperatures for the period 2021-2025 were analyzed (Figures 1 and 2).

The data show substantial interannual variability, particularly during the summer months (June-August), which are critical for the differentiation and maturation of walnut buds.

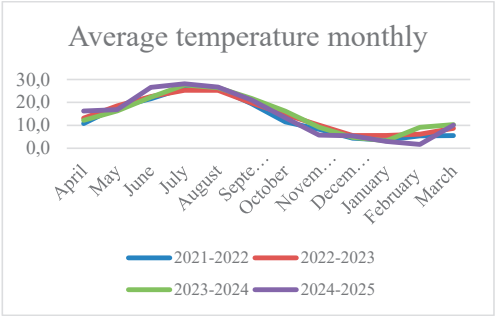

Figure 1. Dynamics of average monthly temperatures for the period 2021-2025

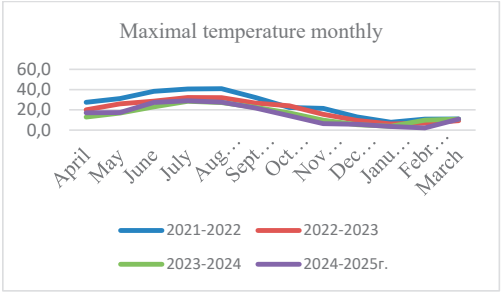

Figure 2. Dynamics of maximal monthly temperatures for the period 2021-2025

The 2025 growing season was characterized by the highest average summer temperatures, with mean values of 26.6°C in June, 28.1°C in July, and 26.7°C in August. Although maximal

temperatures in 2025 did not reach the extreme peaks recorded in 2022 (up to 40.9°C), the prolonged exposure to elevated average temperatures suggests a sustained heat load on developing shoots. This prolonged heat stress is likely more physiologically damaging than short-term temperature extremes.

According to Mitov et al. (2008), optimal temperatures for walnut shoot development range between 15-25°C, while temperatures above 35°C can cause tissue damage. Walnut bud differentiation occurs over a 10-month period beginning in late spring; therefore, persistent summer heat may impair bud vitality, reduce carbohydrate accumulation, and increase the chilling requirement needed for proper dormancy release. These physiological disruptions may explain the markedly lower graft take observed in 2025.

The results align with the findings of Hassankhah et al. (2017), who reported that increased temperatures accelerate phenological development and may lead to insufficient fulfillment of chilling requirements. Similarly, Charrier et al. (2011) emphasize that budburst depends on the balance between accumulated cold and subsequent heat units. In the present study, the combination of warmer summers and relatively mild autumn-winter periods (October-January) likely contributed to incomplete dormancy progression and reduced scion quality. Additional supporting evidence from the literature further confirms the sensitivity of walnut propagation to temperature anomalies. Karadeniz (2005) and Erdogan (2006) report that walnut grafting success often declines sharply when environmental conditions are suboptimal, highlighting the importance of scion physiological status. Rotondo Walnuts (2007) also observed inconsistent results in patch budding under warm climatic conditions, reinforcing the idea that high summer temperatures can negatively affect bud development. These findings support the hypothesis that scions harvested from heat-stressed mother plants may exhibit reduced vitality, leading to lower graft take under controlled callusing conditions.

Vegetative Growth of Grafted Plants (2023-2024)

The vegetative performance of grafted walnut plants grown in containers during 2023 and 2024 is presented in Tables 2 and 3. Plant height, stem diameter, and root system volume were significantly influenced by mineral nutrition. In 2023, plants receiving the higher fertilizer dose (Variant II) reached a height of 36.3 cm, compared with 31.6 cm in Variant I and 25.4 cm in the control. Stem diameter ranged from 6.3 mm in the control to 7.8 mm in Variant II, while root system volume varied between 76.7 cm³ (Variant I) and 126.7 cm³ (Variant II).

Table 2. Vegetative behaviour of grafted walnut plants during winter dormancy in 2023

Variant	Height, cm	Thickness, mm	Root system volum, cm^3	Number of leaves
Control	25.4 b	6.3 b	111.7 ab	10.7 a
Variant I	31.6 ab	7 ab	76.7 b	9.7 a
Variant II	36.3 a	7.8 a	126.7 a	11.7 a

The same trend was observed in 2024, where Variant II again produced the most vigorous plants, with a height of 35.1 cm, stem diameter of 7.1 mm, and root system volume of 121.7 cm³. Control plants exhibited the lowest values for all measured parameters, confirming the positive effect of mineral nutrition on vegetative development.

Table 3. Vegetative behavior of grafted walnut plants during winter dormancy in 2024

Variant	Height, cm	Thickness, mm	Root system‘s volume, cm^3	Number of leaves
Control	24.2 b	5.6 b	106.7 ab	10.7 a
Variant I	30.4 ab	6.3 ab	71.7 b	9.7 a
Variant II	35.1 a	7.1 a	121.7 a	11.7 a



Picture 2. Container growing of walnut planting material

The number of compound leaves did not differ significantly among treatments in either year, indicating that leaf formation is less responsive to fertilization than stem and root growth. Overall, the results demonstrate that increasing fertilizer rates enhances plant vigor, particularly in terms of height, stem thickness, and root system development. These findings are consistent with previous studies on container-grown walnut seedlings, which also report improved vegetative performance under balanced mineral nutrition.

Importantly, this experiment is independent of the graft take analysis and is not influenced by the temperature - related factors discussed earlier. Instead, it provides complementary information on the post - grafting development of successfully propagated plants. Therefore, the vegetative growth results should be interpreted separately from the environmental effects affecting scion quality and grafting success.

The combined analysis of graft take dynamics, temperature patterns, and vegetative growth provides a comprehensive understanding of the factors influencing walnut propagation under controlled callusing conditions. The most striking result is the sharp decline in graft take in 2025, which coincides with the highest average summer temperatures recorded during the study period. This supports the hypothesis that prolonged heat stress during the growing season negatively affects scion physiological quality, reducing their ability to form callus and successfully unite with the rootstock.

The literature strongly reinforces this interpretation. Hassankhah et al. (2017) and Charrier et al. (2011) demonstrated that temperature-driven shifts in phenology can disrupt the balance between chilling and heat requirements, leading to premature or incomplete dormancy transitions. Karadeniz (2005), Erdogan (2006) and Rotondo Walnuts (2007) further show that walnut grafting is highly sensitive to environmental anomalies, with success rates declining sharply under suboptimal climatic conditions.

In contrast, the vegetative growth experiment (2023-2024) revealed that mineral nutrition strongly influences post-grafting development, but these effects are independent of the environmental factors affecting graft take. This distinction highlights the importance of separating scion physiological quality (determined by the previous year's climate) from nursery management practices (such as fertilization), which influence plant performance after successful grafting.

Overall, the study demonstrates that temperature conditions in the preceding growing season are a major determinant of grafting success in walnut, while fertilization primarily affects subsequent vegetative growth. These findings underscore the need for careful selection of scion sources, monitoring of climatic conditions, and adaptation of propagation practices in regions experiencing increasing temperature variability.

CONCLUSIONS

A decrease in the percentage of graft take was found in walnut propagation by both methods (Stratification Room and Heated Tunnel) under controlled conditions for callus formation after grafting during the period 2022-2025. Regarding the propagation method, no statistically significant differences were found between the average graft take values, indicating that the observed variations are determined by the annual temperature conditions and not by the technology used. Probably, the cool autumn and early winter (October-January period) are the main prerequisite for successful propagation in walnut. We assume that the warm summer has a negative effect by reducing the vitality of the buds, affecting the development of tissues, and increasing the need for cooling in winter. The spring months (March-April) also create a prerequisite for a negative impact, as they can provoke premature activation of the cambium. This study assumes that high summer temperatures may have a major impact on the grafts used for walnut propagation under controlled conditions. Further studies are needed to establish stronger relationships and dependencies between the individual factors influencing callus formation and attachment.

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