# Preliminary studies on grapevine asexual propagation by using Deep Flow hydroponics

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## Abstract
The fact that vine nurseries very often face difficulties in finding arable land, free of vine pests and diseases, with appropriate soil characteristics, sufficient quantities of good quality irrigation water etc, led us to investigate the possibility of using the Deep Flow Technique (DFT) in the grapevine asexual propagation process, as it is well known that growing plants with Deep Flow hydroponics contributes to higher production, reuse of water and fertilizers, precocity, better control of the nutrient solution composition and temperature, lower cost for disease control, as well as greater labor productivity.
In this way, hardwood cuttings of the rootstocks 110R, 1103P, 140Ru and 41B, bench-grafted with the table-grape variety Sultanina, were placed for rooting in fog-system benches converted into DFT after the removal of the solid substrate, the sealing of the basin with a plastic sheet and its filling with nutrient solution up to 20 cm. The cuttings were placed into pre-cut holes of polystyrene (styrofoam) panels that floated in the nutrient solution of DFT. The spacing between the cuttings was either 10 x 10 cm or 15 x 10 cm. The rooting percentage after twenty days of rootstock cuttings remaining in the DFT, was 44.4% for R110, 42.2% for 1103P and 140 Ru and 26.7% for 41B. The experiment was repeated the next year and the percentage of the successful development of the transplanted rootstocks (previously rooted with DFT) in the field, was recorded as well.

## Introduction
It is well known that the development of plants with hydroponic DFT system contributes to precocity, higher yield, reuse of water and fertilizers, better control of plant growth, nutrient solution composition and temperature, reduced cost for disease control and greater labor productivity (Goto et al. 1996, Both et al. 1999, Watanabe et al. 2001, A. Assimakopoulou et al. 2011).
The aforementioned advantages of DFT in combination with the ongoing difficulties in finding arable land free of vine pests and diseases, with appropriate soil characteristics, sufficient quantities of good quality irrigation water etc, led us to investigate the possibility of using DFT in the grapevine asexual propagation process and specifically, in the rooting procedure of hardwood, bench-grafted, cuttings of grapevine rootstocks. The relative scientific works in the literature are very few, including the review of Corrêa et al. (2012) which presented the hydroponic production of fruit tree and grapevine seedlings in Brazil by using several hydroponic systems, one of which was DFT.

## Materials and Methods
Three experiments were conducted in consecutive years in a glasshouse of the Technological Educational Institute of Kalamata, Greece, in order to determine the percentage of the successful rooting of hardwood cuttings of the rootstocks 110R,

1103P and 140Ru (Vitis berlandieri x V. rupestris) and 41B (V. vinifera L. x V. berlandieri ), bench-grafted with the table-grape variety Sultanina, by using Deep Flow Technique hydroponics and fogging. The experimental material was supplied by the grapevine nurseries of Bakasieta, in Leontio Nemea, Greece.
After the the removal of the solid substrate from two benches (I and II) equipped with fog system, 2.50 m long and 1.15 m wide, the sealing of the basins with plastic sheet and their filling with nutrient solution up to 20 cm, 12 polystyrene (styrofoam) panels, 40x55x5 cm (width x length x height) and 2.5 cm diameter holes, were floated in the nutrient solution of DFT. The spacing between holes where the cuttings were placed was 10 x 10 cm in bench I and 15 x 10 cm in bench II.
Inserting cutting bases in appropriate concentrations of a-naphthalene acetic acid (NAA) solution preceded their placement in the DFT system.
The fog system operated every 10 min for 10 sec during the day (Walker & Golino, 1999) whereas the air circulation in the nutrient solution operated every 5 min for 40 sec throughout the 24 hours. The nutrient solution used was Hoagland No. 2, ½ in macronutrients and full in micronutrients. The electric conductivity and pH of the nutrient solution during the experiments ranged between 1.3-1.5 mS/cm and 7.3-7.4, respectively, whereas the concentration of the dissolved oxygen was between 3.5-4.0 ppm.
The first experiment was designed as a completely randomized block with four rootstocks x two densities of the cuttings on the panels x three replicates. In bench I, every replicate consisted of a Styrofoam panel with 15 cuttings per rootstock (spacing 15x10 cm) while in bench II, every replicate consisted of a panel with 20 cuttings per rootstock (spacing 10x10 cm). In this way, 180 grafted cuttings were placed in total for rooting in bench I (45 per rootstock) whereas in bench II, 240 (60 per rootstock). Throughout the experiment, the average temperature of the nutrient solution in both benches was $28^0$C whereas the average temperature and RH inside the greenhouse was $26.0^0$ C and 57.2%, respectively.
In the 2nd experiment (next year), only bench I had been converted into the DFT system. Every one of the 12 panels floated, included all the rootstocks tested. The spacing of the cuttings in the first six panels was 15x10 cm whereas the spacing of the other six ones was 10x10 cm, and they were placed alternately in the DFT. The average temperature of nutrient solution was $25^0$C. In bench II, an equal number to bench I of grafted cuttings were placed for rooting into individual pots, 10x15 cm, containing a mixture of compost:perlite (1:1, v/v). In this experiment, the percentage of the successful transplanting in the field both of the cuttings rooted with DFT and in the individual pots with the solid substrate was further examined.
In the third experiment, the effect not only of the rootstock on the rooting percentage, but also two different temperature levels of the nutrient solution of DFT were studied. Specifically, the temperature of the nutrient solution in bench I was maintained at $20^0$C whereas in bench II at $28^0$C. At the same time with the placement of the cuttings in the DFT system, similar plant material was transferred for rooting in the field. The establishment of plant material in both DFT and the field in this experiment was done on 3rd of April, i.e. 40 days earlier than in the previous two experiments. The average temperature in the greenhouse environment was $19.8^0$C.

*Statistical analysis*
The statistical analysis of the results of all experiments was based on the analysis of variance (ANOVA) and the comparison of the means by using the Least Significant Difference (LSD), at $P<0.05$.

## Results-Discussion

*1st experiment*

The results of the first experiment showed that the percentage of the rooted cuttings with DFT, in the case of the spacing 10x15 cm, was 27% for 41B, 42% for 1103P and 140Ru, and 44% for the 110R (Figure 1). The lower rooting percentage of the rootstock 41B in relation to 1103P, 110R and 140Ru, has repeatedly been reported (Stavrakakis, 2004).

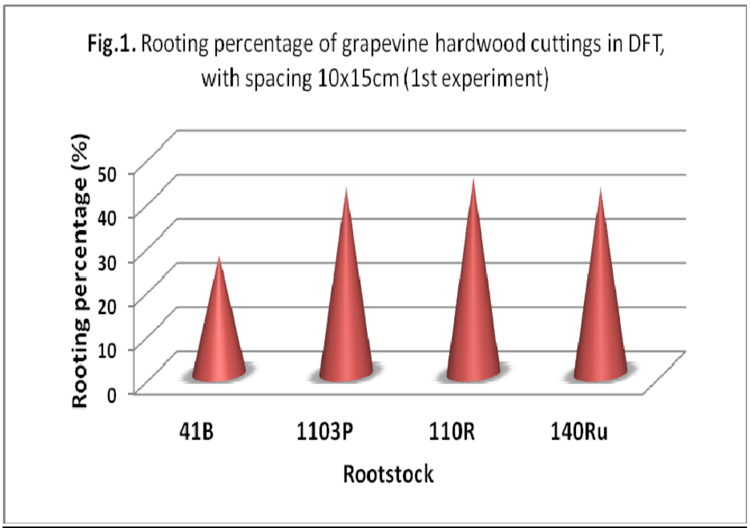


**Figure1.** Rooting percentage of the bench-grafted, with the table-grape variety Soultanina, hardwood cuttings of the rootstocks 41B, 1103P, 110R and 140Ru, by using Deep Flow Technique and fog system, and spacing 10x15 cm (1st experiment).

Comparing the rooting percentage between the density of 180 cuttings in total in bench I (cutting spacing 10x15 cm) and that of 240 cuttings in bench II (cutting spacing 10x10 cm), the higher percentage was found to be in the case of the lower density (bench I). The difference was significant in the case of the cuttings of the rootstocks 41B and 140Ru, but not between 1103P and 110R (Table 1).

**Table 1.** Rooting percentage of the bench-grafted, with the table-grape variety Soultanina, hardwood cuttings of the rootstocks 41B, 1103P, 110R and 140Ru, placed with two densities, (spacing 10x15 cm and 10x10 cm), by using Deep Flow Technique and fog system (1st experiment).

| Υποκείμενο | Spacing of cuttings | Rooting percentage |
|---|---|---|
| | *cm* | % |
| **41B** | 10x15 | 27.0 bc |
| " | 10x10 | 7.0 a |
| **1103P** | 10x15 | 42.0 cd |
| " | 10x10 | 35.0 cd |
| **110R** | 10x15 | 44.0 cd |
| " | 10x10 | 47.0 d |
| **140Ru** | 10x15 | 42.0 cd |
| " | 10x10 | 10.0 ab |

*$2^{nd}$ experiment*

In the second experiment, the results showed that the percentage of the rooted cuttings with DFT was 76.2% for R110, 28.6% for 1103P, 14.3% for 41B and 9.5% for 140 Ru. The different spacing of the cuttings between the two groups of the six panels, both placed in the same bench, did not significantly alter the rooting percentage. Non-significant differentiation of the rooting percentage of the cuttings because of the two different types of spacing in this experiment should be due to the lower total number of the cuttings in the bench as compared to the relevant one of bench II of the $1^{st}$ experiment. The recording of the rooting percentage of the cuttings in the individual pots with the solid substrate showed that in the case of 41B it was 50.0%, 1103P 54.2%, 110R 75.0% and 140Ru 62.5%.

The percentage of the cuttings that had previously rooted in the individual pots with the solid substrate and thereafter were successfully transplanted in the field, was high for all the rootstocks tested; it was 90.0% for R110, 80.0% for 1103P, 90.0 % for 140 Ru and 75.0% for 41B. On the contrary, the percentage of the successful transplantation in the field of the cuttings rooted with DFT and fog system was 55.6% for 1103P and 16.7% for the 110R, while the respective percentages of 41B and 140Ru were not determined due to the very limited number of rooted 41B and 140Ru with DFT.

*3rd experiment*

In the third experiment, the rooting percentage of the cuttings was recorded five weeks after their placement in the DFT system. The percentage of the cuttings of the rootstocks rooted in the nutrient solution at a temperature of $20^0$C was found to be significantly higher compared to that of rootstocks rooted in the nutrient solution with the temperature of $28^0$C. The only exception was 41B, none of whose cuttings rooted, either with DFT at any temperature level or in the field. It should be noted that the buds of 26% of 41B cuttings had burst before the root emergence resulting in their desiccation. The rooting percentage, at nutrient solution temperature of $20^0$C, was 58% for 110R, 56% for 1103R and 24% for 140Ru, with the percentage of rooted 1103R and 110R cuttings significantly higher than that of 140Ru. The percentages of rooted cuttings of 1103R, 110R and 140Ru at nutrient solution temperature of $28^0$C were found to be significantly lower than the relevant ones of $20^0$C; 1103P showed significantly greater percentage than 140Ru whereas 110R presented intermediate values (Figure 2).

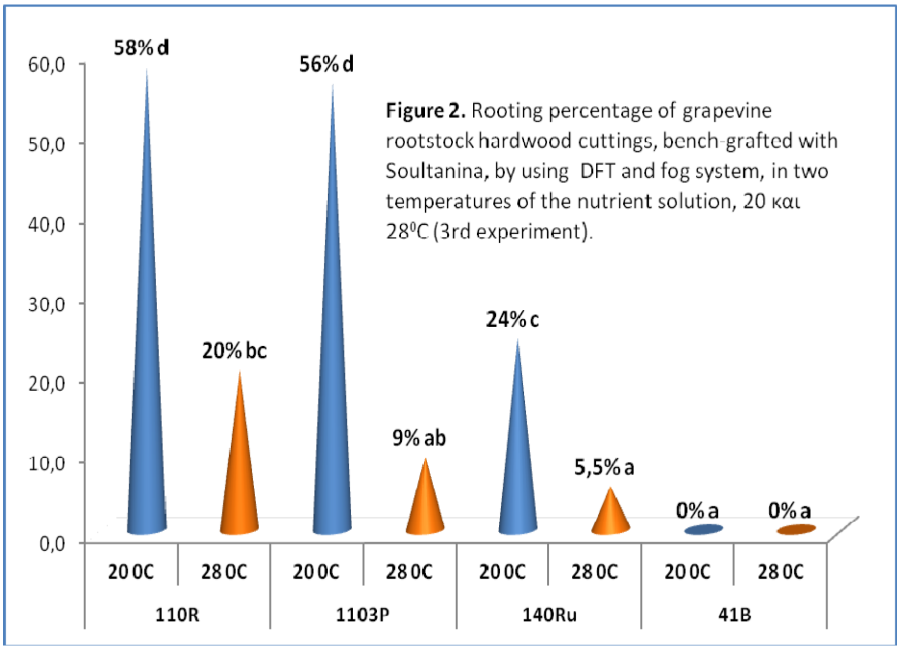


**Figure 2.** Rooting percentage of the bench-grafted with the table-grape variety Soultanina, hardwood cuttings of the rootstocks 41B, 1103P, 110R and 140Ru, by using Deep Flow Technique and fog system, in two temperatures of the nutrient solution, $20^0$C και $28^0$C ($3^{st}$ experiment).

Maintaining the temperature of the nutrient solution at higher levels ($28^0$C) negatively affected the rooting process of the cuttings and further plant development. It is widely known that the higher temperatures prevailed in the root environment increase their respiratory activity with the consequent accumulation of greater amounts of $CO_2$ and the decrease of dissolved $O_2$ (Goto et al. 1996; Drew, 1997), resulting in the further water and nutrient uptake inhibition by plants. Similar to the 3rd experiment lower rooting percentages at the nutrient solution temperature of $28^0$C, were obtained in the first two experiments, as the relevant temperatures also ranged between 25-$28^0$C, due to their later establishment, the consequent prevalence of higher temperatures inside the glasshouse, which in turn increased the nutrient solution temperatures. Apart from the unfavorable root growth conditions, the higher temperature of the glasshouse environment caused the earlier bud break of the cuttings in relation to root emergence, resulting in their desiccation and death (Stavrakakis 2004).

## Conclusions

- The rooting percentages of the bench-grafted, with the table-grape variety Sultanina, hardwood cuttings by using the hydroponic Deep Flow Technique in combination with fog system, were encouraging in the case of the grapevine rootstocks 1103P, 110R and 140Ru, but not in the case of 41B.
- A significant decrease of the time duration of the rooting process as well as an over increase of about three times the number of rooted cuttings per unit area of facilities as compared to the relevant one in the nursery field, were achieved by using DFT system.
- A more appropriate time period for the establishment of the cuttings in the DFT system is early spring as the temperature of both the environment and the

nutrient solution is maintained in more favorable conditions for root emergence levels.

- However, further investigation has to be carried out in relation to several factors, such as nutrient solution dissolved oxygen concentration and air circulation techniques, nutrient solution composition and temperature, etc. affecting the rooting process of hardwood cuttings of every rootstock separately by using DFT.
- In the case of hardly rooted rootstock cuttings with DFT, such as 41B, the possibility of using other hydroponic systems, i.e. aeroponics, should be tested.

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## Προκαταρκτικές εργασίες αγενούς πολλαπλασιασμού της αμπέλου με υδροπονικό σύστημα επίπλευσης (Deep Flow hydroponics)

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Περίληψη

Οι δυσκολίες συνεχούς ανεύρεσης κατάλληλων, από εδαφολογικής και φυτοϋγειονομικής άποψης, αγροτεμαχίων για τις ανάγκες των φυτωριακών επιχειρήσεων αμπέλου, επαρκούς και καλής ποιότητας νερού άρδευσης, αντιμετώπισης εχθρών και ασθενειών κ.ά. οδήγησαν στη διερεύνηση της δυνατότητας χρησιμοποίησης του υδροπονικού συστήματος επίπλευσης (Deep Flow Technique-DFT) στη διαδικασία παραγωγής αγενούς πολλαπλασιαστικού υλικού της αμπέλου, καθώς είναι γνωστό ότι η ανάπτυξη φυτών με DFT συμβάλλει σε πρωίμιση, μεγαλύτερη παραγωγή, μικρότερη κατανάλωση νερού, καλλίτερο έλεγχο σύστασης και θερμοκρασίας υποστρώματος ανάπτυξης, μειωμένες ανάγκες φυτοπροστασίας και μεγαλύτερη απόδοση εργασίας.

Κατ' αυτόν τον τρόπο, άρριζα μοσχεύματα των υποκειμένων 110R, 1103P, 140Ru και 41B, εμβολιασμένα με την επιτραπέζια ποικιλία αμπέλου Σουλτανίνα, τοποθετήθηκαν για ριζοβολία σε πάγκους υδρονέφωσης, που είχαν μετατραπεί σε υδροπονικά συστήματα επίπλευσης μετά την αφαίρεση του στερεού υποστρώματος, τη στεγανοποίηση της λεκάνης με πλαστικό και την πλήρωσή τους μέχρι βάθους 20 cm με θρεπτικό διάλυμα (ΘΔ). Τα μοσχεύματα στηρίζονταν πάνω σε πλάκες εξηλασμένης πολυστερίνης (Styrofoam) που επέπλεαν στο ΘΔ και οι αποστάσεις μεταξύ των ήταν 10 cm x 10 cm ή 15 cm x 10 cm. Η καταγραφή του ποσοστού ριζοβολίας των μοσχευμάτων, που πραγματοποιήθηκε είκοσι ημέρες μετά την τοποθέτησή τους στο ΘΔ, έδειξε ότι ήταν 44,4% για το R110, 42,2% για τα 1103P και 140 Ru και 26,7% για το 41B. Το πείραμα επαναλήφθηκε την επόμενη χρονιά και εξετάστηκε επιπλέον η επιτυχία της μεταφύτευσης των ριζοβολημένων με σύστημα επίπλευσης υποκειμένων στον αγρό.