Agricultural Academy - Sofia
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„New techniques for propagation, container growing of grafted plants and studying of introduced cultivars for sustainable development of walnut production.”

Extended Abstract
on a PhD Thesis for awarding educational and scientific degree "Doctor"

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The research was conducted at the Fruit Growing Institute - Plovdiv during the period 2018-2023.

The dissertation is written on 127 typewritten pages, which include 14 tables and 45 figures. The list of literary sources includes 266 titles, of which 232 are in Latin.

The public defense of the dissertation will be held on……. from ……hours in the hall of the Institute of Fruit Growing - Plovdiv.

I. Introduction

According to official data, in 2022, walnuts occupy 23.44% of the area of fruit crops in Bulgaria. Currently, production in the country is mainly concentrated in two regions - North-Eastern and South-Central. These are the lands around the cities of Shumen, Razgrad, Targovishte, Stara Zagora, Plovdiv, Kardjali, Haskovo and Sliven.

In the last decade, the annual production of walnuts in Bulgaria has been characterized by significant fluctuations from 1,670 to 5,116 tons, with 4,731 tons produced in 2021. The average yields for the period 2012-2021 are in the range of 587 - 1382 kg/ha (Agro statistics 2012-2021). The main reason for the unsatisfactory yields is the still prevailing outdated varietal structure. In orchards over 40 years old, representing a significant part of the existing plantings, the local varieties Sheynovo, Silistrenski, Dryanovski, etc. are mainly represented. They are characterized by an apical and intermediate fruiting type and are sensitive to anthracnose /Gnomonia leptostyla (Fr.) Ces. Et de Not/. These two factors and the seed origin of not a small number of trees predetermine to a large extent the unsatisfactory yields obtained.

The investment in a walnut plantation begins to pay off later compared to other fruit species due to the slower entry of the walnut into a period of full fruiting. This feature of the species can be overcome by using grafted planting material, growing early-fruiting varieties with a short juvenile period and applying modern technologies in the cultivation of this fruit species. Studies in these areas have been conducted in recent years in our country. The suitability of a number of varieties introduced from France, Hungary and the USA to our climatic conditions has been studied. New propagation methods and techniques have been developed and implemented in the production of walnut planting material. In practice, a new technology for growing walnuts, developed at the Institute of Fruit Growing - Plovdiv, is also applied.

The development of walnut production in Bulgaria requires the application of the obtained scientific results in the production of fruits and planting material and the continuous renewal of knowledge, based on experimental work. In this regard, the dissertation examines some new aspects of the propagation and cultivation of walnut, with the aim of sustainable development of walnut production in our country.

II. Aim and tasks of research

This dissertation aims to gain new knowledge and establish effective methods and practices leading to sustainable development of walnut production in propagation, growing grafted plants in containers and cultivar research. To achieve this aim, it is necessary to solve the following specific tasks:

1. Development of a method for propagating walnut, during its winter dormancy, in the conditions of a heated tunnel with water nutritional solution;

2. Optimizing the growth of grafted walnut plants grown in containers;

3. Assessment of the biological and economic qualities of newly introduced walnut varieties.

III. Material and methods

III. 1. Propagation of walnut

III.1.1. Propagation of walnut by the "*bench grafting*" and "*epicotyl grafting*" methods in the conditions of a heated tunnel with an aqueous nutrient solution

The experiment was conducted at the Institute of Fruit Growing - Plovdiv. Propagation was carried out in March by *bench grafting* using the "*cleft*" technique. The local walnut variety Izvor 10 was used, grafted on one-year-old common walnut (*Juglans regia L.*) seedlings. The central root was shortened to about 20-25cm from the grafting site, and the lateral ones were shortened to a length of 5-8cm. Cuttings were collected from mature trees immediately before grafting. After grafting, all plants were planted in polystyrene trays. Each tray contains 8 sockets, each of which has a volume of 3l. A mixture of peat and perlite was used for planting in a ratio of 2:1 parts by volume. The trays prepared in this way with grafted plants were placed in a heated tunnel with water nutritional solution at a temperature of 27°C for 30 days.

The heated tunnel is a steel plot with a height of 1m above the ground. Its length is 4m, width - 1.2m, and depth - 15cm. The bottom of the steel plate is covered with 2 layers of polyethylene, tightly attached to the sides, so there is no nutrient solution leakage. A roof structure made of arc-shaped curved steel rods with a height of 1m covered with polyethylene is built next to the table. In the upper part of the structure above the polyethylene, additional thermal insulation with a thickness of 5 mm has been added. Maintaining the required temperature of 27oC is done by heating with an electric heater attached to the roof structure. For air circulation, the equipment was equipped with a fan. Air humidity during the callus formation process was 90%. To ensure the need for nutrients in the solution, a combined fertilizer N:P:K 20:5:10 MgO-2% SO3-25% + trace elements (Fe-0.07%, Mn-0.04%, Mo- 0.004%, Zn-0.025%, B-0.025%, Cu-0.01%) in a dose of 1g/l solution.

In *epicotyl grafting*, common walnut (*Juglans regia* L.) seeds were immersed in water in mid-December. The water was changed daily to protect the embryo from the harmful effects of phenols released into the water (Vahdati and Hoseini, 2006). Five days later, the seeds were removed from the water and added for stratification in polythene bags that were tightly tied. The maximum stratification was between 3-6°C. All cracked seeds (from mid to late February) were sealed in PVC containers with sand and added at a temperature of 10-15°C. After the seeds formed a well-developed root and new growth, they were grafted into a *cleft.* The cuttings were taken from the mother variety Izvor 10 during their winter regime and stored in a refrigerator until grafting. Binding was done with Teflon tape. After grafting, the root tip of each rootstock was shortened 2-3cm to increase the formation of lateral roots.

After grafting, all plants were planted in polystyrene trays and added under the same conditions as the table grafted plants described above.

The experiment was carried out in three consecutive years during the period 2021-2023, and the propagated plants by the two separate methods were grouped into the following variants:

I. Bench grafted plants placed for callus formation in a heated tunnel with water nutritional solution;

II. Epicotyl grafted plants were placed for callus formation in a heated tunnel with water nutritional solution.

Each variant was represented by 32 plants, each of which was a separate replicate. After the end of callus formation, the intercepted plants were planted in containers with a volume of 10 l, in a mixture of peat and perlite in a ratio of 2:1 parts by volume. Their cultivation continued in a shaded field with a net throughput of 50%.

The obtained results were processed statistically according to Duncan's method (Steele and Torie, 1980) through the program "R studio" (R Core Team, 2020), using the packages "agricolae" (Mendiburu, 2021), "readxl" (Wickham, 2019), “Rcpp” (Eddelbuettel, 2018) and “rstatix” (Kassambara, 2021).

III.2. Container growing of grafted plants

III.2.1. Vegetative behavior of walnut plants grown in containers, grafted with the grafting techniques "cleft" and "whip-tongue"

The research was conducted during the period 2019-2022. The walnut variety Izvor 10 was used, grafted on one-year seed mats of common walnut (Juglans regia L.).

Variants of the experiment:

I. Plants grafted on "patch budding" (window) during the growing season and grown outdoors in a nursery (control);

II. Plants grafted during winter dormancy on "cleft" and grown in containers under 50% shading;

III. Plants grafted during winter dormancy of "whip-tongue" and grown in containers under 50% shade.

The plants of var. I. are grown outdoors according to the adopted "window" technology (Nedev et al., 1976), and those from var. II and var. III are propagated in a stratification room and planted in 10l containers in a mixture of peat and perlite in a ratio of 2:1 and grown two vegetations in a shaded field. The following indicators were recorded: tree height (cm), stem cross-section (mm2), volume of the root system (cm3). Each variant is presented in 25 repetitions.

Determination of leaf chlorophyll content was performed with a CL-01 portable chlorophyll meter (Hansatech Instruments Ltd., UK). The field-portable hand-held device determines relative chlorophyll content using optical absorbance measurements of leaf samples using dual-wavelength (660nm and 940nm wavelength). The relative chlorophyll content is displayed in the range 0 – 2000 units.

Chlorophyll fluorescence (OJIP test)

For a more detailed assessment of the physiological state of the plants, an analysis of chlorophyll fluorescence was performed. Using the HandyPEA Fluorimeter (Hansatech Instruments Ltd., UK), the structure and functional state of the photosynthetic apparatus is analyzed to detect early symptoms of stress and various disorders - OJIP test (Strasser et al., 2000, 2004). The method is non-destructive and is applied without damaging or destroying the analyzed plants. Measurements were taken on the first fully developed leaf from the tip (3rd leaf) after a minimum of 30 minutes of blackout with a special clip. For each variant, 5 plants were measured.

The determination of leaf chlorophyll content and the OJIP test were performed on the same petioles in the middle part of the compound leaf.

The obtained results were processed statistically according to Duncan's method (Steele and Torie, 1980) through the program "R studio" (R Core Team, 2020), using the packages "agricolae" (Mendiburu, 2021), "readxl" (Wickham, 2019), “Rcpp” (Eddelbuettel, 2018) and “rstatix” (Kassambara, 2021).

III.3. Study of cultivars

III.3.1. Study of the biological and economic qualities of introduced walnut varieties

The experimental plantation was created in the spring of 2013, and the study covers the period 2018-2020, i.e. sixth - eighth vegetation of the walnut trees. The planting distance is 10 x 9 meters. The introduced cultivars Sebin, Yalova 1, Valmit and Valeris were studied, and their agrobiological characteristics were compared with those of the standards adopted in our country - Silistrenski and Izvor 10. All varieties were grafted on a rootstock of common walnut (Juglans regia L.). The trees are formed according to the improved layered crown system. The experiment was carried out under irrigation conditions using a micro-sprinkler system. The inter-rows in the plantation are naturally grassed, and the inter-row strip is kept clear of weeds by means of herbicides.

Each variety is represented by three trees, located randomly in the plantation. Each tree is considered a separate repetition. The obtained results were processed statistically according to Duncan's method (Steele and Torie, 1980) through the program "R studio" (R Core Team, 2020), using the packages "agricolae" (Mendiburu, 2021), "readxl" (Wickham, 2019), “Rcpp” (Eddelbuettel, 2018) and “rstatix” (Kassambara, 2021).

Indicators:

1. Duration of flowering of female and male flowers - according to

the onset of full flowering cultivars are grouped:

- very early blooming – flowering is 4 or more days before the standard

Source 10;

- early blooming, when they start blooming 2-3 days before the standard;

- medium flowering, when their flowering coincides with the Izvor 10 standard;

- late blooming, when they start blooming 2-3 days after the standard;

- very late blooming – flowering is 4 or more days after the Izvor 10 standard.

The abbreviation of the separate phenophases is presented in the table. 1.

Table 1. Phenophases of flower development in walnut (Dzuvinov et al., 2013).

Female flowers	Male flowers
Winter dormancy - Af	Differentiation of catkin - Amr
Separation of the scales – Af2	Catkin in late summer – Amv
Swelling – Bf	Winter dormancy – Amg
Bud break - Cf	Catkin growth – Bm
Show leaves – Cf2	Development of male flowers – Cm
Leaf separation – Df	Separation of male flowers – Dm
Dissolving the Leaves – Df2	Dissolving the male flowers – Dm2
Display of female buds – Ef	Separation of the anthers – Em
Paint Show – FF	Beginning of flowering – Fm
Flowering start – Ff1	Mass flowering – Fm2
Mass flowering – Ff2	End of flowering - Gf
End of flowering – Ff3	Fringe Dripping – Hm
Dried licks - Gf	

2. Strength of growth - according to the vegetative growth of the leader and the continuation of the skeletal branches and branches, the cultivars are grouped:

- weak-growing - with an increase of 10-20 cm;

- moderately growing - with an increase of 20-30 cm;

- vigorous-growing - with growth over 31cm.

3. Habitus of the crown - it is established by the shape, density and volume of the crown, as a mandatory sign the angle of the first three skeletal branches relative to the vertical (leader) is taken into account. The volume of the crown is calculated according to the following formula:

$$V = \frac{d^2.\pi.h}{12}$$

V - crown volume in m ^{3};

d -diameter, averaged over the two mutually perpendicular directions, excluding individual protruding branches;

π – 3.14;

h – height of the crown (m), /without the stem/, measured from the level of the first skeletal branch of the lowest branches to the top of the tree (the massed top branches).

The shape of the crown is:

- upright;

- semi-erect;

- splits;

- hung up.

.4. Type of fruiting.

- terminal (apical);

- intermediate;

- lateral.

5. Ripening period - the varieties are grouped:

- with a very early ripening period – 20-25.VIII;

- with an early ripening period - from 26.VIII to 5.IX;

- with medium wound - from 6.IX to 15.IX;

- with mid-late - from 16.IX to 25.IX;

- with a late ripening period - after 26.IX.

6. Fruit yield (kg) – average per tree:

- low (1) – under 15kg;

- medium (2) – from 15.1 to 30kg;

- good (3) – 30.1 to 50kg;

- very good (4) – from 50.1 to 70kg;

- excellent (5) – over 70.1kg.

7. Biometric measurements:

- fruit dimensions - length (height), thickness and width are measured, and the thickness is measured through the flanks of the fruit, and the width through the fruit suture.

- average weight – determined by 30 fruits grouped into the following groups:

very small < 8.5g;

small - from 8.5 to 10, g;

medium - from 10.5 to 12.5g;

large - from 12.5-14.5g and

very large > 14.5g.

8. Shell thickness – defined as:

- thin – up to 1.2mm;

- medium thickness - from 1.3 to 1.7 mm;

- thick – over 1.8mm.

9. Description of the fruits - appearance and yield.

a) Shape - spherical and oblong.

b) Surface - according to the degree of striation, the cultivars are grouped:

- creased, when dimples and furrows are deepened;

- slightly folded, when they are less deep;

- almost smooth when they hardly show.

c) Color - according to the color of the shell, the cultivars are classified as:

-straw yellow (light);

- ash gray;

- brown earth.

d) Main diameter - it is determined whether it is large, medium or small.

e) Color of the kernel skin - it should be:

- light colored;

- light colored with slightly pronounced yellowish shades;

- tiled colored.

f) Percentage of the kernel - after separating the kernel from the shell, its weight is determined. According to it , the cultivars are grouped:

- very low - below 40%;

- low – 40-44%;

- average - from 45 to 49%;

- high - from 50 to 55%;

- very high - over 55%.

10. Resistance to late spring frosts - determined on a percentage basis, counting 100 buds. After counting, they are divided into healthy and damaged flowers. The number of damaged flowers forms the frost percentage from late spring frosts. The calculation is carried out separately for the two types of colors - male and female.

11. Reaction of walnut cultivars to the economically important diseases anthracnose (*Gnomonia leptostyla*) and bacteriosis (*Xantomonas. arboricola* pv. *Juglandis*)

Monitoring was carried out according to the diagonal method, fruits and leaves were visually analyzed or with a magnifying glass for the detection of anthracnose. From each variety, 100 leaves from different parts of the canopy were counted. For each variety, the spread of the disease in the vegetation and the degree of attack are calculated. The observation covers the period 2019-2021. The degree of attack on leaves and fruits was determined according to the scale of Andreevski and Richter (1967) as follows:

0 – no signs of the disease
1 – attacked up to 5% surface
2 – attacked from 6-26% surface
3 – attacked by 26-50% surface
4 – attacked 51-75% surface
5 – attacked more than 75% surface

Cultivar's reaction was determined according to the scale of Andreevski and Richter (1976):

- healthy - without symptoms;

- weakly sensitive - up to 20% attack rate;

- moderately sensitive - from 20-50% and highly sensitive over 50%.

The detection of pathogens was carried out according to standard phytopathological methods (Tafradjiyski et al. 1978, Dimitrov et al. 2000) from infected fruits, shoots, petioles and leaves, from which small pieces of tissue were cut at the border of the diseased and healthy part. They are washed with running water for about half an hour, disinfected in ethyl alcohol and sterile water. Washed tissue pieces were transferred to petri dishes with catophene-dextrose agar (KDA).

The dishes are placed in a thermostat at 23 °C. After the development of the bacterium, it is isolated in pure culture. The pathogenicity of the isolates thus obtained is proven according to Koch's rules (Dimitrov 2000, Tafradjiyski et al. 1978).

The indicators in points 1, 2, 3, 5, 6, 8, 9a, 9b, 9c, 9d and 9e are presented according to the methodology for studying plant resources in fruit plants (Nedev et al., 1979), and in point 1 the number of the groups depending on the time of flowering was increased from three to five, and the variety Izvor 10 was determined as the standard. The indicators in points 4, 7 and 9e are according to the international standard for the description of genetic resources in walnut (Germain, 2004).

III.3.2. Pomological characterization of standard and introduced walnut cultivars

A pomological description of the varieties was prepared based on the summarized data from task IV.3.1. It also complies with the Methodology for the study of plant resources in fruit plants (Nedev et al., 1979) and with the international standard for the description of genetic resources in walnut (Germain, 2004).

IV. Results and discussion

IV.1. Propagation of walnut

IV 1.1 Propagation of walnut by the bench grafting and epicotyl grafting methods in the conditions of a heated tunnel and aqueous nutrient solution.

The results for the average percentage of successfully propagated plants in a heated tunnel with water nutritional solution during the period 2021-2023 are presented in table 2. The obtained percentage of interception - 46.9% in bench - grafted plants (var.I) and 50% in epicotyl-grafted plants (var.II) is proof that walnut propagation is possible in the conditions of a heated tunnel with an water nutritional solution. In the statistical processing of the data, no proven difference was found between the two experimental variants. It is noteworthy that the reported interception rate at var. I and var.II is lower compared to the literature data regarding mass grafting and epicotyl grafting. This shows that not the mode of reproduction, but others factors negatively affected the percentage of successfully propagated plants. The main factors influencing the callus formation of the walnut are: cultivar characteristic, temperature for callus formation and air humidity (Gandev, 2007; 2014, Dzuvinov et al., 2013). Our experiment was conducted with the cultivar - Izvor 10, and the results of other experiments with this variety show similar data (Gandev, 2016, 2017). We also rule out the possibility that temperature had a negative effect on the graft success. The daily temperature measurements made during the study show that at the time of callus formation, it was in the optimal conditions specified by a number of authors - 27°C (± 1°C). In our study, the air humidity in the heated tunnel was between 90 and 95%, i.e. about 10-15% above the optimal

humidity for callus formation indicated in the literature, which is about 80%. We believe that it was the high air humidity in our experiment that negatively affected the interception rate. In 15% of the grafted experimental plants, sporulation was reported on the grafted buds, leading to their death.

Table 2. Average percentage of successfully propagated plants in a heated tunnel with water nutritional solution for the period 2021-2023 year.

Variant	Number of grafted plants	Number of graft taken plants	Percentage of graft take success, %
I. Plants grafted on table and placed for callus forming in heated tunnel with water nutritional solution	32	15 a	46,9 a
II Epicotyl grafted plants placed for callus forming in heated tunnel with water nutritional solution.	32	16 a	50,0 a

During the visual examination of the dead buds, profuse sporulation was found, which covered the entire bud, and necrosis was found on the grafted scion when cut. Two types of sporulation were observed, and the dense mycelium formed was of different color. Parts of the infected buds were placed on potato-dextrose agar, for a more detailed study of the two fungal pathogens.

During laboratory research, characteristic features were found for representatives of the genus "*Penicillium*" Link and the genus "*Fusarium*" Link.

IV 2. Container growing of grafted plants

IV.2.1. Vegetative behaviour of container-grown walnut plants grafted with the "cleft" and "whip- tongue" grafting techniques

The data on the vegetative behavior of walnut plants grafted with different techniques are presented in table 3. Regarding the plant height indicator, it was found that the plants from var. I (control) have higher values compared to those of var. II and var. III, the differences being statistically proven. The average height of the plants at var. I is 121.2cm vs. 95.55cm at var. II and 89.81cm at var. III. No proven differences between var. II and var. III in the average height of the plants. This trend is also observed when examining the cross-section indicator of the stem, for which the highest average value was also recorded in the control variant - 361.6mm^2 for the period of the study compared to var.II - 162.97mm^2 and var.III - 136, 34mm^2.

According to the statistical analysis, between the varieties grafted on "cleft" (var. II) and "whip-tongue" (var. III), there was no proven difference in plant height during the study period. For the plants grafted on a "cleft" (var. II), the following values were recorded during the individual experimental years: 80.1 cm in 2019, 95.54 cm in 2020. and 111cm in 2021. Plants grafted with the "whip-tongue" technique (var. III) reached a height of 76.2cm in 2019; 89.82cm in 2020 and 103.4cm in 2021. In 2019 and 2020, no statistically proven differences were found between the plants of variants II and III in the cross-section of the stem. Plants grafted on a "cleft" have a stem cross-section of 103.8mm^2 in 2019, 163.3mm^2 in 2020. and 221.8mm^2 in 2021. Plants grafted with the "whip-tongue" technique in 2021 have the lowest value for this indicator – 183.5mm^2, and the difference is statistically proven.

Table 3. Vegetative behavior of grafted walnut plants during the period 2019 – 2021 year.

Variant	Height (cm)				Cross section of the stem (mm^2)				Root system volume (cm^3)			
	2019	2020	2021	средно	2019	2020	2021	средно	2019	2020	2021	средно
I.Plants grafted on patch budding (control)	122 a	121,22 a	120,4 a	121,2 a	349,9 a	355,4 a	379,7 a	361,6 a	166 b	219,6 b	273 a	219,53 b
II.Plants grafted by cleft grafting technique	80,1 b	95,54 b	111 b	95,55 b	103,8 b	163,3 b	221,8 b	162,97 b	286 a	294,4 a	302,8 a	294,4 a
III.Plants grafted by whip tongue technique“	76,2 b	89,82 b	103,4 b	89,81b	88,2 b	137,34 b	183,5 c	136,34 b	283 a	292,4 a	302 a	292,42 a

A significant increase in the root system volume was found in plants grown in containers. In control variant I, the lowest values for this indicator were recorded - 166 cm^3 in 2019. and 219.6cm^3 in 2020. In 2021 in plants grafted on a bud, a volume of the root system of 273cm^3 was reported and no statistically proven differences were found between the experimental variants. During the study period, there was no statistically proven difference between plants grafted on "cleft" (var. II) and "whip - tongue" (var. III) for this indicator. Those of var. II has a root system volume: 286cm^3 in 2019; 294.4cm^3 in 2020 and 302.8cm^3 in 2021. For plants from var. III, the reported values are respectively: 283cm^3 for 2019; 292.4cm^3 for 2020; 302cm^3 for 2021.

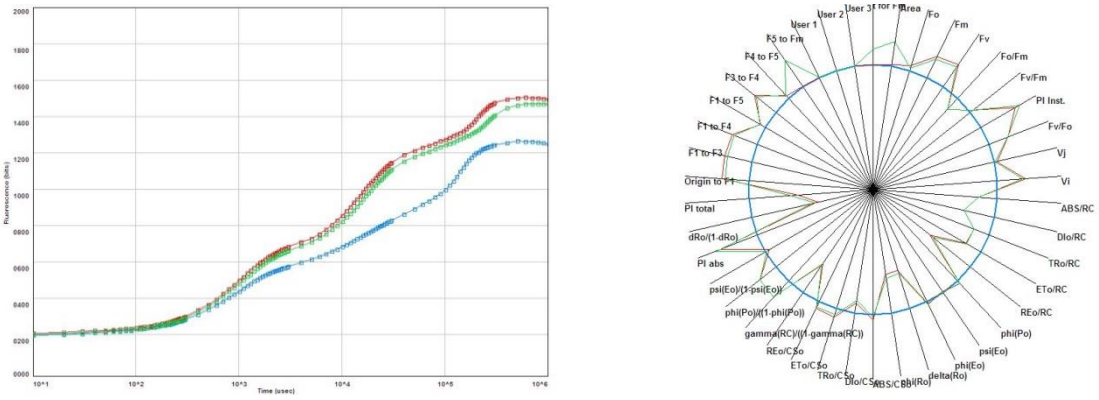

Fig. 1. Induction curves of fast chlorophyll fluorescence (OJIP test) (left) and radar diagram of parameters (right) of walnut plants grafted in 2020 by different methods: "patch budding"; "whip-tongue"; "cleft".

The analysis of fast chlorophyll fluorescence, in all three observed variants, shows that the walnut plants analyzed are photosynthetically active (Yusuf et al., 2010). The minimum (F0) fluorescence of the control plants (grafted on "patch budding") and those grafted on "whip-tongue" and on "cleft" did not differ significantly (Table 5). The maximum (FM) and variable fluorescence of plants from the control variant was lower, compared to that of the two tested variants. This shows that in plants from all three methods of grafting, a normally developed photosynthetic apparatus functions.

Indeed, the most intense growth in height, the largest number of leaves, as well as the highest fresh and dry mass of the leaves was reported in the control (Table 4). Statistically proven differences were found between plants grown in containers and on open field and in relation to other biometrics. For the control variant, higher values were reported for thickness, number of leaves, fresh and dry mass of the root system and the stem. In the walnut planting material grown in containers, higher values were found for the chlorophyll content compared to the control variant.

Table 4. Biometric indicators of grafted walnut plants

Indicators	Plants grafted on patch budding (control)	Plants grafted by „cleft“ grafting technique	Plants grafted by “whip-tongue“ grafting technique
Height, cm	121,22 a	95,54 b	89,82 b
Width, mm	21,26 a	14,40 b	13,2 b
Root system volume, cm^3	219,6 b	294,4 a	292,4 a
Number of leaves	23,8 a	21 ab	20,4 b
Fresh weight of leaves, g	224,82 a	126,94 b	115,56 b
Dry weight of leaves, g	75,96 a	43,62 b	39,68 b
Leaves area, mm^2	655510,9 b	789903,6 a	603991,1 b
Fresh weight of stem, g	222,14 a	112,24 b	112,26 b
Dry weight of stem, g	118,5 a	64,52 b	64,42 b
Fresh weight of root, g	388,24 a	368,28 a	278,44 b
Fresh weight of root, g	167,76 a	156,6 a	113,68 b
Chlorophyll, rel. units	9,98b	15,92 a	14,9 a

IV.3. Varietal study

IV.3.1. Study of the biological and economic qualities of introduced walnut varieties

IV.3.1.1 Flowering period of male and female flowers

The beginning of vegetation and flowering time in common walnut (Juglans regia L.) depend on the genotype of the cultivar and the climatic conditions under which it is grown (Germain et al., 1999). The results are presented in the table. 5 show that on average for the period 2018 – 2020, at the Izvor 10 standard, the phenophase beginning of apical bud bursting (Cf) occurs on March 26. It was established that, of the rest of the studied cultivar, Sebin's vegetation begins the earliest - four days before Izvor 10, followed by Valmit and Yalova 1. The Valeris cultivar begins its development at the latest, with its Cf phenophase occurring three days after the Izvor 10 standard.

Table 5. Average phenological data of walnut cultivars for the period 2018 - 2020 year.

Cultivar	Beginning of Bud burst	*	Flowering of female flowers						Flowering of male flowers						Date of ripening	
			Beginning of flowering	*	Mass flowering	*	End of flowering	*	Beginning of flowering	*	Mass flowering	*	End of flowering	*	Date of ripening	*
Izvor 10	26.03.	0	15.04.	0	21.04.	0	27.04.	0	17.04.	0	22.04.	0	24.04.	0	13.09	0
Silistrenski	27.03.	+1	17.04.	+2	24.04.	+3	01.05.	+4	13.04.	-4	20.04.	-2	23.04.	-1	14.09	+1
Yalova 1	24.03.	-2	18.04.	3	24.04.	+3	30.04.	+3	11.04.	-6	18.04.	-4	18.04.	-6	22.09	+9
Sebin	22.03.	-4	13.04.	-2	20.04.	-1	27.04.	0	9.04.	-8	14.04.	-6	19.04.	-5	18.09	+5
Valmit	23.03.	-3	13.04.	-2	20.04.	-1	26.04.	-1	14.04.	-3	20.04.	-2	26.04.	+2	27.09	+14
Valeris	29.03.	+3	20.04.	+5	26.04.	+5	04.05.	+7	21.04.	+4	28.04.	+6	3.05.	+9	29.09	+16

* Number of days according to Izvor 10

On average for the period, the mass flowering of female flowers (phenophase Ff2) in Sebin and Valmit cultivars occurs only one day before that of Izvor 10. It is found that in Silistrenski and Yalova 1 cultivars the same phenophase occurs three days after the Izvor 10 standard. - late mass flowering of the female flowers was reported in the Valeris cultivar - 5 days after the standard.

The mass flowering of male flowers (catkins) - phenophase Fm2 also occurs differently in individual cultivars (Table 5). It begins the earliest in the Sebin cultivar - six days before the Izvor 10 standard. In the Silistrenski and Valmit cultivars, the flowering of male flowers occurs two days before Izvor 10, and in the Yalova 1 cultivar four days before the standard. With the latest development of the fronds, the Valeris cultivar stands out again - 6 days after the Izvor 10 standard.

According to the occurrence of the mass flowering of male and female flowers in the studied cultivars compared to the control Izvor 10, they are arranged in the following groups:

Flowering of female flowers:
- Early flowering - Shebin and Valmit;
- Late flowering - Silistrenski and Yalova 1;
-Very late flowering- Valeris;

Flowering of male flowers:
- Many early flowering - Silistrenski, Yalova 1, Shebin;
- Early flowering – Valmit;
- Very late flowering – Valeris;

IV 3.1.2. Growth vigor and crown habit

In the table 6, the growth strength and the habit of the crowns of the studied cultivars are presented. During 2018-2020, all cultivars show themselves as strong-growing - with a length of one year's growth of the leader's long stems and skeletal branches over 31cm. This is due to the fact that the trees are in a period of initial and rapidly increasing fruiting. This period of walnut tree development is characterized by vigorous growth that has not yet been reduced by the influence of increased fruiting. The winter pruning to form the crowns has further stimulated the annual growth events of the experienced trees.

It was found that the Silistrenski, Yalova 1 and Valmit cultivars have an upright crown shape. In them, the angle of deviation of the skeletal branches is between 55° and 65°. Izvor 10, Sebin and Valeris have a semi-erect form - with an angle of growth of the skeletal branches between 35° and 45°.

From the results presented in the table. 6 shows that the Yalova 1 cultivar has the largest cross-section of the stem - 271.4cm^2. This variety also has the largest volume of the crown - 34.0m^3. Regarding these two indicators, there is a statistically proven difference between the Yalova 1 cultivar and the other cultivars. With them, the reported values for crown volume and cross-section are, respectively, Izvor 10 - 15.9m^3, 136.6mm^2; Silistrenski - 12.2m^3, 154.8mm^2; Sebin – 14.4m^3, 153.2mm^2; Valmit – 12.7m^3, 103.9mm^2 and Valeris – 9.9m^3, 99.2mm^2.

Table 6. Average data for growth vigor and crown habit for the period 2018-2020 year

Cultivar	**Growth vigor**	**Crown habit**			
		Shape	**Angle of growth of skeletal branches**	**Cross-section of the stem (cm^2)**	**Crown volume (m^3)**
Izvor 10	Vigorous	Semi - erect	35 - 45°	136,6 b	15,9 b
Silistrenski	Vigorous	upright	55 - 65°	154,8 b	12,2 b
Yalova 1	Vigorous	Upright	55 - 65°	271,4 a	34,0 a
Sebin	Vigorous	Semi - erect	35 - 45°	153,2 b	14,4 b
Valmit	Vigorous	Upright	55 - 65°	103,9 b	12,7 b
Valeris	vigorous	Semi – erect	35 - 45°	99,2 b	9,9 b

Ripening period

Regarding the ripening period of the cultivars studied, the obtained results confirm the research of Nedev et al. (1983) on the ripening period of the local cultivars Izvor 10 and Silistrenski. It was found that the two cultivars have an early ripening period (6-15. IX). All other studied varieties ripen later than the Izvor 10 standard. Yalova 1 and Sebin cultivars have a medium-late ripening period (16-25. IX), and Valmit and Valeris cultivars have a late ripening period (after 26. IX).

Due to the first fruiting of the studied cultivars and the damage from late spring frosts described below, results regarding the yield and type of bearing cannot be discussed at this stage of the research.

IV.3.1.3. Biometric fruit measurements and percentage of kernel

The results presented in the table. 7 show that the fruits' height, width and thickness are different in individual cultivars, which also determines their different shape (presented in section IV.3. Cultivar’s study). The highest values for the first two indicators were reported for the variety Yalova 1, 45.7mm height and 35.4 mm width of the fruit, respectively. The lowest value for fruit height was found at Sebin - 36mm. Regarding the width of the fruits, the lowest values were reported for the varieties Sebin - 33.1mm and Izvor 10 - 31mm. The thickness of the fruit has the highest value in the Romanian Valmit variety - 39.8mm, and the lowest in the Sebin variety - 31.5mm.

Table 7. Average biometrical measurements of the fruits for the period 2018-2020 year

Cultivar	Nut height (mm)	Nut width (mm)	Nut thickness (mm)	Nut weight (g)	Kernel weight (g)	Percentage of kernel (%)	Shell thickness (mm)
Izvor 10	40,0 bc	31,0 b	31,9 cd	11,3 c	6,2 b	54,8 a	1,4 c
Silistrenski	38,9 c	35,0 a	34,9 c	13,5 b	6,1 b	45 b	1,6 bc
Yalova 1	45,7 a	35,4 a	36,2 b	16,8 a	6,8 a	40,3 c	2,0 a
Sebin	36,0 c	33,1 b	31,5 d	9,6 d	5,2 c	53,7 a	1,4 c
Valmit	39,8 bc	34,5 a	39,8 a	14,8 b	6,4 ab	43,0 bc	2,1a
Valeris	41,5 b	35,3 a	37,9 b	16,1 ab	6,0 b	35,1 d	2,2a

The statistically proven difference was found in the fruit and nut weight between Yalova 1 and the other varieties observed in the study (Table 7). The value of 16.8 g. weight of one fruit confirms the claims from foreign sources (Akca and Ozongun 2004) regarding the size of the fruits in this cultivar. The Valeris cultivar is also distinguished by very large fruits with an average weight of one fruit - 16.1g. The lowest value of this indicator was found for the Sebin cultivar, which has small fruits - of 9.6g. Izvor 10 is characterized by an average fruit size of 11.3g. Silistrenski cultivar has large fruits - 13.5g. The weight of 14.8g places the Romanian cultivars Valmit in the group of cultivars with very large fruits.

The highest percentage of kernel was found in the cultivar Izvor 10 – 54.8%, which confirms the research of Nedev et al., (2002). The Turkish cultivar Sebin also has a high percentage of kernel - 53.7%, corresponding to external literature reports (Keles et al., 2014). The lowest kernel percentage was reported for the Valeris cultivar, 35.1%. For the other cultivars, the kernel percentage is as follows: Silistrenski - 45%, Yalova 1 - 40.3% and Valmit - 43%.

Regarding the weight of the nut, the highest value - 6.8g - was reported for Yalova 1, and the difference with the other cultivars is statistically proven. The nut has the lowest weight for the Sebin cultivar - 5.2g. The weight of the nut was determined for the remaining 4 cultivars, respectively: Valeris – 6.4g, Valmit – 6.4g, Izvor 10 – 6.2g and Silistrenski – 6.1g.

The average shell thickness of the control cultivar Izvor 10 was found to be 1.4 mm. The same values were reported for the Sebin cultivar. At Silistrenski, the shell is 1.6mm thick. For Yalova1 - the thickness of the shell is 2mm, for Valmit - 2.1mm and for Valeris - 2.2mm.

IV.3.1.4. Description of fruits - shape and appearance

When considering the pomological characteristics of the fruits presented in table. 8 cultivars Izvor 10, Silistrenski and Yalova 1 are characterized by an oblong shape, slightly wrinkled surface and gray-ash color of the shell, medium-sized main opening. Sebin, Valmit and Valeris have a globular shape and a large main opening, which can be a prerequisite for an attack by fruit worms. The fruits of the Sebin variety have a wrinkled surface and a brown color of the shell. Valmit and Valeris are characterized by their slightly folded surface and shell ash-grey colour. It is noteworthy that the fruits of all studied cultivars have a light kernel.

Table 10. Organoleptic qualities of walnut varieties

Cultivar	Fruit shape	Shell surface	Shell color	Main opening	Kernel color
Izvor 10	Oblong	Slightly wrinkled	Grey - ash	Medium	Light
Silistrenski	Oblong	Slightly wrinkled	Grey - ash	Medium	Light
Yalova 1	Oblong	Slightly wrinkled	Grey – ash	Medium	Light
Sebin	Globular	wrinkled	Brown	Large	Light
Valmit	Globular	Slightly wrinkled	Grey – ash	Large	Light
Valeris	Globular	Slightly wrinkled	Grey – ash	Large	Light

IV.3.1.5. Resistance to late spring frosts

At the beginning of the spring of 2019, for the period from March 29 to April 5, a sequence of 6 days with minimum air temperatures below 0°C was recorded. The reported negative values ranged from minus 5°C on March 29th to minus 1.6°C on April 4th (fig. 2).

During this period, the cultivars studied were in different stages of formation and development of male and female flowers. The female flowers of the cultivars Izvor 10, Sebin and Valmit were in the leaf dissolution phase (Df2), in Yalova 1 - in the leaf display phase (Cf2), and in Silistrenski Valeris - in the leaf separation phase (Df). The male flowers (collected in a catkin inflorescence) in the varieties Izvor 10 and Yalova 1 were in the growth phase (Bm), those in Silistrenski, Sebin and Valmit - in the division phase (Dm), and in the Valeris cultivar the catkins were in winter dormancy (Amg)

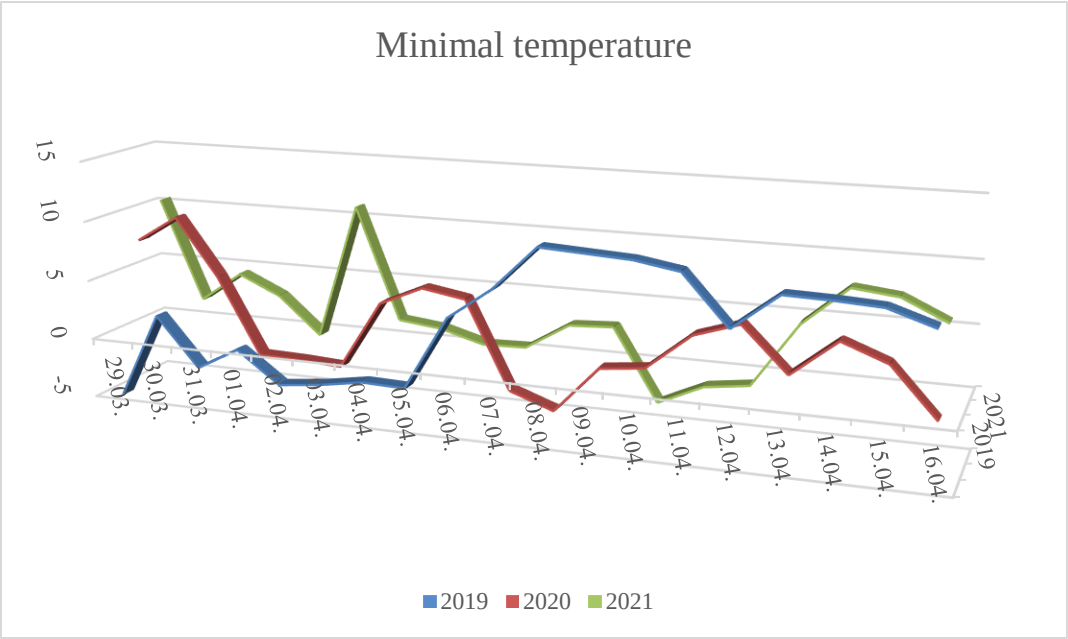

Fig. 2. Minimum temperatures during flower development phenophase of walnut for the period 2019 – 2021 year.

In 2020, with the onset of spring, a prerequisite for damages caused by spring frosts was again created. Again, at the beginning of the growing season, a series of days with negative temperatures were reported, which varied from minus 1°C to minus 3.5°C. Despite the differences in values, compared to the previous year, this time the period of unfavorable days was longer - lasting from April 1st to April 16th (Fig. 2). During this time period, the cultivars studied were in different phenophases. The female flowers of the cultivars Izvor 10, Silistrenski, Yalova 1 and Valmit were in the bud break phase (Cf), in the Sebin cultivar - in the leaf display phase (Cf2), and in the Valeris cultivar the female buds were at rest (Af). The male flowers of the cultivars Izvor 10, Silistrenski and Yalova 1 were in the growth phase (Bm), those of the Sebin and Valmit varieties - in the division phase (Dm), and the catkins of the Valeris cultivar were in winter dormancy (Amg).

During the phenological observations in the spring of 2021 at the end of March and in the first half of April, days with an air temperature below 0 0 C were recorded, with the lowest value recorded on April 10 - (-3.1oC) (fig . 2). Female and male flowers were in different phenophases during the turning spring frosts. The female buds are in the bursting phase for the cultivars Izvor 10, Silistrenski, Yalova 1 and Valeris (Cf). The catkins of Valeris were in the dormancy phase (Amg), and those of Silistrenski, Izvor 10 and Yalova 1- in the growth phase (Bm). The cultivars Shebin and Valmit were at the stage of leaf separation (Df) in female flowers and the development of male flowers (Cm).

The ones presented in the table. 9 results, show that in 2019, in terms of female flowers, the most affected of all cultivars is Valmit with 93% damage. The other Romanian cultivar, Valeris, was also heavily affected - 78%. The lowest damage result was reported for Yalova 1 cultivar - 53%. The lowest percentage of damage to the catkins was reported for the cultivar Izvor 10 - 46%,

and the highest - for the cultivar Valmit - 90%. In the Sebin cultivar, large damages were also observed - 88%. The Valeris cultivar excelled without damage to the catkins from the spring frosts.

Table. 9. Late spring frosts damage (%)

Cultivar	2019		2020		2021	
	Female flowers (%)	Male flowers (catkins) (%)	Female flowers (%)	Male flowers (catkins) (%)	Female flowers (%)	Male flowers (catkins) (%)
Izvor 10	69 c	46 d	43 a	42 d	42 b	70 b
Silistrenski	57 d	54 c	32 b	70 c	40 b	75 ab
Yalova 1	53 d	67 b	37 b	86 a	30 c	73 ab
Sebin	74 bc	88 a	37 b	80 ab	70 a	70 b
Valmit	93 a	90 a	45 a	74 bc	40 b	80 a
Valeris	78 b	0 e	0 c	0 e	10d	10 c

IV.3.1.6. Reaction of walnut cultivars to the economically important diseases anthracnose (*Gnomonia leptostyla*) and bacteriosis (*Xantomonas. arboricola pv. Juglandis*)

The statistical processing of the results indicated in table 10 shows a variation of the values in the individual cultivars for the period of the study. In 2019, for the anthracnose attack, the highest attack index was reported for the Romanian cultivar Valmit - 22.49. The lowest attack index value for the same year was observed in the other Romanian cultivar Valeris – 11.84. In 2020, the highest degree of anthracnose attack was found in the Silistrenski cultivar - 16.04, and the lowest again in the Romanian Valeris cultivar - 3. For 2021, no statistically proven differences were found between the studied cultivars.

Table 10. Reaction of walnut cultivars to economically important diseases

Cultivar	Index of attack on leaves from *Gnomonia leptostyla*		
	2019	**2020**	**2021**
Yalova 1	17,02 abc	9,3 ab	18,19 a
Silistrenski	13,83 c	16,04 a	13,7 a
Valmit	22,49 a	6,7 ab	28,1 a
Izvor 10	21,51 ab	5,31 ab	26,9 a
Sebin	16,28 bc	6,96 ab	28,5 a
Valeris	11,84 c	3 b	25,18 a

In the processing of the data on the attack on the leaves by bacteriosis in 2019, no statistically proven differences were found between the studied Cultivars. In 2020, the Silistrenski cultivar had the highest value compared to the other cultivars - 8.95. The lowest attack rate for the same year was the other local cultivar Izvor 10 – 3.42. For the year 2021, no statistically proven differences were found between the observed Cultivars (Table 11).

Cultivar	Index of attack on leaves from *Xanthomonas arboricola pv. Juglandis*		
	2019	**2020**	**2021**
Yalova 1	16,18 a	5,41 bc	17,42 a
Silistrenski	19,87 a	8,95 a	10,50 a
Valmit	15,76 a	4,56 bc	22,6 a
Izvor 10	23,08 a	3,42 c	25,1 a
Sebin	17,38 a	6,19 b	16,8 a
Valeris	17,27 a	5,65 bc	15,7 a

IV.3.2. Pomological characteristics of the studied walnut varieties

Izvor 10

The cultivar originates from a seed found in the village of Izvor, District- Plovdiv. In 1979, it was adopted as a standard cultivar for the country (Nedev et al. 1983).

The fruits are medium-sized with a weight of 11.3g and an oblong shape. The average height of the fruit is 40mm, the width – 31mm, and the thickness – 31.9mm. The shell is 1.4mm thick, with a gray-ash color and a slightly curved surface. The main hole is medium-sized. The nut is light colored with an average weight of 6.2g and easily separated from the shell. The percentage of kernel is high - 54.8%. When grown in the conditions of southern Bulgaria, it is characterized by an early ripening period - in the first half of September.

The trees show strong growth with a length of the annual growth over 30cm. The shape of the crown is semi-erect, with an angle of deviation of the skeletal branches within 35-45^{o}, and the volume at the end of the seventh vegetation is 15.9m^{3}. The flowering is protogynous, with some overlap with that of the fringes. The cultivar is sensitive to Anthracnose (*Gnomonia leptostyla*) attack and moderately susceptible to bacteriosis (*Xanthomonas arboricola pv. Juglandis*).

Silistrenski

The cultivar originates from a seed found in the area of the village of Kalipetrovo, District Silistra. In 1967, it was adopted as a standard cultivar for the country (Nedev et al., 1983).

The fruits are large, weighing 13.5g and oblong in shape. The average height of the fruit is 38.9mm, width – 35mm, and thickness – 34.9mm. The shell is 1.6 mm thick, with a gray-ash color and a slightly curved surface. The main hole is medium-sized. The nut is light colored with an average weight of 6.1g and easily separated from the shell. The percentage of kernel is on average high - 45%. When grown in the conditions of southern Bulgaria, it is characterized by an early ripening period - in the first half of September.

The trees show strong growth with a length of the annual growth over 30cm. The shape of the crown is semi-erect, with an angle of deviation of the skeletal branches within 55-65^{o}, and the volume at the end of the seventh vegetation is 12.2m^{3}. Flowering is protandrous. The cultivar is susceptible to Anthracnose (*Gnomonia leptostyla*) attack and bacteriosis (*Xanthomonas arboricola pv. Juglandis*).

Yalova 1

The cultivar originates from local forms in the province of Yalova and is widespread in Turkey.

The fruits are very large, weighing 16.8g and oblong in shape. The average fruit height is 45.7mm, width – 35.4mm, and thickness – 36.2mm. The shell is 2mm thick, with a gray-ash color and a slightly wrinkled surface. The main hole is medium-sized. The nut is light colored with an average weight of 6.8g and easily separated from the shell. The percentage of kernel is low - 40.3%.

When grown in the conditions of southern Bulgaria, it is characterized by a mid-late ripening period - in the second half of September.

The trees show strong growth with a length of the annual growth over 30cm. The shape of the crown is upright, with an angle of deviation of the skeletal branches within 55-65^{o}, and the volume at the end of the seventh vegetation is 34m3. Flowering is protandrous. The cultivar is slightly sensitive to Anthracnose (*Gnomonia leptostyla*) attack and slightly sensitive to bacteriosis (*Xanthomonas arboricola pv. Juglandis*).

Sebin

Turkish cultivar, originating from Eastern Anatolia, distributed throughout the territory of Turkey.

The fruits are small, weighing 9.6g and spherical in shape. The average fruit height is 36mm, width – 33.1mm, and thickness – 31.5mm. The shell is 1.4mm thick, with a brown color and a wrinkled surface. The main hole is big. The nut is light colored with an average weight of 5.2g and easily separated from the shell. The percentage of kernel is high - 53.7%. When grown in the conditions of southern Bulgaria, it is characterized by a mid-late ripening period - in the second half of September.

The trees show strong growth with a length of the annual growth over 30cm. The shape of the crown is semi-erect, with an angle of deviation of the skeletal branches within 35-45^{o}, and the volume at the end of the seventh vegetation is 14.4m^{3}. Flowering is protandrous. The cultivar is slightly sensitive to Anthracnose (*Gnomonia leptostyla*) attack and slightly sensitive to bacteriosis (*Xanthomonas arboricola pv. Juglandis*).

Valmit

The cultivar originates from local forms in the region of Valcea, Romania.

The fruits are very large, weighing 14.8g and spherical in shape. The average height of the fruit is 39.8mm, width – 34.5mm, and thickness – 39.8mm. The shell is 2.1mm thick, with a gray-ash color and a slightly curved surface. The main hole is big. The nut is light colored with an average weight of 6.4g. and easily separated from the shell. The percentage of kernel is low - 43%. When grown in the conditions of southern Bulgaria, it is characterized by a late ripening period - after September 26.

The trees show strong growth with a length of the annual growth over 30cm. The shape of the crown is upright, with an angle of deviation of the skeletal branches within 55-65^{o}, and the volume at the end of the seventh vegetation is 12.7m^{3}. Flowering is protandrous. The cultivar is slightly sensitive to Anthracnose (*Gnomonia leptostyla*) attack and slightly sensitive to bacteriosis (*Xanthomonas arboricola pv. Juglandis*).

Valeris

Romanian cultivar.

The fruits are very large, weighing 16.1g and spherical in shape. The average height of the fruit is 41.5mm, width – 35.3mm, and thickness – 37.9mm. The shell is 2.2 mm thick, with a gray-ash color and a slightly curved surface. The main opening is big. The nut is light colored with an average weight of 6 g and is easily separated from the shell. The percentage of kernel is very low - 35.1%. When grown in the conditions of southern Bulgaria, it is characterized by a late ripening period - after September 26.

The trees show strong growth with a length of the annual growth over 30cm. The shape of the crown is semi-erect with a deviation angle of the skeletal branches within 35-45^{o}, and the volume at the end of the seventh vegetation is 9.9m^{3}. Flowering is protandrous. The variety is slightly sensitive to Anthracnose (*Gnomonia leptostyla*) attack and slightly sensitive to bacteriosis (*Xanthomonas arboricola pv. Juglandis*).

V. Conclusions

V.1. Conclusions from the propagation section

1. The use of a heated tunnel with water nutritional solution is a suitable method for propagating the walnut during its winter dormancy.

2. When applying the heated tunnel method with water nutritional solution, the graft take percentage with the epicotyl grafting technique is 50.0%, and with bench grafting 46.9%.

3. Maintaining an air humidity of 90-95%, during the callus formation of the walnut in the conditions of a heated tunnel with water nutritional solution, is a prerequisite for the emergence and reproduction of fungal pathogens, which negatively affect the interception rate.

V.2 Conclusions from section container growing of grafted plants

1. It is possible to produce winter dormancy-propagated walnut planting material in containers by the "cleft" and "whip-tongue" grafting techniques.

2. Plants grafted on a bud in a nursery have a greater stem height and cross-section than those grafted on a scion during the winter dormancy and grown in containers.

3. The volume of the root system of plants grown in containers is greater compared to those grown in a nursery.

4. The grafting of "cleft" and "whip - tongue" does not lead to differences in the vegetative behavior of plants grown in containers.

5. A normally developed photosynthetic apparatus functions in the plants grafted during the winter dormancy of "cleft" and "whip-tongue", grown in containers. No differences in chlorophyll fluorescence parameters were reported between bud-grafted and container-grown plants.

V.3 Conclusions from the cultivar study section

1. In relation to the flowering of female flowers, the cultivars Sebin and Valmit are early flowering, Silistrenski and Yalova 1 are late flowering, and Valeris is very late flowering.

2. Regarding the flowering of the catkins, Silistrenski, Yalova 1 and Sebin are very early flowering. Valmit is an early flowering and Valeris is a very late flowering.

3. The cultivars Izvor 10, Sebin and Valeris have a semi-erect shape of the crown, and Silistrenski, Yalova 1 and Valmit - with an upright shape.

4. Yalova 1 has the largest crown volume of the studied cultivars, which reached a value of 34 m 3 during the study period.

5. Based on the fruit weight indicator, the cultivars Yalova 1, Valmit and Valeris have very large fruits, Silistrenski - large, Izvor 10 - medium, and Sebin - small.

6. The cultivars Izvor 10 and Sebin have a high percentage of kernel, Silistrenski have a medium, Yalova 1 and Valmit have a low, and Valeris have a very low percentage of kernel.

7. The Turkish cultivar Sebin and the Romanian Valmit are the most sensitive to late spring frosts.

8. Yalova 1, Sebin, Valmit and Valeris cultivars are weakly sensitive to Bacteriosis (*Xanthomonas arboricola pv. Juglandis*) and Anthracnose (*Gnomonia leptostyla*), while Izvor 10 and Silistrensky are sensitive.

9. The cultivars Shebin and Valmit are sensitive to cultivation in the conditions of southern Bulgaria, due to their early flowering, leading to spring frosts.

10. The Romanian cultivar Valeris has valuable economic qualities - late flowering, large fruits and low sensitivity to Bacteriosis (*Xanthomonas arboricola pv. Juglandis*) and Anthracnose (*Gnomonia leptostyla*) when grown in the conditions of southern Bulgaria.

VI. Contributions

VI. 1. Scientific contributions

VI.1.1 Contributions of an original nature

1. A new method of propagating the walnut, called propagating in a heated tunnel with water nutritional solution, has been developed during the winter dormancy.

2. It was established that the increase of humidity above 90% during the callus formation of the walnut leads to the development of phytopathogens of the genus Penicillium Link and the genus Fusarium Link and to the death of a part of the grafted plants.

3. The formation of better-developed overgrown (fibrous) roots was established in plants grown in containers compared to those grown in a nursery.

4. The agrobiological characteristics of the introduced Turkish cultivars Yalova 1 and Sebin and the Romanian varieties Valmit and Valeris were studied in the conditions of the Plovdiv region, Southern Bulgaria.

5. A pomological description of the introduced cultivars Yalova 1, Sebin, Valmit and Valeris was made.

6. A high sensitivity of the Sebin and Valmit cultivars to late spring frosts, when grown in the conditions of southern Bulgaria, was found.

VI. Scientific and applied contributions

VI.2.1. Contributions of a confirmatory nature

7. The agrobiological characteristics of the cultivars Izvor 10 and Silistrenski when grown in the conditions of southern Bulgaria have been confirmed.

8. It has been confirmed that the degree of late spring frost damage in walnut depends on both temperature values and the stage of flower development.

VI.2.2. Contributions to practice

9. The possibility of applying the walnut propagation method in practice during the winter dormancy in a heated tunnel with water nutritional solution was studied.

10. The possibility of producing walnut planting material grown in containers has been established.

Publications related to the dissertation

1. Dimitrov, A., V.Akova, V.Nikolova . 2021.” Pomological characteristic of local and introduced walnut cultivars in South Bulgaria”. Fruit Growing Research, Vol. XXXVII pp 14-18.
   https://publications.icdp.ro/publicatii/lucrari%202021/02.Angel%20Dimitrov.pdf
2. Dimitrov, A., V. Akova 2021."Late spring frost damage to local and introduced walnut cultivars", Journal of Mountain Agriculture on the Balkans, vol.24, (1), 209-217
   https://jmabonline.com/en/article/jFsW5vZ2KAKweFLj4PTQ
3. Dimitrov, A., Y. Yonchev, V. Akova & S. Gandev, 2023. „Propagation of Walnut in Conditions of Heated Tunnel with Water Nutritional Solution”**,** Journal of mountain agriculture on the Balkans, vol. 26, (1), pp. 347-359.
   https://jmabonline.com/en/article/YA9YHe68Jw3ixkWzENA4

SUMMARY

This dissertation aims to gain new knowledge and establish effective methods and practices leading to sustainable development of walnut production in propagation, growing grafted plants in containers and varietal research.

The experiments were conducted at the Fruit Growing Institute - Plovdiv during the period 2018-2023 year. The cultivars Izvor 10, Silistrenski, Yalova, Shebin, Valmit, Valeris were observed.

As a result of the study, it was found that it is possible to propagate walnut in the conditions of a heated tunnel with water nutritional solution. The possibility of producing walnut planting material grown in containers has been confirmed. The introduced varieties from Turkey - Yalova 1 and Shebin and Romania - Valmit and Valeris were studied, and their agrobiological and economic qualities were evaluated.