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EVALUATION OF THE ROLE OF CALLUSING MEDIA AND PARAFFIN WAX IN SUCCESSFUL GRAPEVINE PROPAGATION

THESIS OF DOCTORAL (Ph. D) DISSERTATION

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1 SHORT INTRODUCTION AND OBJECTIVES

Grohs et al. (2017) review, divides the 130-year history of grapevine grafting into three parts. Until 1950, the focus was on the exploration of rootstock varieties and rootstock effects, after which until 2000 the emphasis was on the development and dissemination of grafting practices. Finally, since 2000, the diagnosis and management of pathogens that spread by nurseries have come to the fore. In international and national publication activities, the focus has shifted to phytosanitary topics (Gramaje et al., 2017). Waite et al. (2014) in Guideline for Grapevine Propagation shows step-by-step methodology to produce high quality propagating material. Further clarification and development research in grape propagation technology is necessary.

Objectives

In order to increase the efficiency of grafting production, one of the basic and critical technological processes is callusing, which results in the formation of the wound tissue, the callus, creating fusion and coexistence. The two basic pillars of callusing are the forcing media and the use of paraffin. Although both the use of paraffin and the callusing media have a significant history in graft production practice, results supported by up-to-date experiments are not available. We aimed to examine and observe these two crucial aspects in order to document and transfer into the propagation practice.

- Based on comparative studies, we sought answers to certain technological steps widespread in modern propagation practice in the early 1980s, as well as if this technology is suitable for cultivating a wide range of scion-rootstock combinations.

- In our studies, we aimed to make a comparative analysis of the callusing media of grape grafts for three consecutive years, specifically based on the graft yield results and considering durability of the technologies.

- We aimed to examine the justified usage of paraffin, a technological step that has detrimental effect on the environment in cultivation technology, which has a significant input material in the sector.
2 MATERIAL AND METHODS

In all experimental year, the Kun Szőlő Nursery and Table Grape Plantation, our own estate, was used to carry out the experiments. During performing of the experiments, we strictly followed to uniform and officially accepted methodologies in producing grapevine grafting.

2.1 Callusing media experiment

In the three-year (2013-2015) series of experiments, in total 15 varieties (13 wine grapes and 2 table grapes) and 4 rootstocks were set up in 11-11 scion-rootstock combinations serving real customer needs (Table 1.). The variety names are abbreviated below for easier of understanding. The analysis of the varieties is always meant for the studied scion-rootstock combination.

<table>
<thead>
<tr>
<th>Variety</th>
<th>T. 5C</th>
<th>T-K 125 AA</th>
<th>T-F. S.O.4</th>
<th>Börner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabernet sauvignon</td>
<td>2013-2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cabernet franc</td>
<td>2013-2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Csanád</td>
<td>2013-2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Castellum</td>
<td>2013-2015</td>
<td>2013-2015</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Kadarka</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2013-2015</td>
</tr>
<tr>
<td>Italia</td>
<td>-</td>
<td>-</td>
<td>2013-2015</td>
<td>-</td>
</tr>
<tr>
<td>Hibernal</td>
<td>2013 és 2015</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Borsmenta</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2013 and 2015</td>
</tr>
<tr>
<td>Andor szőlő</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2013 and 2015</td>
</tr>
<tr>
<td>Pinot regina</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2013 and 2015</td>
</tr>
<tr>
<td>Merlot</td>
<td>2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zweigelt</td>
<td>2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zinfandel</td>
<td>2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moldova</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2014</td>
</tr>
</tbody>
</table>

The three medias which are most commonly used in Hungary medium were included in the experiment: sawdust, perlite and aqueous media, that is “medium-free”. A paraffin-waxed and a non-waxed test group were also set up in the sawdust media to verify the presence of waxing. Thus, in the experiment, a total of 4 treatments were applied in 4 replicates (Table 2.).
Table 2.: Name and code of the treatments in callusing media experiment

<table>
<thead>
<tr>
<th>Code</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.lan</td>
<td>Unwaxed, callused in sawdust (waxing not before callusing, but before nursery)</td>
</tr>
<tr>
<td>f.por</td>
<td>Waxed, callused in sawdust (waxed twice: before callusing and before nursery)</td>
</tr>
<tr>
<td>perlit</td>
<td>Waxed, callused in perlite (waxed twice: before callusing and before nursery)</td>
</tr>
<tr>
<td>víz</td>
<td>Waxed, callused in water (waxed twice: before callusing and before nursery)</td>
</tr>
</tbody>
</table>

96 grafts were grafted for each variety, with the exception of ‘Cabernet sauvignon / T. 5C’. A total of 400 grafts were produced from the buds of ‘Cabernet sauvignon’. After grafting the scion-rootstock combinations were divided into four treatments.

During the callusing, we followed to the industrial practice. We took care after callusing, to ensure that each batch retained its independence and integrity. In the nursery the plants were planted in serial block arrangement. They received a uniform maintaining at the graft school. The conditions of the experiment, both during the callusing period and in the nursery, were suitable for carrying out the experiments.

In 2013, after planting the grafts into the nursery, the temperature remained consistently warm and free of extremes. Heat days only started in mid-June, a month and half after planting.

In 2014, the weather was not favorable for the initial graft development. The average was around 10 °C for more than a week after planting, which is considered to be cool for the development of the grafts. Warming up was not even afterwards either. Heat days already appeared at the end of the first ten days of June.

In 2015, after planting in early May, the temperature began to rise rapidly. There was no intermittent cooling, however, the daily temperature almost reached 30 °C. The first serious heat appeared in the first ten days of June.

2.2 Paraffin waxing experiment

In the experiment, 7 paraffin waxes which are commonly used in the Hungarian nursery practice, were used and examined on the ‘Pintes’ and ‘Kadarka’ grape varieties (Table 3.). The callusing media was sawdust conforming to the basic technology.

In the paraffin–usage experiment, the ‘Pintes’ grape variety was grafted on ‘T. 5C’ rootstock, while ‘Kadarka clone P.147’ on ‘T-F. S.O.4’.
Table 3.: Data of the paraffin waxes tested in the experiment

<table>
<thead>
<tr>
<th>Producer</th>
<th>Proagriwax G-Mediterranean</th>
<th>Proagriwax RH-Ester</th>
<th>Staehler Rebwachs Pro</th>
<th>Staehler Optiwax Red Slabs</th>
<th>Staehler CPT Rouge</th>
<th>Starwax</th>
<th>Cirka Blanche</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suggested technological step</td>
<td>for nursery and for storage</td>
<td>for callusing for callusing</td>
<td>for nursery and for storage</td>
<td>for storage for nursery and for storage</td>
<td>for storage</td>
<td>for nursery or nursery</td>
<td></td>
</tr>
<tr>
<td>Special additive</td>
<td>-</td>
<td>0.0035 % 2.5 dichloro-benzoate</td>
<td>0.0035 % 2.5 dichloro-benzoate</td>
<td>-</td>
<td>-</td>
<td>micro particles of light metals</td>
<td>-</td>
</tr>
<tr>
<td>Congealing (°C)</td>
<td>65 – 69</td>
<td>60 – 65</td>
<td>73</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>Oil content (%)</td>
<td>&lt; 2.0</td>
<td>&lt; 2.0</td>
<td>&lt; 2.0</td>
<td>&lt; 1.2</td>
<td>&lt; 1.5</td>
<td>&lt; 2.0</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>Colour</td>
<td>red or green</td>
<td>red</td>
<td>red</td>
<td>red or green</td>
<td>red or green</td>
<td>silver</td>
<td>white v. green</td>
</tr>
<tr>
<td>Needed quantity (kg/1000 plants)</td>
<td>4.0</td>
<td>1.5</td>
<td>0.7 – 0.8</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0 – 2.5</td>
<td>2.0 – 2.5</td>
</tr>
</tbody>
</table>

The paraffin–usage experiment was set up in 2014 in 4 replicates, 25-25 plants per each replicate. The number of treatments was 8, as an untreated control was also set up. The untreated control was not waxed before callusing or before nursery development. The layout of the experiment in the nursery was a serial block. The waxed batches, had two dips, therefore two treatments: the first one was on 9th April, before callusing, immediately after grafting, at a depth of 7-10 cm from the top of the grafts, and the second one was on 8th May, after callusing, before nursery development dipped to the expected implantation depth. During dipping, the temperature of the paraffins was adjusted between to 80 °C and 83 °C in accordance with the recommended working temperature for each paraffin. After dipping, the grafts were placed into wooden forcing boxes in sawdust medium.

2.2.1 Assessments of the experiments

The grafts were evaluated three times in the experiments, firstly after callusing, secondly at the nursery, while tied to phenology, when the shoot tips appeared uniformly, and thirdly after picking up the rooted vines.

After opening the forcing boxes, the first evaluation was performed on the grafts based on the methodological description of Kocsis and Bakonyi, 1994.

Evaluation of grafting and basal callus development during callusing on scale 0-5: Value 0 is for no visible callus formation; Value 1 is for a 1-2 mm callus less than 10% of the contour
of the join; *Value 2* is for a 1-2 mm callus less than 50% of the contour of the join; *Value 3* is for a 1-2 mm callus more than 50% of the contour of the join; *Value 4* is for an elliptical callus with 1-2 mm interruptions in less than 80% of the contour of the join; *Values 5* is for an elliptical callus with the same thickness in more than 80% of the contour of the join.

**Evaluation of the buds after callusing on scale 0-3:** *Value 0* is for no shoot development; *Value 1* is for a thin and short shoot; *Value 2* is for an etiolated and medium-thick shoot; *Value 3* is for a strong and well-developed shoot.

**Evaluations in nursery:** After planting the grafts into the nursery field, when the shoots were visible a numbers of viable grafts (pre-success rate in %) were established, at stage BBCH 16-18, and the length of the longest shoots was measured (“shoot length”).

**Evaluation after nursery:** The third evaluation happened once the grafts were selected and classified after being picked up in autumn. First, we recorded the number of those grafts that were viable and reached the regulated standard quality and then a final success rate (%) was established by comparing these results to the numbers of grafted plants. The diameter of the viable grafts was measured (mm).

Data were summarized per each replicate and the mean and standard deviation of replicates were calculated. Data sets were compared by two-tailed Student t-test, looking for the effects of the treatments annauly, then the effect of varieties per treatment, and finally the correlations between years. If the precondition was not met on the data pair, a p-value was calculated with a non-parametric test (Mann-Whitney test) (Sváb, 1981).

The correlation between grafting callus values and pre-success or final success rate was examined using correlation analysis. Discriminant analysis was used to determine the segregation of the data sets.

Data analysis were run and evaluated using IBM SPSS version 25.0 and Microsoft Excel.
3 RESULTS OF THE EXPERIMENTS

3.1 Results of the callusing media experiment

3.1.1 Results in 2013

The development of the grafting callus in 2013 was adequate for all 4 callusing media, thus the grafts with the weakest callused variety were also suitable for nursery rearing. The results of unwaxed in sawdust treatment differed slightly from those of waxed in sawdust batches and showed only one variety (‘Borsmenta’) significant difference. In medium-free (water) callusing, the callus development was good. The callus was green and harder to the touch, in contrast to perlite and sawdust medium.

The basal callus developed differently in the tested medias and in addition there were differences between the varieties. In the case of ‘Castellum / T. 5.C’ and ‘Csanád’, the varietal effect is partially significant. It is striking in the values, that at the basal part of the canes in water did not formed callus on any of the varieties. In contrast, in perlite medium showed mainly better callus development compared to sawdust medium for all tested scion rootstock combinations.

In 2013, during callusing, the shoots of the varieties developed the most in the case of the medium-free substrate, which difference can be statistically verified for all scion-rootstock combinations. Beside of that the shoots of the batches callused in sawdust or perlite with waxing developed more traction. The more prosperous shoots were developed during unwaxed callusing.

The pre-success data evaluated in the nursery partially predicted the tendency of the final success after picking. Values were found to be significantly lower from medium-free grafts with the exception of one scion-rootstock combination. Observing the effect of the callusing medias, all grafting combinations callused either in perlite or in water gave the worst yields. In the average of the 11 studied cultivars, the unwaxed sawdust yielded 69.5%, waxed sawdust yielded 70.4%, while waxed perlite yielded 50.0% and medium-free yielded only 38.8%.
3.1.2 Results in 2014

Irrespective of the varieties, in 2014 the grafting callus development in medium-free media significantly lagged behind from the callus development in sawdust control and any other treatments. This meant that on most of the grafts callused in medium-free environment not or only partial callus developed. Several plants of some cultivars showed such weak callus formation (2 or less callus formation value) that in practice the plants are not able to develop in nursery.

Paraffinized grafts also had weaker callus initiation compared to grafts without paraffin waxing, with the exception of ‘Csanád’. Callusing in perlite showed balanced grafting callus development compared to paraffin-sawdust treatment.

In 2014, there was also no development of callus on the base of the grafts if they were callused in water. In contrast, the good basal callus development observed in 2013 in the perlite medium could not be observed in 2014. The strongest basal callus of most varieties was developed by batches callused in sawdust without waxing.

In the nursery, the data of pre-success and yield reflected the weak grafting callus development of the grafts. The weakest grafting callus development, and in this connection the weakest pre-success and yield, were observed on treatment medium-free.

3.1.3 Results in 2015

In 2015, we recorded excellent and uniform grafting callus development independently from medium and variety.

Basal callus did not develop in 2015 in the case of medium-free callusing. The pre-success in the nursery also had promising results in the case of medium-free (aqueous) callusing, although in none of the varieties did exceed the results of control treatment waxed in sawdust selected from grafting practice.

Similar to the trend observed at pre-success (in ascending order), ‘Castellum / T-K. 125AA’;‘Castellum / T. 5C’;‘Csanád’;‘Hibernal’ and ‘Andor szőlő’ varieties were found to have lower yields. Overall, the best yields were obtained for ‘Cabernet franc’ and ‘Borsmenta’ varieties.
3.1.4 Results of the three experimental years

From the data of the three experimental years, it can be seen that the results of the callusing media gave significantly different results according to the vintages.

The yield of the ‘Castellum’ for both rootstock combinations, on average were 38%, significantly lower than the average of all the studied varieties (55.3%), while the strongest was ‘Borsmenta / T-F. S.O.4’ (70.8%).

The average of the three-year data set shows that among the tested callusing media, the sawdust and the perlite provided very good callus formation (Fig. 1.). In four of the six cultivars, the waxed grafts in sawdust gave the best grafting callus formation value.

![Figure 1.: Results of grafting callus development on the average of three experimental years](image)

It should be noted that value 4 on grafting callus development with a small standard deviation indicates “contiguous callus” on “80% of the perimeter of the grafting point”, is excellent in practice. The results of callus development of grafting point callused without media indicated a lower rate with a large standard deviation. It has a practical meaning that callusing in water may yield more variable results for vintages.

The summarized data reflects that basal callus were not developed in water medium in any of the years and in any of the cultivars (Fig. 2.). However, in the case of callusing in medium, both in sawdust or perlite, the base of the plant begins to develop partial basal callus thus providing a more favorable root development in the nursery. During assessing the
development of the basal callus, we did not observe root formation in any year and in any variety.

The studied varieties showed varying basal callus development thus, it can be seen that the grafted variety has an influence on the nature of the basal callus.

Shoot development in the forcing chamber act uniformly, both in the rate of bud development and in the variance values. In some varieties, the medium-free callusing shows a more dynamic shoot development compared to the callusing in medium.

The retraction or lag of the shoot development of paraffinized grafts during callusing compared to the shoot development of unwaxed batches was observed only in 2013, and it was not observed in the other years and in the average of the varieties.

The final yield values reflect that the callusing in perlite (with the exception of ‘Italia / T-F. S.O.4’) and in medium-free media resulted significantly worse results for all varieties compared to the callusing paraffinized sawdust (Fig. 3.). The effect of waxing the grafts in callusing sawdust medium is not clear.

To get an idea of the influencing effect of grafting callus formation on nursery and final production results the distribution of grafting callus was summed to values 5-3 and 5-4. Based on the statistical analysis, neither the distribution of values 5-4 nor the distribution of values 5-3 is related to the pre-success or even to the yield. In contrast, the pre-success in the nursery is related to the final yield result at 1% significance level. During the years 2013-2015, we experienced a decrease of 3.6%, 10.9% and 8.9%, respectively, in the average of the varieties, between the results of pre-successes and the final yields.
3.2 Results of paraffin waxing experiment

In the ‘Pintes’ variety, the two paraffin with callus stimulating hormone additive (Proagriwax RH-E and Rebwachs Pro) advanced more developed, the other paraffins, which are not specifically recommended for callusing advanced less developed grafting callus. This difference in grafting callus development between hormonal and non-hormonal paraffins was also statistically well separated, although it was not significant compared to the untreated control. The data of the basal callus and the shoot development of the waxed bathes were significant compared to untreated control. In the untreated control, the basal callus was significantly more developed and the shoot development was significantly less developed. In the basal callus development data set there was a statistical discrepancy with hormonal paraffins and Proagriwax G-Mediterranean, Staehler CPT Rouge, and partially other paraffins too. According to this, when the paraffins with callus stimulating hormone were applied at the grafting point, the basal callus were less developed.

The waxed treatments of the ‘Pintes’ variety reflected significantly higher pre-success rate compared to treatment without paraffin. This trend was followed by the final yield results as well.

The yield of the nursery was very weak, only 13% of the grafts were viable (Fig. 4.). If we look at the p-values of the comparison between the paraffin products, it appears that Starwax resulted significantly the highest yield than all other paraffin products. While Staehler CPT Rouge resulted significantly higher results than almost all other paraffin products. Compared to the average yield Staehler CPT Rouge resulted 7.14% better and Starwax resulted 14.14%
better yield. The Proagriwax G-Mediterranean product which has Official Authorization Use in Hungary yielded 3.87% worse result than the average data of all paraffin products.

In the case of Kadarka, among the paraffins, Proagriwax RH-E, Rebwachs Pro, and Staehler CPT Rouge showed better grafting callus formation at the grafting union. Both products which has callus stimulating additive (Proagriwax RH-E, Rebwachs Pro) were significant compared to untreated Grafts which were waxed with Cirka Blanche resulted significantly 0.2 weaker callus formation.

The development of the basal callus in treatment Starwax was significantly better compared to the untreated control, while the two hormonal paraffins showed significantly weaker basal callus development compared to Starwax treatment.

The shoot development during callusing and the pre-success in the nursery were uniform and promising, even for the unwaxed control batches.

The final yield after picking the nursery was significantly better for Optiwax Red Slabs and Starwax paraffins (Fig. 4.). The Proagriwax G-Mediterranean product which has Official Authorization Use in Hungary yielded slightly better yield compared to the average yield of all treatments.

Figure 4.: Effect of the paraffin waxes on final success on 'Pintes / T. 5C' and 'Kadarka / T-F. S.O.4'

Note: * 5% significant difference
4 DISCUSSION

4.1 Comparison of callusing medias

In a three-year experiment, comparing 4 callusing methods in 11 scion-rootstock combinations per year, evaluating the results, it is obvious that callusing media has different effect among the varieties in callusing and yield. In general, world varieties were less susceptible, while newly bred varieties were more susceptible to the use of different callusing method. No significant varietal susceptibility has been observed consistently over the years in grafting callus formation.

In 2013 we observed uniform and strong, in 2014 variable and weaker, while in 2015 we observed excellent grafting callus formation on the tested varieties, which can be mainly influenced by the vintages.

Perlite medium has favourable effect of the basal callus development in 2013, while in 2014 the sawdust favoured more, and in 2015 the perlite and the sawdust resulted alternatively better basal callus formation. The development of the basal callus indicates the viability of each graft, but does not necessarily indicate the rooting ability of plants.

The development of the basal callus was not influenced only by the callusing medias or the vintages, but also by the grafted varieties on the rootstock. Namely, in the case of grafting different varieties on the same rootstock, callused in the same medium, the basal callus developed differently. That is, after grafting, not only the rootstock have an influencing effect on the scion, but the scion influences the characteristics of the rootstock.

Looking back at the results of the three years, it can be seen that, callusing without medium, nursery owners can expect outstandingly good performance in some years, but very different in other vintages, both in terms of grafting callus development and yield.

It can be clearly stated, as described in the literature (Fallot, 1973; Füri, 1982), that in the case of callusing in medium-free environment, basal callus did not develop due to lack of oxygen. In the case of callusing in medium (substrate), the basal callus begins to develop already during the callusing, providing a more favorable base for root development starting in the nursery.

Medium-free callusing showed the weakest er-success and yield ratio in all three years, in almost all scion-rootstock combinations. These experiences unanimously support the findings of Megyeri (2019) and Miklós (2014) in comparison of callusing medias. However, due to the more favorable investment and waste generation characteristics of medium-free
callusing, producers are looking to learn more about this technology (Szabó, 2017a; Szabó et al., 2017a; Szabó et al., 2018; Szabó, 2019).

About the nursery period of the grafts, it can be told that the per-success (recorded at the time when the shoot tips are clearly visible) conclude the final yield after picking. Though the development of the grafting callus is not correlated with the yield so it is not related to the per-success or the final yield.

It does not appear a main difference on the average data, the callus formation without waxing is not safe in every vintage and when on all varieties. In addition, Zilai's (1964) statement cannot be confirmed with these data in all three tested years, whereas the paraffin coating during callusing hinder the budbreak and shoot development, thus it has a positive effect on the elongation and overgrowth of the shoots.

On average in the experimental years, the most effective callusing media was sawdust for all varieties. Callusing in sawdust does not accidentally look back on a stable history of several decades. Experimental data reflects the fact that this technology can be said to be stable, and the side effects of the vintages are less perceptible (Köse, 2015).

The difference between the paraffin waxed and the non-paraffinized batches in yield, it did not exceed 8% on average over several years data. Beside of that the positive effect of waxing was not observed in half of the tested cultivars.

It can be told that in the vast majority of the studied cultivars a better yield can be expected in the case of sawdust callusing than in perlite or medium-free callusing.

Although the goal was not to compare the reproducibility of the studied varieties, the yield data reflects that the further varieties can be propagated better than the average is ‘Cabernet sauvignon / T. 5C’, ‘Cabernet franc / T. 5C’, ‘Kadarka / TF. S.O.4’, ‘Andor szőlő / T-F S.O.4’ and ‘Pinot regina / T-F. S.O.4’. Very easy to propagate ‘Borsmenta / T-F. S.O.4’. Less well able to be propagated were ‘Csanád / T. 5C’, ‘Moldova / Börner’. The latter combination may have an unfavourable rootstock effect. It was particularly difficult to propagate ‘Castellum / T. 5C’ and ‘Castellum / T-K. 125AA’ which is stated by nursery owners.
4.2 Comparison of paraffin waxes

The development of the grafting callus was the best in case of callus stimulating additives in case of both varieties. The callus-stimulating effect of Rebwachs Pro and Proagriwax RH-Ester can be statistically demonstrated in both cultivars.

Dimitrova et al. (2008) and Calugar et al. (2019) found a significantly better grafting callus development in case of callus stimulating additives compared to treatment without waxing. In contrast, in our studies, the significantly more advanced grafting callus forced by hormone additive paraffins is not really important in practical point of view, since all products and unwaxed control showed well developed callus formation. The effect of hormone additive paraffins can be used for a shorter interval of callusing or at a lower temperatures (Zink and Eder, 2005; Corbean et al., 2009; Corbean et al., 2011).

In order to form a thicker callus, it is clearly advisable to use paraffin containing hormone-additive to form a wound-welding tissue. However, the benefits during callusing of the paraffins with hormone additive are no longer able to be experienced at autumn after picking the nursery. These experiences are in paralell with the observations of Miklós (2014).

Is because the first waxing only was performed at the point of grafting, we did not expect a large difference in the development of the basal callus. In contrast, the results of the evaluations show that all paraffins observed in the ‘Pintes’ variety caused significantly worse callus development at a base. This tendency was not observed on ‘Kadarka’.

In case of both studied varieties, the paraffins with hormone-additive gave the best grafting callus development but the less well-formed basal callus compared to untreated or any other paraffin products. These correlations are statistically proven. On ’Kadarka / T-F. S.O.4’, Starwax showed significantly the best basal callus development and also the best yield results.

The observations of Zilai (1964) was not able to be verified in paraffin testing experimental. This was that paraffin waxing before callusing delays the bud break and reduces the elongation of the shoots compared to unwaxed grafts.

Comparing the results of bud development at the time of callusing with the pre-success of the nursery, it appears that the buds of the ‘Pintes / T. 5C’ variety were maybe damaged during callusing in the absence of paraffin waxing. This did not occur in the case of unwaxed ‘Kadarka / T-F. S.O.4’, thus the lack of the paraffin waxing had less effect on pre-success and yield, than in ‘Pintes / T. 5C’.
In the nursery, paraffin performs its main protective function mostly in the first few weeks after transplanting (Zink and Eder, 2005; Dimitrova et al., 2008; Tuncel and Dardeniz, 2013). It can be assumed that callusing paraffins were less resistant to lower temperatures and higher temperature fluctuations. So, grafts treated with such paraffin for callusing could not be adequately protected. Their yields (Proagriwax RH-E., Rebwachs Pro) have declined mainly from calluing to nursery yiled.

In terms of final yield, the paraffins for nursery use performed better in both varieties. Starwax produced significantly the best yields on both varieties. 72% for ‘Pintes / T. 5C’ and 90% for ‘Kadarka / T-F. S.O.4’.

The discriminant analysis of the paraffin waxes (Fig. 5.) shows well that the untreated control (1.) was well separated from the group mean, thus it had a particularly strong effect on the studied parameters if the grafts were not waxed in either for callusing or for nursery.

![Figure 5.: Discriminant analysis of paraffin waxes in the mean of varieties and evaluated parameters](image)

Similarly, treatment 7 was also separated, which shows that paraffin treatments with Starwax affected the measured results, that are, mainly increasing the yield of grafting, although it did not perform well in case of other evaluations. Treatments 3 and 4 were similarly separated, reflecting the influence of paraffin with callus stimulating hormone additive (Proagriwax RH-E., Rebwachs Pro) on grafting callus development and, in parallel, basal callus formation during
callusing. Despite the fact that the development of grafts started well, they remained among the last in yield. The separation of treatment 2 shows the influencing effect of the only paraffin type currently allowed in Hungary, Proagriwax G-Mediterranean, on the measured parameters, which can be said to be acceptable in all evaluations.

Although the results of each paraffin were well separated, all products gave well acceptable and applicable results for practice. Only one treatment, ‘Pintes / T. 5C’ without paraffin were significantly lagging behind the 50% yield as an industrial norm.
5 THESIS POINTS

1. It was determined that grafting components are the most important for successful stratification. There are neutral and sensitive grapevine varieties for the medium of stratification. We have set it up in an order among the stratification medium according to the successful propagation.

2. The forcing media, the scion and the applied paraffin at the grafting union notably determine the development of the basal callus.

3. The waxing in the forcing chamber does not delaying the sprout of the buds or the development of the young shoots. The waxing during stratification is optional if there is forcing media but essential before planting the grafts into the nursery. The development of the callus at the grafting union during stratification is not correlate with the final grafting success after nursery.

4. Among the newly bred varieties 'Castellum' is requiring more attention in propagation process and has lower grafting success in addition perform differently on variable rootstocks.

5. Among the tested paraffin products Starwax for nursery use resulted the best final grafting success on both 'Pintes / T. 5C' and 'Kadarka / T-F. SO.4'.
6 LIST OF PUBLICATIONS

6.1 Publications in hungarian language in refereed scientific journal


6.2 Publications in foreign language in refereed scientific journal


6.3 Refereed full text conference proceedings


6.4 Presentations, posters


6.5 Other publications


REFERENCES


