COMPARISON OF THE EFFECTS OF DIFFERENT PARAFFIN PRODUCTS APPLIED DURING GRAPEVINE PROPAGATION

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This study investigates the effectiveness of alternative paraffin products on the callusing ability and grafting success of two Hungarian grapevine varieties: 'Kadarka' on SO4 and 'Pintes' on Teleki 5C. Seven different paraffin-based waxes were applied twice during the grapevine propagation process: before callusing and before the nursery period. After callusing, the development of the grafting callus and the basal callus were assessed on a scale of 0 to 5 and the development of the buds was assessed on a scale of 0 to 3. In the nursery the vegetative performance was evaluated. Final grafting success was calculated based on the percentage of well-developed graftings. The result showed that different waxes have significant effect on the evaluated parameters but varying with the two tested varieties. Callus formation at the grafting point showed the highest value with the Rebwachs Pro (4.9) and Proagriwax RH-E (4.7) in the case of 'Pintes' and 'Kadarka', respectively. Although the paraffins with callus-stimulating additive yielded the best grafting callus formation in both varieties, this did not manifest itself in grafting success as expected. Vegetative performance also showed significant differences influenced by the waxes for both varieties. The highest rate of final success was provided by the Starwax paraffin with 72 % and 90 % for the 'Pintes' and 'Kadarka', respectively.
Keywords: paraffin wax, waxing, grapevine grafts, callusing, Hungarian grapevine varieties

Vergleich verschiedener Paraffine in der Rebveredlung. In dieser Arbeit wird die Wirkung von verschiedenen Paraffinen auf Kallusbildung und Veredlungserfolg bei zwei ungarischen Weinrebsorten ('Kadarka' auf SO4 und 'Pintes' auf Teleki 5C) untersucht. Sieben Rebwachse wurden zweimal während der Rebveredlung angewendet: vor der Kallusbildung und vor der Auspflanzung in der Rebschule. Nach der Kallusbildung wurden der Kallus an der Veredlungsstelle und der an der Basis mittels einer Skala von 0 bis 5 und das Knospenwachstum mittels einer Skala von 0 bis 3 bewertet. In der Rebschule wurde die vegetative Leistung bewertet. Der finale Veredlungserfolg wurde mittels der Anzahl gut entwickelter Pfropfreben berechnet. Die Ergebnisse zeigten, dass die Rebwachse deutliche Wirkung auf die untersuchten Parameter haben, jedoch in unterschiedlichem Maß bei den zwei Sorten. Die Kallusbildung an der Veredlungsstelle war am besten bei Rebwachs Pro (4,9 bei 'Pintes') und Proagriwax RH-E (4,7 bei 'Kadarka'). Obwohl die besten Ergebnisse an der Veredlungsstelle mit den Wachsen mit kallusfördernden Zusätzen erreicht wurden, war diese Wirkung beim finalen Veredlungserfolg nicht wie erwartet feststellbar. Die vegetative Leistung beider Sorten war auch deutlich von den Rebwachsen beeinflusst. Der beste Veredlungserfolg wurde mit Starwax erreicht (72 % bei 'Pintes' und 90 % bei 'Kadarka').
Schlagwörter: Rebwachs, Paraffinierung, Pfropfrebe, Kallusbildung, ungarische Rebsorten

Until the middle of the 20th century, grapevine propagation was performed in ridged nurseries. The soil of the ridge protected the callused grafts and cuttings against environmental effects, e. g. sunburn, drought stress, or late frost. However, the ridge technology was costly, required large amounts of manual labour and was problematic if soil properties were unfavourable (Eifert, 1981).

These reasons forced nurseries to develop an alternative method of protection. In the 1950s, Italian and Swiss publications first referred to the application of paraffin wax on grafts above the soil surface (Eifert, 1981). Further experiments on paraffin application were conducted by Eifert in 1960 and 1965 (Eifert et al., 1980) that proved the cost-effectiveness and efficiency on grafting success. Based on global trials, referred to in the studies reported by Eifert and Eifert (1981a), the usage of paraffin wax rapidly spread in large-scale production in the 1980s in Hungary.

In industrial practice Winkler et al. (1974) also suggested using paraffin wax pre- and post-callusing incubation. Later Becker and Hiller (1977) and Becker et al. (1982) reported that the graft union can be protected from dehydration during callusing by dipping into grafting wax. Besides the benefits of using paraffin wax, Becker et al. (1982) also reviewed the environmental and soil conservation aspects of paraffin residues in the agricultural field.

The only common disadvantages of using paraffin wax instead of ridging in the nursery is the susceptibility of the grafts to late spring frost at the beginning of May. Despite this, the use of paraffin wax quickly spread in the early 1990s (Eifert, 1981; Kozma, 1993; Jeszenszky, 1996).

However, covering the surface of the graft with paraffin wax needs to be a prudent technological step. The working temperature (70 to 85 °C) of the melted wax should be carefully adhered to as stipulated in the instructions of the respective product, as the melted paraffin can burn the grafting and shoot tissues. Therefore, the dipping must be quick (~1 second) and the graft ought to be cooled to ambient temperature directly after. During a longer dipping (5 seconds) the temperature of the plant tissue can reach 50 °C, and also overheat can last for a longer period, if it is stored at ambient temperature (Willhöft, 1983; Becker et al., 1982; Gramaje and Armengol, 2012).

The grafting material should have optimal water content (Eifert and Eifert, 1981b), otherwise if it is dehydrated, the wax can diffuse into the desiccated tissues thereby transmitting chemicals which are toxic to the plants. After hydration and before waxing, the surface of the grafts must be dried, because the paraffin will not adhere to a wet surface (Willhöft, 1983; Becker et al., 1982).

Due to different technological needs, specialized waxes were developed for the forcing period (wax for callusing), for nursery use (wax for nursery or greenhouse) and for storage during the winter period (wax for cold storage or/and planting) (Willhöft, 1983; Smith et al., 2012).

The main point of using the paraffin wax at the first stage, i. e. after bench grafting, is to gently stabilize the scion on the rootstock and stimulate the healing wound to produce a callus. The callusing wax should be flexible to follow the growth of the callus. Although, during callusing the paraffin wax can work as a protective physical barrier. It may have a disadvantage if heavy waxing occurs, because graft healing requires high levels of oxygen. On the other hand, the usage of paraffin that is overheated and too fluid can cause partial healing as wax penetrates the graft union and forms a barrier between rootstock and scion (Hartmann et al., 2014; Waite et al., 2015). Callus tissue must encapsulate the grafting union, but it should be moderate for uniform vascularization. To achieve a good join and a circular, uniform callus, Corbean et al. (2009) used paraffin with hormones. Compared to normal paraffin without stimulators, they observed a 12 to 20 % increase in grafting success with a reduced forcing duration (14 to 15 days). Dimitrova et al. (2008) and Iliev et al. (2014) also showed that using paraffin with hormones can boost the level of callus formation during the forcing period. Zink and Eder (2005) proved that the use of paraffin with hormone additives demonstrates the greatest advantages when implementing shorter forcing periods or employing cold callusing technologies.

Callusing wax which contains hormones can cause rapid and copious callus formation at the grafting point, but overabundant wound tissue can flow and push apart the scion, which is fragile, etiolated, and can be easily damaged (Hegedűs, 1991).

Corbean et al. (2011) reported that paraffin with additives results in better grafting success, but they recommended a shorter callusing duration because it is more cost-effective.
Wax is applied for the second time when the freshly joined plant is transplanted from the forcing box into the nursery fields or greenhouses. Paraffin wax is added to seal the callus for the first days and weeks until the vascular tissues are connected and the young plant union can establish a root system and draw water.
Rough paraffin creates a varying thickness of seal, easily melts at higher temperature and cracks in cooler nights (Kozma, 1993). Hair-cracks on the paraffin shelter mainly reduce the protection of the coating against desiccation. To avoid this, waxes contain polymer additives to tolerate large temperature fluctuations. Thus, the new generation of nursery waxes have a good heat resistance and, consequently, are flexible (Willhöft, 1983; Smith et al., 2012).
An undesirable effect of the paraffin seal was documented by Zilai and Tompa (1981). Following a good start, the grafts unexpectedly died. This was attributed to irregular warm, long-lasting weather conditions that resulted in the paraffin wax melting on the surface of the callused grafts. Paraffin elements and wax substances were found between the tissue cells of the grafts where they were covered by paraffin wax. The blocked tracheas that resulted in necrosis were observed in optical microscopy studies. As a consequence, paraffin wax producers now warn that melted wax molecules can migrate into the plant and destroy the living tissue if the wax encounters higher temperatures.
Zink and Eder (2005) established during a 10-year-trial, that the scion and rootstock combination and the parameters of the vintage have a higher impact on callus development during the forcing and nursery period, than the applied wax. Furthermore, in the nursery, the benefit of the paraffin cover is realised if the temperature is hot after transplanting.
Finally, paraffin waxes are also applied after harvesting the one-year-old plant from the nursery field during cold storage (3rd wax). Wax is applied to improve the storage properties of the plant and to ensure a successful plantation in the vineyard.

Using paraffin for waxing the grapevine grafts during the propagation process is a common technological process, but its use has several advantages and disadvantages. More recently, economical questions regarding its use have been raised (Calugar et al., 2019). There are different traditions and requirements in each grape grafting region and as a consequence different paraffin waxes are produced all over Europe.
In this study two grapevine varieties were investigated: 'Kadarka' grafted onto SO4 and 'Pintes' on Teleki 5C. 'Kadarka' is widely used with its several clones and subclones in most Hungarian wine districts (Hajdu, 2013; Werner, 2013). 'Pintes' is autochthonous and produced for unique white or sparkling wine (Csepregi and Zilai, 1988; Péter, 2015). There is a difference between these varieties in their ability to form callus (Bényei et al., 1999). Amongst the Hungarian Grapevine Graft Producers (Association of Hungarian Nurseries), who have experience in grafting 'Kadarka', it is declared that this convar. *pontica* variety has difficulties concerning grafting. It also needs a longer forcing period, and the bud break in the nursery is commonly delayed. There is less experience with 'Pintes' in propagation, but it has been declared like a usual convar. *pontica* variety. Two commonly used rootstock varieties were tested in this experiment. Both Teleki 5C and SO4 are considered as middle term and well-callusing rootstocks (Hegedűs et al., 1966; Romberger et al., 1979; Tangolar et al., 1997).
The focus of this study was therefore to observe and assess the effects of different paraffin types on two grapevine varieties, 'Pintes' and 'Kadarka', with respect to callus development during callusing, viability in the nursery period and final grafting success.

MATERIALS AND METHODS

PLANT MATERIALS

The study was conducted with two Hungarian grapevine varieties: 'Pintes' on rootstock Teleki 5C and 'Kadarka' (clone P.147) on rootstock SO4. The phytosanitary status of both rootstock propagation materials was 'certified material' and both scions were 'standard material' with respect to Directive 68/193 EEC. All grafting materials were prepared as to general practice. Rootstocks were disbudded and the basal node was cut. Scions were chopped into one-bud cuttings. Both were soaked in a solution of water and disinfectant (a concentration of 0.5 % 8-hydroxyquinoline), and stored in polyethylene bags between 1 and 3 °C in a cold storage unit.

TESTED PARAFFIN WAXES

Seven commonly used paraffin products (Table 1) were tested in this study. These products were compared with each other and with an untreated control. The untreated control had no paraffin applied during the entire trial process (neither in the forcing chamber, nor in the nursery).

EXPERIMENTAL DESIGN

Each treatment comprised 25 grapevine grafts in 4 replications. After bench grafting (April, 9th, 2014) plant material was nipped in paraffin to a length of 7 to 10 cm from the top of the scion and placed for callusing.

After callusing, but before nursery (May, 8th, 2014), the waxing was conducted for a length of 20 to 25 cm. The callusing was performed in sawdust, between April, 9th and 30th, at an average temperature and humidity of 28.5 °C and 95.6 %, respectively. The conditioning after callusing was performed from May, 1st to 8th.
Irrespective of callus development and viability, all the callused grafts were transplanted into the nursery field. The experimental conditions were excellent for

Table 1: Characteristics of the tested paraffin waxes

	Proagriwax G-Mediterranean	Proagriwax RH-Ester	Staehler Rebwachs Pro	Staehler Optiwax Red Slabs	Staehler CPT Rouge	Starwax	Cirka Blanche
Producer	Norsk Wax Stavern, Norway		Chauvin Agro (Ser Wax brand) Sarrians, France			Quimiwax 2000 S.L. Bordeaux, France	
Technological steps	both for nursery and storage	for callusing	for callusing	both for nursery and storage	for storage	both for nursery and storage	for nursery
Special additives	-	0.0035 % 2.5 dichloro-benzoate	0.0035 % 2.5 dichloro-benzoate	-	-	micro particles of light metals	-
Congealing point (°C)	65-69	60-65	73	76	76	70-74	77-80
Working temp. (°C)	80-83	80	76-80	83-86	83-86	85-90	85-90
Oil content (%)	< 2	< 2	< 2	< 1.2	< 1.5	< 2	< 2
Colour	red/green	red	red	red/green	red/green	grey	white/green
Required quant. (g/plant)	4.0	1.5	0.7-0.8	2.0	2.0	2.0-2.5	2.0-2.5

the development of the plants in the nursery field but the weather conditions were not always optimal. From the beginning of May the daily average temperature was ~15 °C but it dropped to 10 °C for 7 to 8 days which is less than optimal for the root development of the young grafts. The hot days (daily average above 25 °C) started from mid-June which is advantageous as heat stress can damage the grafting callus at an early period of the nursery cultivation.

The first assessments were recorded after callusing according to the methodology of Kocsis and Bakonyi (1994). The grapevine grafts were replaced from the callusing boxes and a 0 to 5 scale was employed to describe the development of the callus at the basal node and the grafting union:

0 ... no callus formation;
1 ... 1 to 2 mm callus under 10 % of the contour of the join;
2 ... 1 to 2 mm callus under 50 % of the contour of the join;
3 ... 1 to 2 mm callus more than 50 % of the contour of the join;
4 ... elliptical callus with 1 to 2 mm interruptions in under 80 % of the contour of the join;
5 ... elliptical callus with the same thickness in more than 80 % of the contour of the join.

A scale of 0 to 3 was used to describe the development of the buds of the scions.

0 ... no bud development;
1 ... bud began to expand but leaves are unfolded;
2 ... etiolated and thin or medium-thick shoot (at maximum 3 cm);
3 ... well-developed green shoot (longer than 3 cm).

After planting the grafts into the nursery field at stage BBCH 16 to 18 (Lorenz et al., 1995) the numbers of viable grafts (pre-success rate) were evaluated. The length of the longest shoots was measured. Grafts were removed from the nursery field and well-developed ones according to Directive 68/193 EEC were selected and percentage of successful grafting was subsequently established.

STATISTICAL ANALYSIS

Results are presented in statistical comparisons conducted between the different treatments tested for 'Pintes' and 'Kadarka', using IBM SPSS Statistic 25.0 (IBM Corp. Released 2017; IBM SPSS Statistics for Windows, Version 25.0; IBM Corp., Armonk, USA) and Microsoft Excel (Microsoft Corporation, 2018; Microsoft, Redmond, USA). Normality of the data was inspected based on the Saphiro-Wilk test. In the case of a normal distribution, the two-sample Student's t-test, and in the case of not-normally distributed data the Mann-Whitney U-test were conducted in order to compare all treatments to each other. Finally, a Canonical Discriminant Functions Analysis was also performed.

RESULTS AND DISCUSSION

Our results showed that the use and type of paraffin significantly modifies classification values of callusing, bud and shoot development, thereby influencing the final grafting success. Results suggest that the effect of paraffin is not uniform between the two scion × rootstock combinations (Table 2 and 3).

Data regarding the grafting callus from the untreated control and treatment with any of the tested paraffin types showed only a small variance with both varieties, with an average value between 4.1 and 4.9. This indicates that callus development was sufficient for creating a good join between rootstock and scion when employing any of the treatments.

But callus formation at the grafting union showed significant ($p < 0.05$) differences in both 'Pintes' and 'Kadarka' caused by the use of different paraffin. The two paraffins containing hormone additives (Proagriwax RH-E and Rebwachs Pro) had the greatest influence on the development of the grafting callus. This result was as expected because a wax with an added callus-stimulating hormone can influence and improve callus development better than waxes without additive.

In the case of 'Pintes' the highest average classification value for callusing (4.9) was observed with Rebwachs Pro, while the lowest value was obtained after application on of Cirka Blanche and Proagriwax G-Med. (4.1). In case of the variety 'Kadarka' the formation of the callus was more intense after the application of Proagriwax

Table 2: Mean values of callus formation, bud development and nursery production with 'Pintes' according to the applied paraffin types

	Untreated	Proagriwax G-Med.	Proagriwax RH-E	Rebwachs Pro	Optiwax Red Slabs	Staehler CPT Rouge	Starwax	Cirka Blanche
Callus formation at grafting union	4.5[ab]	4.1[a]	4.7[b]	4.9[b]	4.2[a]	4.3[a]	4.2[a]	4.1[a]
Callus formation at basal section	3.3[d]	2.5[c]	1.8[ab]	1.6[a]	2.2[bc]	2.5[c]	1.9[ab]	2.4[bc]
Bud development at callusing	0.5[a]	0.9[b]	1[bc]	1.1[bc]	1.3[c]	0.9[ab]	1[b]	0.9[ab]
Pre-success rate in the nursery (%)	20[a]	83[bc]	75[bc]	93[c]	88[bc]	88[bc]	84[b]	82[bc]
Shoot length in the nursery (cm)	58.5[c]	48.7[bc]	57[bc]	46.7[bc]	37[ab]	50.9[bc]	24.8[a]	65.9[bc]
Final grafting success (%)	13[a]	54[bc]	50[b]	50[b]	57[b]	65[c]	72[d]	57[bc]

Different letters indicate significant differences between the paraffin types based on Student's t-test and Mann-Whitney U test ($p \leq 0.05$).

Table 3: Mean values of callus formation, bud development and nursery production with 'Kadarka' according to the applied paraffin types

	Untreated	Proagriwax G-Med	Proagriwax RH-E	Rebwachs Pro	Optiwax Red Slabs	Staehler CPT Rouge	Starwax	Cirka Blanche
Callus formation at grafting union	4.4[b]	4.2[ab]	4.7[d]	4.6[cd]	4.2[ab]	4.6[bcd]	4.4[bc]	4.2[a]
Callus formation at basal section	2.3[a]	2.6[ab]	2.7[a]	2.4[a]	2.7[ab]	2.9[ab]	3.2[b]	2.7[ab]
Bud development at callusing	1.1	1.0	1.0	1.1	1.0	1.0	0.9	1.0
Pre-success rate in the nursery (%)	78	90	78	86	90	92	90	84
Shoot length in the nursery (cm)	33.7[a]	64.1[cd]	43[ab]	64.5[d]	51.8[bcd]	54.7[c]	40.9[ab]	50.6[abcd]
Final grafting success (%)	68[a]	74[ab]	73[ab]	68[a]	81[bc]	67[a]	90[c]	64[a]

Different letters indicate significant differences between the paraffin types based on Student's t-test and Mann-Whitney U test ($p \leq 0.05$).

RH-E (4.7) whilst the lowest classification value occurred in the cases of Proagriwax G-Med, Optiwax Red Slabs and Cirka Blanche (4.2).
Callusing at the basal section also showed significant differences ($p < 0.05$) irrespectively of the scion × rootstock combination. 'Pintes' grafted onto SO4 showed higher variability in callusing than 'Kadarka' grafted onto Teleki 5C. The former combination showed the highest value of callusing without paraffin (3.3) while the lowest value was recorded in the case of Rebwachs Pro (1.6). In the latter combination the highest value of callusing was observed with Starwax (3.2) whilst the lowest was without paraffin.
When the tested parameters of this study were chosen, the hypothesis was that waxing the grafted union with paraffin has no (or limited) effect on the basal callus development, because the first paraffin application only covers the grafting union. As we assessed the bud development, 'Kadarka' yielded a mean value of 1 indicating that most of the buds were alive and had begun to form short and small shoots. There was no difference compared to the untreated, nor even between the used paraffin waxes. In the case of 'Pintes', however, the untreated variant yielded a mean value of 0.5. This could have been caused by dried or unvigorous buds that were unable to commence growth (Tables 2 to 3.).
Vegetative performance in the nursery was described according to shoot length of the scion. The highest average shoot lengths were recorded with the application of Cirka Blanche (65.9 cm) and Rebwachs Pro (64.5 cm) while the shortest shoots were developed with the use of Starwax (24.8 cm) and in the case of untreated control (33.7 cm) for 'Pintes' and 'Kadarka', respectively.
The grafting pre-success of the un-waxed 'Pintes' (untreated control) specimens was significantly lower than that with the treated ones. The pre-success percentages of the treated samples were between 75 and 93 % (average 84.7 %), while it was only 20 % for the untreated samples. However, the few untreated samples yielded longer shoots.
The pre-success of 'Kadarka' was between 78 and 92 % (average 87.1 %) with the treated samples, but also 78 % with untreated ones. In the case of 'Kadarka', the shoot length with the untreated variant was much shorter than with the treated ones. Proagriwax G-Med, Rebwachs Pro, Optiwax Red Slabs, and Staehler CPT Rouge treatments resulted in significantly longer shoots.
The final grafting success percentage was evaluated on November, 2nd. Almost all treated specimens showed better results compared to the untreated control for each assessment except 'Kadarka' in the case of Staehler CPT Rouge (1 % less), Cirka Blanche (4 % less), and Rebwachs Pro (no difference). Optiwax Red Slabs and Starwax were outstanding and significant with 'Kadarka' (Tables 2 and 3).
With respect to final grafting success, poor results for the untreated 'Pintes' specimens were obtained. All treatments with paraffin waxes gave acceptable final grafting success (50 to 72 %). Comparing the final success rate to the evaluated nursery pre-success, the results for the former declined by 23 to 43 %. 'Kadarka' typically showed a much smaller decline (between 5 to 25 %). In the case of Starwax the pre-success and the final success were equal (90 %).
The highest final grafting success was achieved with Starwax for both variety combinations, even though lower results were observed for other parameters, for example shoot length in the nursery. This paraffin wax was also outstanding in the Canonical Discriminant Functions Analysis (Fig. 1). Based on our results the paraffin types were grouped in accordance to the technological steps that they are offered for. The hormone additive waxes (Proagriwax RH-E, Rebwachs Pro (No. 3, 4.)) for callusing are separated from the waxes for nursery and storage. The Proagriwax G-Mediterranean (No. 2.) is offered for all technological steps and is thus separated from the groups which are offered for specified use. The unwaxed control (No. 1.) is grouped away from the treated tests.
Considering that the varieties were grafted on different rootstocks, the final success on average, including treated and untreated samples, is much higher with 'Kadarka' (73.9 %) than with 'Pintes' (52.7 %).

CONCLUSION

Based on our results, it has been demonstrated that using different types of paraffin for waxing grapevine grafts during callusing and in the nursery, revealing that waxed grafts have a higher success rate compared to those that are unwaxed. As typically reflected by the untreated 'Pintes', if there is any environmental stress factor (temperature, humidity), there can be a significant loss in the number of surviving grafts and buds.
Callus formation of the grafting union after the forcing

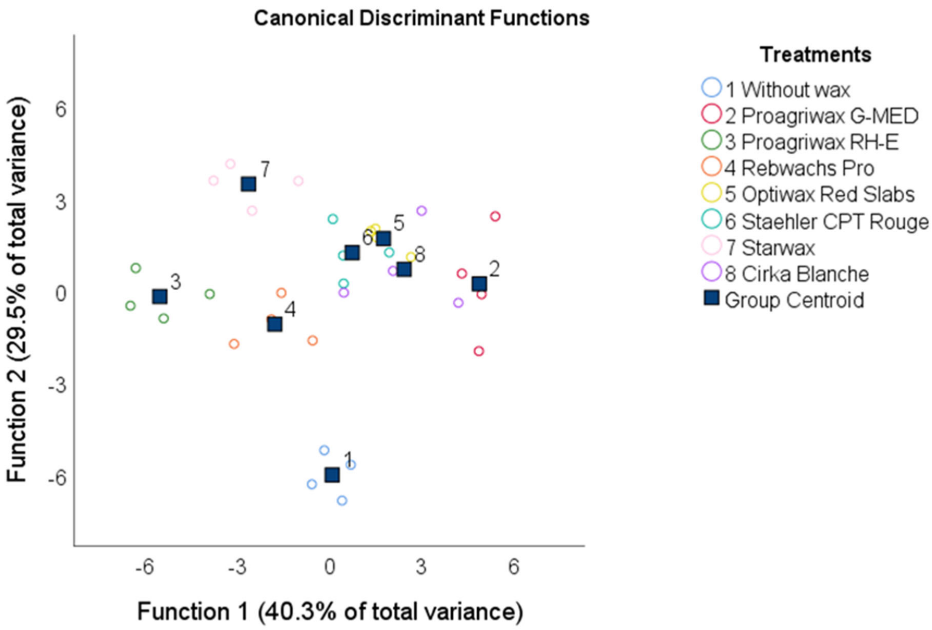

Fig. 1: Discriminant analysis of each parameter with both varieties

period is the key to produce successful grapevine grafts but in our study there is no significant correlation between callus formation and final success rate after nursery. In case of the callus stimulating paraffins after the forcing period, Proagriwax RH-E and Rebwachs Pro yielded the best results concerning callusing at the grafting union. Although the grafting success in the early nursery period appeared to be high with these paraffins, at the time of collection in autumn it was much lower. Thus, paraffin products with callus development additive should be selected for callusing only.

Besides this, Proagriwax G-Mediterranean is the only authorized paraffin product in Hungary, and this presented good uniform results with both tested varieties.

The best paraffin wax of this trial was Starwax yielding an outstanding final success (in 'Pintes' 72 %, in 'Kadarka' 90 %). This product gave the lowest drop-back between pre- and final success. It can thus be deduced that the main purpose of Starwax is to protect the join of the young grafts effectively in the nursery, but according to our observations it delays bud break at the beginning of the nursery period. This product contains micro-particles of light metals, which reflect UV rays. So, it therefore has a special effect in order to protect callus tissue from overheat and sunburn in the nursery environment.

It can be concluded that choosing the correct paraffin wax and using it according to the technological steps can increase the final success rate of the grafts. The producers highlight the special regulations and instructions, which must be clarified before the use of the product. Consequently, further studies are needed to determine the influence of waxing technology on other varieties.

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