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ADVANCES IN PROPAGATION OF GRAPEVINE IN THE WORLD[1]

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ABSTRACT - Grapevine production by classical grafting methods and in commercial scale emerged over 130 years. This system remained handmade until the mid-1950s, when emerged the first international certification programs aimed at obtaining mother plants with high viral sanity. The necessity to increase the scale of production on industrial model and plant material production based on minimum morphological standards appeared at the end of the 1960s. Along the 1970s, research unlocked knowledge on semi-automated grafting, process hygiene, use of plant growth regulators and understanding of physiological events of rootstock-scion compatibility, callus formation and rooting. So, until the mid-2000s, certification schemes and propagation processes advanced little in technical standard. However, grapevine growing areas were expanded and demands for plant material increased, and new diseases emerged from contaminated nurseries. These new diseases (new viral complexes, phytoplasmas, bacteria and grapevine trunk diseases) were discovered by high-sensitivity diagnostic methods. Today, there is a new discussion on the nursery segment worldwide. The propagation techniques have been reviewed from the perspective of reducing the incidence of new diseases and minimum physiological damage of nursery plants during the production stages. Therefore, technological innovations regarding equipment, practices and production inputs have been incorporated in new certification schemes. However, despite these advantages, these schemes have become more complex and multidisciplinary than previous ones, bringing difficulties in adaptation of nurserymen.
Index-terms: *Vitis* grafting, nursery, certification, grapevine cuttings.

AVANÇOS OBSERVADOS NA PROPAGAÇÃO DE VIDEIRAS NO MUNDO

RESUMO – A propagação de videiras a partir dos métodos clássicos de enxertia e em escala comercial teve origem há mais de 130 anos. Este sistema permaneceu artesanal até meados da década de 1950, quando se iniciaram os primeiros programas internacionais de certificação com foco na obtenção de plantas básicas com elevada sanidade para vírus. No fim da década de 1960, surgiu a necessidade de aumentar a escala para produção em um modelo industrial em que a muda apresentasse um padrão morfológico mínimo. Ao longo da década de 1970, aprofundaram-se as pesquisas relacionadas à automatização da prática de enxertia, à higienização do processo, ao uso de reguladores de crescimento e ao entendimento dos eventos fisiológicos da compatibilidade entre enxerto e porta-enxerto, formação de calos e enraizamentos. Assim, até meados dos anos 2000, os esquemas de certificação e o processo de propagação pouco evoluíram em termos técnicos. Porém, a medida que a área vitícola foi expandindo e a demanda por mudas aumentando, verificou-se que novas doenças se alastravam em escala global a partir de viveiros contaminados. Estas novas doenças (complexos virais, fitoplasmas, bactérias e fungos causadores de podridões vasculares) foram sendo descobertas à medida que os métodos de diagnose avançaram em sensibilidade de detecção. Hoje, surge nova discussão no segmento viveirista mundial fundamentada no fato de que o processo de propagação está sendo revisto sob o foco da redução de incidência das novas pragas e mínimo dano à muda ao longo das etapas da produção. Surgem, assim, inovações tecnológicas, tanto em equipamentos quanto em práticas e insumos, sendo incorporadas aos novos modelos de certificação. Mas, se por um lado, estes esquemas tornam-se cada vez mais multidisciplinares, por outro, a complexidade gerada pode trazer dificuldades para a adesão pelos viveiristas.
Termos para indexação: *Vitis* enxertia, viveiro, certificação, mudas de videira.

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INTRODUCTION

The new scenario of grapevine propagation

The history of world viticulture is closely linked to the production of propagating materials. From the late seventeenth century, the epidemic of the root aphid known as phylloxera (*Daktulosphaira vitifoliae*) spread throughout Europe, decimating vineyards of varieties of *Vitis vinifera* species, which were commonly planted without the use of rootstocks (RILEY, 1891). Thus, growers started importing rootstocks of American origin resistant to this disease, and from this event, the vegetative propagation of grapevine by grafting became part of the modern viticulture activity (SPOERR, 1902). Initially, the process evolved in the technique, seeking higher yield in number of viable cuttings, resulting in automation by table grafting (ALLEY, 1957); the adjustment of paraffin types for grafting; the application of stratification for callus formation; storage of cold propagating material; the use of plant growth regulators for the formation of roots and calluses (ALLEY; PETERSON, 1977) and basic measures of process hygiene (BECKER; HILLER, 1977).

In a second stage, the process evolved to the phytosanitary quality, as several diseases, especially those caused by viruses were transmitted to cuttings from mother plants. Official entities responsible for obtaining, cleaning and availability of foundation plant stocks of high phytosanitary quality for nurseries were founded in several wine countries (MARTELLI, 1999). Certification schemes standardize quality protocols to obtain cuttings; however, there is no unified standard for these schemes. Some of them check the presence of viruses in materials produced in basic stocks, which is the case of EPPO Certification, which regulates the certification of agricultural crops in the European Union and is applied in countries such as Germany, Portugal, Spain and Italy (OEPP, 2008). Other countries have broader programs, with verification protocols and technical recommendations from the production of nuclear material to commercial nurseries. This is the case of APFIP in Australia, PPECP in Canada, Entav-Inra in France, FPS in the United States and Vine Improvement Scheme in terms of the Plant Improvement Act in South Africa. In Brazil, the application of the certification term in grapevine fields awaits official regulation of technical conformity standards (ALMEIDA, 2002). The nurseryman only needs to comply with minimum legal criteria for the commercialization of production, and there is no official control of the phytosanitary quality (BRASIL, 2004).

Currently, regardless of whether the scheme is certified or not, there is a growing movement of technical transformation of the nursery segment on a global scale. Although large gains have been obtained with current schemes, there is an urgent need to innovate them against new phytosanitary threats (FILO et al., 2013). In recent years, new diseases have been introduced in mother plants and widely disseminated among producing regions from propagation materials (GRAMAJE; DI MARCO, 2015). Due to little consideration in the current certification schemes, these diseases have generated a new stage for the advancement of technologies in propagation of grapevine cuttings (FILO et al., 2013).

New diseases transmitted by vegetative propagation

Traditionally, the focus of phytosanitary quality of mother plants lies on virus detection. Grapevine species and hybrids used as rootstocks are hosts of about 65 viruses, eight viroids and 13 phytoplasmas. This amount increases frequently due to the continuous development of techniques for the detection of these pathogens, especially next generation sequencing (NGS) (MARTELLI, 2014; ROOSSINCK et al., 2015).

The following are among the several reasons why grapevine is so affected by these pathogens: it is inherently susceptible to several pathogens; it is cultivated in different geographic regions and under different environmental conditions and is vegetatively propagated, which is the main way of transmission of viruses, viroids and phytoplasmas. As a consequence, grapevine is able to acquire, maintain and accumulate such infectious agents, perpetuating them during the vegetative cycle. In addition, some insects and nematodes are efficient natural vectors of certain viruses and phytoplasmas, making the situation even more complex. Despite this context, grapevine remains one of the most important fruit crops in the world (FIORE, 2015).

The four main grapevine viruses in the world are grapevine leafroll, rugose wood complex, fanleaf degeneration, and fleck disease due to the economic importance and incidence that they present in several grape-growing countries (MARTELLI et al., 2007; MAREE et al., 2013; BASSO et al., 2014; NAIDU et al., 2015). However, recently, some new viruses have been discovered in grapevines and quickly became known as “emerging viruses” for the expression and relevance they have acquired in the world (MARTELLI; SALDARELLI, 2015). Some examples are *Grapevine Syrah virus 1* (GSyV-1)

associated with the *Syrah decline* (AL RWAHNIH et al., 2009); *Grapevine red blotch-associated virus* (GRBaV), which causes the red blotch disease (SUDARSHANA et al., 2015); *Grapevine vein clearing virus* (GVCV), which causes "vein clearing" (ZHANG et al., 2011) and *Grapevine Pinot gris virus* (GPGV), which causes chlorotic mottling and leaf deformation (SALDARELLI et al., 2015).

However, the criteria of phytosanitary quality in mother plants and cuttings need revision due to the increase in the incidence of fungi causing trunk diseases that cause the decline and death of young vineyards (STAMP, 2001). In a survey carried out in 146 nurseries in Europe, Gramaje and Di Marco (2015) verified that in 25% of vineyards, the replanting of mother plants younger than 10 years was carried out due to the occurrence of this decline. Likewise, Liminana et al. (2009) had already pointed out that even mother plants with healthy external appearance had internal necrotic lesions. These findings are examples that some of these fungi can release spores in the air, presenting potential for infection through the wounds that occur in mother plants (LARIGNON; DUBOS, 2000; ROONEY-LATHAM et al., 2005; GRAMAJE, ARMENGOL, 2011; BALOYI et al., 2013). For this reason, since the year 2000, a group of researchers has sought to redesign the process of cutting production with a focus on protecting against fungi that cause trunk diseases.

The pathogens associated with this complex have as main representatives: Esca, caused by *Phaeomoniella chlamydospora, Phaeoacremonium* spp. and *Fomitiporia* spp.; Petri Disease, caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.; Botryosphaeria dieback and canker, caused by Botryosphaeriaceae fungi; Eutypa dieback, caused by *Eutypa lata* and Black foot disease, caused by *Cylindrocarpon* spp., *Campylocarpon* spp., *Dactylonectria* and *Ilyonectria* spp. (LARIGNON; DUBOS, 2000; VICENT et al., 2001; HALLEEN et al., 2004; AROCA et al., 2006; HALLEEN et al., 2006a; HALLEEN et al., 2006b; MOSTERT et al., 2006; ZANZOTTO et al., 2007; REGO et al., 2009; ÚRBEZ-TORRES et al., 2012; BERTSCH et al., 2013; BILLONES-BAAIJENS et al., 2013; WHITELAW-WECKERT et al., 2013; LOMBARD et al., 2014). In addition to these species, new genera have been continuously reported in literature. HALLEEN et al. (2007) conducted pathogenicity studies in nurseries and vineyards with declining plants and found that symptomatic plants were contaminated by *Cadophora luteo-olivaceae*, *Phialemonium* cf. *curvatum* and *Pleurostomophora richardsiae*, whereas Moyo et al. (2014) identified species of arthropods also acting in the transmission of trunk diseases.

In addition to viruses and trunk diseases, bacterial diseases are another phytopathogenic group that can infect host plants and be transmitted via propagative material. In Brazil, there is occurrence of bacterial canker caused by *Xanthomonas campetris* pv. *viticola*. Its occurrence has already been reported in Pernambuco, Bahia, Piauí, Ceará, Roraima and São Paulo (RODRIGUES NETO et al., 2011). The main measure for the control of this bacterium is to avoid its entry into areas where the disease does not occur and, if necessary, to eliminate the inoculum present in contaminated plants (SILVA et al., 2012). Another bacterial disease is Pierce's disease, caused by *Xylella fastidiosa*, which is very important in grapevines in countries such as the United States, Mexico, Costa Rica, Venezuela and Chile, causing loss of quality and quantity of grapes, as well as the death of plants. Care for this disease should be at the time of introduction of vegetative vine materials in the country, and legal measures must be followed (KUHN, 2006).

Advances observed in phytosanitary diagnostic methods

Obtaining the mother plant is one of the most critical stages in the process of cutting production. Its sanitary condition will define the quality of cuttings over the years. Diagnosis is a fundamental step, together with epidemiological studies for the implementation of effective control of diseases caused by viruses, viroids and, recently, phytoplasmas, bacteria and fungi related to trunk diseases. Field observation is the first step in the phytosanitary checking process. However, latent infections and the presence of nonspecific symptoms, that is, symptoms common to several diseases, not necessarily caused by a specific pathogen, are frequent (FIORE, 2015).

In the case of the diagnosis of viruses, viroids and phytoplasmas, technology has evolved over the years, starting with biological indexing, followed by serological and molecular tests. Biological indexing by mechanical transmission of the pathogen to herbaceous hosts or graft indexing in woody indicators is the oldest diagnostic technique in use, although still important, especially due to its high sensitivity and in cases of diagnosis of unknown diseases. However, serology-based diagnosis has enzyme-linked immunosorbent assay (ELISA) as the most commonly used test. Other techniques are based on the detection of pathogenic nucleic acids,

such as molecular hybridization and polymerase chain reaction (PCR) with its "conventional" type variants, in which the results are visualized on gels after electrophoresis or "real-time or quantitative" type (AL RWAHNIH et al., 2012, DUBIELA et al., 2013, OSMAN et al., 2013).

Molecular diagnosis has advanced significantly in recent years. The restriction in the use of a certain molecular test is due to the fact that it is only directed to the detection of pathogens with known nucleotide sequences and to some pathogens that are targets of the reaction. This makes it impossible to detect pathogens with unknown nucleotide sequence or that are not pre-established targets of detection. These two restrictions are overcome when using Next Generation Sequencing (NGS). This technique allows identifying all viruses, viroids and phytoplasmas present in a plant tissue sample (AL RWAHNIH et al., 2015). With the continuous improvement of this technique, there is a permanent expansion of knowledge about the viroma (viral population) present in grapevines (BURGER; MAREE, 2015). Thus, the generated information can be used to define better and more efficient virus control and management strategies. However, the relatively high cost still does not allow the use of NGS as a routine technique, but it is economically feasible, for example, when compared to the costs of biological indexing (considering that for some pathogens there is a need to wait up to two years for the final result) and considering that biological tests are needed to incorporate selected mother plants in certification programs. In relation to quarantine controls, the NGS technique also allows reducing costs aiming the introduction of a new genetic material in a country (GIAMPETRUZZI et al., 2015). For the implementation of this new technique, it is essential to have support from the field of bioinformatics allied to the executor laboratory, as it will be necessary to process and analyze a large number of nucleotide sequences (WU et al., 2015).

Currently, the classical approach to phytosanitary testing of the mother plant based only on the presence of viruses has been reviewed, as studies have shown that fungi that cause trunk diseases are also transmitted to cuttings through these plants (GRAMAJE; ARMENGOL, 2011). For the diagnosis of fungi, the availability of tools for detection is the key factor and, in recent years, it is the area that has most advanced. Some phytopathogens such as *Plasmopara viticola*, *Botrytis cinerea* and *Elsinoe ampelina* are easy to detect as it is possible to verify typical visual symptoms. For trunk diseases, symptoms are not so obvious and detection in asymptomatic propagation material may be critical. Currently, the most widely used method for detection is isolation in culture medium. It is a slow method and usually presents difficulty in identification. Molecular diagnosis, as a faster and more efficient tool, using DNA extraction and specific tools is an alternative.

Conventional PCR, nested-PCR and real time-PCR are being developed for the identification and detection of the causal agents of Black foot disease, Petri disease and Botryosphaeriaceae species in plant tissues, water and soil samples obtained from nurseries and vineyards. In some cases, specific primers are designed to identify one or a few species (ALANIZ et al., 2009). However, in other cases, tools that allow the simultaneous identification of the largest possible number of species have been developed. Weir and Graham (2009), using t-RFLP were successful in the differentiation of *Cylindrocarpon, Eutypa, Botryosphaeria, Phaeomoniella* and *Phaeoacremonium* species. It is noteworthy that these techniques also require care. PCR inhibitors may be present in samples, mainly in lignified tissues, limiting the use of this method (GRAMAJE; ARMENGOL, 2011). Lummerzheim et al. (2009) tested multiplex-PCR from fungi-extracted DNA for the simultaneous identification of *Botryosphaeria dothidea*, *Diplodia seriata, Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora* and did not obtain conclusive results.

Advances observed in the management of new diseases

Procedures to control viruses, viroids and phytoplasmas in the process of cutting production are exclusively based on the prevention of infection. The use of tested propagation material, free of major diseases, and preferably from a clonal and phytosanitary selection program that includes clonal cleaning by thermotherapy and / or meristem culture is recommended (MALIOGKA et al., 2009). Currently, Brazil does not yet have an official certification system for grapevine cuttings (BRASIL, 2004), similar to those in some countries of the European Union (Italy, France, etc.), as well as in countries such as the USA, Argentina, Chile, and South Africa. These countries have official certification standards defining levels of tolerance for the presence of pathogens, especially viruses in grapevine planting materials according to categories (MALIOGKA et al., 2015).

Another option, not yet commercially available for grapevine, is the use of plants whose resistance has been incorporated as a result of

genetic transformation, considering the absence of sources of natural genetic resistance to viruses in grapevines (LAIMER et al., 2009; OLIVER; FUCHS, 2011). In addition, the control of vectors (nematodes, mealybugs such as *Pseudococcidae*, etc.) and weeds (hosts of pathogens and vectors) contribute to reduce the spread of viruses, viroids and phytoplasmas, together with the adoption of effective quarantine measures able to prevent the introduction of pathogens that are not yet present in a country or that are restricted to demarcated regions of territory (TSAI et al., 2010; ALMEIDA et al., 2013).

In the case of fungi related to trunk diseases, the procedure of recommended control is systemic to the entire production process of the cuttings with critical points of possible sources of inoculum. Thus, in the management of mother blocks, the need to protect wounds caused to plants after pruning of propagation material has been emphasized (VAN NIEKERK et al., 2011). The susceptibility of these wounds to trunk diseases varies from four weeks to more than four months, depending on the pathogen, the cultivar, the region and the climatic conditions (SERRA et al., 2008). Currently, the recommendation to protect the wound with chemical or biological fungicides (DI MARCO et al., 2004; FOURIE; HALLEEN, 2006; HALLEEN et al., 2010) is unanimous. Mutawila et al. (2016a) recommend the application of *Trichoderma* after six hours from the end of sap exudation from cuts and Kotze et al. (2011) observed that the use of *Trichoderma* showed better results than the use of chemicals. Mutawila et al. (2016b) found that the 6-pentyl-a-pyrone (6PP) component produced by *Trichoderma harzianum* and *Trichoderma atroviride* was shown to inhibit trunk pathogens. In addition, the cleaning of tools is another practice that significantly reduces the infection of plant pathogens in mother plants. Agustí-Brisach et al. (2011) observed that the transmission of *Phaeomoniella chlamydospora, Cadophora luteo-olivacea, Diplodia seriata, Eutypa lata* and *Phaeoacremonium aleophilum* decreased after disinfection of pruning instruments.

After the entry of the propagation material into the nursery, there are a number of practices that are performed. Hydration of the plant material is widely recommended (WAITE et al., 2015). However, Gramaje and Di Marco (2015), in a survey conducted in 146 nurseries in Europe, pointed out that hydration was performed prior to cold storage, but that the hydration time was variable, from one hour to more than 24 hours. Different species of trunk fungi have been detected in this water, and contamination can come from the plant material that brings the inoculum from the field or by the poor hygiene of tanks (RETIEF et al., 2006; AROCA et al., 2010; AGUSTÍ-BRISACH et al., 2011; WAITE et al., 2013). Gramaje and Di Marco (2015) also observed that the use of chemicals in hydration varied among nurseries. When used, they were fungicides based on iprodione and 8-hydroxyquinoline sulfate or biocides based on chlorine dioxide.

The place of cuttings production, when not disinfected, is related to fungal infection. The cold chamber, where temperatures range from 2ºC to 6ºC and humidity around 90%, is the ideal environment for the development of fungi adapted to cooler conditions such as *Penicillium* spp. (GRAMAJE; ARMENGOL, 2011). In stratification, higher temperatures (27ºC to 29ºC) and humidity above 90% favor the development of *Botrytis cinerea*. Aroca et al. (2010) found viable *Phaeoacremonium* spp., *Phaeomoniella chlamydospora* and *Cadophoraluteo-olivaceae* propagules in pruning shears, grafting machines and peat used for callus formation. Likewise, Retief et al. (2006) verified that the increase in the incidence of *Phaeomoniella chlamydospora* at the rooting site occurred due to contamination in the pre-storage hydration phase. Waite and Morton (2007) verified that contamination at this stage was derived from fungal structures from mother plants.

Rooting is the last step of the process of cuttings production. When in the field, a practice performed in certain regions is grounding the grafting point for protection against severe cold or maintenance of the grafting moisture. However, this can drastically increase the incidence of fungi such as *Cylindrocarpon* spp. (HALLEEN et al., 2003; FOURIE; HALLEEN, 2006). Also, the planting of cutting with incomplete formation of the basal heal callus exposes xylem tissues to colonization by fungi present in the rooting beds, especially Black foot disease (HALLEEN et al., 2003; DÍAZ et al., 2009). The management of sprinkler irrigation used in the production of cuttings by table grafting to increase the grafting union may favor the release of fungal spores causing trunk diseases, in addition to other shoot diseases (GRAMAJE; DI MARCO, 2015). Regarding the control of invasive species, Agustí-Brisach et al. (2011) observed that several plant species were potential hosts of fungi of trunk diseases such as Black foot and Petri diseases.

Finally, the phytosanitary quality of cuttings, in general, is represented by their morphological conformity at the time of marketing. According to Díaz et al. (2009), deficient processes that include inhibition of basal callus formation, decreased root emission, poor formation of the grafting callus,

grafting failure and symptoms of incompatibility result in cutting highly susceptible to several fungi in future vineyards.

Regarding the use of fungicides throughout the process of cuttings production, although widespread, its use in the management of trunk diseases is recent. Among the chemical fungicides reported in literature, the most used in nurseries worldwide are: 8-hydroxyquinoline sulfate, thiophanate-methyl, captan, mancozeb, thiram, benomyl, didecyldimethylammonium chloride (FOURIE; HALLEEN, 2006). However, the performance of each product depends on how it is used in the process. For example, for the control of Botryosphaeriaceae fungi, fungi causing Black foot and Petri diseases, carbendazim and thiophanate-methyl have the potential to reduce infections in rooting beds (FOURIE; HALLEEN, 2006; REGO et al., 2006; GRAMAJE et al., 2009; ALANIZ et al., 2011; BILLONES-BAAIJENS et al., 2013). The use of thiophanate-methyl and thiram is efficient to reduce infections by *Phaeomoniella chlamydospora* and *Phaeacremonium minimum* when used in different stages of cutting hydration (KUN; KOCSIS, 2014).

However, the use of biological control agents to reduce trunk diseases has increased significantly in recent years (GRAMAJE; DI MARCO, 2015). *Trichoderma* species have been used to reduce the incidence of *Phaeomoniella chlamydospora, Phaeacremonium* spp*., Eutypa lata, Phomopsis viticola, Lasiodiplodia theobromae, Neofusicoccum australe, Neofusicoccum parvum* and *Diplodia seriata* (KOTZE et al., 2011; MUTAWILA et al., 2011). This control agent is used in the immersion process of whole cuttings, roots and basal ends of cuttings and whole grafted cuttings (FOURIE; HALLEEN, 2006; DI MARCO; OSTI, 2007; MOUNIER et al., 2014). Pertot et al. (2016) tested the use of *Trichoderma atroviride* at different stages of the grafting process. The best results were obtained in the pre-storage, stratification and pre-planting hydration stages. Fourie and Halleen (2002) applied *Trichoderma harzianum* for 5 seconds before grafting, after grafting and monthly during rooting, obtaining 41.7% more root mass. Another option currently studied is the combination of chemical (short-term effect) with biological fungicides (long-term effect). Mutawila et al. (2015) generated benzimidazole-resistant *Trichoderma atroviride* mutants through the use of gamma radiation. When tested in vineyard field trials, these mutants were successful in protection against *Phaeomoniella chlamydospora*. Another group of microorganisms being tested are mycorrhizal fungus. Grapevine roots inoculated with *Glomus intraradices* presented lower number of root lesions and lower severity by fungi causing Black foot disease (PETIT; GUBLER, 2006). Similarly, Jones et al. (2014) observed that the inoculation of *Acaulospora laevis* and *Funneliformis mosseae* decreased the susceptibility of three grapevine rootstocks to *Ilyonectria* spp.

Another phytosanitary practice that has been validated in recent years is treatment with hot water (HWT) for the control of fungi of trunk diseases (FOURIE; HALLEEN, 2006; WAITE; MORTON, 2007; GRAMAJE et al., 2009). Basically, HWT is performed in two periods: before grafting (FOURIE; HALLEEN, 2004a), and after collection of cuttings from the nursery and before planting in the vineyard (HALLEEN et al., 2007). Gramaje et al. (2009) observed that the mycelial growth of *Cadophora luteo-olivaceae* isolates was not viable from 50ºC to 54ºC for 30 minutes, but *Cylindrocarpon liriodendri* and *Cylindrocarpon macrodidymum* isolates were already unviable from 43ºC to 47ºC. However, in some situations, the treated material may present failure in budding, root formation and sealing of the grafting site due to physiological damage caused by high temperatures (GRAMAJE et al., 2009; WAITE; MORTON, 2007). For example, in Spain, a protocol of 53ºC for 30 minutes has been used; however, these temperatures have caused damage to budding of traditional grape cultivars used in New Zealand (GRAHAM, 2007; BILLONES-BAAIJENS et al., 2015). This variability of recommendations and the fact that the treatment does not present efficiency against 100% of fungi (GRAMAJE et al., 2010) have made the use of this technology not widespread and unpopular.

Advances observed in propagation physiology

The knowledge of the physiological effects involved in grapevine propagation, such as hormonal balance in grafting events has already been explored and reviewed (ALONI et al., 2010). However, studies related to the biochemical and molecular understanding of the different stages of the technique in interaction with the production environment have been carried out. For example, overcoming the incompatibility between rootstock and grafting is derived from the understanding of cytological events that involve the formation of vascular connections and plasmodesmas during grafting (PINA; ERREA, 2005). Similarly, Cookson et al. (2013) characterized at molecular level the sequence of hormonal signaling events related to the rootstock / grafting relationship, observing that the expression of this signalization is

differentiated in time and specific for the rootstock, the grafting and the grafting interface.

These advances have allowed us understanding how the stages of cutting production are closely related throughout the entire process and cannot be considered in isolation. Todik et al. (2005) monitored the hormonal balance between auxin and cytokinin throughout grafting and verified positive effects on the use of paclobutrazol and chlormequat still in the vegetative growth stage of mother plants, increasing the number of first-class cuttings after stratification. Analyzing the relationship between zinc and auxin synthesis throughout grafting, Somkuwar et al. (2013) increased the uniformity and the amount of roots after the addition of the micronutrient in mother plants of rootstocks. Another advance derived from the understanding of interactions is the association or refinement of already consolidated techniques, seeking to maximize the results that would be obtained in isolation. Gökbayrak et al. (2010) obtained large numbers of roots in rootstocks of 41B after 24 hours of immersion in water followed by exogenous application of AIB for 20 seconds before planting. Corbean et al. (2009) compared different hormones added to grafting paraffin and obtained 91% of grafts on 12 days of stratification using 8-quinolinol and Dobrei et al. (2013) reduced cuttings mortality from differentiated stratification temperature management (gradual reduction from 28°C to 26°C).

The quantification of the damage caused by the environment (especially climate) and by the process on the propagation material has also influenced the definition of the most specific techniques (HUNTER et al., 2003). Works relating damage to the plant material from its origin with loss of physiological vigor throughout the process are increasingly frequent. Currently, loss of physiological vigor has been related to the increase of the phenomenon of "abnormal grafting" (HUNTER et al., 2013). This phenomenon has its initial symptoms still in the nursery, with the typical graft thickening (GARDIMAN et al., 2007). However, plant death usually occurs only after planting. Thus, a number of studies on the physiological quality indicators have been conducted in recent years (HUNTER et al., 2003) such as: accumulation of carbohydrates, phenolic substances, nitrogenous components and anti-oxidant enzymes. These indicators are used to monitor the transport of nutrients and water (BAVARESCO; LOVISOLO, 2000) or pathogens (GAMBETTA et al., 2009) in xylem and phloem throughout the process of cutting production. After multicriteria analysis, Hunter et al. (2013) tested different rootstocks, stratification methods, soil types and irrigation methods and verified that there are macro-and micro-factors determining the damage to the mother plant and to the propagation material throughout grafting. According to these authors, the major consequence of stress was the poor formation of intervascular connections of the graft, resulting in the activation of the incompatibility or abnormality mechanism.

The effect of damage on cutting formation has been considered in the field and process scale. Popescu et al. (2014) characterized mother plants in France and Romania and concluded that the climatic and soil conditions of each site were determinant in the accumulation of sugars and carbohydrates for the same clones. At the process level, Iliescu et al. (2012) observed that when the carbohydrate content of the plant material was below 12%, it became unviable for cutting production. These authors verified that the prolonged use of hydration in pre-planting for more than 24 hours resulted in the reduction of carbohydrates and, therefore, a higher percentage of dead cuttings. A similar situation was observed by Gramaje and Di Marco (2015), who verified extreme oxygen reductions in sealed packages to avoid moisture loss of the propagation material stored in cold chamber. Up to certain levels, reduction of oxygen levels stimulates budding; however, at very low levels, there is production of toxic substances, leading to loss of physiological quality of the material.

From the physiological understand of cutting process, it has been verified that common practices are disregarded by nurserymen, but present a high impact on the physiological quality of plant material. For example, in a survey conducted by Gramaje and Di Marco (2015), 51% of nurseries took up to 4 hours between collection and arrival at the nursery. The permanence of the propagating material in transit for excessive time resulted in greater dehydration and risks of contamination by pathogens.

Advances observed in certification schemes

The need to reduce the physiological damage of the propagating material throughout propagation and the tendency of the combined use of technologies for the prevention of trunk diseases at different stages has evidenced shown the urgent need of evolution of certification schemes. Halleen and Fourie (2016) proposed an integrated management strategy focusing on trunk disease management for South African nurseries. Initially, plant material collected from mother blocks is disinfested in a broad-spectrum fungicidal solution such as benomyl or carbendazim before cold storage. Prior

to grafting, the material is submitted to HWT (50°C for 30 minutes) and then cooled by immersion in a solution of water with didecyldimethylammonium chloride for 30 minutes. After grafting, the material is immersed for one minute in a *Trichoderma* solution prior to placement in stratification boxes, which is carried out before planting. In the nursery, cuttings are kept free from foliar diseases, nematodes, soil diseases, etc. in order to reduce stress. Finally, before marketing, cuttings are again submitted to HWT in order to eradicate soil diseases such as black foot, *Pythium* and *Phytophthora* that may eventually have infected them. This process was validated by the authors and proved to be highly efficient with significant improvement of the phytosanitary quality of cuttings produced.

With regard to transfer and control of clonal plants that compose mother blocks, new business models are being designed. In countries where there is official certification, there is an appreciation for the preservation of local clones, restricted importation of plant materials and guarantee of intellectual property. In this way, business models have sought to increase the control of the distribution of basic material to nurseries (ALLEY; GOLINO, 2000). In countries that do not have an official certification model, there is a search for alternative solutions through voluntary schemes for the constitution of healthy stocks of mother plants. For example, in Brazil, plants obtained after *in vitro* thermotherapy associated with *in vitro* cultivation were indexed by molecular method for diseases such as grapevine leafroll complex, corky bark, fanleaf degeneration, fleck disease and stem pitting. These plants are systematically transferred to nurseries after confirming their technical capacity and meeting minimum cutting propagation requirements (GROHS et al., 2015).

CONCLUSION

Although the practice of grapevine grafting originated in antiquity, with records from the beginning in the Christian era, but it was in the last 130 years that the modern technique was developed. In this period, it is possible to subdivide it into three cycles: 1) from 1900 to 1950: emphasis on the compatibility of grafting for different phylloxera-resistant rootstocks; 2) from 1950 to 2000: emphasis on standardization of the production process and programs for phytosanitary certification with a focus on viruses); 3) from the 2000s: emphasis on the diagnosis and management of new diseases, focusing on trunk diseases, in adjusting production protocols for local specificities and in new certification models.

Regarding this new cycle, recent scientific discoveries have been accompanied by innovations in automation such as: specific machinery for grafting, stratification and collection of plant material; equipment for physical phytosanitary treatments; availability of specific chemical and biological inputs for cuttings. However, the increase in the complexity of the current certification programs and production costs are disadvantages, leading to a selection of nurseries. Thus, it is expected that in the medium term, there will be a gain in quality of vineyards with greater phytosanitary status, longevity and production. However, in the short term, there will be a tendency of limitation in the capacity of meeting the demand for nurserymen, restriction in the international trade and consequent increase in the cutting cost.

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