CHEMICAL ENGINEERING TRANSACTIONS
VOL. 58, 2017

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Remigio Berruto, Pietro Catania, Mariangela Vallone

ISBN 978-88-95608-52-5; **ISSN** 2283-9216
DOI: 10.3303/CET1758034

Evaluation of Graft Success of Grapevine after Incubation Room by means of Thermographic, Electrical and Mechanical Techniques

Antonino Pisciotta*[a], Santo Orlando[a], Rosario Di Lorenzo[a], Leonardo D'Acquisto[b]

[a] Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze, Edificio 4, Ingresso H, 90128 Palermo.
[b] Dipartimento dell'Innovazione Industriale e Digitale, Università degli Studi di Palermo, Viale delle Scienze, Edificio 8, 90128 Palermo.
antonino.pisciotta@unipa.it

Grafting is an important technique for getting good yields in plant multiplication. Understanding the success of the graft after the incubation stage is important to the evaluation of the suitable grafting for the open field (nursery). Successful grafting in vines requires the development of a functional vascular system between the scion and the rootstock. The graft compatibility and its augmentation depend upon various natural factors like environment, soil conditions and protective measures. The present study examines the capability of thermographic, mechanical and electrical techniques to assess the graft quality and success after the incubation stage. The trial was carried out at Vivai Mannone, (Petrosino, Western Sicily, 37°42'26.28''N – 12°29'09.57''E). After the different tests, various grafting combinations were planted in the nursery and followed for the vegetative season. Before the evaluation procedure was performed graft unions have been subjected to a moderate heating from ambient temperature, then the thermal transient toward ambient temperature was monitored by means of a thermal imaging camera. As far as the electrical testing procedure was concerned, a sinusoidal voltage was applied through the grafts-cuttings, and the voltage attenuation at different points at increasing distances from the source was measured by an oscilloscope. The mechanical strength of the graft undergoing a controlled rate flexural loading was monitored by a PC remote controlled digital dynamometer. Experimental results show that we were able to distinguish the successful grafting only with the thermographic test. Moreover, this technique was the only non-destructive test from which it was possible to derive quantitative parameters, useful to provide successful nursery forecast. Engraftment results detected at the nursery showed a 15% error in forecast based on the proposed thermal image method, which is a satisfactory value for a feasibility study.

1. Introduction

Routine grafting of grapevine in the agricultural systems of Europe began at the end of the 19th century to combat the devastating yield losses caused by the introduction of phylloxera (*Daktulosphaira vitifoliae* Fitch), a soil-dwelling insect pest introduced from the USA (Ravaz, 1930). This practice has now been adopted in approximately 80% of vineyards world-wide (Pouget, 1990).
Despite the generalization of grafting in viticulture and the increase in vegetable grafting worldwide, little is known about the early mechanisms involved in grafting and how the structure of the graft union develops during graft union formation in any plant species. Grafting knowledge and techniques are essentially based on practical experience rather than on scientific study. The general cellular events which occur after the grafting are quite well known and common to woody and non-woody plants. Successful grafting is a complex biochemical and structural process that begins with an initial wound response, followed by callus formation, creation of a continuous cambium and the establishment of a functional vascular system between the two grafting partners (reviewed by Pina and Errea, 2005; Martinez-Ballesta et al., 2010).
The inability of the two different plants parts, when grafted together, to produce such union and the difficulties of the resulting single plant to develop satisfactorily is termed "incompatibility (Coombe et al., 1992; Hartman

Please cite this article as: Pisciotta A., Orlando S., Di Lorenzo R., D'Acquisto L., 2017, Evaluation of graft success of grapevine after incubation room by means of thermographic, electrical and mechanical techniques, Chemical Engineering Transactions, 58, 199-204
DOI: 10.3303/CET1758034

et al., 1983).Grafting success requires several developments at the graft interface such as cell recognition and communication, the initiation of cell cycle, cell proliferation, cell differentiation and plasmodesmata formation (Estrada-Luna et al., 2002; Ermel et al., 1997; Pina et al., 2009; Kollmann and Glockmann, 1991). The cellular events at the graft interface have been well characterized by histological studies in various woody plants, such as, Picea spp., apples and Prunus spp. (Weatherhead and Barnett, 1986; Soumelidou et al., 1994; Olmstead et al., 2006). To date graft union morphology has been studied with magnetic resonance imaging (MRI) in pine trees (Leszczynski et al., 2000) and in grapevine (Bahar et al., 2010). In order to study the internal structures and the 3D organization of graft zone in grapevine, X-ray tomography was applied in young vines with differing degrees of grafting success (Milien et al., 2012).
Although graft incompatibility is rare in grapevine, grafting success can present a great variability depending upon various natural factors like environment, soil conditions and protective measures (as reviewed by May, 1994 and Pisciotta et al., 2016). Grafting is an important technique for getting good yields in plant multiplication. Understanding the success of the graft after the incubation stage is important to the evaluation of the suitable grafting for the open field (nursery). The nursery production process is characterized by a partial engraftment of the grafts cuttings (normally is between 50% and 70%).This entails a significant loss of viability, as well as being due to waste in terms of semi-processed materials, labor and use of equipment and space. From these advances belong the need to develop, economic, standardized, and automated simple technologies to improve the efficiency of the production process. The present study examines the capability of thermography, mechanical and electrical techniques to assess the graft quality and success after the incubation stage, starting a multi-disciplinary approach which uses scientific expertise in the sectors of viticulture, the mechanization of agriculture and the mechanical and thermal measurements.

2. Materials and Methods

During the 2014 and 2015 vintages, three different samples each one composed by 200 cuttings (Figure 1a), coming out from three successive incubation rooms (Figure 1b) at "Mannone nursery" (Petrosino, Western Sicily, 37°42'26.28''N – 12°29'09.57''E) have been used for mechanical strength, thermal transient and electrical tests.
The results obtained were analyzed in order to predict the grafts-cuttings that would not have taken root after plantation in nursery field.

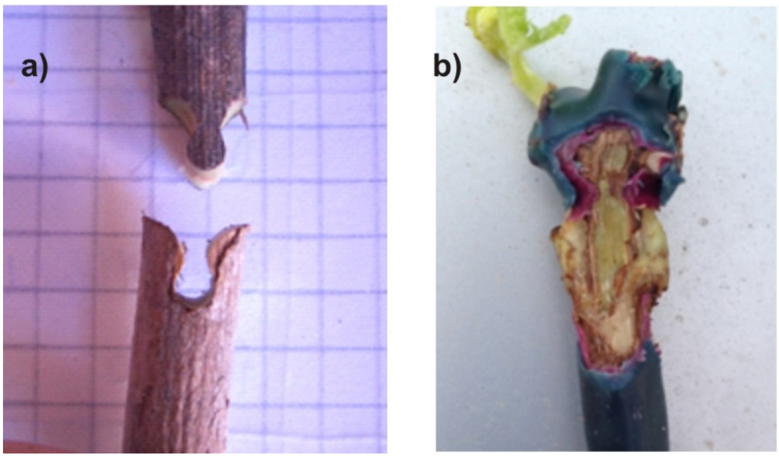

Figure 1: Detail of the Ω graft union (a) and detail of the grafting point after the callus room (b).

The protocol developed to perform the thermographic analysis consists in positioning 10 different grafting cuttings in a frame, heat them by a halogen lamp to get them to 30-32 ° C, then after removal of the heat source, let them cool back to the laboratory ambient temperature. During the two phases, the radiative flux emitted by graft-cuttings was monitored through a FLIR X6540sc camera remotely controlled by a PC equipped with a dedicated software to control the image acquisition and processing. Thermal images (Figure 2), formed by 640 x 512 pixels were acquired with a frame rate of 0.2 Hz, and on the basis of camera-subject distance was possible to obtain a spatial resolution of about 0.5 mm. So at the end of each test it was available about 9000-10000 frames that could be processed providing point by point information on the evolution of the thermal status.

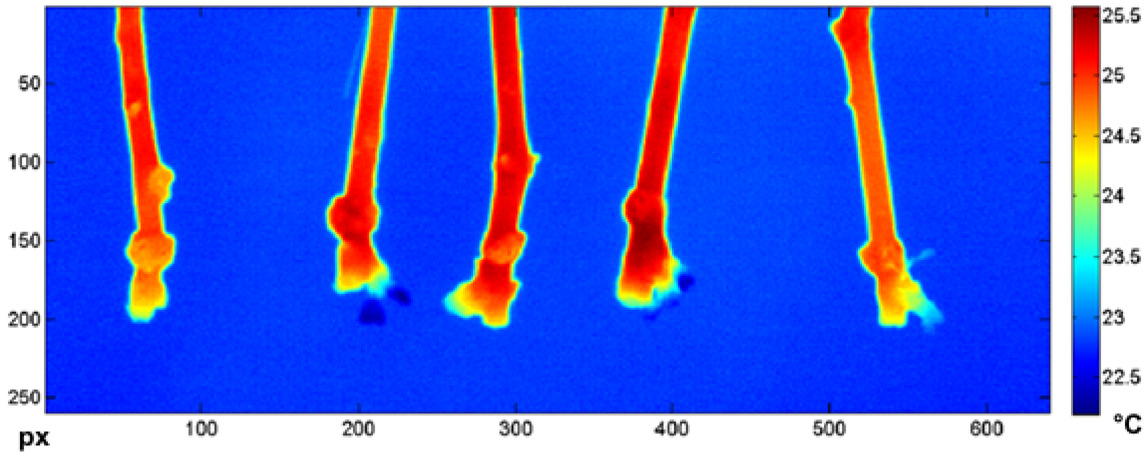

Figure 2: Sample of thermal image acquired (dimension in pixel, temperature in °C)

A different evaluation approach was based on electrical test consisted in the application of a sinusoidal voltage through the grafts-cuttings generated by a signal generator Philips PM5134, and the measurement of the voltage attenuation at different points located at increasing distances from the source by an oscilloscope (Tektronix TDS 520D). The tests have been carried out at the sinusoidal excitation voltage of 20 Vpp, at different frequencies between 18 Hz and 180kHz, either by connecting the electrodes to the graft-cuttings extremes, and then observing the longitudinal attenuation, either by entering the excitation electrodes in various diametrically opposite points and measuring at different distances from these attenuation values.
The mechanical strength was evaluated by subjecting the graft union zone to a mechanical bending test. The graft-cuttings was blocked so as to leave overhanging the engagement zone and the scion on which the load is applied. The positioning of the "specimen" was adjusted so as to always ensure the same geometry between constraints and grafting point. The tests were conducted using a IMADA Vertical Motorized Test Stand MX2-500N-L system, which allowed to impose a constant and controlled strain rate, equipped with Digital Force gauge DS2 series connected to PC equipped with acquisition software that allows to monitoring load - time history along the application of the bending displacement (Figure 3).

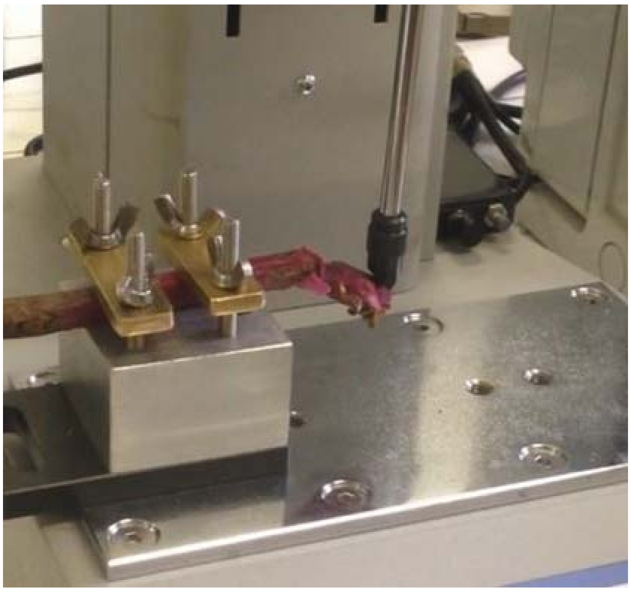

Figure 3: Mechanical strength tests

After the various non-destructive tests, the graft cuttings were planted during March-April in the nursery in open field, following the best practices adopted by the “Vivaio Mannone”. The plant distances were 6 cm in the row and 100 cm between the rows. Young vines were followed during the growing season from bud-break to November. Percentage of success was calculated on the 600 vines, two months after plantation (Figure 4). Vines with green shoots and in vegetative development were positive evaluated while vines with dry shoots were negative evaluated (dead vines).

Figure 4: Nursery overview during the vegetative season

3. Results and discussion

The processing of the cooling transient data sets acquired by means of the FLIR thermal camera enabled to compare the thermal response from different regions in the surroundings of the callus area.
Figure 5 shows the results of the thermometric analysis performed on 6 ROIs (region of interest) in two grafts.
In particular two different thermal response were observed on the heating and cooling curves recorded near the callus, mainly depending on the maturity of the callus (Figure 5). This result was congruent to tactile observations by which grafts are distinguished in a well-formed callus (hard to the touch), when the tissues forming the callus reach a good state of maturity (graft ready) and in a callus young (soft to the touch) when the tissue where not well-differentiated (not ready graft).
The advantage of thermographic technique is to reach the same assessment without requiring physical manipulation of the callus that could bring damage.
The electrical tests have not shown any systematic result: in fact, the measured voltage values do not find any correlation with the grafting success outcomes and the results obtained by the thermographic test.
The mechanical tests were carried out before the thermographic tests, in order to verify the mechanical strength of graft belonging to the different categories. The results obtained are correlated with the predictions made by the camera; graft specimen with "mature callus " showed a behavior similar to that of brittle materials, while subjects with "cold callus " had a behavior similar to that of a ductile material.
Definitely, a "mature callus " characterized by a well-woody tissues, provides a higher resistance to creep considered by instantaneous structural failure while a "cold callus", whose tissues are still under formation and differentiation process, showing greater aptitude for relative sliding of the adjacent fibers and is characterized by a more gradual break (Figure 6).
Before uprooting (two months after plantation) in the nursery and after calculation of the percentage of grafting success, the forecast error showed by the thermographic technique was only the 15%, a satisfactory value for a feasibility study (Table 1).

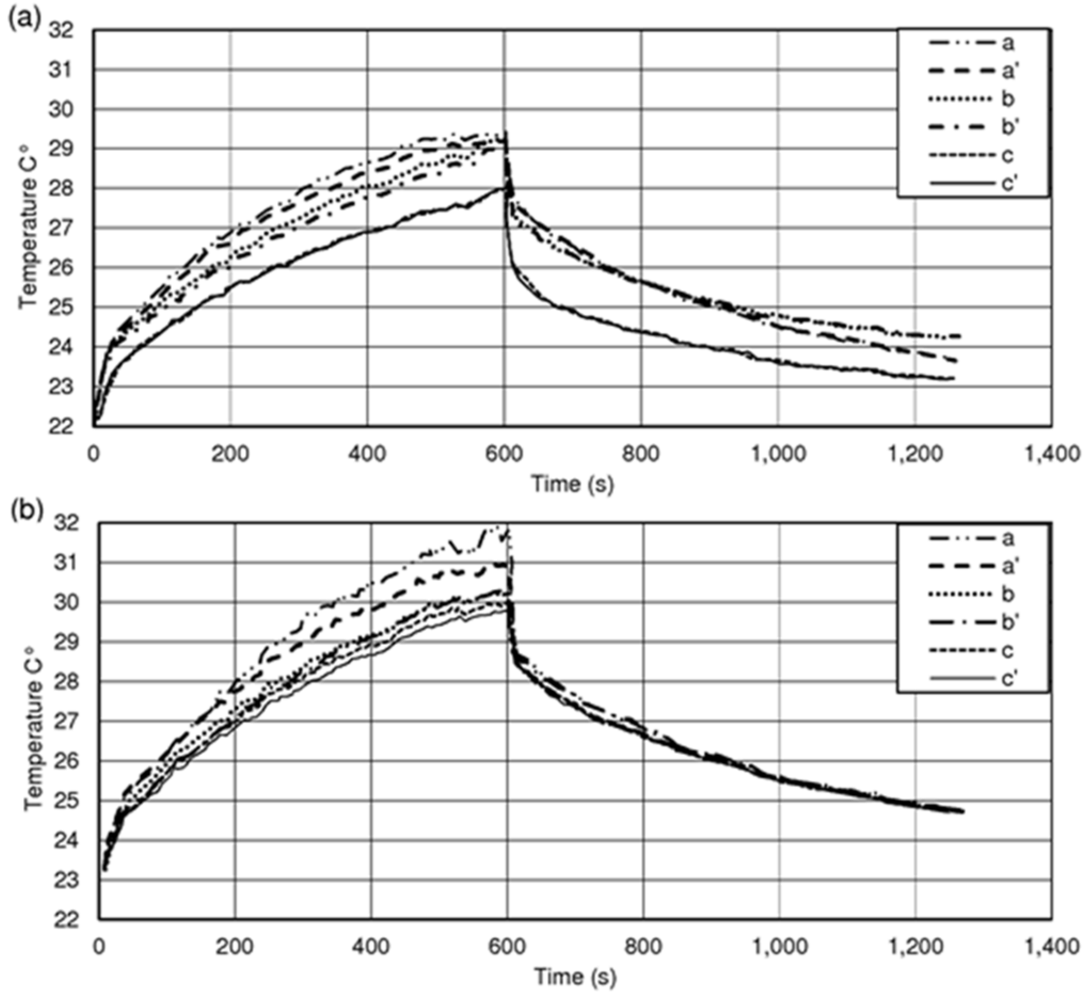

Figure 5: Curves of temperature variation in 6 ROIs near the callus: (a) "not ready" graft; (b) graft ready

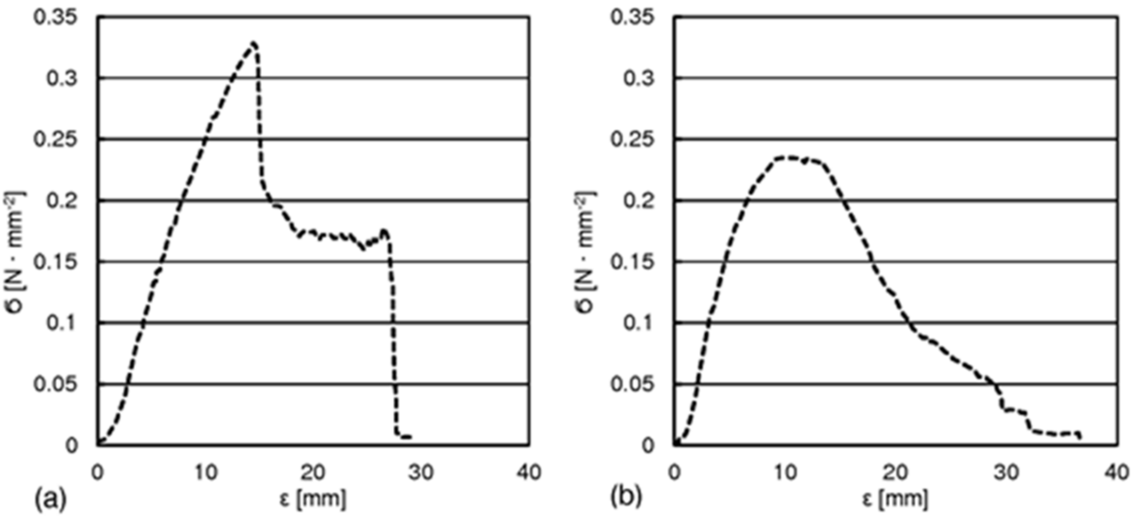

Figure 6: Three point bending loading test: I) higher initial peak, more resilient, "brittle" failure; II) lower initial peak, "ductile" failure

Table 1: Error percentage of grafting success by thermographic technique

Class	Not ready	Ready	Unclear	Total
N	94	352	64	510
Estimated failure (n)	28	54	n.a.	82
Estimated failure (%)	29.8	15.3	n.a.	16.1

4. Conclusions

Results show that we were able to distinguish the successful grafting only with the proposed thermographic method. Moreover, this technique was the only non-destructive test from which it was possible to derive quantitative parameters, useful to do the nursery successful forecast. Engraftment results detected at the nursery showed a 15% error in forecast based on the proposed thermal image method, which is a satisfactory value for a feasibility study.

Future studies are expected to eliminate the error due to the presence of lesions in paraffin, providing solutions that enable the standardization, automation and engineering of the control process.

Acknowledgments

Thanks to “Vivai Mannone” Nursery and their technicians for assistance and technical advice.

References

Bahar E., Korkutal I., Carbonneau A., Akcay G., 2010. Using magnetic resonance imaging technique (MRI) to investigate graft connection and its relation to reddening discoloration in grape leaves. J. Food Agric. Environ. 8, 293–297.

Coombe B. G. and Dry, P. R., 1992, Viticulture, Vol. 2. Practices, Winetitles, Austria.

Ermel F.F., Poessel J.L., Faurobert M., Catesson A.M., 1997. Early scion/stock junction in compatible and incompatible pear/pear and pear/quince grafts: a histo-cytological study. Ann. Bot. 79, 505–515.

Estrada-Luna A.A., Lopez-Peralta C., Cardenas-Soriano E., 2002. In vitro micrografting and the histology of graft union formation of selected species of prickly pear cactus (Opuntia spp.). Sci. Hortic. 92, 317–327.

Hartman H.T. and Kester D.E., 1983, Plant Propagation, Principles and Practices, 4th edn., Prentice Hall Inc., New Jersey, 727.

Kollmann R., Glockmann C., 1991. Studies on graft unions. 3. On the mechanism of secondary formation of plasmodesmata at the graft interface. Protoplasma 165, 71–85.

Leszczynski R., Byczkowski B., Jurga S., Korszun S., 2000. NMR microimaging studies of the union between stock and scion. Appl. Magn. Reson. 18, 147–153.

Martinez-Ballesta M.C., Alcaraz-Lopez C., Muries B., Mota-Cadenas C., Carvajal M., 2010, Physiological aspects of rootstock–scion interactions, Sci. Hortic. 127, 112–118.

May P., 1994. Using Grapevine Rootstocks – The Australian Perspective. Grape and Wine Research Corporation, Adelaide.

Milien M., Renault-Spilmont A.S., Cookson S.J., Sarrazin A., Verdeil J.L., 2012. Visualization of the 3D structure of the graft union of grapevine using X-ray tomography. Sci. Hortic. 144 (2012) 130–140.

Olmstead M.A., Lang N.S., Ewers F.W., Owens S.A., 2006. Xylem vessel anatomy of sweet cherries grafted onto dwarfing and non-dwarfing rootstocks. J. Am. Soc. Hortic. Sci. 131, 577–585.

Pina A., Errea P., 2005, A review of new advances in mechanism of graft compatibility–incompatibility, Sci. Hortic. 106, 1–11.

Pina A., Errea P., Schulz A., Martens H.E., 2009. Cell-to-cell transport through plasmodesmata in tree callus cultures. Tree Physiol. 29, 809–818.

Pisciotta A., Orlando S., Di Lorenzo R., Fazio G., Barbaccia V., D’Acquisto L., 2016. Verifica dell’attecchimento degli innesti-talea di vite dopo la forzatura mediante tecniche termografiche e meccaniche. Italus Hortus, 19, 135-136.

Pouget R., 1990, Histoire de la lutte contre le phylloxera de la vigne en France: 1968–1895, O.I.V., 157-158.

Ravaz L., 1930, De l’influence spécifique réciproque du sujet et du greffon – effets de la greffe, Extrait des Annales de l’Ecole nationale d’Agriculture de Montpelllier 20, 1–15.

Soumelidou K., Battey N.H., John P., Barnett J.R., 1994. The anatomy of the developing bud union and its relationship to dwarfing in apple. Ann. Bot. 74, 605–611.

Weatherhead I., Barnett J.R., 1986. Development and structure of unusual xylem elements during graft union formation in Picea sitchensis L. Ann. Bot. 57, 593–598.