Note

http://doi.org/10.1590/1678-992X-2022-0224
ISSN 1678-992X

SCIENTIA AGRICOLA
Crop Science

Hydraulic conductivity and native embolism of Geneva® Series apple rootstocks measured by the XYL'EM-*Plus*® embolism meter

Tâmara Foster Acosta[1]*, Paulo Celso de Mello-Farias[1], Flavio Gilberto Herter[1], Carlos Danilo Cabrera Bologna[2], Léo Omar Duarte Marques[1], Camila Schwartz Dias[1]

[1]Universidade Federal de Pelotas/Faculdade de Agronomia Eliseu Maciel, C.P. 354 – 96010-900 – Pelotas, RS – Brasil.
[2]Instituto Nacional de Investigación Agropecuaria – Estación Experimental "Wilson Ferreira Aldunate", Las Brujas, Ruta 48, km 10, Rincón del Colorado – 90200 – Canelones – Uruguay.
*Corresponding author <tamaraacosta1986@gmail.com>

Edited by: Sergio Tonetto de Freitas

Received October 26, 2022
Accepted January 25, 2023

ABSTRACT: Most commercial apple orchards are established on either Marubakaido (*Malus prunifolia* Borkh.) (high vigor), M-9 (*Malus pumila*) (low vigor), or a combination of both rootstocks through the intergrafting technique. The Geneva® Series rootstocks were developed as an alternative to orchards modernization. The vigor of rootstocks influences the anatomical xylem formation and, therefore, the canopy cultivar's hydraulic conductivity. When affected by embolism, hydraulic conductivity harms plant metabolism, reducing crop yield and fruit quality. This study aimed to evaluate four Geneva® Series rootstocks with potential use in southern Brazil, in terms of hydraulic conductivity (K) and percentage loss of conductance (PLC), during the winter period in two different years. The G.213 rootstock presented the best performance for the variables analyzed; however, higher values of xylem functionality loss were observed in G.814.
Keywords: *Malus domestica* Borkh, PLC, water content, hydraulic functioning, cavitation

Introduction

One of the most significant challenges of modern agriculture is to meet the growing demand for food, while the area allocated for crops has mostly stayed the same. In recent decades, apple orchards have shown a significant increase. Therefore, developing genotypes that combine high yield and soil and water use optimization is crucial (Reig et al., 2018; Reig et al., 2020). These genotypes allowed for the increase in plant density in commercial crops (Robinson et al., 2011).

The grafting technique is essential in pomiculture, mainly in high density systems, as it presents greater initial economic return; however, there are also risks (Autio et al., 2017). Thus, combining traits, such as resistance to biotic and abiotic stresses, has become significant (Reig et al., 2018). Besides, vigor control of the canopy cultivar and root system efficiency improves fruit quality (Fazio and Robinson, 2008; Reig et al., 2019; Reig et al., 2020). In this sense, the choice of scion cultivar, rootstock, and conduction system should meet the edaphoclimatic requirements of the producing region for an economic return (Reig et al., 2019).

Apple orchards in Brazil follow the trend of the main genotypes used worldwide, predominantly Marubakaido (*Malus prunifolia* Borkh.) and 'M-9' (*Malus pumila*) rootstocks, as well as the combination of these through the intergrafting technique. The Geneva® Series rootstocks have attributes that best meet Brazilian production's demands; some have already been introduced to the market (Denardi et al., 2017).

Water availability is a determining factor in the resumption of tissue growth in temperate species, as it influences carbohydrate mobilization during the hibernal rest phase. Many variables, including vigor, affect the olerance of rootstocks to water stress (Corso and Bonghi, 2014). In vigorous plants, the xylem is composed by cells with a larger diameter, optimizing water transport (Xu and Ediger, 2021); however, these cells are prone to cavitation and thus to embolism under water deficit conditions. Embolism impairs xylem functionality and transportation of water and solutes, affecting sprouting and buds differentiation. Assuming that the water flow in the xylem is not interrupted during winter, this study aimed to evaluate four rootstocks of Geneva® Series in terms of hydraulic conductivity and percentage loss of conductance.

Materials and Methods

Plant material and experimental design

The study was conducted during the winter cycle of plants in the years 2019 and 2021, during July, Aug, and Sept. Plant material was collected in an experimental orchard (34°40′04″ S, 56°20′27″ W, altitude 36 m), located in the district of Rincón del Colorado, department of Canelones, Uruguay, with an average annual temperature of 15 °C and average annual precipitation of 1100 millimeters (mm). The site has an average accumulation of chill hours (CH) of 565 CH. At the same time, the climate of the region is Cfa, a subtropical climate with hot summers, according to Köppen-Geiger climate classification (Peel et al., 2007). Samples were collected on 18 July, 15 Aug, and 12 Sept in 2019 (accumulation of 578 CH and 1053.5 mm until the last collection) and on 16 July, 13 Aug, and 14 Sept in 2021 (accumulation of 548 CH and 1011 mm until the last collection) (INIA, 2022). The measures to fight the SARS-CoV-2 and closing Uruguayan borders to foreigners did not allow collecting and evaluations in 2020. The target rootstocks of this study were G.41, G.202, G.213, and

G.814, from a sow farm. The experimental design was completely randomized and consisted of four treatments (rootstocks), each with four branches from which two segments were obtained for hydraulic conductivity (K) and percentage loss of hydraulic conductance (PLC) readings, thus composing eight replications per treatment (n = 32).

Semi-woody branches, approximately 0.5 m in length and 0.4 cm in diameter, were collected between 07h00 and 08h00 in the morning. The branches were chosen randomly regarding their position in the plant., Immediately after cutting, they were placed in a container with enough water to cover the basal portion and prevent air entrance. Subsequently, the bases of the branches were placed in contact with wet newsprint, all wrapped in plastic film to prevent dehydration. There, the branches were stored and packaged, as described above, in a thermal box with ice and then evaluated for their hydraulic conductivity. The data obtained were submitted to the analysis of variance (ANOVA) using the Student's t test at 0.05 probability and tested for normality using the Shapiro-Wilk test. When significant, the means were submitted to the Tukey test ($p < 0.05$).

Hydraulic conductivity and PLC measurements

The Xylem Embolism Meter (XYL'EM-*Plus*, Bronkhorst) was used for this activity. Branches were sectioned in a water container with pruning shears, in 5-cm segments from the internode portion. The ends were cleaned with a blade in a thin and precise cut and the samples were attached to the device, using thread-seal and Hellermann tapes on the basal portion to prevent the solution flow from escaping from the sides of the branches. Two segments were used per branch, totaling eight readings per genetic material for each collection period. The solution was composed of Milli-Q water added with KCl and $CaCl_2$ (0.745g L^{-1} and 0.111g L^{-1}, respectively), degassed and filtered (0.22 µm) with the aid of a stirrer and a vacuum pump for 10 min. Firstly, the reading was performed at a pressure of 0.06 MPa to measure the initial hydraulic conductivity of the segments ($K_{initial}$). Then, xylem vessels saturation was carried out with a pressure of 0.2 MPa until the emissions of air bubbles ceased at the end of the samples. This process, called flushing, is intended to remove native xylem embolism. Afterward, four new readings were performed at a pressure of 0.06 MPa, obtaining the mean value for K_{max}. With the difference between K_{max} and $K_{initial}$, the percentage loss of hydraulic conductance was calculated according to the following formula:

$$PLC = \left(1 - \frac{K_{initial}}{K_{max}}\right) * 100$$

where: PLC: percentage loss of hydraulic conductance. $K_{initial}$: initial xylem hydraulic conductance and it can also be understood as native conductance (K_{native}); K_{max}: maximum hydraulic conductance obtained after flushing.

K_{max} is a function of water flow over the difference between the inlet and outlet hydrostatic pressure in the sample. It is expressed in mmol m s^{-1} MPa^{-1} (SI), where the loss of hydraulic conductivity is the difference between the initial and final reading after the flushing, and is expressed as a percentage (Bronkhorst, 2021).

Results

In general, the higher the value obtained for PLC, the greater the xylem embolism degree. During the first year of evaluations, G.213 rootstock showed the highest values of $K_{initial}$ in Aug and Sept, while G.202 presented the lowest values for the three collections in 2019 (Figure 1). However, for the PLC variable, in the three dates evaluated, G.814 had the highest values and G.213, the lowest (Figure 2), meaning that G.213 rootstock had the best indices for hydraulic conductivity and PLC. Higher volumes of $K_{initial}$ also mean a greater water flow transported by xylem cells. At the same time, lower PLC values (Figure 2) represent a lower occurrence of native embolism and, thus, greater xylem functionality.

In 2019, there was a shortage of branches with a length of 0.5 m; therefore, it was impossible to standardize the samples'diameters and lignification degree. This hindered the precision of cuts, so in 2021,

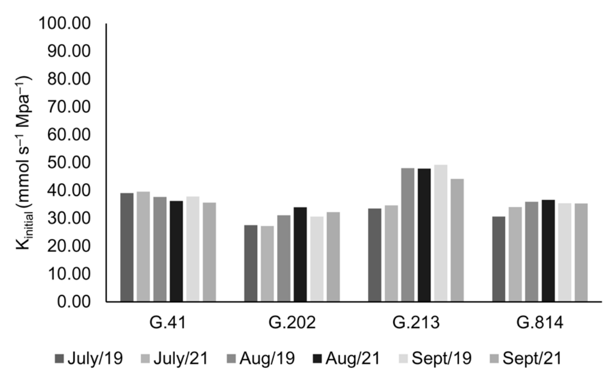

Figure 1 – $K_{initial}$, in mmol s^{-1} MPa^{-1}, during July – Sept period.

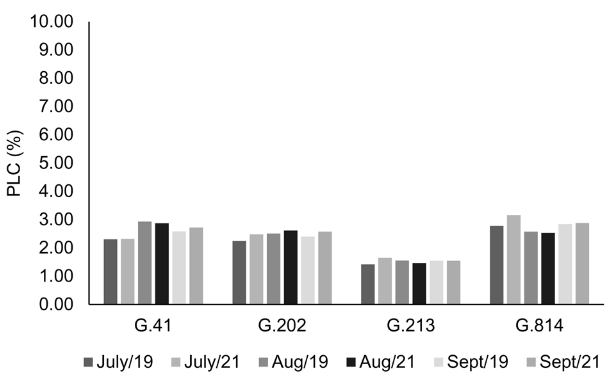

Figure 2 – PLC, in %, during July – Sept period.

the authors decided to adjust the methodology used to obtain greater standardization in the samples and reduce measurement errors. The adjustments consisted of collecting only branches with greater flexibility and a basal diameter smaller than 0.4 cm, which avoided wood splitting at the time of the first cut of the samples. In addition, the disposal of the basal and apical five centimeters of the branches was standardized, as these portions could be easily damaged and thus influence the results.

Data obtained in 2021 showed that the methodological adjustments allowed for a trend toward more consistent values for both variables analyzed. However, G.202 kept the lowest average values for $K_{initial}$, while G.213 had the highest averages for the same variable. The behavior observed for PLC in 2019 was also kept in 2021, as G.814 showed the most significant loss of xylem functionality, while G.213 had the lowest PLC values in the three periods observed.

Discussion

K_{max} is the best parameter to evaluate xylem transport capacity which is affected by several reasons (Cochard et al., 2013). PLC can determine the embolism degree, representing an indirect estimate of the cavitation percentage in xylem vessels (Cochard et al., 2000). Generally, the higher the value for $K_{initial}$, the greater the efficiency in transporting water from the plant. Therefore, given that hydraulic conductivity and PLC are inversely proportional parameters, higher PLC values mean a greater occurrence of embolism in the xylem, implying a deficient water movement in plants. The same evaluation method showed that plants gradually increased hydraulic conductance loss with successive freezing and thawing in high-yield apple cultivars (Beikircher and Mayr, 2017). This resulted in accumulation during winter and conductivity was not fully reestablished before the sprouting period, reaching PLC values close to 60 % in the 'Braeburn' cultivar. This increase in PLC was not observed in the present study, which can be explained by the difference in climatic conditions between the assessment sites, since the authors used plants from the Vinschgau Valley region (Italy), where winter conditions are too severe when compared to the climatic conditions in the southern region of Uruguay. In addition, the branches evaluated were obtained from canopy cultivars and branches from rootstock were not evaluated.

The relationship between water flow and vigor in 'Rocha' pear trees established on three different rootstocks in the state of Santa Catarina – Brazil – showed PLC values of 6.22 % and 19.36 % for plants on *Pyrus calleriana* and 'EMA', respectively (Abreu et al., 2021). The authors observed that hydraulic conductivity loss was inversely proportional to plant vigor, since the rootstock with greater vigor had a lower PLC value. The Geneva® Series rootstocks were classified in increasing order: G.11, G.41, G.16, G.213, and G.814 vigor compared to M-9, and G.935, G.222, and G.202 vigor compared to M-26 (Fazio et al., 2013). In the Brazilian conditions, G.202, G.213, G.214, and G.814 have been evaluated (Petri et al., 2019). Plant materials of less vigor tend to have lower plasticity of xylem vessels, suggesting a lower capacity to circumvent vessel obstruction caused by embolism. The data obtained for rootstocks of this study showed that PLC values remained very close and with slight variation between the months evaluated. Average temperatures during the winter period in the region of this study may explain this minimal variation, since Geneva® rootstocks were developed in the northern hemisphere, where winter conditions are somewhat different from those in South America.

Outside the winter period, plants use the stomatal closure mechanism to keep the water flow balance in the xylem and avoid significant variations in pressure along the conducting vessels. This balance is crucial for the occurrence of physiological and metabolic events in plants (Zeiger et al., 1987). Some species can reestablish flow in the xylem during spring after an embolism formation in the hibernal period. However, for species where this is not possible, conductivity loss during winter affects plant growth and development, hindering important metabolic processes (Christensen-Dalsgaard and Tyree, 2014). Therefore, sprouting is affected by xylem functionality and capacity of species to form new vessels in spring (Hacke and Sauter, 1996; Ameglio et al., 2002).

Under adverse conditions, plants may accumulate solutes for root formation to increase their water absorption capacity or originate xylem cells more resistant to high negative pressures (Taiz et al., 2017). Crop yield is often limited by the hydraulic conductance of species (Brix, 1962; Cowan, 1986). Native embolism in apple trees decreased in spring, according to Christensen-Dalsgaard and Tyree (2014). The authors attributed this decrease to the capacity to reestablish flow in thexylem and to the growth of new conducting vessels, leading to increased resistance to cavitation. A positive correlation between the K_{max} and the number of flower primordia in apple trees has been reported (Lauri et al., 2008).

Plant water movement occurs by diffusion (Angelocci, 2002) through the gradient created in the soil-plant-atmosphere system. Water moves from the soil to the plant, from the plant to the atmosphere through transpiration, and from the highest to the lowest water potential (Ψ_w). In mild winter conditions, interruptions in water transport due to $_w$ gradient that forms in the soil-plant-atmosphere system are more recurrent during the spring or summer period. Vapor pressure deficit (VPD) is directly proportional to air temperature, decreasing Ψ_w in the atmosphere (Pimentel, 2004) and, therefore, influencing the gradient formed. Water deficit probably impacts a cultivar response in different ways according to the rootstock vigor, preferentially affecting fruit size in the dwarf genotypes and the number of fruits in the vigorous ones (Xu and Ediger, 2021).

The xylem is directly influenced by rootstocks vigor, as the greater the vigor, the greater the xylem area. This is the vascular system structure, which is most influenced by the tissue differentiation rate in the apical meristems and vascular cambium. Hydraulic resistance is a parameter to be considered when choosing a particular genotype as a rootstock, since the anatomical characteristics of the xylem vessel elements are decisive for water transport plants (Olmstead, 2006; Lucas et al., 2013). A positive relationship has been reported between hydraulic conductivity and leaf biomass (Rodríguez-Gamir et al., 2010). The authors evaluated leaf/root ratio and transpiration in citrus plants on different rootstocks.

Low hydraulic conductivity can also influence the concentration of hormones in the sap, such as abscisic acid (ABA) (Tworkoski and Fazio, 2015). ABA modulates plant responses to water stress and extreme temperatures, such as stomatal opening and closing. High concentrations of ABA also have a negative impact on breaking dormancy by buds.

Conclusion

The rootstocks studied displayed a consistent pattern of hydraulic conductivity and PLC during the winter period, showing that mild winter conditions do not paralyze flow in the xylem. The genotype G.213 rootstock performed best, with the highest $K_{initial}$ and the lowest PLC values. Similarly, G.814 showed less hydraulic efficiency with the highest PLC values and the lowest $K_{initial}$ indicators, showing reduced water transport in the months evaluated.

Acknowledgments

The authors wish to thank the financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant process number 158767/2018-7. We gratefully thank Prof. Dr. Fabiano Simões (*in memoriam*) for the support and knowledge provided during the development of this research.

Authors' Contributions

Conceptualization: Mello-Farias P, Herter FG, Bologna CDC. **Data curation:** Acosta TF, Marques LOD, Dias CS. **Formal analysis:** Acosta TF, Marques LOD, Dias CS. **Funding acquisition:** Acosta TF. **Investigation:** Acosta TF, Marques LOD. **Methodology:** Mello-Farias P, Herter FG, Bologna CDC. **Project administration:** Mello-Farias P. **Resources:** Acosta TF. **Supervision:** Mello-Farias P, Herter FG, Bologna CDC. **Writing-original draft:** Acosta TF, Mello-Farias P, Herter FG. **Writing-review & editing:** Acosta TF, Mello-Farias P.

References

Abreu ES, Carra B, Dini M, Silva TA, Simões F, Pasa MS, et al. 2021. Native embolism in "Rocha" pear under different rootstocks and their relationship with plant vigor. Acta Horticulturae 1303: 221-226. https://doi.org/10.17660/ActaHortic.2021.1303.32

Ameglio T, Bodet C, Lacointe A, Cochard H. 2002. Winter embolism, mechanisms of xylem hydraulic conductivity recovery and springtime growth patterns in walnut and peach trees. Tree Physiology 22: 1211-1220. https://doi.org/10.1093/treephys/22.17.1211

Angelocci LR. 2002. Plant water and gaseous/energetic exchanges with atmosphere: introduction to biophysical treatment = Água na Planta e Trocas Gasosas/Energéticas com a Atmosfera: Introdução ao Tratamento Biofísico. Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil (in Portuguese).

Autio W, Robinson T, Black B, Blatt S, Cochran D, Cowgill W, et al. 2017. Budagovsky, Geneva, Pillnitz, and Malling apple rootstocks affect 'Honeycrisp' performance over the first years of the 2010 NC-140 'Honeycrisp' apple rootstock trial. Journal of the American Pomological Society 71: 149-166. Available at: https://pubhort.org/aps/71/v71_n3_a4.htm [Accessed Dec 21, 2022]

Beikircher B, Mayr S. 2017. Annual patterns of xylem embolism in high-yield apple cultivars. Functional Plant Biology 44: 587-596. https://doi.org/10.1071/FP16048

Brix H. 1962. Effect of water stress on rates of photosynthesis and respiration in tomato plants and loblolly pine seedlings. Physiologia Plantarum 15: 10-20. https://doi.org/10.1111/j.1399-3054.1962.tb07982.x

Bronkhorst. 2021. XYL'EM-Plus. Instruction manual and tutorial for xylem embolism measurements: version 2.2. Available at: http://herve.cochard.free.fr/pdf/xyl'emplusmanual.pdf [Accessed May 1, 2022]

Christensen-Dalsgaard K, Tyree M. 2014. Frost fatigue and spring recovery of xylem vessels in three diffuse-porous trees *in situ*. Plant, Cell Environment 37: 1074-1085. https://doi.org/10.1111/pce.12216

Cochard H, Bodet C, Améglio T, Cruiziat P. 2000. Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles. Facts or artifacts? Plant Physiology 124: 1191-1202. https://doi.org/10.1104/pp.124.3.1191

Cochard H, Badel E, Herbette S, Delzon S, Choat B, Jansen S. 2013. Methods for measuring plant vulnerability to cavitation: a critical review. Journal of Experimental Botany 64: 4779-4791. https://doi.org/10.1093/jxb/ert193

Corso M, Bonghi C. 2014. Grapevine rootstock effects on abiotic stress tolerance. Plant Science Today 1: 108-113. https://doi.org/10.14719/pst.2014.1.3.64

Cowan IR. 1986. Economics of carbon fixation in higher plants. p. 133-170. In: Givnish TJ, ed. On the economy of plant form and function. Cambridge University Press, Cambridge, UK.

Denardi F, Kvitschal MV, Hawerroth MC. 2017. Apple rootstocks: past, present and future. Agropecuária Catarinense 28: 89-95 Available at: https://publicacoes.epagri.sc.gov.br/RAC/article/view/167 [Accessed May 3, 2022] (in Portuguese, with abstract in English).

Fazio G, Robinson TL. 2008. Modification of nursery tree architecture with apple rootstocks: a breeding perspective. New York Fruit Quarterly 16: 13-16.

Fazio G, Aldwinckle H, Robinson T. 2013. Unique characteristics of Geneva apple rootstocks. New York Fruit Quarterly 1: 25-28.

Hacke U, Sauter JJ. 1996. Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous and ring-porous trees. Oecologia 105: 435-439. https://doi.org/10.1007/BF00330005

Instituto Nacional de Investigación Agropecuaria [INIA]. 2022. INIA agroclimate and informations systems unit = Unidad de Agroclima y Sistemas de Información del INIA. INIA-GRAS, Montevideo, Uruguay. Available at: http://www.inia.uy/gras/Unidad-de-Agroclima-y-sistemas-de-informaci%C3%B3n-del-INIA [Accessed June 3, 2022] (in Spanish).

Lauri PE, Bourdel G, Trottier C, Cochard H. 2008. Apple shoot architecture: evidence for strong variability of bud size composition and hydraulics within a branching zone. New Phytologist 178: 798-807. https://doi.org/10.1111/j.1469-8137.2008.02416.x

Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav SR, Helariutta Y, et al. 2013. The plant vascular system: evolution, development and functions. Journal of Integrative Plant Biology 55: 294-388. https://doi.org/10.1111/jipb.12041

Olmstead MA, Lang NS, Ewers FW, Owens SA. 2006. Xylem vessel anatomy of sweet cherries grafted onto dwarfing and nondwarfing rootstocks. Journal of American Society of Horticultural Science 131: 577-585. https://doi.org/10.21273/JASHS.131.5.577

Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology and Eart System Sciences. 11: 1633-1644. https://doi.org/10.5194/hess-11-1633-2007

Petri JL, Hawerroth FJ, Fazio G, Francescatto P, Leite GB. 2019. Advances in fruit crop propagation in Brazil and worldwide-apple trees. Revista Brasileira de Fruticultura 41: 1-14. https://doi.org/10.1590/0100-29452019004

Pimentel C. 2004. The plant's relation with water = A Relação da Planta com a Água. Seropédica, Rio de Janeiro, RJ, Brazil (in Portuguese).

Reig G, Lordan J, Fazio G, Grusak MA, Hoying S, Cheng L, et al. 2018. Horticultural performance and elemental nutrient concentrations on 'Fuji' grafted on apple rootstocks under New York climatic conditions. Scientia Horticulturae 227: 22-37. https://doi.org/10.1016/j.scienta.2017.07.002

Reig G, Lordan J, Sazo MM, Hoying S, Fargione M, Reginato G, et al. 2019. Long-term performance of 'Gala', Fuji' and 'Honeycrisp' apple trees grafted on Geneva® rootstocks and trained to four production systems under New York State climatic conditions. Scientia Horticulturae 244: 277-293. https://doi.org/10.1016/j.scienta.2018.09.025

Reig G, Lordan J, Hoying SA, Fargione M, Donahue DJ, Francescatto P, et al. 2020. Long-term performance of 'Delicious' apple trees grafted on Geneva® rootstocks and trained to four high-density systems under New York State climatic conditions. HortScience 55: 1538-1550. https://doi.org/10.21273/HORTSCI14904-20

Robinson TL, Hoying SA, Fazio G. 2011. Performance of Geneva rootstocks in on-farm trials in New York state. Acta Horticulturae 903: 249-256. https://doi.org/10.17660/ActaHortic.2011.903.31

Rodríguez-Gamir J, Intrigliolo DS, Primo-Millo E, Forner-Giner MA. 2010. Relationships between xylem anatomy, root hydraulic conductivity, leaf/root ratio and transpiration in citrus trees on different rootstocks. Physiologia Plantarum 139: 159-169. https://doi.org/10.1111/j.1399-3054.2010.01351.x

Taiz L, Zeiger EE, Møller IM, Murphy A. 2017. Plant physiology and development = Fisiologia e Desenvolvimento Vegetal 6ed. Artmed, Porto Alegre, RS, Brazil (in Portuguese).

Tworkoski T, Fazio G. 2015. Effects of size-controlling apple rootstocks on growth, abscisic acid, and hydraulic conductivity of scion of different vigor. International Journal of Fruit Science 15: 369-381. https://doi.org/10.1080/15538362.2015.1009973

Xu H, Ediger D. 2021. Rootstocks with different vigor influenced scion-water relations and stress responses in Ambrosia™ Apple Trees (*Malus domestica* var. Ambrosia). Plants 10: 614. https://doi.org/10.3390/plants10040614

Zeiger Z, Farquhar GD, Cowan IR. 1987. Stomatal Function. Stanford University Press, Palo Alto, CA, USA.