

Polyethylene glycol damages grafted citrus plants based on biometric, physiological, and biochemical responses

Eduardo Augusto Girardi¹, Andréa Dias Brandão², Rubens Duarte Coelho³, Hilton Thadeu Zarate do Couto³, Marcos Silveira Buckeridge⁴, Francisco de Assis Alves Mourão Filho³

Abstract – Drought tolerance is a major trait for the selection of citrus rootstocks in breeding programs. PEG 4000 solution at 110 g L⁻¹ (osmotic potential of -376 kPa) was used to evaluate biometric, physiological, and biochemical responses of grafted citrus plants in pots. ‘Valencia’ sweet orange plants grafted onto ‘Rangpur’ lime (drought tolerant) rootstock showed greater plant growth compared to that on ‘Swingle’ citrumelo (drought sensitive) rootstock, regardless of PEG-induced stress; however, biological responses to PEG were similar on both rootstocks. Plant dry weight, gas exchange, and leaf and root starch concentrations were reduced by PEG. Water potential at 2:00 p.m. and Fe and Mn leaf concentrations increased with severe phytotoxic symptoms. The results corroborate the limitation of PEG for assessing drought tolerance of citrus plants at the whole plant level.

Index terms: *Citrus limonia*, *Citrus paradisi x Poncirus trifoliata*, osmotic agent, rootstock, water deficit.

Polietileno glicol prejudica plantas enxertadas de citros com base em respostas biométricas, fisiológicas e bioquímicas

Resumo – A tolerância à seca é um dos principais atributos para a seleção de porta-enxertos de citros em programas de melhoramento genético. Solução de PEG 4.000 a 110 g L⁻¹ (potencial osmótico de -376 kPa) foi aplicada para avaliar respostas biométricas, fisiológicas e bioquímicas de plantas enxertadas de citros em recipientes. Laranja ‘Valencia’ enxertada em limão ‘Cravo’ (tolerante à seca) apresentou maior crescimento vegetativo do que sobre citrumelo ‘Swingle’ (intolerante à seca), independentemente do estresse por PEG. Contudo, as respostas biológicas ao agente osmótico foram similares para ambos os porta-enxertos. Matéria seca, trocas gasosas e concentração de amido em folhas e raízes foram reduzidas pela aplicação de PEG. Potencial hídrico às 14 h e concentrações foliares de Fe e Mn aumentaram com sintomas severos de fitotoxicidade. Esses resultados corroboram a limitação de PEG para avaliar a tolerância à seca de citros em plantas inteiras.

Termos para indexação: *Citrus limonia*, *Citrus paradisi x Poncirus trifoliata*, agente osmótico, deficiência hídrica, porta-enxerto.

Corresponding author:
francisco.mourao@usp.br

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¹Agronomist, graduate student in Crop Science - University of São Paulo, Piracicaba-SP, Brazil. Email: eduardo.girardi@embrapa.br.

²Biologist, graduate student at University of São Paulo, São Paulo-SP, Brazil. Email: brandao.a.d@bol.com.br

³Agronomist, professor at the University of São Paulo, Piracicaba-SP, Brazil. Emails: rdcoelho@usp.br, htzcouto@usp.br; francisco.mourao@usp.br

⁴Biologist, professor at the University of São Paulo, São Paulo-SP, Brazil. Email: msbuck@usp.br

Citrus trees are cultivated in regions subjected to seasonal drought that constrains fruit yield under rain-fed cultivation or in irrigated orchards with limited water supply (CARR, 2012). Drought tolerance is a major trait for rootstock breeding and selection (CASTLE, 2010), and each scion-rootstock combination may show several distinct biological responses under water deficit (RIBEIRO et al., 2014).

Osmotic agents, such as polyethylene glycol (PEG), are commonly added to the soil or potting mix to assess drought tolerance in potted plants, since field experiments are more costly, time-consuming and involve exposure to other abiotic and biotic stresses (COMEAU et al., 2010; PENELLA et al., 2014). A given soil osmotic potential can be indefinitely set with the use of PEG, which cannot be reproduced by other water deficit-inducing methods.

Drought tolerance in citrus plants has been evaluated with PEG as an osmotic stress agent in hydroponic and *in vitro* cultures, and in potted seedlings of different species (ZEKRI; PARSONS, 1990; ZEKRI, 1991, 1993; MADEMBA-SY et al., 2003; BOSCARIOL-CAMARGO et al., 2007; FORNER-GINER et al., 2011). PEG solutions with molecular weights from 4000 to 6000 have been extensively used, in spite of their well-documented detrimental effects and phytotoxic effects (PLAUT; FEDERMAN, 1985). The putative influence of the citrus scion-rootstock combination on responses to PEG has not yet been described, although it was demonstrated in other crops (PENELLA et al., 2014).

In this study, the hypothesis that polyethylene glycol (PEG) solution can be applied on grafted citrus plants as a method for selecting drought resistant rootstocks was tested.

This study was carried out in a screen-protected commercial citrus nursery in Araras, state of São Paulo, Brazil (22° 25' 15" S, 47° 23' 03" W, 611 m). Daily maximum and minimum average air temperatures were 40.5°C and 18.6°C, respectively, and average relative humidity was 70% during the assessment period.

'Valencia' sweet orange (VO) [*Citrus sinensis* (L.) Osbeck] plants were budded onto 'Rangpur' lime (RL) (*C. limonia* Osbeck) or 'Swingle' citrumelo (SC) [*C. paradisi* cv. Duncan Macf. X *Poncirus trifoliata* (L.) Raf.]. These rootstocks are considered more tolerant and less tolerant to drought stress, respectively (RIBEIRO et al., 2014).

Eight-month-old nucellar RL or SC seedlings cultivated in 4.5-L polyethylene bags filled with commercial pine bark potting mix (Rendmax™ Citrus) were grafted with VO buds at 15-cm height in December 2006. Cultural practices usually recommended for container-grown citrus nursery plants were followed (CARVALHO et al., 2005) and, upon grafting, a controlled-release fertilizer (22-04-08) (Osmocote™) was applied at 11 g plant⁻¹.

PEG-induced stress was initiated after unwrapping of the budding tape with the application of PEG 4000 (Oxiteno Sibol Ltda.) at 110 g L⁻¹, which corresponds to

solution with -376 kPa osmotic potential (ZEKRI, 1991). Each plant was daily irrigated with 300 mL of this solution from 15 to 120 days after grafting (DAG). Control plants were irrigated with the same volume of tap water that maintained the container moisture at field capacity. The pH of the potting mix was also weekly monitored by testing the leaching solution.

The experiment was installed in a 2 x 2 factorial design (rootstocks x PEG levels) with five replicates and three grafted plants per plot. A preliminary evaluation was conducted with different osmotic concentrations and periods of PEG application, and the combination of -376 kPa and 15–120 DAG was selected as the most representative to be used in this study.

Leaf and root dry weights were measured at 120 DAG. Samples (all leaves harvested from each plant and the entire root system per plant including the taproot) were dried at 65 °C for 72 h. The water potential was measured at 120 DAG at 6:00 a.m. and 2:00 p.m. in a pressure chamber (model 3005, Soil Moisture Equipment Co.), using mature fully-expanded leaves from the apex. Gas exchange was measured on the same type of leaves at 120 DAG, with infrared gas analyser (LI-6400, Li-Cor Inc.) adjusted to 1000 μmol photons m⁻² s⁻¹, air flow of 450 μmol s⁻¹ and ambient relative air humidity and CO₂ concentration. During assessments, average air temperature in the greenhouse was about 23 °C, vapour pressure deficit of approximately 1.75 kPa, and PAR ≈ 150 μmol photons m⁻² s⁻¹.

Leaf nutrient N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn concentrations were determined at 120 DAG (samples comprised six mature leaves per plant, 18 leaves per plot, and three replicates) according to standard analysis (SRIVASTAVA, 2012). Starch concentrations were determined at 120 DAG according to Amaral et al. (2007) in three replicates, with plants separated into root system and leaves. As described for dry weight, samples were dried at 80 °C for 72 h, ground and sieved (0.8 mm mesh); 30 and 50 mg of dried leaf and root samples, respectively, were separated from the total material obtained per replicate.

Data were submitted to analyses of variance ($P \leq 0.05$) and results presented as relative percentages to facilitate comparison of variables within the same figure, with RL control plants as 100 %. Original data on leaf nutrient concentrations were presented for the main rootstock effect.

Leaf and root dry weights were higher for VO/RL regardless of treatment, approximately 2.25- and 1.33-fold higher, respectively. In contrast, there was approximately 55% and 33% reduction in leaf and root dry weights, respectively, with PEG application with both rootstocks (Figure 1 A and B).

Leaf water potential at 6:00 a.m. (predawn) was reduced by approximately 33% by PEG with both rootstocks (Figure 1 C). Conversely, at 2:00 p.m., PEG application resulted in water potential 33% higher (Figure

1 D). SC and RL were more sensitive to PEG-stress at 6:00 a.m. and 2:00 p.m., respectively. Gas exchange variables showed response similar to PEG; only the net CO₂ assimilation rate (*A*) is presented (Figure 1 E). PEG drastically reduced *A*, notably in plants with RL rootstock (-80%).

SC led to higher leaf N, P, Ca, Mg and B concentrations, whereas RL resulted in higher Fe, Cu, Mn and Zn concentrations in VO leaves regardless of treatment (Table 1). Leaf mineral composition varied according to the duration and intensity of PEG-induced stress (data not shown); consistent results were observed only for Fe and Mn, which were increased by 28%, on average, with both rootstocks (Figure 1 F and G).

Starch concentration was higher in the leaves and roots of control VO/RL (Figures 1 H and I). PEG-stress decreased the starch concentration in leaves by 75% and 40% with RL and SC, respectively, in relation to control. In contrast, starch depletion in the roots was similar for both rootstocks (approximately 30%). This decrease in starch concentration within plant organs was related to the reduced *A*, resulting in depletion of this energy reservoir under PEG stress (Figure 1 E).

PEG-stressed plants showed phytotoxic effects, regardless of rootstock used (Figure 2). Initially, there was mild internodal leaf chlorosis, similar to Mn deficiency, with no change in leaf size or shape (Figure 2 A). These symptoms developed into severe chlorosis, which began to fold over the ventral side (mild curling) (Figure 2 B). With the progress of PEG application, leaves became heavily deformed, with severe curling towards the ventral side and a large reduction in blade area, including margin necrosis, and leaf abscission (Figure 2 C and D). The apex was more damaged and vegetative growth was continuous, making it difficult to distinguish different shoot flushes. Towards the end of assessments, water irrigation was restored and new shoots were visually healthy (Figure 2 E). Non-stressed plants were symptomless throughout the evaluation.

The high growth and starch content of plants with RL rootstocks, especially the root system even under severe stress caused by PEG, corroborated studies on potted plants under water deficit (PEDROSO et al., 2014). Plants grafted onto RL usually acclimate to drought stress in a faster way and benefit from a more efficient carbohydrate partitioning induced by this rootstock. However, the relative biological responses to PEG stress observed in this study were very similar between plants grafted onto RL and SC, although being well-known drought tolerant and sensitive rootstocks, respectively, in both field and greenhouse conditions (PEDROSO et al., 2014; RIBEIRO et al., 2014). Therefore, there was no clear association between drought tolerance of evaluated rootstocks and PEG-induced stress.

PEG application at -376 kPa clearly led to detrimental effects on grafted citrus plants by decreasing plant growth, gas exchange and starch concentrations in leaves and roots. The same sharp decline was observed

in 'Cleopatra' mandarin (*C. reshni* Hort ex Tanaka), sour orange (*C. aurantium* L.), *P. trifoliata* and 'Carrizo' citrange rootstock seedlings (*C. sinensis* × *P. trifoliata*) in pots irrigated with PEG solutions (ZEKRI; PARSONS, 1990; ZEKRI, 1991, 1993; FORNER-GINER et al., 2011). The higher leaf water potential of PEG-stressed plants at 2:00 p.m. in relation to the control also suggests a different physiological mechanism in response to water deficit.

Phytotoxic effects in grafted citrus plants observed in this work are similar to those described for seedlings of several citrus species by ZEKRI (1991, 1993), who proposed that the phytotoxic effects of PEG in citrus plants arise from the higher Mn and Zn accumulation in the roots and leaves. In the present study, the potting mix in control and PEG-stressed plants had pH values of approximately 6.3 and 4.5, respectively, throughout evaluation. As pH interferes in the absorption and accumulation of cationic micronutrients (KUMIKO; TORU, 2004), the effect of PEG elevating the acidity may have favoured Fe and Mn imbalance and phytotoxic effects (Figure 1 F and G; Figure 2). PEG is also considered an efficient adsorptive substance for Fe and Mn ions in aqueous solutions, with higher adsorption capacity at pH range of 2.9 – 5.9 (REIAD et al., 2012).

The observed phytotoxic symptoms and physiological effects are not expected to be directly caused by the low pH induced by PEG application, since the physiological apparatus of different citrus rootstocks were not affected by pH values as low as 4.0 in nutritive solutions without Al (PEREIRA et al., 2000). On the other hand, 'Volkamer' lemon (*C. volkameriana* Ten. & Pasq.) seedlings showed reduction in photosynthetic efficiency when Mn concentrations in leaves were high (PAPADAKIS et al., 2007), which was similar to PEG application results obtained in the present study.

In conclusion, the initial hypothesis of this study, "feasibility of the use of polyethylene glycol (PEG) solution in grafted citrus plants as a method for selecting drought resistant rootstocks", was rejected by the results obtained in this experiment. 'Valencia' sweet orange grafted onto 'Rangpur' lime (drought tolerant) rootstock was more vigorous than with 'Swingle' citrumelo (drought sensitive) rootstock, regardless of PEG-induced stress. PEG solution causes phytotoxic symptoms and detrimental plant performance in grafted citrus plants, irrespective of the drought tolerant properties of the rootstock variety. PEG-induced toxic stress and water deficit stress occurred at the same time in citrus plants, and it was not possible to separately quantify these effects.

Table 1. Leaf nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) concentrations in ‘Valencia’ sweet orange grafted onto ‘Rangpur’ lime (RL) and ‘Swingle’ citrumelo (SC) rootstocks, grown in pots and irrigated with polyethylene glycol (PEG) at -376 kPa from 15 to 120 days after grafting.

Rootstock	(g kg ⁻¹)					(mg kg ⁻¹)				
	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
RL	27.4 b	1.39 b	21.2 a	24.9 b	2.89 b	46.9 b	123 a	108 a	107 a	37.2 a
SC	31.3 a	1.58 a	21.5 a	26.6 a	3.91 a	53.4 a	83 b	103 b	82 b	33.1 b
CV%	4.2	6.2	6.0	5.2	6.6	7.3	10.0	8.8	9.1	11.8

*Mean values followed by the same letters are equivalent by the F-test ($P \leq 0.05$).

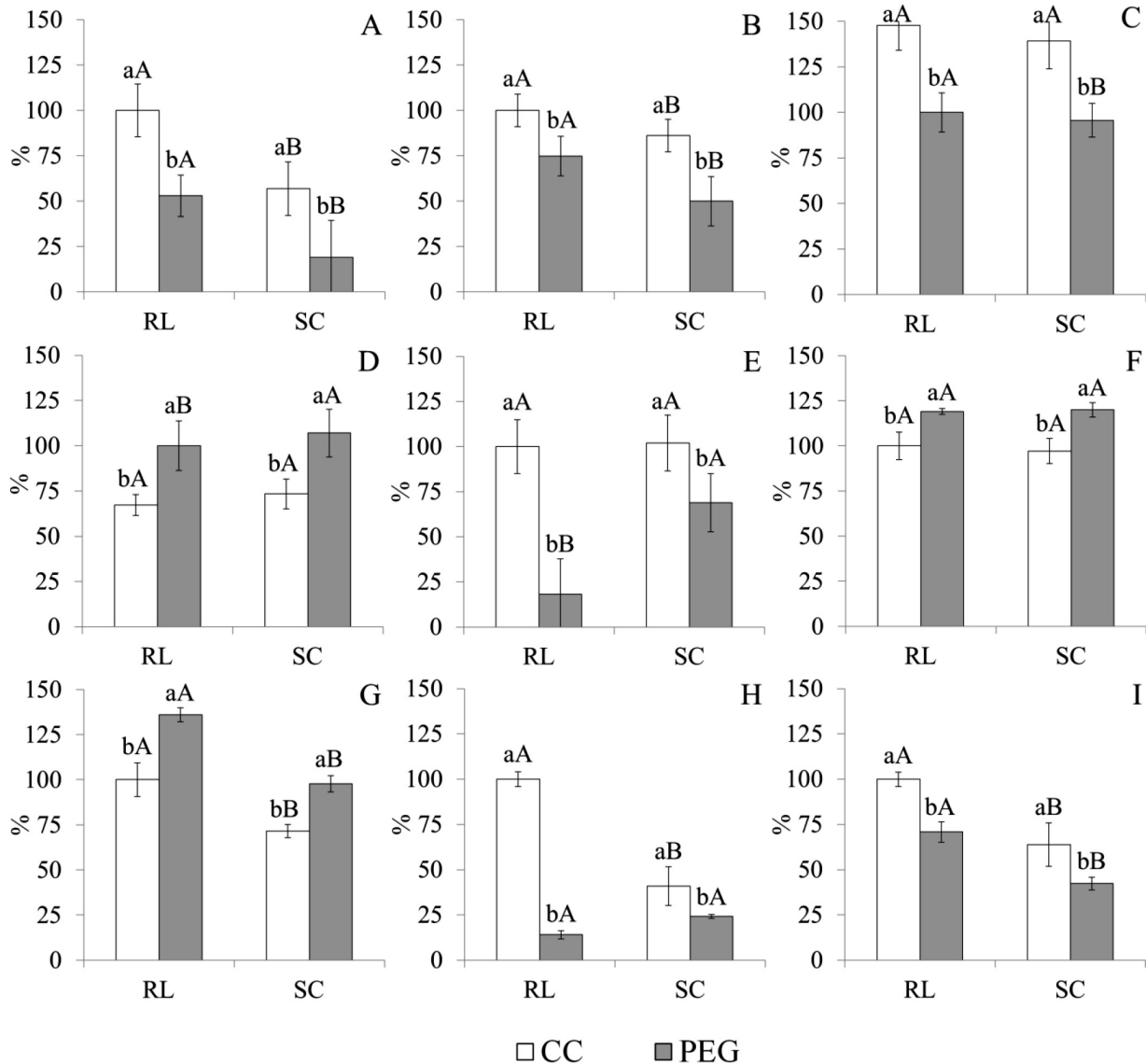


Figure 1. Relative mean values (%) of leaf (A) and root (B) dry weights, leaf water potential at 6:00 a.m. (C) and 2:00 p.m. (D), net CO₂ assimilation rate (E), leaf Fe (F) and Mn concentrations (G), and leaf (H) and root (I) starch concentrations of ‘Valencia’ sweet orange (VO) grafted onto ‘Rangpur’ lime (RL) and ‘Swingle’ citrumelo (SC) rootstocks in pots, and irrigated with polyethylene glycol (PEG) at -376 kPa from 15 to 120 days after grafting. CC = plants daily irrigated at container capacity (control). Relative mean values ± relative standard errors followed by the same lowercase and capital letters, respectively, between treatments within each rootstock and between rootstocks within each treatment, are not different by the F-test ($P \leq 0.05$).



Photographs: E. A. Girardi

Figure 2. Initial symptoms of phytotoxic effects in grafted citrus plants irrigated with polyethylene glycol (PEG) solution (A and B); advanced phytotoxic symptoms in grafted citrus plants irrigated with PEG solution at -376 kPa (C and D); healthy aspect of new shoot flush after restoring regular water irrigation without PEG solution (E).

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