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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL)

ASPECTOS FISIOLÓGICOS E NUTRICIONAIS DO ALUMÍNIO EM DUAS ESPÉCIES CÍTRICAS E UMA DE CERRADO EM CULTIVO COM SOLUÇÃO NUTRITIVA

OTÁVIA FARIA DOS ANJOS ABDO BANHOS

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Vegetal)

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TÍTULO DA TESE: ESTUDO DO METABOLISMO MINERAL DO AI3+ EM ESPÉCIES DO CERRADO E AVANÇOS NO CONHECIMENTO SOBRE SUA TOXICIDEZ EM ESPÉCIES CULTIVADAS

AUTORA: OTAVIA FARIA DOS ANJOS ABDO BANHOS
ORIENTADOR: GUSTAVO HABERMANN

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Prof. Dr. GUSTAVO HABERMANN
Departamento de Botânica / Instituto de Biociências de Rio Claro - SP

PROF. DR. FRANCISCO ANTONIO MONTEIRO
Departamento de Solos e Nutrição de Plantas / ESCOLA SUPERIOR DE AGRICULTURA

Prof. Dr. VICTOR ALEXANDRE VITORELLO
Centro de Energia Nuclear na Agricultura / Universidade de São Paulo

Prof. Dr. JOSÉ LAVRES JUNIOR
Centro de Energia Nuclear na Agricultura / Universidade de São Paulo

Prof. Dr. PAULO EDUARDO RIBEIRO MARCHIORI
Centro de Pesquisa e Desenvolvimento em Ecofisiologia e Biofísica / Instituto Agronômico de Campinas - SP

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Ao meu esposo Marcelo

Minha querida mãe “Lú”

Por todo apoio, carinho

e incentivo nessa trajetória!

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"Que minha coragem seja maior que
meu medo e que minha força seja
tão grande quanto minha fé. "

SUMÁRIO

RESUMO

O alumínio (Al^{3+}) é tóxico para a maioria das plantas e é um fator limitante para o crescimento das plantas e para a produtividade das culturas em todo o mundo. Os efeitos do Al foram estudados em plantas de laranjeira-doce (*Citrus sinensis* L. Osbeck) enxertadas em porta-enxerto sensível ao Al, o limoeiro (*Citrus limonia* cv. 'Cravo'), em uma espécie nativa do Cerrado não-acumuladora de Al (*Styrax camporum*) e em limoeiro 'Cravo' não enxertado. Foram estudadas doses de Al (0, 185, 370, 555 e 740 μM Al) em plantas cultivadas em areia e em solução nutritiva pH 4.0 contendo 0 e 1480 μM, o acúmulo de Al nos órgãos desses genótipos, as trocas gasosas, o desempenho fotoquímico da clorofila *a* e o potencial da água na folha. A composição química nominal dessa solução foi testada pelo software Geochem-EZ e resultou em mais de 85% de Al^{3+} livre disponível. Nas raízes, estudamos as alterações anatômicas. Todos esses parâmetros foram estudados em resposta à perturbação causada pelo Al no ambiente radicular. Para causar perturbação em laranjeiras enxertadas em limoeiro 'Cravo', a disponibilidade de Al no substrato deve ser maior que 55%. Solução nutritiva contendo até 1480 μM Al causa alterações fisiológicas em limoeiro 'Cravo' após 45 dias e esta concentração de Al em solução é toxica para as plantas de *Styrax camporum*.

ABSTRACT

Aluminum (Al^{3+}) is toxic to most plants and is a factor limiting plant growth and crop yields throughout the world. Aluminum effects were studied in sweet-orange (*Citrus sinensis* L. Osbeck) plants grafted on 'Rangpur' lime (*Citrus limonia*), an Al-sensitive plant, in a non-Al-accumulating plant (*Styrax camporum*) from the Cerrado vegetation, and also in non-grafted 'Rangpur' lime plants. Plants under increasing Al concentrations (0, 185, 370, 555 and 740 μM Al), which were cultivated in sand and plants cultivated directly in nutrient solutions containing 0 and 1480 μM Al were used for studying the accumulation of Al in their organs, gas exchange, chlorophyll photochemical performance and leaf water potential. The nominal chemical composition of this solution was tested on Geochem-EZ software, and it resulted in more than 85% free Al^{3+} available. Anatomical alterations were also studied in roots. All these parameters were studied in response to the Al disturbance in the root environment. Aluminum availability in the substrate must be higher than 55% to cause disturbances in sweet-orange plants grafted on 'Rangpur' lime rootstocks. Nutrient solution containing up to 1480 μM Al cause physiological alterations in non-grafted 'Rangpur' lime plants after 45 days, and this Al concentration is toxic to *Styrax camporum* plants.

INTRODUÇÃO GERAL

O Cerrado abriga uma grande biodiversidade, especialmente quando se consideram as espécies lenhosas (Mittermeier et al., 1999; Guarim Neto & Morais, 2003). A vegetação das fisionomias do Cerrado ocorre sobre solos profundos, bem drenados, distróficos, latossolos ácidos (pH ≤ 4,0) e com saturação por alumínio (Al) acima de 60% (Haridasan, 1992; Haridasan, 2008).

Os minerais de alumínio das rochas são solubilizados à forma Al^{+3} pelo intemperismo natural das rochas (Rengel, 1992; Hartwig et al, 2007), pelo clima e também pela atividade humana (Ma et al., 2001). Na solução do solo pode se apresentar sob diferentes formas, dependendo do pH e de outros fatores do solo. Em valores de pH abaixo de 5,0, predomina o íon trivalente positivo (Al^{3+}), aparentemente mais tóxico (Echart & Molina, 2001). Porém, à medida que o pH aumenta, formam-se as espécies $Al(OH)^{2+}$ e $Al(OH)^{+}_{2}$. Em pH próximo da neutralidade, o Al assume forma pouco solúvel de $Al(OH)_3$ e, em condições alcalinas, predomina a forma $Al(OH)^{-}_{4}$ (Delhaize & Ryan, 1995).

Muitas espécies de plantas são sensíveis à presença do Al^{3+} no ambiente radicular. Desta forma, a toxicidade por Al^{3+} é das principais limitações na produtividade de plantas em áreas cultiváveis para diversas espécies de importância agronômica (Delhaize & Ryan, 1995), limitando o alongamento radicular e a produtividade (Machado & Pereira, 1990). Um dos primeiros efeitos negativos do Al^{3+} se desencadeia na zona de transição do ápice radicular (Sivaguru & Horst, 1998) e pode ser detectado em poucos minutos após a exposição das raízes ao Al^{3+} (Kochian et al., 2004). Inicialmente o Al^{3+} inibe o alongamento e a expansão celular e, posteriormente, a divisão celular, prejudicando a absorção de água e nutrientes. Devido à sua carga elétrica positiva, o Al reage com inúmeros sítios nas células, como a parede celular, o citoesqueleto, o núcleo e, principalmente, a membrana plasmática (Kochian et al., 2004). Também interfere em algumas enzimas orientando a deposição de polissacarídeos na parede celular e aumentando a rigidez através de ligações cruzadas de pectinas (Foy et al., 1978).

Vários mecanismos adaptativos tais como dimorfismo de raiz (Sternberg et al., 2005), transpiração noturna (Bucci et al., 2004), realocação/redistribuição de nutrientes antes da senescência das folhas (Nardoto et al., 2006), reciclagem de nutrientes da serrapilheira (Silva, 2001) e associações micorrízicas (Reis, 1999) podem explicar porque as comunidades de plantas nativas do Cerrado são mais eficientes que as plantas agrícolas cultivadas em solos do Cerrado. Assim, algumas espécies (geralmente lenhosas e perenes) nativas do Cerrado desenvolveram características de acumular altas concentrações de Al^{3+} nas folhas, com concentrações superiores a 1000 mg kg^{-1} de Al^{3+} de folha seca. Estas espécies também são chamadas de acumuladoras de Al^{3+} e são

frequentemente das famílias Euphorbiaceae, Myrtaceae, Rubiaceae, Melastomataceae e Vochysiaceae (Haridasan, 1982; Cuenca et al., 1991; Jansen et al., 2002 a, b).

Apesar das espécies da família Styracaceae não acumularem altas concentrações de Al^{3+}, *Styrax camporum* Pohl., um arbusto do Cerrado que acumula cerca de 1300 mg kg^{-1} de Al^{3+} de massa seca de folhas (Bressan et al., 2016), se desenvolve melhor na presença de Al^{3+} (Souza & Habermann, 2012). Sua floração ocorre de setembro a outubro e os frutos amadurecem de maio a agosto e são dispersos por pássaros (Lorenzi, 1992).

Algumas espécies (variedades botânicas de espécies, sendo muitas de interesse econômico, como trigo, milho, soja, dentre outras) desenvolveram mecanismos para a complexação do Al^{3+} com ligantes orgânicos, como Al^{3+}-oxalato e também com ligantes inorgânicos, como Al^{3+}-silicatos (Ma et al., 2001). Duas categorias de mecanismos de tolerância ao Al têm sido propostas: mecanismos de exclusão e mecanismos de tolerância. As plantas que utilizam mecanismos de exclusão do Al são capazes de evitar a entrada desse íon no simplasto, enquanto aquelas que possuem mecanismos de tolerância interna são capazes de manter seus processos metabólicos próximo da normalidade, mesmo na presença de concentrações relativamente elevadas de Al no simplasto (Gonçalves et al., 2000).

As razões para a diferença na mobilidade do Al^{3+} entre espécies agrícolas extrusoras de Al^{3+} e as espécies do Cerrado acumuladoras de Al^{3+} ainda não são bem compreendidas e o papel do Al^{3+} no metabolismo destas espécies de Cerrado ainda não está resolvido (Haridasan, 2008; Horst et al., 2010; Bressan et al., 2016). Altas concentrações de Al^{3+} no simplasto pode ser indicativo de 'resistência', como sugerido por Va´zquez et al. (1999), que observaram a internalização do Al^{3+}, contribuindo para a 'resistência' ao Al^{3+} em um genótipo de milho. O termo tolerância ou resistência, no caso do Al^{3+}, pode ser relativo. Plantas extrusoras de Al^{3+} na raiz podem ser consideradas 'tolerantes' (Magalhães, 2010) ou 'resistentes' (Ryan et al., 2011). Talvez a falta de padronização do termo seja porque nas espécies cultivadas sem genes de extrusão ativados, o Al^{3+} é tóxico, enquanto para as espécies acumuladoras do Cerrado, o Al^{3+} não parece ser tóxico (Haridasan, 2008).

A toxicidade ao Al^{3+} e outros problemas causados pela acidez do solo podem ser contornados com o uso de técnicas como a calagem, porém a utilização de corretivos de acidez do solo nem sempre é viável sob o ponto de vista econômico, principalmente, devido aos custos crescentes dos processos de obtenção, transporte e aplicação desse insumo. A calagem é uma das práticas que mais contribui para o aumento da eficiência dos adubos e consequentemente, da produtividade e da rentabilidade de espécies de importância agronômica (Sousa et al., 1989), mas seu uso deve ser anual ou, no mínimo, bienal, dependendo do tipo de solo, da cultura e do manejo utilizado.

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Capítulo 1

Aluminum-induced decrease in CO_2 assimilation in 'Rangpur' lime is associated with low stomatal conductance rather than low photochemical performances[1]

Otavia F. A. A. Banhos[1], Brenda M. de O. Carvalho[2], Eduardo B. da Veiga[1], Anna C. G. Bressan[1], Francisco A. O. Tanaka[3], Gustavo Habermann[4]*

[1]Programa de Pós-Graduação em Ciências Biológicas (Biologia Vegetal), Univ Estadual Paulista, Unesp, Instituto de Biociências, Departamento de Botânica, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil; [2]Centro de Ciências Agrárias, Universidade Federal de São Carlos, UFSCar, Rodovia Anhanguera, km 174, Araras, SP, Brazil; [3]Escola Superior de Agricultura "Luiz de Queiróz" - Universidade de São Paulo, ESALQ-USP, Núcleo de Apoio à Pesquisa em Microscopia Eletrônica Aplicada à Pesquisa Agropecuária (NAP/MEPA), Departamento de Fitopatologia e Nematologia, Av. Pádua Dias, 11, 13418-900, Piracicaba, SP, Brazil; [4]Univ Estadual Paulista, Unesp, Instituto de Biociências, Departamento de Botânica, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil

*Corresponding author: ghaber@rc.unesp.br; phone: 0055 (19) 3526-4210

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Abstract

Aluminum (Al) is toxic to most plants. Although inhibition of root elongation can occur even under 10 μM Al, above 1000 μM damage to photochemical performances has been reported, reducing the CO_2 assimilation rate (*A*). However, Al is retained in the roots of most plants, including *Citrus limonia* ('Rangpur' lime) with no direct explanation for the low *A*. In this study, 'Rangpur' lime plants were cultivated hydroponically in a nutrient solution with 1480 μM Al, and we expected to determine the time range within which Al-induced decrease in *A* starts. To our surprise, low stomatal conductance (*gs*) rather than low photochemical performances was evident 45 days after exposing the roots to Al. Aluminum was mostly retained in the roots and histochemically observed in the stele, suggesting reduction in water uptake/transport in the xylem that became fibrous. We also found a 35% reduction in the midday leaf water potential in plants exposed to Al, indicating association between the fibrous xylem vessels and low *gs*, which could explain the Al-induced decrease in *A* in 'Rangpur' lime plants.

***Keywords*:** Aluminum allocation; *Citrus limonia*; Histochemical characteristics; Leaf gas exchange parameters; long-distance Al effects

1. Introduction

Aluminum (Al) is the third most abundant element in the Earth's crust and, in the soil, it naturally occurs as Al_3SiO_4; however, in acidic soils (pH < 5.0) it is hydrolysed to Al^{3+}, which is toxic to most plant species (Foy, 1988; Horst et al., 2010). Acidic soils occupy approximately 30 to 45% of the world's ice-free land (von Uexküll and Mutert, 1995), therefore, Al is a factor limiting plant growth (Horst et al., 2010) and crop yields (Foy, 1988; Vitorello et al., 2005) throughout the world.

Inhibition of root elongation is the first and most conspicuous symptom observed in plants exposed to Al (Horst et al., 2010; Sun et al., 2010). Low root growth can be detected within hours at Al concentrations as low as 10 μM, causing ruptures on the rhizodermis (Kopittke et al., 2008; Blamey et al., 2011). Aluminum is retained in the roots of most Al-sensitive species (Vitorello et al., 2005), including 'Rangpur' lime (*Citrus limonia*) (Santos et al., 1999), 'Sour Pummelo' (*C. grandis*) and sweet orange (*C. sinensis*) (Yang et al., 2011). In the roots, the Al seems to bind to the cell walls (Rangel et al., 2009), where it can be histochemically evidenced with chrome azurol S (Wehr et al., 2010). Besides the local, rapid and direct effect of Al on root elongation, it also exerts long-distance effects on the shoots, such as reduced shoot growth (Jiang et al., 2009).

Another long-distance indirect effect of Al on shoots is an Al-induced decrease in CO_2 assimilation rate (*A*) observed in many species, including *Citrus spp* (Pereira et al., 2000; Chen et al., 2005a,b; Jiang et al., 2008, 2009; Yang et al., 2011), coffee plants (*Coffea arabica*) (Konrad et al., 2005), maize (*Zea mays*) (Lidon et al., 1999), and rye (*Secale cereale*) (Silva et al., 2012). In 'Cleopatra' tangerine (*Citrus reshni*) (Chen et al., 2005a,b) and in 'Sour Pummelo' (Jiang et al., 2008; 2009), Al presence in the root environment is associated with low photochemical performance in leaves, as a result of low electron transport rate (ETR) of photosystem II (PSII), and attenuated photochemical quenching (qP), reducing the energy and electron transporters for maintaining carboxylation reactions. This low photochemical performance is also reported for coffee plants exposed to Al (Konrad et al., 2005). Another reason for Al-induced decrease in *A* could involve a reduced nitrogen (N) uptake by leaves, since N is a constituent of many photosynthetic enzymes. In maize, increasing Al to up to 2960 μM in the root environment decreased shoot N concentration (Lidon et al., 1999), but in 'Rangpur' lime, leaf N concentration was unchanged when the roots were exposed to up to 1110 μM Al (Santos et al., 1999). Given the relationship between N and photosynthesis (Kumar et al., 2004), it is noteworthy that the rate of carboxylation reactions are not decreased by Al in 'Cleopatra' tangerine (Chen et al., 2005b), coffee plants (Konrad et al., 2005) or

rye (Silva et al., 2012). On the other hand, most of these studies show 30 to 80% decrease in stomatal conductance (*gs*) when the roots are exposed to Al, reiterating a multi-factor effect of Al on *A*.

Citrus is cultivated in humid and sub-humid areas of temperate, tropical and subtropical regions of the world, mostly on acidic soils that are usually rich in Al. However, there are different species and cultivars of *Citrus*, and specific combinations of scions/rootstocks for each region may determine fruit yields (Carr, 2012). The effects of Al on *A* are rarely studied in the 'Rangpur' lime rootstock, which is largely used in subtropical areas of the Americas due to its high drought resistance (Ribeiro and Machado, 2007; Magalhães Filho et al., 2008) and, therefore, this rootstock is useful in rain-fed *Citrus* plantations.

Studies of the effects of Al and its movement in the plant with the objective of determining the time range within which Al induce a decrease in *A* are rare for *Citrus*, a plant that drives its photosynthetic responses according to factors that shift every season (Ribeiro et al., 2012). Therefore, using rootstocks in hydroponic system with a nutrient solution containing more than 1000 μM Al, as performed by Chen et al. (2005a,b) and Jiang et al. (2008, 2009), we measured *A*, ϕPSII, ETR and qP in 'Rangpur' lime plants growing in 1480 μM Al, over a 45-day experiment. We predicted that Al-induced decrease in *A* is triggered by an early reduction in photochemical performance. Following this prediction, we sought to detect the time range within which the low photochemical performance occurs in 'Rangpur' lime plants. In addition, histochemical analyses of roots and leaves were performed and provided evidence to support the functional analyses.

2. Material and Methods

2.1 Plant material and experimental conditions

We used three-month-old and 13 ± 0.6 cm-high 'Rangpur' lime (*Citrus limonia*) plants for studying the effects of Al and its movement in the plant within a 45-day period. The plants were maintained in hydroponic system and grew directly on an aerated nutrient solution (Furlani and Furlani, 1988) inside opaque plastic boxes (50 cm in length x 30 cm in width x 15 cm in height; 20 L).

The nutrient solution shows a chemical composition based on Clark's solution (Clark, 1975) that has been used to test Al resistance in *Citrus* rootstocks (Santos et al., 2000). It contained the following macronutrients (in mM): NO_3^- 0.96; NH_4^+ 0.41; P, 0.013; K, 0.86; Ca, 1.43; Mg, 0.33; S, 0.22; and micronutrients (in μM): Cl, 214.1; Fe (EDTA), 23.3; B, 8.33; Mn, 2.91; Zn, 0.76; Cu, 0.32; Mo, 0.31. Besides macro and micronutrients, this solution contained 0 and 1480 μM (40 mg/L) Al

provided through $AlK(SO_4)_2$ 12 H_2O. This Al concentration was used because most studies have usually observed Al-induced decrease in photosynthetic parameters only when evaluating non-grafted plants under more than 1000 μM Al (Chen et al., 2005a,b; Konrad et al. 2005; Jiang et al. 2008, 2009; Silva et al., 2012). The pH of the solution was maintained at 4.0 ± 0.1 in order to keep the Al as soluble as possible. In addition, the nominal chemical composition of this solution was tested on Geochem-EZ software (Shaff et al., 2010), and it resulted in more than 85% free Al^{3+} available. Solution pH was monitored daily and replaced every ten days. Besides testing the solution on Geochem-EZ software, we noted that nominal 1480 μM Al supply resulted in 1100 ± 5.3 μM Al, which was measured colorimetrically (Sarruge and Haag, 1974). Expanded polystyrene (Isopor®) 50 x 30 cm plates (2-cm thick), with five holes (2.5 cm in diameter) each, were floated on the nutrient solution in the boxes, and the plants were fixed in these holes with polyurethane foam strips that were placed around the plant collar. The boxes were kept on benches, maintained inside a greenhouse, under semi-controlled conditions (air temperature 28.5 ± 0.7°C; relative humidity 63.3 ± 1.3%; 753.4 ± 176.1 μmol photons m^{-2} s^{-1}; approximately 14h of natural photoperiod).

2.2 Experimental design

We measured leaf gas exchange and fluorescence of chlorophyll *a* in light-adapted leaves, after transferring 'Rangpur' lime plants from the nutrient solution with no Al to the solution containing 1480 μM Al. After the transference, these parameters were assessed for three hours (every three minutes), every day for 10 days after the transference (DAT), and at 45 DAT when leaf water potential (Ψw) was measured. At 115 DAT (to ensure a long-term Al stress for plant organs and growth) the plant size, biomass, Al concentration in each organ, and anatomical and histochemical analysis of leaves and roots were performed in the plants maintained in the nutrient solution with 0 and 1480 μM Al.

2.3 Photosynthetic parameters

CO_2 assimilation (***A***) and transpiration (***E***) rates, stomatal conductance (***gs***), and intercellular CO_2 (***Ci***) were measured with an open portable gas exchange system (LI-6400xt, LI-COR, Lincoln, NE, USA). The CO_2 concentration entering the leaf cuvette averaged 390 μmol mol^{-1}, as provided by the 6400-01 CO_2 mixer (LI-COR). Measurements were taken between 9:00 and 11:00h (Feistler and Habermann, 2012) on cloudless days, under the natural fluctuation of air temperature and vapor pressure deficit (VPD) inside the greenhouse. The photosynthetic photon flux density (PPFD) was provided by an artificial light source (6400-40 LCF, LI-COR), which was set to provide 1200 μmol photons m^{-2} s^{-1} in the leaf cuvette, as this value saturates *A* for *Citrus* (Habermann et al., 2003).

The fluorescence of chlorophyll *a* was measured with a portable modulated fluorometer (6400-40 LCF, LI-COR, Lincoln, NE, USA), which was integrated into the LI-6400xt gas exchange system. The effective quantum yield of PSII (Φ_{PSII}) was calculated as $(F_m' - F_s)/F_m'$, where F_m' and Fs are the maximum and the steady-state fluorescence in light-adapted leaves, respectively. Apparent electron transport rate (ETR = Φ_{PSII} PPFD 0.5 0.85) was calculated, using 0.5 as the fraction of excitation energy distributed to PSII, and 0.85 as the fractional leaf light absorbance; the proportion of open PSII reaction centers (qP) was measured as $(F_m' - F_s)/(F_m' - F_o')$ (Bolhàr-Nordenkampf and Öquist, 1993), where F_o' is the minimal fluorescence in light-adapted leaves, measured after exposing the leaf to a far red light pulse before the actinic light goes off.

2.4 Al concentration in plant organs

Leaves, shoots and roots were washed under deionized water and oven-dried at 60°C to constant dry mass. These samples were then ground and digested in a nitric:perchloric acids solution. The concentration of Al was quantified colorimetrically (Sarruge and Haag, 1974).

2.5 Biometric parameters

The lengths (cm) of stems (from the plant collar to the shoot apex) and roots (from the plant collar to the root tip) were measured with a ruler, and the number of leaves was counted. At 0 and 115 DAT plants were separated into leaves, stems (plus petioles) and roots, which were dried at 60°C until constant mass. The biomasses (g) of organs and total plant biomass were evaluated, and the leaf area (LA, cm^2) was measured with an area meter (LI-3100C, LI-COR, USA).

2.6 Anatomical analysis

The leaves and root tips were collected and immediately fixed in FAA 50 (37% formaldehyde, glacial acetic acid, 50% ethanol; 1:1:18 v:v:v) and preserved in 70% alcohol (Johansen, 1940). We also used fresh tissues to histochemically test the Al indicator (chrome azurol S - CAS), and the staining patterns were the same as those obtained when the plant material was fixed (FAA 50) and preserved (70% alcohol).

The anatomical study was based on consecutively sliced cross sections of leaf segments (1 cm^2) from leaf midribs containing part of the leaf lamina, and of root tips (approximately 0.5 cm from the root apex). These sections were hand-made with a razor blade. Non-dye treated cuts were immersed in distilled water for 15 min before mounting semi-permanent glass slides. Dye-treated cuts were stained (one or two drops of the staining solution - Kukachka and Miller, 1980) for 45 min (at room temperature) as no staining difference was observed after 20, 30, 40 or 60 min. After the

staining, the cuts were immersed in distilled water for 15 min three times and mounted in semi-permanent glass-slides. All cuts were observed under light microscope (DMLB, Leica Microsystems, Wetzlar, Germany). The images were captured with a digital camera (DFC-290, Leica Microsystems, Germany) functionally attached to the DMLB.

As Al indicator, we used chrome azurol S (CAS) or 'mordant blue 29'. This indicator is highly specific to detect Al in plant tissues (Bressan et al., 2016) as it strongly complexes Al that is bound tightly to pectate or cell wall material (Wehr et al., 2010). Chrome azurol S (3''-sulpho-2'',6''-dichloro-3,3'-dimethyl-4-hydroxyfuchson-5,5'-dicarboxylic acid), 50% purity (Sigma-Aldrich, St. Louis, MO, USA) solution was prepared dissolving 20 mL of a 41.3 mM CAS solution (25 g/L) into 80 mL of a 760 mM and 4.82 pH sodium acetate solution (Kukachka and Miller, 1980). Therefore, CAS final concentration was 8.3 mM (5 g/L) (pH = 4.76 ± 0.01).

2.7 Leaf water potential (Ψ_w)

Leaf water potential at predawn (Ψ_{pd}) and midday (Ψ_{md}; under maximum VPD) was measured by the pressure chamber method (Turner, 1981), using a DIK-7000 (Daiki Rika Kogyo, Tokyo, Japan) portable chamber.

2.8 Data Analysis

The study was conducted with five plants per treatment. A one-way analysis of variance (Anova) was performed between plants exposed to 0 and 1480 μM Al, testing Ψ_{pd} and Ψ_{md} at 45 DAP, as well as the leaf number, LA, shoot and root lengths, leaf, shoot, root and total biomasses and Al concentrations in plant organs at 0 and 115 DAP. In addition, a repeated measures Anova (RM-Anova) was performed to test for differences in the effect of Al on *A*, *gs*, *E*, *Ci*, ϕPSII, ETR and qP over time on the same group of plants transferred from 0 to 1480 μM Al. In this case, we did not compare plants *maintained* under 0 and 1480 μM Al, because we aimed at studying the *dynamics* of ecophysiological responses on the same individuals after the transference, and RM-Anova is more appropriate for such purposes, when the group of individuals serves as its own control (Girden 1992).

The Tukey test ($\alpha = 0.05$) was used to conduct post-hoc comparisons to estimate the least significant differences between mean results of growth, biomass, Al concentrations in the organs and Ψ_w.

3. Results

Values of *A*, *gs*, *E*, *Ci* and ϕPSII remained unchanged within three hours after 'Rangpur' lime plants were transferred from 0 to 1480 μM Al (Fig. 1). However, these parameters decreased consistently throughout the 45-day period, and significant differences were observed for *A* (Fig. 2A), *gs* (Fig. 2B) and *E* (Fig. 2C) at 45 DAT in relation to day 0; *Ci* (Fig. 2D), ϕPSII (Fig. 3A), qP (Fig. 3B) and ETR (Fig. 3C) remained unchanged over this period.

The 'Rangpur' lime plants cultivated in the nutrient solution with 1480 μM Al showed lower leaf number (Fig. 4A), leaf biomass (Fig. 4B) and LA (Fig. 4C) at 115 DAP, in relation to the control plants. Aluminum significantly reduced plant growth from 0 to 115 DAP, with a conspicuous impact on root length (Fig. 4G) and root biomass (Fig. 4F) when compared to the impact on shoot length (Fig. 4E) and shoot biomass (Fig. 4D).

At 115 DAP, Al concentration was significantly higher in the leaf (Fig. 5A), shoot (Fig. 5B), root (Fig. 5C), and in the whole plant (Fig. 5D) when cultivated at 1480 μM Al in comparison to the control plants. After 115 days at 1480 μM Al, tissue Al concentration was 10 times higher in the roots (Fig. 5C) in relation to the shoots (Fig. 5B) and leaves (Fig. 5A).

Chrome azurol S did not indicate the presence of Al in the roots or leaves of 'Rangpur' lime plants under 0 μM Al (Fig. 6B, C). However, this dye indicated the presence of Al in the roots of plants exposed to 1480 μM Al (Fig. 6E), but not in their leaves (Fig. 6F). In these plants, Al was noted in the root vascular cylinder (Fig. 6H) and, compared to other tissues, Al was noted more intensely in the cytoplasm of the phloem and inside the lumen of xylem vessels (Fig. 6H, I). However, no positive reactions were observed on the cell wall of xylem vessels (Fig. 6H, I). The roots of plants exposed to Al also showed structural modifications to the pericycle and phloem, as well as fibrous xylem vessels (Fig. 6I), which did not occur in the roots of plants not exposed to Al (Fig. 6G).

Predawn leaf water potential was the same for plants at 0 and 1480 μM Al, whereas Ψ_{md} was 35% lower in plants exposed to Al when compared to the control plants (Fig. 7).

4. Discussion

When we transferred 'Rangpur' lime plants from a nutrient solution with 0 to 1480 μM Al, we expected to determine the time range within which Al-induced decrease in photochemical performances would start influencing *A*. Nonetheless, Al did not affect photochemical performances

throughout the study. Stomatal conductance, however, consistently decreased throughout the study and was significantly low after 45 days. We observed 90% lower *gs* at 45 DAT in relation to the first day that the plants were transferred to 1480 μM Al (Fig. 2B). This suggests that *gs* reduction may be more important than low photochemical performances for explaining the Al-induced decrease in *A* in 'Rangpur' lime plants. Aluminum-induced decrease in *gs* by 30% in 'Cleopatra' tangerine (Chen et al., 2005b), 40% in 'Sour Pummelo' (Jiang et al., 2008), 65% in coffee plants (Konrad et al., 2005), and 80% in rye (Silva et al., 2012) has already been reported.

Since *A* decreased steadily through a 45-day Al treatment (Fig. 2A), and *Ci* stayed unchanged throughout this period (Fig. 2D), one may argue that non-stomatal factors could *also* be involved in the Al-induced decrease in *A* because unchanged *Ci* might imply reduced carboxylation rates. However, we found a large correspondence between *A* (Fig. 2A), *gs* (Fig. 2B) and *E* (Fig. 2C), indicating that *A* was under diffusive control, *i.e.*, it was dependent on *gs*. In addition, in 'Cleopatra' tangerine (Chen et al., 2005b) and rye (Silva et al., 2012) the Al-induced decrease in *A* is unaccompanied by reduced carboxylation rates, weakening the non-stomatal factor hypothesis. Low mesophyll hydration must be considered as one of the mechanisms that might explain this association between low *A* and *gs* in plants exposed to Al. Samac and Tesfaye (2003) and Vitorello et al. (2005) defend that Al induces the stunting of the primary root and inhibition of lateral root formation, which would lead to reduced water uptake. In the present study, however, the plants exposed to Al showed reduced leaf number (Fig. 4A) and low leaf area (Fig. 4C) at 115 DAP; their leaf biomass did not increase (from 0 to 115 DAP) as it did in the control plants (Fig. 4B), therefore compensating for the decrease in root length (Fig. 4G) and root biomass (Fig. 4F) caused by Al. In addition, plants exposed to Al exhibited lower Ψ_{md} in relation to the control plants (Fig. 7), indicating a greater difficulty for these plants to hydrate their mesophyll during the day. These Ψ_{md} values represent a mild but important water deficit when compared to Ψ_{md} of sweet orange grafted on 'Rangpur' lime plants grown in the field during the dry season under subtropical conditions (-2.0 to -2.5 MPa; Ribeiro and Machado, 2007; Habermann and Rodrigues, 2009), or after 12 days of drought under semi-controlled conditions (-1.6 MPa; Magalhães Filho et al., 2008), or to Ψ_{md} values of sweet orange plants grafted on 'Rough' lemon (*C. jambhiri*) exhibiting leaf wilting after 10 days under drought (-2.0 MPa; Vu and Yelenoski, 1988). Therefore, the reduced hydration in mesophyll evidenced by the low Ψ_{md} (Fig. 7) could be associated with a lack of functional capacity of the fibrous xylem vessels (Fig. 6H, I) and, consequently, explain the low *gs* (and *A*) found in 'Rangpur' lime plants exposed to Al.

Our results also show that Al caused an indirect/long-distance effect on *A* because Al was mostly retained in the roots (Fig. 5C), and was *not* anatomically evidenced in the leaves (Fig. 6F). Aluminum immobilization in the roots has already been reported for most crop species (Vitorello et al., 2005), including 'Rangpur' lime (Santos et al., 1999), 'Sour Pummelo', and sweet orange (Yang et al., 2011) plants. Our results demonstrate that Al is associated with structural modifications to the pericycle and phloem; modifications to the xylem may also be noted as this tissue became fibrous in the presence of Al (Fig. 6H, I) when compared to plants not exposed to Al (Fig. 6G). This illustrates how a long-distance effect of Al may have an influence on *gs* and *A* in the leaves. CAS reacts positively with Al bound to pectin from the cell wall (Wehr et al., 2010), and it evidenced the presence of Al on the phloem cell walls (Fig. 6H, I), where pectins are known to be abundant. It also reacted positively with the phloem contents of roots exposed to Al (Fig. 6I). This corroborates the Al localization in phloem cells of *leaves* of Al-accumulating plants (Haridasan et al., 1986; Tolrà et al., 2011). We did not note positive reaction between CAS and xylem cell *walls* (where lignin is more important than pectin), but we observed thick xylem cell wall, characterizing them as fibrous vessels (Fig. 6H) when compared to those from plants not exposed to Al (Fig. 6G). In cowpea, the first 2-3 mm of the primary root is completely recovered from Al stress after 24 h, except for the already damaged cell roots (Blamey et al., 2011). That is, once Al is bound (to the primary cell wall, rich in pectins) during the process of cell development, it is unlikely to be released (Rangel et al., 2009). Therefore, it is possible that the xylem of 'Rangpur' lime plants became fibrous during cell development in response to the presence of Al, and this might have affected water uptake/transport through these vessels, as indirectly evidenced by the low *gs* (Fig. 2B) and Ψ_{md} (Fig. 7).

One could argue that CAS staining the lumen of the xylem vessels of plants exposed to Al (Fig. 6H, I) could be due to an insufficient washing after staining, since this dye is dependent on pectin-bound Al (Wehr et al., 2010). It is uncertain that insufficient washing has occurred because cuts were immersed in distilled water for 15 min three times. On the other hand, we have no further evidence, such as X-ray spectra using scanning electron microscope (Bressan et al., 2016), that Al was *somehow* present in the lumen of these vessels. However, this does not affect the main purpose of our study. We show that low mesophyll hydration is more important than a photochemical limitation. This limited hydration suggests reduction in water uptake/transport in the roots. Despite showing an indirect evidence of such reduction (low *gs* and Ψ_w), it is assumed that Al-induced callose deposition in the symplast inhibits not only the cell-to-cell flow through plasmodesmata but also the apoplastic bypass flow in root cortical cell walls (Horst et al., 2010). Therefore, further investigation of root water flow and xylem thickening over time of Al exposure merits attention.

In this study, we observed structural modifications to the pericycle and cambial cells, as well as fibrous xylem vessels, suggesting that Al "invaded" the stele, resulting in reduction in water uptake/transport in the roots, as indirectly evidenced by the low *gs* and Ψ_w after a 45-day Al treatment. Therefore, in 'Rangpur' lime plants, the Al-induced decrease in *A* seems to be an indirect (long-distance) effect of Al that is mostly retained in the roots, with limited involvement of photochemical parameters.

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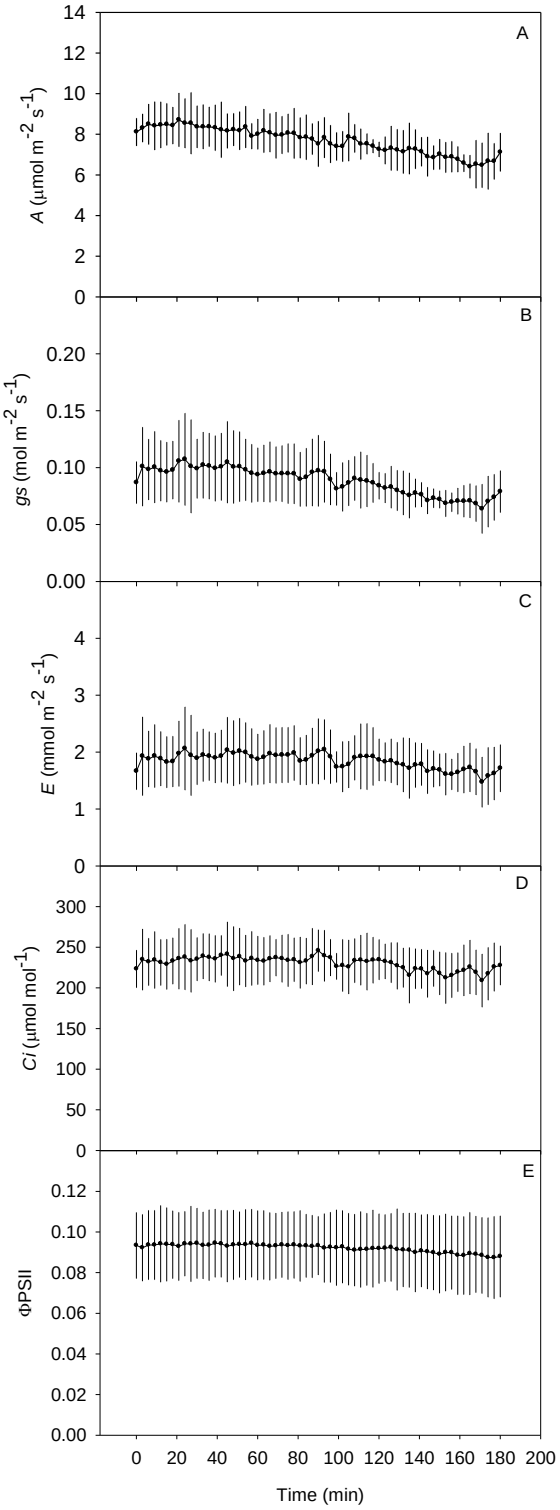

Fig 1. Leaf gas exchange rates (A, B, C, and D) and effective quantum yield of photosystem II (E) of leaves of 'Rangpur' lime plants measured for three hours after being transferred to a nutrient solution containing 1480 μM Al. Absence of letters indicates lack of significant difference (RM Anova; $p > 0.05$) between mean values (n = 5 plants) and values previously obtained. Bars are s.d.

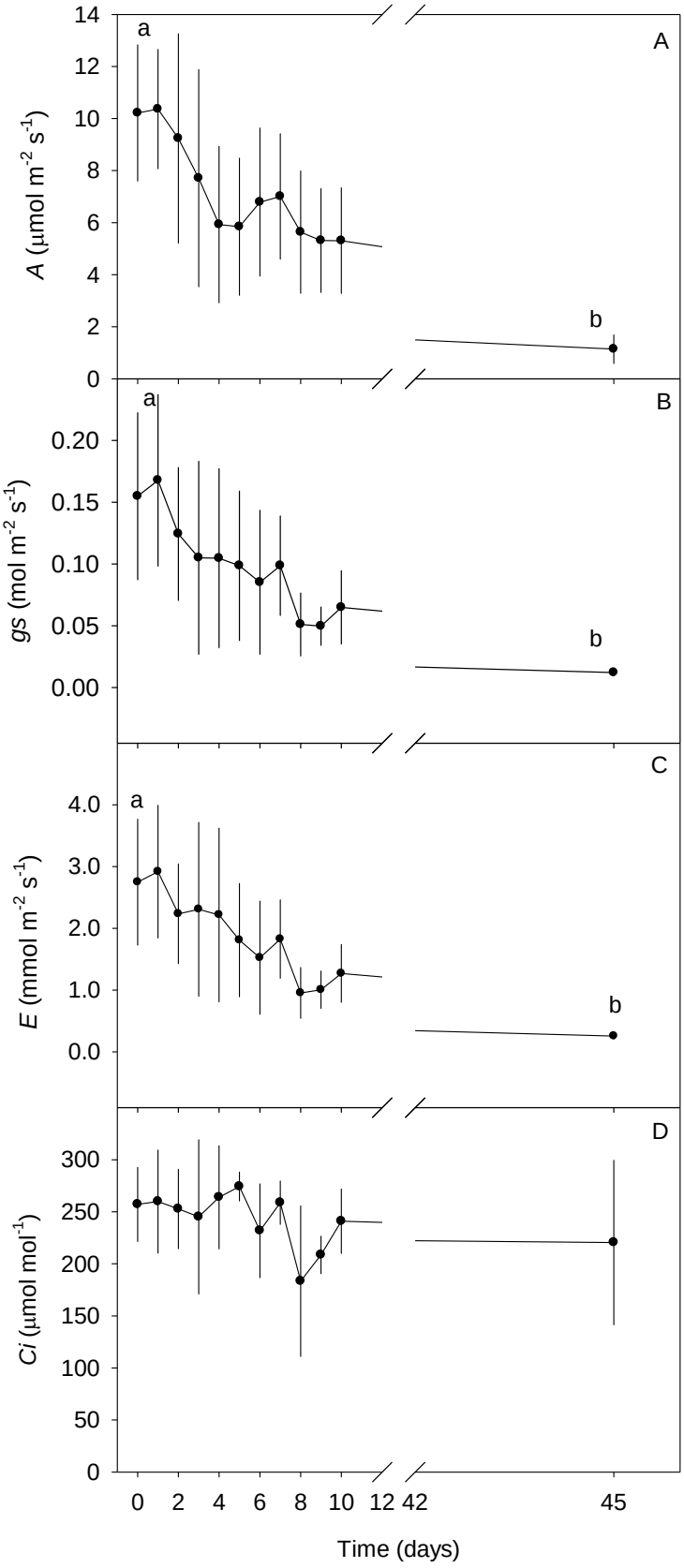

Fig 2. Leaf gas exchange rates of 'Rangpur' lime plants measured for 45 days after being transferred to a nutrient solution containing 1480 μM Al. Absence of letters indicates lack of significant differences (RM Anova; $p > 0.05$), and distinct letters indicate significant differences (RM Anova; $p < 0.05$) between mean values (n = 5 plants) and values previously obtained. Bars are s.d.

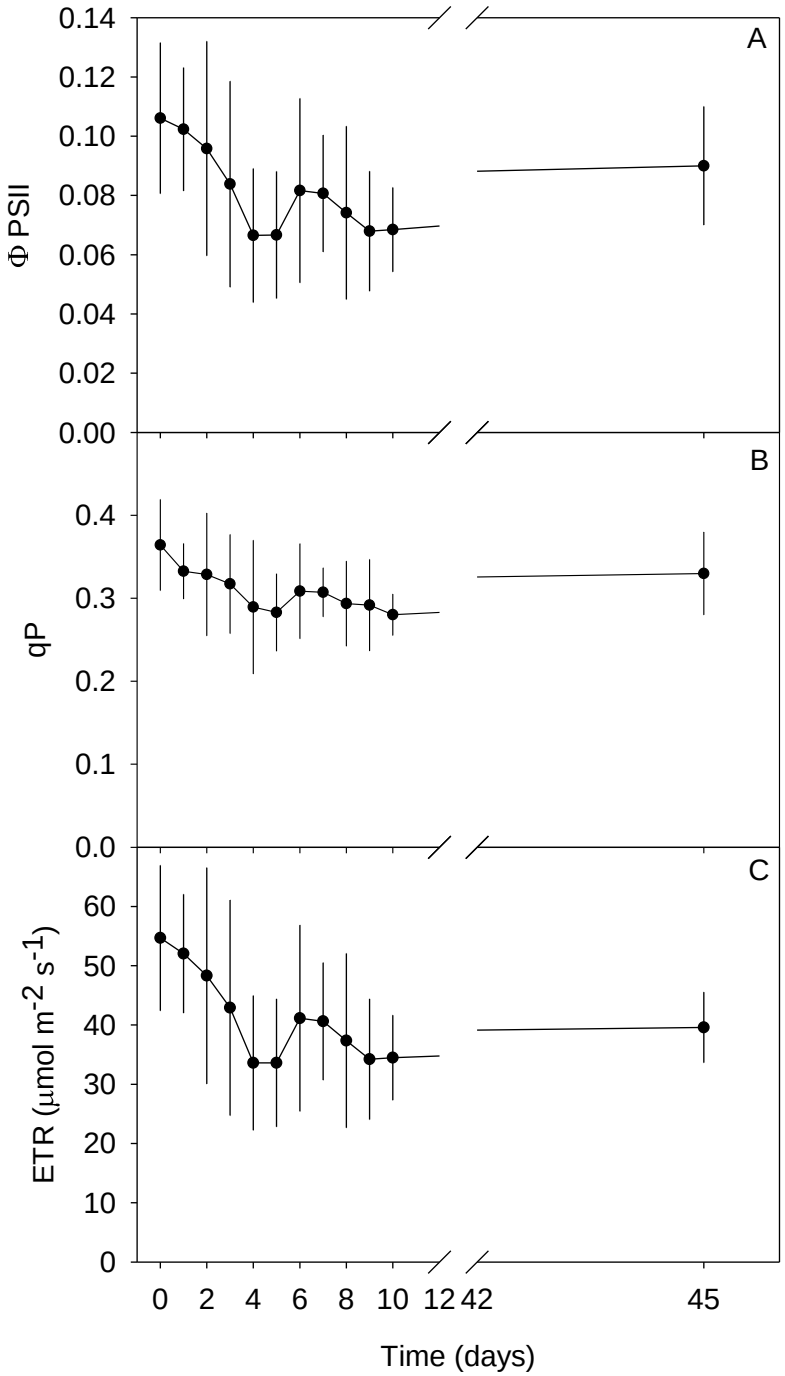

Fig 3. Photochemical parameters in leaves of 'Rangpur' lime plants, measured for 45 days after being transferred to a nutrient solution containing 1480 μM Al. Absence of letters indicates lack of significant differences (RM Anova; $p > 0.05$) between mean values ($n = 5$ plants) and values previously obtained. Bars are s.d.

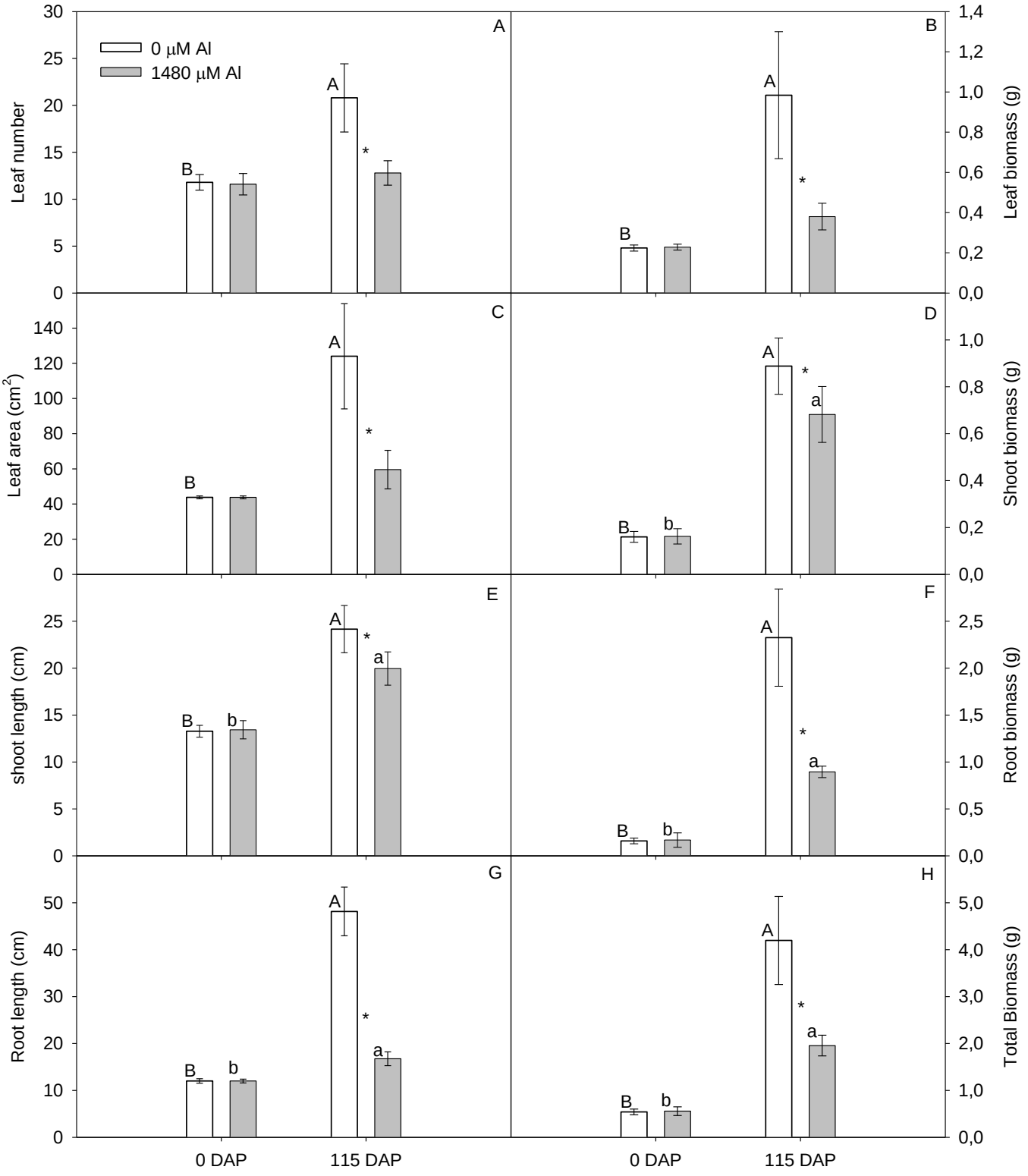

Fig 4. Growth and biomass of organs of 'Rangpur' lime plants cultivated for 115 days in nutrient solutions containing 0 and 1480 μM Al. Asterisks indicate significant differences by the Tukey test ($\alpha = 0.05$) between Al treatments. For the control plants, distinct uppercase letters indicate significant differences ($p < 0.05$) between 0 and 115 DAP; for plants exposed to Al, distinct lowercase letters indicate significant differences ($p < 0.05$) between 0 and 115 DAP. Columns are mean values (n = 5 plants), and bars are s.d. DAP = days after planting

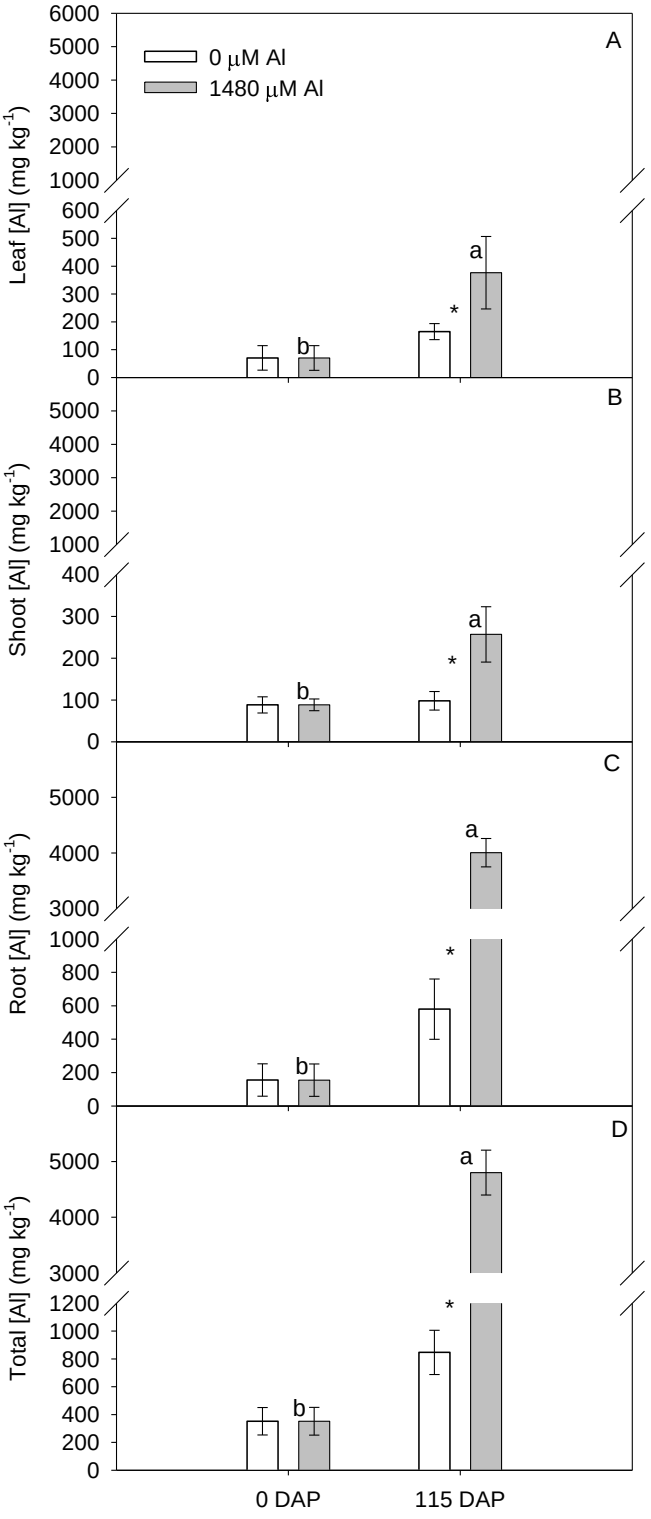

Fig 5. Mean values (n = 5 plants) of Al concentration in leaves (A), shoots (B), roots (C), and in the whole plant (D) of 'Rangpur' lime rootstocks cultivated for 115 days in nutrient solutions containing 0 and 1480 μM Al. Asterisks represent significant differences by the Tukey test ($\alpha = 0.05$) between Al treatments. For each treatment, absence of letters indicates lack of significant differences ($p > 0.05$) and distinct letters indicate significant differences ($p < 0.05$) between 0 and 115 DAP. Bars are s.d. DAP = days after planting

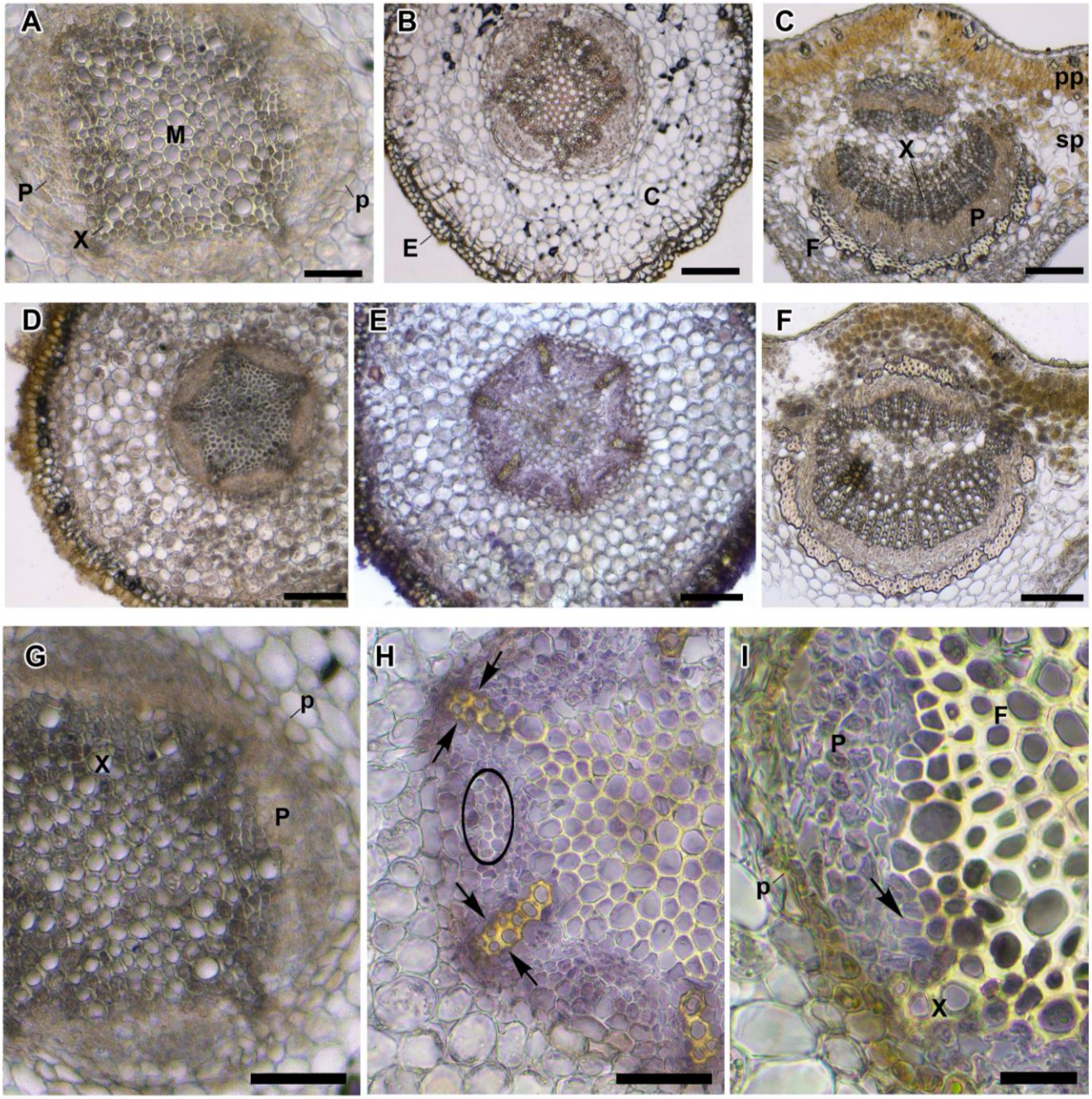

Fig 6. Anatomical analyses of roots (A, B, D, E, G, H, and I) and leaf midribs (C and F) of 'Rangpur' lime plants. A, B, C and G: cultivated in nutrient solutions containing 0 μM Al; D, E, F, H and I: cultivated in nutrient solutions containing 1480 μM Al; A and D: non-stained; B, C, E, F, G, H and I: stained with chrome azurol S. C = cortex; E = epidermis; F = fibers; M = medulla; p = pericycle; P = phloem; pp = palisade parenchyma; sp = spongy parenchyma; X = xylem. Double-opposite arrows: fibrous xylem; Simple arrows: positive reaction of cellular contents. Scale bars (μm): A = 50; B-F = 100; G-H = 50; I = 20.

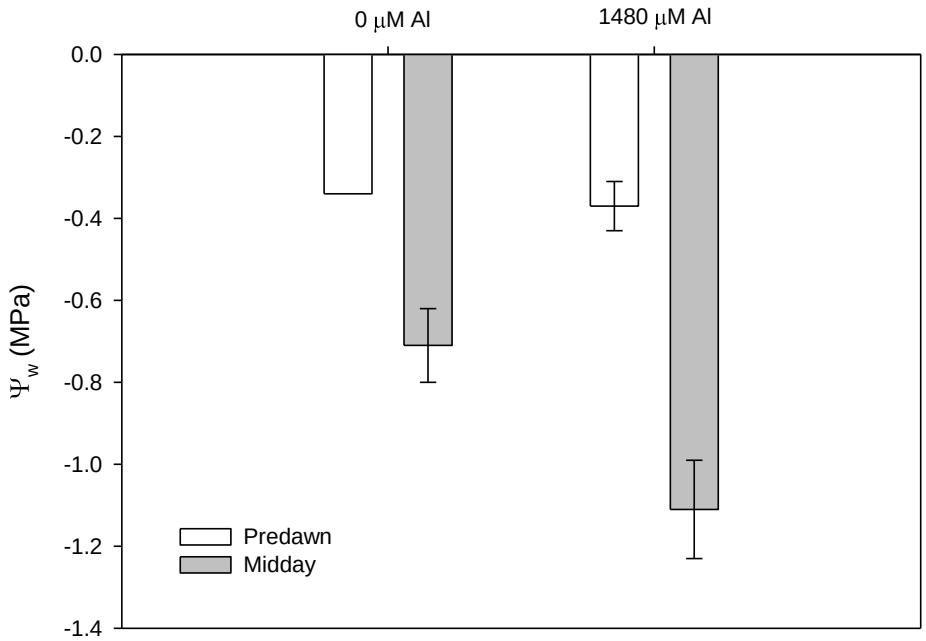

Fig 7. Mean values (n = 5 plants) of leaf water potentials of 'Rangpur' lime plants cultivated for 45 days in nutrient solutions containing 0 and 1480 μM Al. For each time of day (predawn or midday), absence of letters indicates lack of significant differences by the Tukey test ($p > 0.05$), and distinct letters indicates significant differences ($p < 0.05$) between 0 and 1480 μM Al. Bars are s.d.

Capítulo 2

O alumínio interfere no crescimento e acúmulo de nutrientes de laranjeira 'Valencia' enxertadas em limoeiro 'Cravo'?

Otavia F. A. A. Banhos[1], Eduardo B. da Veiga[1], Anna C. G. Bressan[1], Gustavo Habermann[2]*

[1]Programa de Pós-Graduação em Ciências Biológicas (Biologia Vegetal), Universidade Estadual Paulista, Unesp, Instituto de Biociências, Departamento de Botânica, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brasil; [2]Univ Estadual Paulista, Unesp, Instituto de Biociências, Departamento de Botânica, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brasil

*Autor correspondente: ghaber@rc.unesp.br; telefone: 0055 (19) 3526-4210

Resumo

Alumínio (Al^{3+}) causa fitotoxicidade e limita a produtividade dos *Citrus*. No Brasil, a combinação de laranjeira-doce (*Citrus sinensis*) enxertada em limoeiro (*Citrus limonia*) cv. 'Cravo' é utilizada em mais de 80% dos pomares. Contudo, na maioria dos estudos sobre toxicidade ao Al, porta-enxertos não enxertados são cultivados diretamente em solução nutritiva. Foram estudamos os efeitos do Al no crescimento e acúmulo de nutrientes em mudas de laranjeira 'Valencia' enxertadas em limoeiro 'Cravo' cultivadas em tubos de PVC contendo areia. As plantas foram irrigadas com solução nutritiva (pH = 4,0) contendo 0, 185, 370, 555 e 740 μM Al. A disponibilidade trocável de Al foi crescente, atingindo 55% quando a areia foi irrigada com 740 μM Al. Apesar disso, o crescimento do caule e das raízes foi semelhante entre as cinco doses de Al aos 90, 200 e 260 dias após o plantio. A biomassa de folhas, de raízes, biomassa total e até o número de folhas, área foliar e área foliar específica seguiram este mesmo padrão de resposta. 90% do Al absorvido foi retido no sistema radicular e houve pouca interferência do Al no acúmulo dos macro e micronutrientes nos diferentes órgãos. Portanto, para estudos em que laranjeiras 'Valência' enxertadas em limoeiro 'Cravo' são testadas em substratos inertes como a areia, é necessário elevar a disponibilidade trocável de Al no substrato para além de 55%, utilizando-se mais do que 740 μM Al na solução de irrigação. Caso contrário, o Al parece não interferir no crescimento e nutrição mineral dessas plantas.

***Palavras chave*:** Citrus, porta-enxerto, *Citrus sinensis*, *Citrus limonia*, toxicidade e nutrição mineral

1- INTRODUÇÃO

O Brasil é o principal produtor mundial de citros, destacando-se na produção de suco de laranja concentrado, sendo também o maior exportador desse produto (Agrianual, 2009). O cultivo de citros nos estados de São Paulo e Minas Gerais é um dos mais importantes no país e é realizado em grande parte onde originalmente foi a borda sul da vegetação de Cerrado no Brasil, onde os solos são ácidos, ricos em alumínio (Al) e com fertilidade limitada (Haridasan, 2008; Souza & Habermann, 2012). Porém, o uso da calagem para elevar a saturação de bases no solo (V%), neutralizar o Al e corrigir o pH do solo tem sido a tecnologia adotada para se evitar perdas de produtividade.

A fitotoxicidade por Al tem sido um dos principais fatores que limitam a produtividade agrícola e se manifesta por meio de sintomas com efeitos contínuo e crescente na morfologia e fisiologia das raízes. Esses sintomas incluem reduções no número e comprimento das raízes, resultando na redução da massa seca e volume do sistema radicular (Sivaguru & Paliwal, 1993), redução da absorção de água (Foy et al., 1978) e nutrientes (Mendonça et al., 2003). Com isso, há restrição da exploração do solo pelas raízes e perdas de produtividade.

O Al também interfere na absorção de outros nutrientes, promovendo desbalanço na absorção de nitrogênio (N), enxofre (S) e no ciclo do carbono (Bolan & Hedley, 2003; Tang & Rengel, 2003). Pode interferir também na absorção, transporte e acúmulo de cálcio (Ca), magnésio (Mg), fósforo (P) e potássio (K) (Foy et al., 1978). Em *Citrus* sp, essas interferências se dão pela alta demanda pelo Mg e, principalmente Ca (Oliveira, 1986), além da sensibilidade dos porta-enxertos à acidez e ao Al no solo (Malavolta & Violante Neto, 1988). Em porta-enxertos como limoeiro rugoso (*Citrus jambhiri*), tangerineira (*Citrus reshni*) 'Cleópatra', laranjeira-azeda (*Citrus aurantium*), citrangeiro (*Poncirus trifoliata* x *Citrus sinensis*) 'Carrizo' e citrumeleiro (*Poncirus trifoliata* x *Citrus paradisi*) 'Swingle', o desbalanço de macro e micronutrientes nas raízes e parte aérea já foi relatado quando as plantas foram expostas à até 1655 µM Al (Lin & Myhre, 1991). Em limoeiro (*Citrus limonia*) cv. 'Cravo' e citrumeleiro 'Swingle', mas principalmente no segundo, houve diminuição do acúmulo de K, Mg, S e P quando as plantas foram expostas à até 1110 µM Al em solução nutritiva (Santos et al., 1999).

A interferência do Al na absorção e acúmulo de nutrientes pode estar associada à alta retenção química do Al nos tecidos das raízes, sendo pouco Al transportado para a parte aérea e acumulada nas folhas (Lin & Myhre, 1991; Santos et al., 1999; Banhos et al., 2016). Mesmo assim, o Al também pode interferir em atividades metabólicas na parte aérea, sendo esses efeitos considerados

indiretos (à longa distância). Em limoeiro 'Cravo', limoeiro 'Volkameriano' (*Citrus volkameriana*), tangerineira 'Cleópatra' e tangerineira 'Sunki' (*Citrus sunki*) (Pereira et al., 2000; Chen et al. 2005a,b; Banhos et al., 2016), bem como em toranjeira (*Citrus grandis*) (Jiang et al., 2008), o Al reduz a assimilação de CO_2, a condutância estomática e atenua a fluorescência da clorofila *a*. Todos esses estudos supra-descritos foram feitos com porta-enxertos e, na maioria das vezes a concentração nominal de Al na solução nutritiva foi sempre maior que 1000 μM, concentração acima da qual os sintomas de fitotoxicidade parecem ser mais pronunciados em plantas não enxertadas. Assim, não se encontram estudos da interferência do Al em plantas cítricas enxertadas, tão importantes na produção de laranja-doce.

Na citricultura brasileira e paulista a combinação de diversos cultivares de laranjeira-doce (*Citrus sinensis* L. Osbeck) enxertados sobre o limoeiro 'Cravo' ocorre em mais de 80% dos pomares devido ao pronunciado vigor do limoeiro 'Cravo' conferido à copa, precocidade de produção e principalmente tolerância à seca (Ribeiro & Machado, 2007; Magalhães Filho et al., 2008), importante em pomares não irrigados, como na citricultura brasileira.

Dessa forma, o presente trabalho teve como objetivo avaliar a interferência do Al no crescimento vegetativo e acúmulo de macro e micronutrientes em plantas de laranjeira-doce cv. 'Valencia' enxertadas em limoeiro 'Cravo'. Assim, testamos cinco concentrações de Al a fim de confirmar a sua fitotoxicidade nesta importante combinação copa/porta-enxerto da citricultura brasileira.

2- MATERIAL E MÉTODOS

2.1 Material vegetal e condições experimentais

Foram utilizadas plantas de laranjeira-doce (*Citrus sinensis* L. Osbeck) cv. 'Valência' enxertadas em limoeiro (*C. limonia*) cv. 'Cravo' com 14 meses de idade e 50,2 ± 1,8 cm de altura, obtidas de viveiro comercial certificado. As plantas foram cultivadas em tubos de PVC (90 cm de altura x 15 cm de diâmetro interno; 15L) contendo areia lavada. Na parte inferior dos tubos, uma tela de plástico com malha de 0,2 cm foi fixada com banda de borracha e uma camada de brita foi usada para drenagem da água. Os tubos ficaram na vertical, apoiados por estrutura de madeira, distanciados entre si 0,25 m na linha e 0,80 m nas entrelinhas. Essas estruturas ficaram em casa de vegetação com ventiladores e controle de umidade para evitar o aquecimento excessivo, de modo que a temperatura média durante o estudo foi de 28,12 ± 1,22°C.

As plantas foram irrigadas três vezes por semana com 500ml de solução nutritiva utilizada para estudo de tolerância de plantas de arroz (Furlani & Furlani, 1988), que é baseada na solução de Clark (Clark, 1975) e tem sido utilizada em estudos de toxicidade de Al em *Citrus* (Santos et al., 1999; Banhos et al., 2016). Esta solução continha a seguinte composição de macronutrients (em mM): 0,96 (NO_3^-); 0,41 (NH_4^+); 0,013 (P); 0,86 (K^+); 1,43 (Ca^{2+}); 0,33 (Mg^{2+}); 0,22 (S) e micronutrientes (em μM): 214,1 (Cl^-); 23,3 (Fe-EDTA); 8,33 (B); 2,91 (Mn^{2+}); 0,76 (Zn^{2+}); 0,32 (Cu^{2+}); 0,31 (Mo^{2+}). À esta solução, cinco concentrações de Al de 0, 185, 370, 555 e 740 μM Al (0, 5, 10, 15 e 20 mg L^{-1}), a partir da fonte $AlK(SO4)_2$ 12 H_2O, foram adicionadas à solução básica e, após correção do pH para 4,0, as cinco soluções constituíram os tratamentos aplicados por irrigação nas plantas. O limite superior desses cinco tratamentos (740 μM Al) foi estabelecido arbitrariamente em testes prévios que objetivaram a superação de 50% da saturação em Al (Al^{3+}%) na areia, uma vez que não existem na literatura dados para apoiar essas concentrações. Além disso, como descrito na introdução, na maioria dos estudos sobre toxicidade de Al utiliza-se cultivo de plantas não enxertadas diretamente em solução nutritiva. Para nos certificar de que a areia não iria interferir na disponibilidade de nutrientes da solução nutritiva, a análise de sua fertilidade foi realizada antes e após a lavagem (Tabela 1). Vasos sem plantas, mas com areia, também foram irrigados por 90 dias para avaliação da fertilidade e de Al^{3+}% (Tabela 2).

2.2 Estratégia experimental

As plantas de laranjeira 'Valência' enxertadas em limoeiro 'Cravo' foram irrigadas com solução nutritiva com as cinco concentrações de Al e, aos 0, 90, 200 e 260 dias após o plantio (DAP) tiveram o comprimento do caule e das raízes anotados. As folhas foram contadas, a área foliar (cm^2) medida e área foliar específica (cm^2 g^{-1}) calculada. Os órgãos (raízes, caule + pecíolos e folhas) foram separados e tiveram suas biomassas determinadas, que somadas compuseram a biomassa total da planta. Após a determinação da biomassa em cada época, as concentrações totais de N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn e Al foram determinadas nos tecidos desses órgãos. A partir desses valores, foi calculada a porcentagem da concentração de cada nutriente encontrado em cada órgão em relação à concentração total do nutriente na planta. Este cálculo também foi realizado para o Al.

2.3 Medidas biométricas

O comprimento radicular e comprimento do caule, do coleto da planta até os meristemas apicais caulinares e radiculares, respectivamente, foram medidos com fita métrica (cm). O número de folhas foi contado manualmente. A área foliar (cm^2) foi medida com integrador de área foliar, LI-

3100C (LI-COR, Lincoln, NE, USA). Após secagem em estufa de circulação forçada de ar a 60°C até massa constante, as amostras de raízes, caule + pecíolos e folhas foram pesadas em balança de precisão para determinação da biomassa (g).

A partir da massa seca das folhas (MSF) e da área foliar (AF), foi calculada a área foliar específica (AFE, AF total ÷ MSF), de acordo com Habermann & Bressan (2011).

2.4 Concentração de nutrientes e de Al nos tecidos

As concentrações totais de N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Cu e Al nos tecidos foram determinadas por análise de rotina no Laboratório de Nutrição Mineral de Plantas da Escola Superior de Agricultura Luiz de Queiróz (ESALQ, USP – Piracicaba, SP, Brasil). As amostras foram moídas e digeridas em ácidos nítrico e perclórico. As concentrações de Ca e Mg foram obtidas pelo uso de espectrofotômetro de absorção atômica. O K foi determinado por fotometria de ionização de chama, o S foi medido pelo método turbidimétrico, enquanto que P e Al por colorimetria e o N pelo método de digestão e análise por micro-Kjeldahl (Sarruge & Haag, 1974). Os micronutrientes foram determinados de acordo com Malavolta et al. (1997).

2.5 *Análise da fertilidade da areia*

O pH (em $CaCl_2$), matéria orgânica, P, K, Ca, Mg, Al, soma de bases (SB = [K] + [Ca] + [Mg]), capacidade de troca de cátions (CTC = [K] + [Ca] + [Mg] + [H] + [Al]), saturação de bases [V% = (100 SB) ÷ CTC] e saturação por Al [Al^{3+}% = (100 Al) ÷ CTC] foram determinados por análise de rotina no Laboratório de Ciência do Solo da ESALQ (USP, Piracicaba, SP, Brasil), com métodos de acordo com Raij et al. (1987).

2.6 Análise dos resultados

O delineamento experimental utilizado foi o de blocos casualizados, conduzido com cinco plantas por unidade experimental. Os resultados foram submetidos à análise de variância *One-way Anova*, em que foram testadas, separadamente para cada coleta (0, 90, 200 e 260 DAP), as variáveis biométricas e nutricionais entre as cinco doses de Al. As médias foram comparadas pelo teste de Tukey ($\propto$ = 0,05%).

3 - RESULTADOS E DISCUSSÃO

A areia utilizada como substrato para aplicação das doses de Al mostrou CTC relativamente baixa e desprovida de Al (Tabela 1). A lavagem da areia elevou o pH em uma unidade, diminuindo a concentração de micronutrientes catiônicos (Fe^{2+}, Cu^{2+}, Mn^{2+} e Zn^{2+}). Porém, essa lavagem não alterou a acidez potencial ($H^+ + Al^{3+}$), nem as disponibilidades de P e S, a soma de bases trocáveis e a CTC, SB, V% e o Al^{3+}% (Tabela 1). É importante notar que não encontramos estudos de toxicidade ao Al em que as plantas tenham sido cultivadas em substratos inertes como a areia. Na maioria desses estudos utilizando *Citrus* (Santos et al., 1999; Pereira et al., 2000; Chen et al. 2005a,b; Jiang et al., 2008; Banhos et al., 2016) as plantas foram cultivadas diretamente na solução nutritiva. Assim, o presente trabalho pode trazer informações para futuros estudos de toxicidade ao Al utilizando substrato inerte.

O monitoramento da fertilidade do substrato após aplicação das cinco concentrações de Al mostrou que a irrigação com solução sem Al (0 μM Al) deixou o P disponibilizado no substrato (Tabela 2). Contudo, a presença de Al, sobretudo nos tratamentos de 370, 555 e 740 μM Al, deve ter promovido precipitação de uma parte do Al uma vez que a disponibilidade desses micronutrientes catiônicos foi reduzida nesses tratamentos (Tabela 2). Nos tratamentos com Al, o P também pode ter se precipitado na forma de sais associados ao Al, uma vez que as bases não se alteraram (Tabela 2) e o Al seria um dos poucos candidatos à esta reação de precipitação. Mas mesmo assim, a disponibilidade potencial de Al (Al^{3+}%) foi crescente conforme se aumentou a concentração de Al na solução de irrigação, com exceção dos tubos irrigados com solução contendo 555 μM Al. Nos tubos irrigados com solução contendo 740 μM Al os valores de Al^{3+}% elevaram-se para 55% (Tabela 2). Em pomares comerciais, para se evitar a interferência da acidez do solo e do Al na produtividade, recomenda-se elevar os valores de V% para aproximadamente 70% (Mattos Junior et al., 1987) e reduzir Al^{3+}% para um valor abaixo de 15% (Quaggio et al., 1992). Portanto, valores de Al^{3+}% = 55% e pH ≈ 4,0, como obtido no presente estudo, teoricamente, representa um distúrbio na fertilidade para as plantas estudadas.

Apesar deste distúrbio na fertilidade e crescentes disponibilidades de Al no substrato em que as plantas cresceram, o caule, mas principalmente as raízes cresceram consideravelmente da amostragem inicial (0 DAP) para a segunda amostragem (90 DAP) em todas as doses de Al (Fig 1). Porém, não houve diferença no crescimento desses órgãos entre 90, 200 e 260 DAP quando esta comparação foi feita dentro de cada um dos cinco tratamentos [valores de *p* (caule): 90 DAP = 0,91; 200 DAP = 0,37; 260 DAP = 0,91; (raiz): 90 DAP = 1,00; 200 DAP = 0,99; 260 DAP = 1,00; Fig.

1a, b]. O número de folhas (Fig. 3a) e a área foliar total da planta (Fig. 3b) também seguem este mesmo padrão de resposta em que há aumento das medidas iniciais (0 DAP) para as outras amostragens (90, 200, 260 DAP), mas não entre essas últimas. Apesar do crescimento inicial (0 → 90 DAP), quando o comprimento de caule (Fig. 1a), de raiz (Fig. 1b), bem como a biomassa de folhas (Fig. 2a), de raiz (Fig. 2c), biomassa total (Fig. 2d) e até mesmo o número de folhas (Fig. 3a), área foliar (Fig. 3b) e área foliar específica (AFE; Fig. 3c) foram comparados entre as cinco concentrações de Al, as médias são estatisticamente semelhantes, independentemente das épocas de avaliação. Apenas a biomassa de caule (Fig. 2b) de plantas cultivadas com 740 μM Al por 260 dias mostrou-se superior à das plantas cultivadas com 0 μM Al, sendo que as concentrações de 185, 370 e 555 μM Al tiveram biomassa de caule semelhantes entre si (Fig. 2b).

O fato de a AFE ter sido semelhante entre as cinco concentrações de Al, independentemente da época de avaliação, indica que, até 55% de Al trocável no substrato, este metal não interfere na estrutura foliar, como por exemplo alterações no "custo" de formação da folha. A não interferência do Al na formação da folha também é evidenciada pelo fato de cerca de 60% da concentração do nitrogênio (N) ter sido encontrada nas folhas, e ter apresentado diferença entre as doses de Al somente aos 260 DAP (Fig. 4a; Tabela 3). Corroborando estas evidências, também observamos que aproximadamente 90% do Al ficou retido na raiz das plantas tratadas com as cinco concentrações de Al, nas três épocas de avaliação (Fig. 5f). Portanto, além do Al não interferir na absorção, transporte e acúmulo de N nas folhas (com exceção aos 260 DAP; Tabela 3), os efeitos do Al na parte aérea parecem ser indiretos (a longa distância). A retenção de Al no sistema radicular já foi reportada para muitas espécies sensíveis ao metal (Vitorello et al., 2005). No presente estudo, as laranjeiras 'Valência' foram enxertadas em limoeiro 'Cravo', porta-enxerto que quando exposto isoladamente à solução nutritiva com mais de 1000 μM Al também retém cerca de 80% do Al na raiz, transportando muito pouco para as folhas, onde testes histoquímicos para Al são negativos (Banhos et al., 2016). Em limoeiro 'Cravo' exposto à até 1110 μM Al em solução nutritiva, a concentração de N nas folhas também não se altera, e 80% do Al é retido no sistema radicular (Santos et al., 1999), corroborando nossos resultados. Esses autores também sugeriram usar mais que 14 dias entre as avaliações da interferência do Al na absorção dos nutrientes para confirmar seus resultados. Nós usamos 110 e 60 dias entre as avaliações (90, 200 e 260 DAP) e corroboramos seus resultados, reiterando que o Al (é retido na raiz) e o N (não sofre interferência do Al nas folhas) também seguem o mesmo comportamento em plantas enxertadas.

A retenção do Al nas raízes deve-se às evidências de que o Al liga-se covalentemente às pectinas (Wehr et al., 2010) da parede celular primária das células (Bressan et al., 2016), o que

também foi evidenciado na raiz de limoeiro 'Cravo' (Banhos et al., 2016). Assim, para muitas plantas, incluindo as do gênero *Citrus*, o Al parece não ser (ou é muito pouco) transportado para a parte aérea (Santos et al., 1999; Vitorello et al., 2005), produzindo poucos sintomas diretos nas folhas e ramos, como defenderam Banhos et al. (2016). Porém, nas raízes os sintomas são conspícuos e rápidos (Kopittke et al., 2008), contrastando com o efeito indireto, lento e à longa distância de redução do crescimento da parte aérea, já observado para *Citrus* (Chapman,1968).

Apesar de nossos resultados mostrarem que o crescimento vegetativo não se altera quando Al^{3+}% foi de até 55% no solo, o aumento de Al^{3+}% foi associado à redução da SB e V% em 72% para ambos os parâmetros (Tabela 2), mostrando interferência na disponibilidade das bases, incluindo Ca e Mg, que são nutrientes absorvidos e acumulados em grande concentração pelos *Citrus* (Oliveira, 1986). Contudo, mais de 60% do Ca foi encontrado nas folhas (Fig. 4d), com interferência do Al aos 200 e 260 DAP (Tabela 3). No caso do Mg, houve aumento de concentração nas folhas à medida que as plantas se desenvolveram (90 → 260 DAP) (Fig. 4e), evidenciado de forma mais marcante ainda para o K (Fig. 4c). Esses resultados sugerem que, de forma geral, o Al não interferiu na absorção e acúmulo de bases, como K, Ca e Mg pelas folhas. Citrumeleiro 'Swingle' e citrangeiro 'Carrizo' (não enxertados) expostos à até 1655 μM Al em solução nutritiva também mostram aumento da concentração de Ca nas folhas e diminuição nas raízes (Lin & Myhre, 1991). Esses autores concluíram que as variações das concentrações de Ca nas plantas cítricas podem não ser o principal fator relacionado aos efeitos tóxicos do Al.

A concentração foliar de P não variou aos 90 e 200 DAP, mas aos 260 DAP houve interferência do Al nos três órgãos da planta (Tabela 3). Aproximadamente 40-50% do P permaneceu na folha ao longo do estudo, mas plantas cultivadas com 740 μM Al aos 90 DAP e todas as doses aos 200 e 260 DAP mostraram cerca de 20% de P alocado no caule (Fig. 4b). Desta forma, nota-se que a retenção de P no sistema radicular diminuiu dos 90 DAP para 200 e 260 DAP (Fig. 4b). Aos 260 DAP, as plantas irrigadas com solução contendo 555 μM Al reduziram a concentração de P em todos os órgãos (Fig. 4b). O Al pode se ligar ao P, tanto na membrana quanto no interior das células, formando compostos de baixa solubilidade que precipitam na zona de incorporação de P nas raízes (Clarkson, 1966; Yang et al, 2011). Portanto, esperava-se que ocorresse redução na absorção de P e aumento de sua retenção nas raízes, restringindo seu transporte para a parte aérea. No entanto, à medida que as plantas ficaram mais tempo expostas ao Al (200 e 260 DAP) o P se redistribuiu mais para o caule e manteve sua proporção nas folhas (Fig. 4b). A maior eficiência no transporte de P para a parte aérea de plantas expostas ao Al pode estar relacionada à sua capacidade de neutralizar o Al na raiz (Yang et al., 2011). Esses autores demonstraram que em *Citrus grandis* o P poderia atenuar os

efeitos tóxicos do Al por meio da imobilização do metal na raiz. No presente estudo, o Al foi consideravelmente retido na raiz (Fig. 5f), mas o P só sofreu interferência do Al (comparação entre as cinco doses) aos 260 DAP (tabela 3), sugerindo baixa interferência do P com o Al e necessidade de maior tempo de exposição ao Al para evolução do desbalanço de P.

Mais de 90% do Fe se concentrou nas raízes (Fig. 5c), possivelmente associado ao Al, que também manteve a mesma proporção de retenção nas raízes (Fig. 5f). Aos 260 DAP, a interferência do Al no acúmulo de Fe foliar e radicular é notável (Tabela 3; Fig. 5c). Contudo, sob deficiência de Fe, ocorre clorose nas folhas uma vez que se trata de um elemento de baixa mobilidade metabólica (Jeong & Guerinot, 2009), mas no presente estudo não observamos este sintoma. Além disso, a baixa interferência do Al no acúmulo de N (Fig. 4a) também sugere que a interferência do Al no acúmulo de Fe não deve ter sido importante, dada a complexa relação nutricional entre N e Fe (Marschner, 2011). À medida que as plantas se desenvolveram, dos 90 aos 260 DAP, houve aumento das concentrações de Cu (Fig. 5b) e Mn (Fig. 5d) nas folhas das plantas de todas as doses de Al, embora com interferência do Al nos órgãos das plantas (Tabela 3). Isso sugere que em laranjeiras enxertadas as reações fotoquímicas da fotossíntese não devem ser desfavorecidas pelo Al. Isso porque o Mn compõe a enzima que promove a fotólise da água (Yano et al., 2006) e o Cu compõe a plastocianina, proteína cúprica que transfere os elétrons entre o complexo do citocromo b_6f e o fotossistema I (PSI) (Yruela, 2005). De fato, valores da eficiência quântica potencial e efetiva do fotossistema II, transporte eletrônico de elétrons nos tilacóides e extinção fotoquímica da luz aumentaram com o incremento da concentração de Al na solução de irrigação das plantas de todos os tratamentos aos 260 DAP (dados não mostrados). Isso reforça a pouca interferência do Al no metabolismo mineral do Fe, Cu e Mn, com também reduzidas consequências fisiológicas para o desempenho fotoquímico, conforme já observado por Banhos et al. (2016) para plantas de limoeiro ‘Cravo’.

A concentração foliar de Zn sofreu interferência do Al nas três épocas de avaliação (Tabela 3). Mas é na raiz que se espera maior interferência do Al no acúmulo de Zn, que é cofator enzimático da biossíntese de triptofano, precursor do ácido indol acético (IAA) (Bartel, 1997). O desbalanço na distribuição de auxinas, como o IAA, é um dos candidatos à explicação do porquê o Al reduz o crescimento radicular (Sun et al., 2010). Dos 90 DAP até os 200 e 260 DAP, a proporção de Zn acumulado nas raízes reduziu de aproximadamente 60% para 40-50% (Fig. 5e), mas a interferência do Al só ocorreu aos 90 e aos 260 DAP (Tabela 3). Por outro lado, estas interferências não parecem ter sido importantes, principalmente quando se comparam as doses de 0 e 740 μM Al (fig. 5e). Além disso, no presente estudo, as raízes cresceram consideravelmente entre 0 e 90 DAP e as diferenças (do crescimento de raízes) entre as cinco doses de Al também não foram significativas ($p > 0,05$)

independentemente da época de avaliação. Esses resultados demonstram que aspectos importantes do metabolismo de Zn não parecem ter sido afetados de maneira relevante pelo Al.

4 - CONCLUSÕES

Quando a disponibilidade de Al na areia (Al^{3+}%) é de até 55%, parece não haver prejuízo para o crescimento, desenvolvimento e nutrição mineral de laranjeiras 'Valência' enxertadas em limoeiro 'Cravo'. Além disso, ao contrário da maioria dos estudos de fitotoxicidade ao Al em que as plantas são cultivadas diretamente na solução nutritiva, nosso estudo mostrou que para produzir efeitos tóxicos do Al em laranjeira 'Valência' enxertada em limoeiro 'Cravo' cultivada em substrato inerte como a areia, é preciso irrigar o substrato com mais de 740 μM Al (20 mg Al L^{-1}).

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Tabela 1. Análise química e concentração de macro e micronutrientes em areia grossa bruta e após peneiramento e lavagem para uso no estudo com plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo'

Amostra	pH	P	S	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+Al^{3+}	CTC	SB	V%	Al^{3+}%	BO_3^-	Cu^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}
		--mg dm^{-3}--		----------mmol$_c$ dm^{-3}----------							-------%------		----------mg dm^{-3}----------				
Bruta	5,2	16	< 4	< 0,7	4	< 1	< 1	8	12,6	4,8	38	1	<0,12	0,4	32	4,2	0,4
Lavada	6,1	16	< 4	< 0,7	4	< 1	< 1	8	12,6	4,8	38	1	<0,12	< 0,4	18	2,6	< 0,4

Resultados de análise de fertilidade, obtida do laboratório do Departamento de Ciência do Solo, Escola Superior de Agricultura Luiz de Queiroz, USP, Piracicaba, SP.

Tabela 2. Índices de fertilidade e concentração de macro e micronutrientes em areia grossa peneirada e lavada, após 90 dias sob irrigação com soluções nutritivas com 0, 185, 370, 555 e 740 μM Al, usadas no estudo com plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo'

Trat. $[Al^{3+}]$	pH	P	S	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+Al^{3+}	CTC	SB	V%	Al^{3+}%	BO_3^-	Cu^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}
--μM--		--mg dm^{-3}--		----------mMol$_{carga}$ dm^{-3}----------							-------%------		----------mg dm^{-3}----------				
0	5.1	44	*	< 0.7	4	< 1	0	11	15.8	5	32	1	<0.12	<0.4	32	6.0	3.1
185	4.4	6	*	< 0.7	< 3	< 1	1	12	15.1	3.2	21	20	<0.12	<0.4	28	5.0	0.6
370	4.3	7	*	< 0.7	< 3	< 1	2	11	13.3	2.6	19	37	<0.12	<0.4	27	2.8	0.7
555	4.4	7	*	< 0.7	< 3	< 1	1	11	13.7	3.0	22	22	<0.12	0.4	25	3.7	0.5
740	4.2	7	*	< 0.7	< 3	< 1	2	13	14.6	1.4	9	55	<0.12	<0.4	26	2.1	0.5

Resultados de análise de fertilidade, obtida do laboratório do Departamento de Ciência do Solo, Escola Superior de Agricultura Luiz de Queiroz, USP, Piracicaba, SP.

* (Elemento enxofre não determinado na análise)

	P		
	90 DAP	**200 DAP**	**260 DAP**
NITROGÊNIO			
Folha	0.7704	0.2290	0.0000
Caule	0.1111	0.7540	0.9273
Raiz	0.2865	0.1288	0.0031
FÓSFORO			
Folha	0.1287	0.3257	0.0002
Caule	0.0000	0.3737	0.0100
Raiz	0.1204	0.6001	0.0000
POTÁSSIO			
Folha	0.8179	0.0101	0.0000
Caule	0.4711	0.7515	0.0701
Raiz	0.3005	0.0147	0.1344
CÁLCIO			
Folha	0.8552	0.0484	0.0246
Caule	0.2092	0.5393	0.2300
Raiz	0.0000	0.8413	0.0562
MAGNÉSIO			
Folha	0.6299	0.1786	0.0366
Caule	0.2594	0.0019	0.1656
Raiz	0.0001	0.7683	0.9225
ENXOFRE			
Folha	0.0114	0.9899	0.0000
Caule	1.0000	0.6047	0.0082
Raiz	0.0000	0.8005	0.1095
BORO			
Folha	0.0971	0.5709	0.0040
Caule	0.0441	0.0335	0.0098
RAIZ	0.1778	0.0726	0.0689
COBRE			
Folha	1.0000	0.0000	0.0000
Caule	0.7354	0.0000	0.0000
Raiz	0.0061	0.3598	0.0000
FERRO			
Folha	0.0862	0.0676	0.0000
Caule	0.3705	0.7535	0.5265
Raiz	0.0059	0.3318	0.0071
MANGANÊS			
Folha	0.0001	0.0000	0.0000
Caule	0.0002	0.4003	0.0458
Raiz	0.0000	0.4615	0.5987
ZINCO			
Folha	0.0333	0.0006	0.0000
Caule	0.0001	0.7402	0.4235
Raiz	0.0000	0.0828	0.0103
ALUMÍNIO			
Folha	0.3100	0.0311	0.0015
Caule	0.0002	0.6340	0.5963
Raiz	0.0241	0.1757	0.0002

Tabela 3: Valores de *p* obtidos da análise de variância, considerada para cada órgão vegetativo e cada época de avaliação, tendo como causas de variação cinco concentrações de Al na solução de irrigação (0, 185, 370, 555 e 740 μM Al) de plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo', cultivados em areia grossa por até 260 dias.

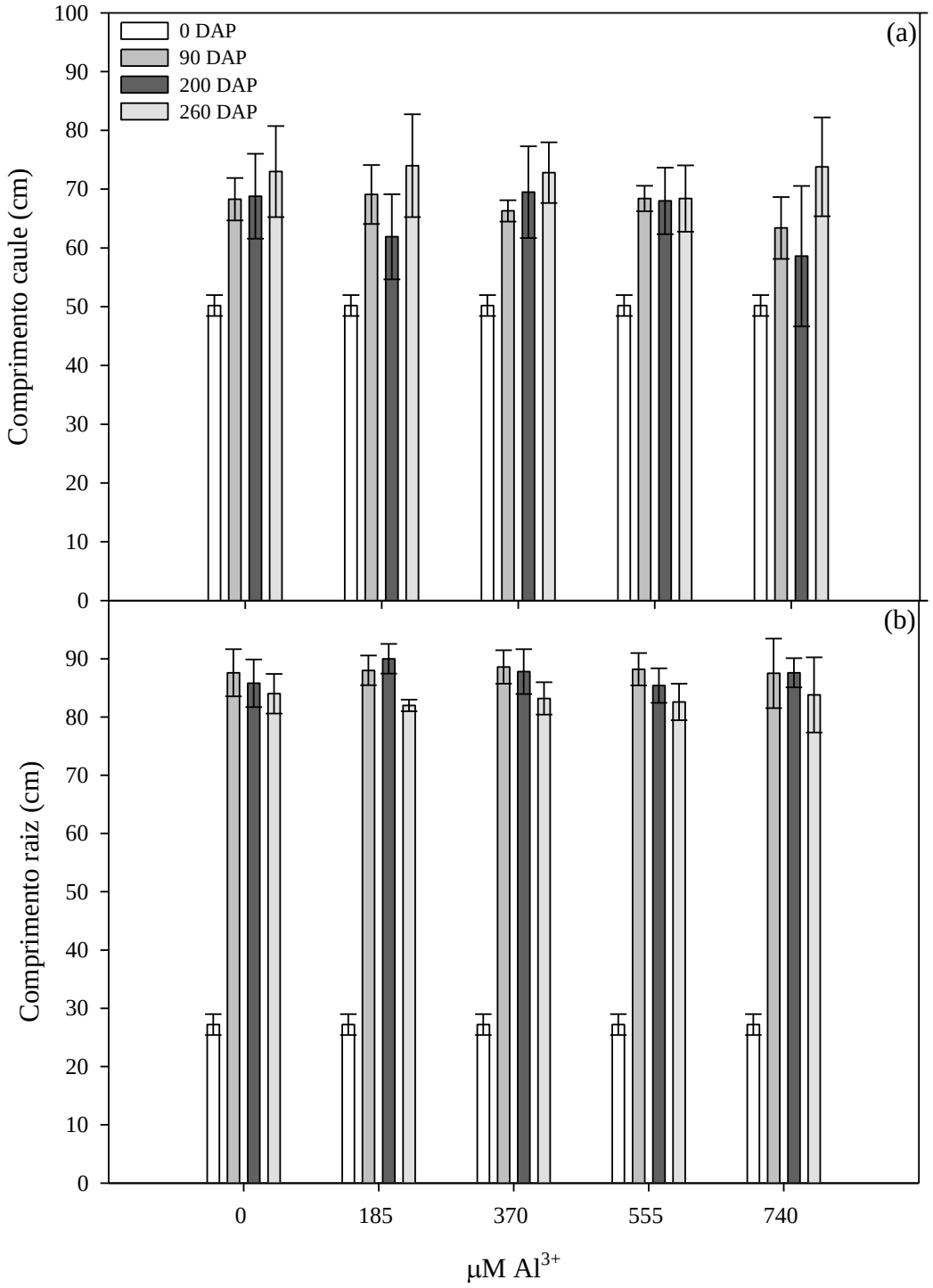

Figura 1. Comprimento do caule (a) e da raiz (b) de plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo', cultivados em areia grossa por 260 dias e irrigados com solução nutritiva contendo 0, 185, 370, 555 e 740 μM Al. Ausência de letras indicam semelhanças entre as concentrações de Al dentro de cada época de avaliação (DAP). Colunas são a média de 5 plantas (repetições) e as barras, o desvio padrão. DAP: dias após plantio.

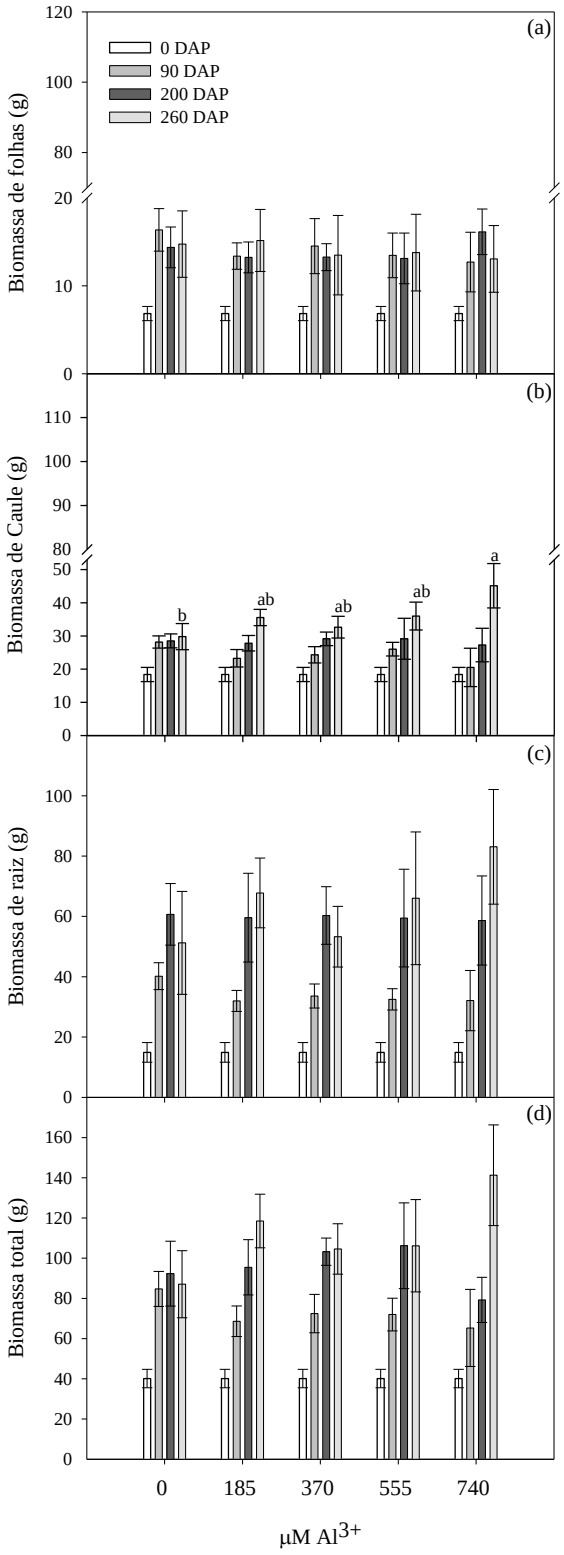

Figura 2. Biomassa de folhas (a), caule (b), raiz (c) e total (d) de plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo', cultivados em areia grossa por 260 dias e irrigados com solução nutritiva contendo 0, 185, 370, 555 e 740 µM Al. Ausência de letras indicam semelhanças entre as concentrações de Al dentro de cada época de avaliação (DAP). Colunas são a média de 5 plantas (repetições) e as barras, o desvio padrão. DAP: dias após plantio.

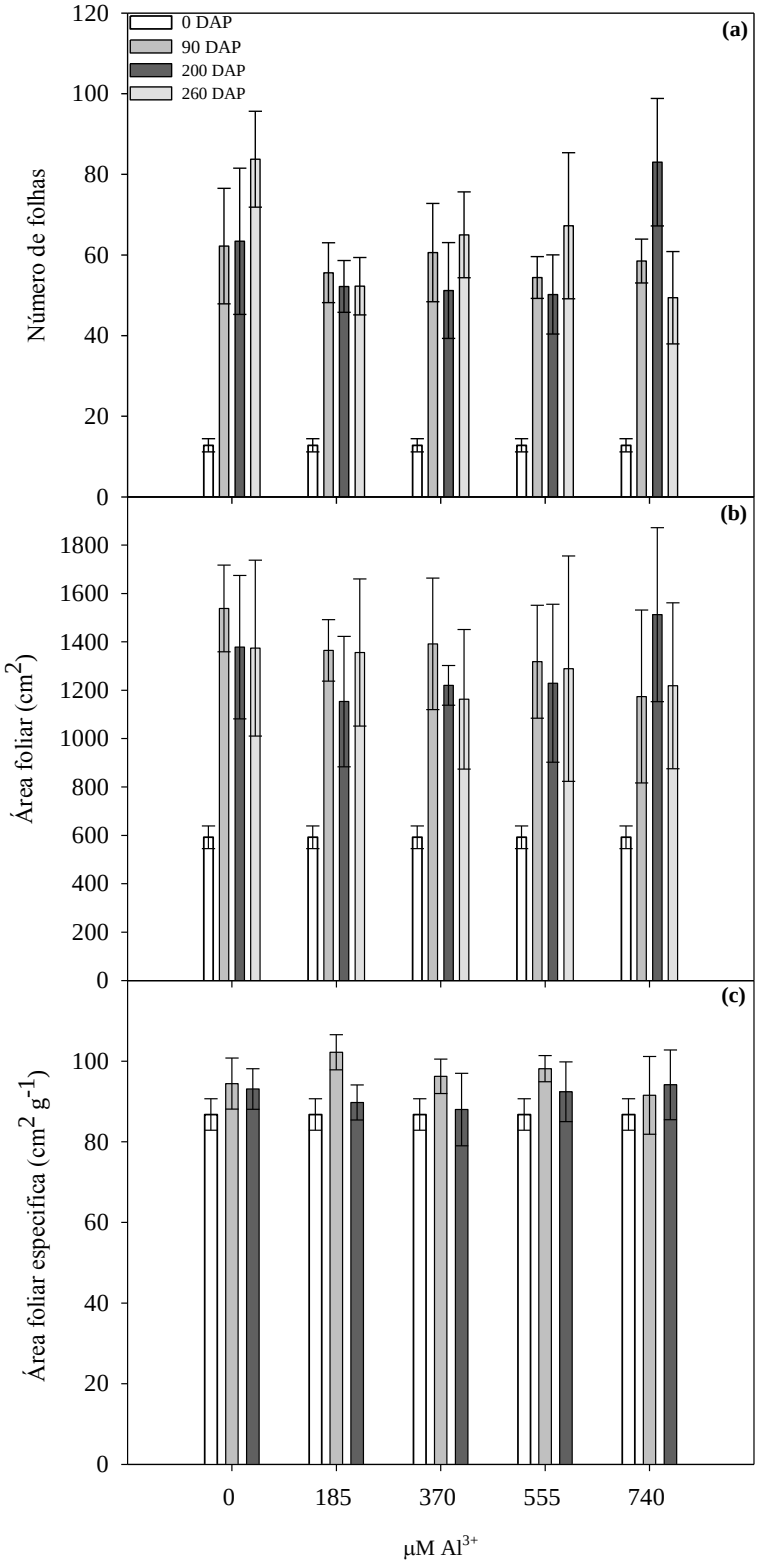

Figura 3. Número de folhas (a), área foliar (b) e área foliar específica (c) de plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo', cultivados em areia grossa por 260 dias e irrigados com solução nutritiva contendo 0, 185, 370, 555 e 740 μM Al. Ausência de letras indicam semelhanças entre as concentrações de Al dentro de cada época de avaliação (DAP). Colunas são a média de 5 plantas (repetições) e as barras, o desvio padrão. DAP: dias após plantio.

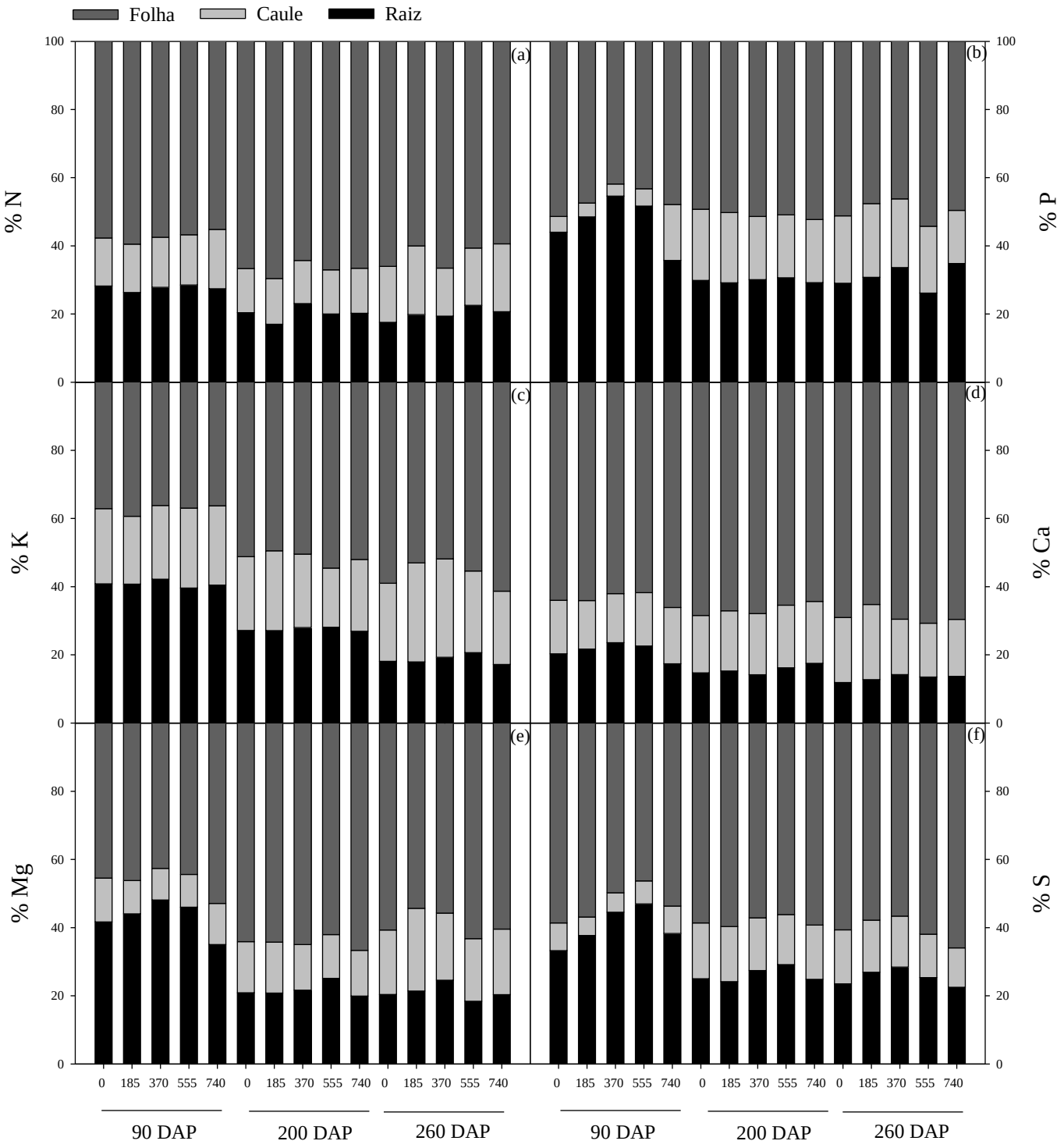

Figura 4. Distribuição de N (a), P (b), K (c) Ca (d), Mg (e) e S (f) em raiz, caule e folhas de plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo', em areia grossa por 260 dias e irrigados com solução nutritiva contendo 0, 185, 370, 555 e 740 μM Al. Colunas são as médias de 5 plantas (repetições). DAP: dias após plantio.

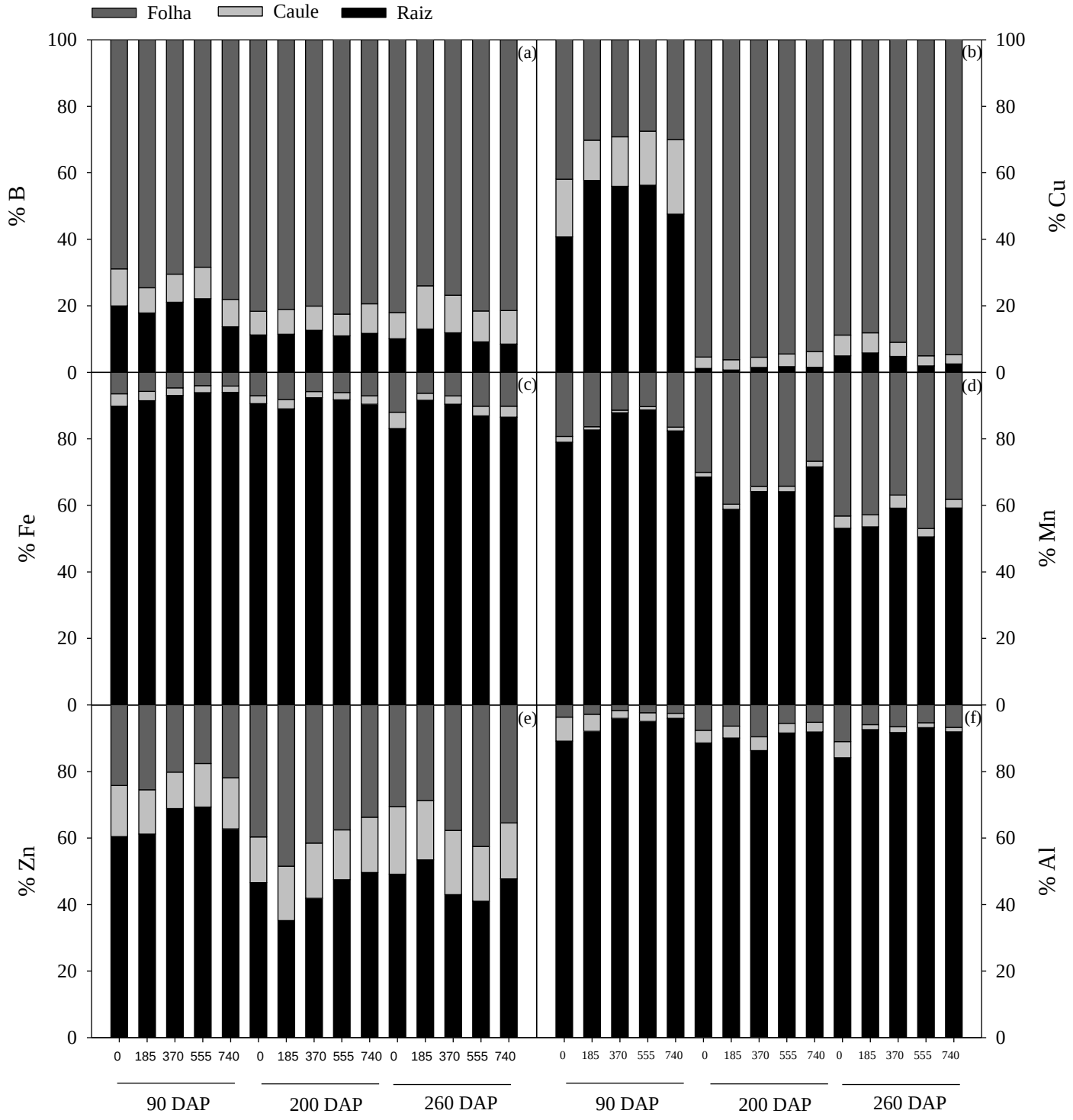

Figura 5. Distribuição de B (a), Cu (b), Fe (c) Mn (d), Zn (e) e Al (f) em raiz, caule e folhas de plantas de laranjeira 'Valência' enxertadas sobre limoeiro 'Cravo', cultivados em areia grossa por 260 dias e irrigados com solução nutritiva contendo 0, 185, 370, 555 e 740 μM Al. Colunas são as médias de 5 plantas (repetições). DAP: dias após plantio.

Capítulo 3

High aluminum availability may affect *Styrax camporum*, an Al non-accumulating species from the Brazilian savanna

Otávia F. A. A. Banhos[1], Marcelo Claro de Souza[2] and Gustavo Habermann[3*]

[1]*Programa de Pós-Graduação em Ciências Biológicas (Biologia Vegetal), Univ Estadual Paulista, Unesp, Instituto de Biociências, Departamento de Botânica, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil;*

[2]*Universidade de São Paulo, USP, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Ciências Farmacêuticas, Av. do Café, s/n; 14040-903, Ribeirão Preto, SP, Brazil;*

[3]*Univ Estadual Paulista, Unesp, Instituto de Biociências, Departamento de Botânica, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil;*

* *Corresponding author. E-mail: ghaber@rc.unesp.br;* Tel.: +0055 19 3526-4210

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Abstract

In the Cerrado vegetation, generally known as 'Brazilian savanna', aluminum (Al) accumulating and non-accumulating plants coexist, growing on soils that are acidic, poor in nutrients and rich in Al. Differing from Al-sensitive species, these plants are not expected to experience Al injuries. Using *Styrax camporum*, a non-accumulating plant, we recorded biometric variations in leaves, shoots and roots of young plants exposed to 0 and 1480 μM Al in a nutrient solution. Photosynthetic responses were measured bi-weekly over 91 days. Plants exposed to Al drastically reduced flushing, indicating that Al interferes with the functioning of the shoot apex. Aluminum caused low CO_2 assimilation rate, largely explained by low stomatal conductance, while Al-induced decrease in photochemical performance occurred only on some dates during the experiment. In addition, the absorbed Al was mostly retained in the roots. Although counter-intuitive, as this species grows on Al-rich soils, we noted that high Al availability impairs lateral root formation, causing an impact on water uptake and gas exchange rates of this species.

Keywords: Al^{3+}; Cerrado woody species; Metal toxicity; Nutrient solution; Photosynthesis; Styracaceae

Introduction

The flora of the Cerrado is comprised of aluminum (Al) accumulating and non-accumulating species (Haridasan 1982; Souza et al. 2015a), which are distributed between savanna-type (cerrado *sensu stricto*) and forest (Cerradão) physiognomies of this vegetation (Ratter et al. 1997). These physiognomies are mostly comprised of shrubs and trees that grow on dystrophic and acidic (pH < 4.0) soils with exchangeable Al saturation (m%) between 60 and 90% (Ratter et al. 1997; Habermann and Bressan 2011, Souza et al. 2015b). In addition, these soils are limited in nutrients, mainly to P, Zn, Cu, and Mn (Haridasan and Araújo 1988; Haridasan 2008; Pivello et al. 2010).

Some Al accumulating plants may show above 15000 mg Al kg^{-1} dry leaves and these species belong to a few families occurring in the Cerrado: Melastomataceae (*Miconia* spp), Rubiaceae (*Palicourea rigida*), Vochysiaceae (*Callisthene sp.*, *Qualea spp*, *Salvertia convallariodora* and *Vochysia sp*) and Loranthaceae (*Passovia ovata* and *Psittacanthus robusthus*) (Haridasan 1982; Haridasan and Araújo 1988; Andrade et al. 2011; Scalon et al. 2013). Al-accumulating species may constitute 35% of the species found in a Cerrado *sensu stricto* remnant (Haridasan 1982), and 18% of the species found in a Cerradão fragment (Haridasan and Araújo 1988). The rest of the non-herbaceous Cerrado plant community may be considered non-accumulating species, which show between 100 and 600 mg Al kg^{-1} dry leaves (Haridasan 1982; Souza et al. 2015a).

On the other hand, most Al-sensitive species are herbaceous (crop) plants (Silva et al. 2012) or trees that are not able to secrete Al-organic acid complexes at the root tip (Brunner and Sperisen 2013). In these sensitive species, the most conspicuous symptom is the inhibition of root growth (Horst et al. 2010; Sun et al. 2010) because the Al binds itself to the rhizodermis, increasing its rigidity while reducing the ability of outer cells to elongate (Kopittke et al. 2008). These plants also show reduced gas exchange rates, such as CO_2 assimilation rate (*A*), which could be considered an indirect/long-distance effect caused by the fact that toxic Al binds itself to root cell walls and can be permanently stored in this organ (Vitorello et al. 2005; Rangel et al. 2009). Sensitive plants exposed to Al accumulate 70-80% more Al in roots than in leaves and shoots (Jiang et al. 2009; Yang et al. 2011). Some studies attribute Al-induced decrease in *A* to photochemical apparatus injuries, as evidenced by low values of electron transport rate (ETR), effective quantum yield of photosystem II (ΦPSII) and photochemical quenching (qP) in plants exposed to more than 1000 μM Al (Chen et al. 2005; Jiang et al. 2008; 2009).

As far as we are aware, there are no studies of Al effects on plant growth or photosynthetic performance in either Al-accumulating or Al-non-accumulating species from the Cerrado. Aluminum has also been suggested to have some unknown positive roles in chloroplasts of Al-accumulating

species, as it has been histochemically evidenced in these organelles of *Q. grandiflora* and *Callisthene major* (Vochysiaceae) (Andrade et al. 2011). Moreover, some Al-accumulating species do not grow well and show leaf chlorosis when cultivated in eutrophic soils with low m% (Haridasan 2008). In this way, although no physiological role has been suggested for Al in these plants, accumulating and non-accumulating species coexist in the Cerrado, growing on acidic soils with m% > 70%, with no apparent damage to their organs or metabolism (Andrade et al. 2011). Therefore, Al is not expected to cause disturbances in growth, physiological responses or morphological changes in these plants.

Styrax camporum Pohl. (Styracaceae) is considered a non-accumulating species (Haridasan 1982), although we have observed between 1000 and 1500 mg Al kg^{-1} dry leaves in field studies (data not shown). It is a tree (3-8 m in height), naturally occurring in Cerrado areas, and it exhibits a wide distribution between Cerrado physiognomies (Kissmann et al. 2012). It has been observed in remnants of Cerradão, cerrado *sensu stricto*, and other forest-influenced environments within Cerrado areas (Nakajima and Monteiro 1987). Its seeds are dispersed during the dry season (April-August) and are relatively easy to germinate (Kissmann and Habermann 2013), growing into five-leaf plants within approximately eight months.

In the present study, we predicted that *S. camporum* plants are not sensitive to high soluble Al concentration (>1000 μM) in nutrient solution. We recorded biometrical variations of leaves, shoots and roots of plants exposed to Al over 91 days and the Al concentration in these organs at the end of the study. During this period, we also measured photosynthetic parameters, such as gas exchange rates, as well as photochemical performances.

Material and methods

Plant material and experimental conditions

Mature fruits of *Styrax camporum* Pohl were collected from adult trees growing in a Cerradão area (37 ha; 22°15'S and 47°00'W) in the municipality of Corumbataí, state of São Paulo, southeastern Brazil. Fifteen eight-month-old plants (26 ± 1 cm in height) were obtained from seeds that germinated in May 2013. The roots of these plants were rinsed under tap water to remove substrate debris composed of organic substrate (Tropstrato florestal®, São Paulo, Brazil), sand and oxisoil (1:1:1; v:v:v), on which these plants grew in 2L (black) plastic bags, inside a greenhouse. The intact plants were transferred to opaque plastic boxes (50 cm in length x 30 cm in width x 15 cm in height; 20 L), containing nutrient solutions with 0 and 1480 μM Al.

As far as we can tell, studies of young non-accumulating plants from the Cerrado under

contrasting Al concentrations are not available, and we have not previously tested other Al concentrations for *S. camporum*. Therefore, we chose 1480 μM Al (40 mg L^{-1}) because most studies testing high [Al] on Al-sensitive plants have used more than 1000 μM: 1480 μM (Konrad et al. 2005; *Coffea arabica*), 2000 μM (Chen et al. 2005; *Citrus reshni*), 1600 μM (Jiang et al. 2008; *C. grandis*), 1200 μM (Jiang et al. 2009; *C. grandis*) and 1850 μM (Silva et al. 2012; *Secale cereale*).

We used a nutrient solution (Furlani and Furlani 1988) with a chemical composition based on Clark's solution (Clark 1975) that has been used to study Al toxicity in Al-sensitive tree species (Santos et al. 2000). However, we diluted its macro- and micronutrient concentrations by seven in order to resemble the nutrient composition of Cerrado soils (Habermann and Bressan 2011; Souza et al. 2015b). For example, Kopittke et al. (2010) also observed that the soil solution from an Australian acidic oxisoil exhibits nutrient concentrations that are approximately seven-fold lower than those in Hoagland & Arnon's nutrient solution. Although nutrient concentrations in solutions (mass per liquid volume) cannot be compared with nutrient *exchangeable contents* measured in soils (ionic charges per volume of a solid matrix), we observed that this final nutrient solution showed no precipitation and induced no nutrient deficiency in *S. camporum* plants. The pH of the aerated solution was maintained at 4.0 ± 0.1. Nominal 1480 μM Al supply resulted in 1100 ± 5.3 μM Al, and nutrient concentrations were as follows. Macronutrients (in mM): NO_3^- 0.137; NH_4^+ 0.058; P, 0.0019; K, 0.123; Ca, 0.204; Mg, 0.047; S, 0.031. Micronutrients (in μM): Cl, 30.58; Fe (EDTA), 3.32; B, 1.19; Mn, 0.41; Zn, 0.10; Cu, 0.04; Mo, 0.04. In addition, when we tested the chemical composition of this solution on Geochem-EZ software (Shaff et al. 2010) it resulted in more than 85% free Al^{3+} available. Solution pH was monitored daily (corrected to 4.0, if necessary) and replaced every ten days.

The boxes stood on benches inside a greenhouse with semi-controlled conditions. During the experiment, the photosynthetic photon flux density (PPFD) inside the greenhouse was 782.03 ± 157.73 μmol photons m^{-2} s^{-1}, with photoperiod of approximately 13h, and air temperature, 29.5 ± 1.9. Expanded polystyrene (Isopor®) 50 x 30 cm plates (2-cm thick) with five holes (2.5 cm in diameter) were floated on the nutrient solution in the boxes, and the plants were fixed in these holes with polyurethane foam strips that were placed around the plant collar.

Experimental design

Five plants were grown in the box with the nutrient solution containing 1480 μM Al, and five plants were grown in the box with the solution containing 0 μM Al. In addition, leaves of five plants were counted and their shoot and root lengths (cm), leaf area (cm^2) and biomasses of leaves, stems

and roots were determined at the beginning of the study. After 91 days, these biometric parameters were thoroughly measured for plants from both boxes, and the Al concentration was also measured in roots, shoots and leaves.

At 0 (on the day of planting), 14, 21, 28, 42, 49, 56, 62, 70, 86 and 91 days after planting (DAP), the gas exchange and chlorophyll fluorescence were measured on the plants' leaves.

Photosynthetic parameters

CO_2 assimilation (*A*) and transpiration (*E*) rates, stomatal conductance (*gs*) and intercellular CO_2 (*Ci*) were measured with an open gas exchange system (LI-6400xt; LI-COR, Lincoln, NE, USA). Water use efficiency (WUE) was calculated as *A*/*E*, according to Habermann et al. 2003. CO_2 concentration entering the leaf cuvette was 390 µmol CO_2 mol^{-1} air, as provided by the 6400-01 CO_2 mixer (LI-COR). Measurements were performed between 9:00 and 11:00h (Feistler and Habermann 2012) on cloudless sky days, under natural fluctuation of air temperature and vapor pressure deficit (VPD) inside the greenhouse. The VPD inside the leaf cuvette was 1.5 ± 0.2 kPa, which means that the relative humidity in the (reference) chamber oscillated around 65%. Photosynthetic photon flux density (PPFD) was supplied by an artificial LED light (10% blue and 90% red) source (6400-40 LCF, LI-COR), which was set to provide 1200 µmol photons m^{-2} s^{-1}, as this value saturates *A* in *S. camporum* leaves (Habermann et al. 2011).

Chlorophyll *a* fluorescence was measured with a portable modulated fluorometer (6400-40 LCF; LI-COR), which was integrated into the LI-6400xt gas exchange system. For calculating maximum quantum yield of photosystem II (PSII) (F_v/F_m), leaves were dark-adapted for 30 min (Bolhàr-Nordenkampf and Öquist 1993) with aluminum foils, before measuring the fluorescence. The saturating light pulse was 7000 µmol photons m^{-2} s^{-1} during 0.7s. F_m and F_v are maximum and variable fluorescence in dark-adapted leaves, respectively. The effective quantum yield of PSII (Φ_{PSII}), was calculated as $(F_m' - F_s)/F_m'$, where F_m' and F_s indicate the maximum and the steady state fluorescence in light-adapted leaves, respectively. Apparent electron transport rate (ETR = Φ_{PSII} PPFD 0.5 0.85) was calculated, using 0.5 as the fraction of excitation energy distributed to PSII, and 0.85 as the fractional light absorbance. The proportion of open PSII reactions centers (qP) was measured as $(F_m' - F_s)/(F_m' - F_o')$ (Bolhàr-Nordenkampf and Öquist, 1993). We also calculated the light fraction used for PSII in photochemistry [$P = ((F_m' - F_s)/ F_m')$], heat dissipation in the antenna [$D = 1 - (F_v'/F_m')$] and heat dissipation in reaction centers [$E = (1 - qP)\ (F_v'/F_m')$], which were in accordance to Demming-Adams (1996). For these calculations, F_v' is the variable fluorescence between the maximal (F_m') and minimal (F_o') fluorescence from light-adapted leaves.

Biometric parameters

Lengths of stems (from plant collar to the shoot apex) and roots (from plant collar to the root tip) were measured with a ruler (cm) and the number of leaves, counted.

The leaves, stems (plus petioles) and roots of the plants were separated. Leaf area (cm^2) was measured with an area meter (LI-3100C, LI-COR). Leaf, stem and root samples were oven-dried at 60°C to constant mass, and biomass of these organs as well as the total biomass were measured using analytical scale.

Aluminum concentration in roots, shoots and leaves

At 91 DAP, after measuring the biomass of plant organs, the samples were oven dried at 60 °C for 72 h, ground and digested in a solution of sulfuric:nitric:percloric acids (1:10:2, v/v/v). After digestion, Al concentrations were determined by using an atomic absorption spectrophotometer (Sarruge and Haag, 1974) and were expressed as mg Al per kg dry plant material.

Data analysis

The variation of *A*, *gs*, *E*, *Ci*, *A/E*, F_v/F_m, ΦPSII, ETR, qP, P, D, and E between both treatments were analyzed using a T-test at 5% level at every evaluation date (0-91 DAP). We used the same T-test at 5% level to check the variation of the number of leaves, leaf area, shoot and root lengths, and biomass of leaf, shoot, root and total biomass between both treatments at 0 and 91 DAP and, for these same biometrical traits, between 0 and 91 DAP for each treatment individually. For leaf, shoot, root and total Al concentrations measured at 91 DAP, the same T-test at 5% level was used to check for differences between both treatments.

We used an allometric bivariate analysis (standard major axis regression – SMA) to test the correlation between *A* x *gs* and *E* x *gs* for both treatments and the variations in slope and intercept between the treatments. Data were $\log_{10}$ transformed (Warton et al 2006; 2012). Statistical procedures were performed in R software (R Development Core Team 2012).

Results

Aluminum affected roots more than stems and leaves because, in plants exposed to Al, leaves were green, fully developed and had the same shape as those from plants not exposed to Al (Fig. 1a, 1b). Roots, however, were visually affected by Al, as there was a lack of lateral (or fine) roots in plants exposed to 1480 μM Al, which appeared to invest more in coarse roots (Fig. 1d) in relation to plants not exposed to Al (Fig. 1c).

Plants exposed to Al did not sprout normally, as their average number of leaves was 55% lower than the control plants at 91 DAP, and plants exposed to Al did not increase the number of leaves from 0 to 91 DAP (Fig. 2a). Consequently, the leaf area of plants exposed to Al was the same between 0 and 91 DAP (Fig. 2b). In addition, the leaf biomass was similar between both treatments at 91 DAP, but while the control plants exhibited a four-fold increase in leaf biomass, plants exposed to Al showed an insignificant rise in this parameter between 0 and 91 DAP (Fig. 3a).

Aluminum also affected shoot growth because plants exposed to Al maintained the same shoot length after 91 days, while the shoot length of plants not exposed to Al increased 20% during the same period (Fig. 2c). Although both treatments showed significant increases in their shoot biomasses from 0 to 91 DAP, plants not exposed to Al had a sharp five-fold increase in shoot biomass during the same period, but the shoot biomass, when comparing both treatments, was the same at 91 DAP (Fig. 3b).

The root length, when comparing both treatments, was the same at 91 DAP, but the roots of plants exposed to Al were 10 cm shorter than those from the control plants (Fig. 2d). Both treatments showed significant increase in their root biomasses from 0 to 91 DAP. In plants exposed to Al this parameter showed a ten-fold increase, while those not exposed to Al had a 16-times increase in root biomass during the same period (Fig. 3c).

Aluminum reduced gas exchange rates. Despite the variability observed for these rates, *A* was lower in plants exposed to Al when compared to those not exposed to Al from 14 until 91 DAP (Fig. 4a). Values of *gs* and *E* also reflected the response pattern observed for *A*. Except for the beginning and the end of the study, *gs* and *E* remained lower in plants exposed to Al when compared to those not exposed to Al (Fig. 4b and 4c). Aluminum did not considerably affect the intercellular CO_2 (*Ci*), except at 21, 62 and 86 DAP when *Ci* was higher in plants not exposed to Al in relation to those cultivated with Al (Fig. 4d). Similarly, although not considerably affected by Al, the water use efficiency (*A*/*E*) was higher at 21, 62 and 86 DAP in plants exposed to Al when compared to those not exposed to Al (Fig. 4e). In addition, we observed significant correlations between *A* x *gs* and *E* x

gs for both treatments (Fig. 5a, 5b). Both correlations differed for intercepts between treatments ($p < 0.05$).

Aluminum did not affect F_v/F_m (Fig. 6a). However, Al caused reductions in ΦPSII (Fig. 6b) and ETR (Fig. 6c), mainly between 14 and 56 DAP. Photochemical quenching was reduced in plants exposed to Al, but only at 14 and 21 DAP (Fig. 6d), and fractions of absorbed light used in photochemistry (P) were lower in plants exposed to Al at the beginning of the study and at 42 and 49 DAP (Fig. 7b). The heat dissipation in the antennas (D) was increased in plants exposed to Al at 21 (+3.5%), 28 (+14%), 49 (+27.5%), 62 (+12%), 86 (+13.8%) and 91 (+13.7%) DAP, while heat dissipation in the reaction centers (E) was higher in plants exposed to Al only at 21 DAP (+9.6%) (Fig. 7).

At the end of the study, the plants exposed to Al showed higher Al concentration in relation to plants not exposed to Al, and this was a reflection of higher Al concentration found in all plant organs (Fig. 8). Interestingly, in plants exposed to Al, 69.5 ± 1.9% of it was retained in the roots, while only 23.2 ± 3.4% and 7.1 ± 1.9% were retained in the shoots and leaves, respectively.

Discussion

Differing from our prediction, the results showed that high Al concentration in the nutrient solution affects the growth of *S. camporum*. Although the leaves of plants exposed to Al were fully expanded and had the same shape as those from plants not exposed to Al (suggesting no apparent toxicity to shoots), the number of leaves of plants exposed to Al remained the same between 0 and 91 DAP (Fig. 2a). Therefore, the smaller leaf number (Fig. 2a), leaf area (Fig. 2b) and leaf biomass (Fig. 3a) found in plants exposed to Al after 91 days, in relation to plants not exposed to Al are likely to be due to low leaf flushing. This indicates that Al may have interfered with the functioning of the shoot apical meristem. It is difficult to find studies of Al effects on leaves and shoots using simple data like number of leaves, mainly in native plants from environments where Al is not expected to be a toxic element, although Al saturation is extremely high in soils from these areas (Haridasan 2008). In *Cedrela odorata*, *Heliocarpus americanus* and *Tabebuia chrysantha*, Al non-accumulating species from tropical forests in Ecuador, healthy leaf area decreased and leaf chlorosis increased with the increase of Al concentration (Rehmus et al. 2014). In rye, an Al-sensitive species, leaves were smaller after three weeks under 1110 μM Al (Silva et al. 2012). Therefore, *S. camporum* plants demonstrate some type of resistance to Al because after being exposed to high Al concentration for 91 days leaves were green and had the same size as those from plants not exposed to Al, despite the damage to their shoot apical meristem (low leaf flushing).

Aluminum is known to cause damage to apical meristems, interfering with cell division (Matsumoto 2000), but not to *shoot* apical meristems. The most important symptom of Al toxicity, in general, is the inhibition of *root* elongation (Horst et al. 2010; Sun et al. 2010). In Al-sensitive species, low root growth can be detected within hours under Al concentrations as low as 10 μM (Kopittke et al. 2008). In these plants, Al-induced decrease in root length may be of 60 to 80% in relation to the root length of plants not exposed to Al (Blamey et al. 1987; Delhaize and Ryan 1995; Kopittke et al. 2008; Sun et al. 2010). In the present study, the roots of plants exposed to Al were only 10 cm shorter than those of plants not exposed to Al (Fig. 2d), representing a 15% reduction in the root length. Therefore, *S. camporum* exhibits some type of resistance to Al, as the typical Al-induced decrease in root growth exhibited by Al-sensitive species was not evidenced in this non-accumulating species from the Cerrado.

On the other hand, the most conspicuous symptom observed in the roots of *S. camporum* plants exposed to Al was the lack of lateral roots (Fig. 1d). Responsible for anchorage to the soil as well as for minerals and water supply (Kramer and Boyer 1995), lateral roots are *not* formed at the root meristem, but at the root maturation zone (Lavenus et al. 2013). Therefore, the root apex of *S. camporum* does not seem to be affected by Al, contrasting with the rapid and permanent damage to root meristems of Al-sensitive species (Kopittke et al. 2008). These results suggest that the root maturation zone is somehow affected by high concentration of Al in this species.

We also demonstrated that 69.5% of the Al found in plants exposed to Al was retained in the roots. Aluminum was also found in plants grown in nutrient solution with no Al (Fig. 8), but this is a common observation in similar studies using (Al-sensitive) crop plants (Jiang et al. 2009; Yang et al. 2011). Root Al retention has already been reported for crop plants (Vitorello et al. 2005). In *Citrus grandis*, 70-80% of the Al was found in roots (Jiang et al. 2009; Yang et al. 2011). *S. camporum* is a non-accumulating plant but, as we have already observed 1000 - 1500 mg Al kg^{-1} dry leaves in field studies, we expected to find Al in the leaves of plants of the present study. We did, but almost 70% of the Al was retained in their roots and only 7.1% in their leaves. In the field, *S. camporum* plants grow on soils showing m% between 60 and 90% (Haridasan 2008; Andrade et al., 2011; Habermann and Bressan, 2011). Although m% and Al concentration in nutrient solutions are not comparable, it is possible that (somehow) Cerrado soils are not toxic to non-accumulating plants when compared to nutrient solution containing Al, as demonstrated in the present study. In addition, no studies have demonstrated, so far, *whether* Al is retained (and in which proportion), or not, in the roots of *S. camporum* trees in the field. We did not anatomically/histochemically investigate possible sites of Al deposition for the Al we found retained in the roots of *S. camporum* plants but, apparently, the Al

stunts lateral root induction in this species. This lack of lateral roots may explain the 60% lower root biomass increment between 0 and 91 DAP in plants exposed to Al when compared to plants not exposed to Al (Fig. 3c). In addition, this lack of lateral roots may have interfered with the water uptake, which could be associated with the low gas exchange rates.

Therefore, our results also suggest that the reason behind the reduced gas exchange rates in *S. camporum* plants exposed to Al is diffusive, *i.e.* dependent on early stomatal closure (Chaves 1991; Chaves et al. 2002). Carbon assimilation was reduced in plants exposed to Al during most of the experiment (Fig. 4a), and the low *gs* could explain their low *A* (Fig. 5a) and *E* (Fig. 5b) values. Thus, it seems that Al inhibits the formation of lateral roots at the maturation zone. Since lateral roots are responsible for water uptake (Kramer and Boyer 1995), the Al-induced decrease in lateral root formation might have caused a lack of water supply to the mesophyll, which eventually led to low *gs*, and had an impact on gas exchange rates (Fig. 4a, b, c). Some studies (Chen et al. 2005; Jiang et al. 2008; Konrad et al. 2005; Silva et al. 2012) carried out on Al-sensitive species have reported 30 to 80% decrease in *gs* in plants exposed to Al. Samac and Tesfaye (2003) and Vitorello et al. (2005) defend that, in Al-sensitive plants, Al stunts the primary root and inhibits lateral root formation, which would lead to reduced water uptake. Therefore, it is possible that a similar sequence of responses (low *lateral* root formation → low water uptake → low *gs* and gas exchange rates) might have occurred with *S. camporum*.

Low photochemical performances, such as reduced ETR, qP and ΦPSII have been observed in Al-sensitive plants when exposed to this metal, which could explain the low *A* observed in these crop plants (Chen et al. 2005; Konrad et al. 2005; Jiang et al. 2008; 2009). In the present study, low photochemical performance in plants exposed to Al included low ΦPSII and ETR (between 14 and 56 DAP), low qP (at 14 and 21 DAP), and increased D (between 21 and 91 DAP). However, (low) F_v/F_m, an indicator of damage to the photochemical apparatus (Baker 2008) was unchanged between the treatments (Fig. 4a). Healthy *S. camporum* plants under water deficit (leaf water potential = -3.2 MPa) also exhibit attenuation of photochemical performances (although F_v/F_m is stable at 0.78 ± 0.2), and under such conditions this species has to cope with relative excessive PPFD (Feistler and Habermann 2012). Moreover, while D increased by 3-27.5% in plants exposed to Al (Fig. 7b), *gs* values decreased by 72% (21 DAP), 64% (28 DAP), 82.5% (49 DAP), 54% (56 DAP), 78.9% (62 DAP), and 78% (at 70 and 86 DAP) in these plants (Fig. 4b). The positive relationships between *A* x *gs* (Fig. 5a) and *E* x *gs* (Fig. 5b) for both treatments also reinforce that *A* was under diffusive (stomatal) control. Therefore, as observed for Al-sensitive species, in *S. camporum* plants the Al might have attenuated the photochemical performance, but the participation in such attenuation is not

so important as the reduced *gs* caused by Al in these plants. Consequently, it is more reasonable to assume that the low photochemical performance in *S. camporum* plants exposed to Al could be a mechanism to dissipate excessive energy due to a lack of water supply to the mesophyll, which led to a significant stomatal closure.

One may still argue that most photosynthetic parameters showed a reduction after 50 DAP, and that there was a considerable variation in gas exchange and photochemical parameters throughout the experiment (Fig. 4 and 6). However, these variations were similar for both treatments. In addition, from 0 to 50 DAP, the mean air temperature was 30.2 ± 1.3 °C, dropping to 27.7 ± 1.5 °C after 50 days. Most importantly, *A*, *gs* and *E* remained higher in plants not exposed to Al when compared to plants exposed to Al (Fig. 4a, b, c), reiterating that gas exchange rates seemed to be largely reduced by Al, even under variable conditions.

This may be the first report of Al effects on a non-accumulating species from the Cerrado, which would not be expected to experience injuries caused by Al. Therefore, we suggest further investigation with this species, such as Al dose-response experiments. Differing from our hypothesis, high soluble Al concentration in nutrient solutions seems to affect the growth of *S. camporum*. It interferes with the shoot apex, as plants exposed to Al drastically reduced flushing. However, in contrast with Al-sensitive species, Al is not a stressful factor to the root tip, but to the root maturation zone. It seems to disturb the formation of lateral roots and, consequently, water uptake is reduced, causing a lack of water supply to the mesophyll, which would explain the low *gs* and reduced gas exchange rates.

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Fig 1 Morphological details of shoots and leaves (a, b) and roots (c, d) of *S. camporum* plants grown for 91 days in nutrient solutions containing 0 (a, c) and 1480 (b, d) μM Al.

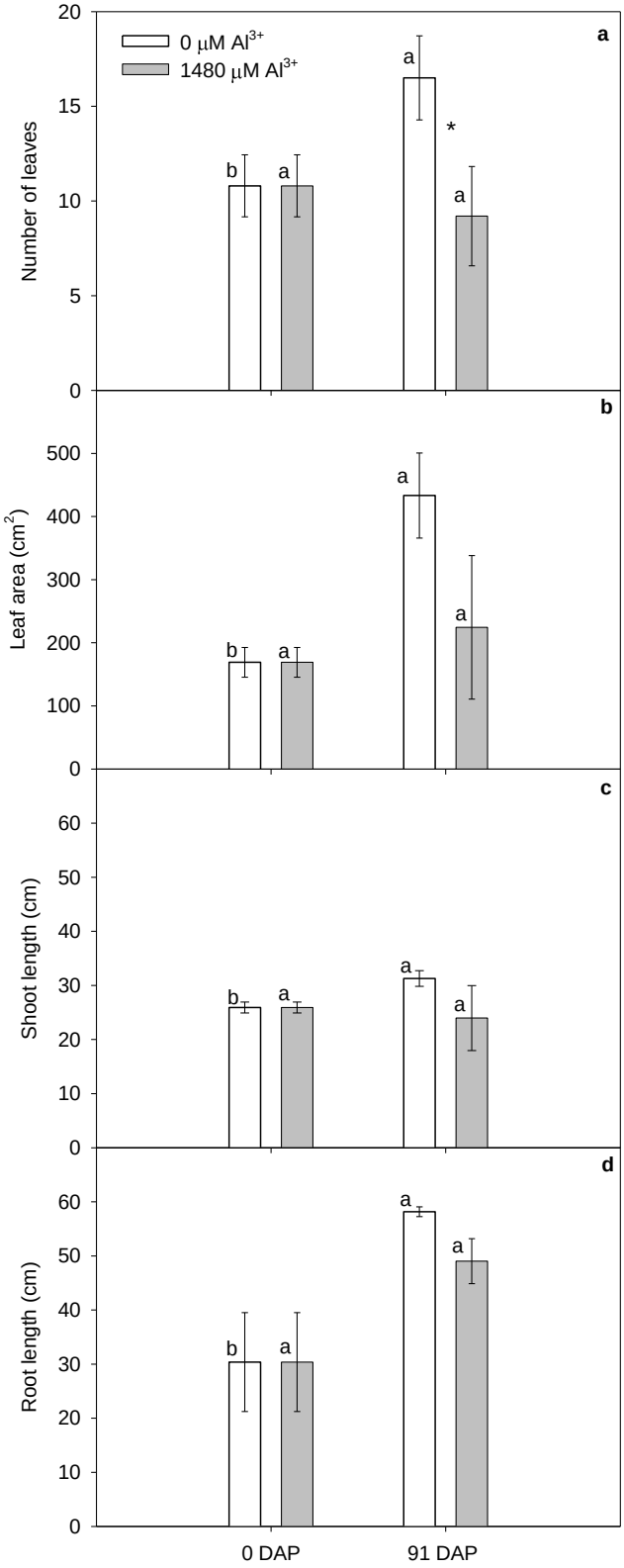

Fig 2 Mean values (n = 5 plants) of biometric parameters (a-d) of *S. camporum* plants at 0 and 91 days after planting (DAP) in nutrient solutions containing 0 and 1480 μM Al. For the same treatment, distinct letters indicate significant differences ($P < 0.05$) between 0 and 91 DAP. Asterisks indicate significant difference ($P < 0.05$) between treatments at 91 DAP (vertical bars = s.d.).

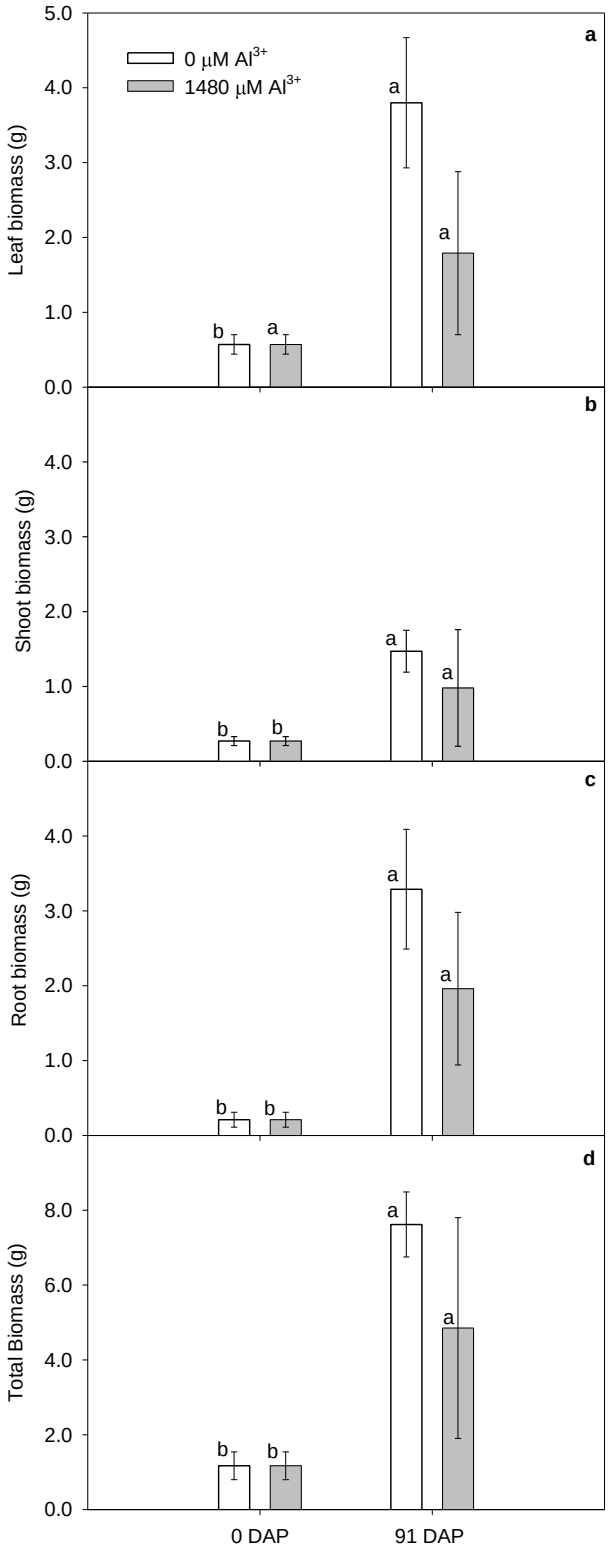

Fig 3 Mean values (n = 5 plants) of biomass of organs (a-c) and total plant biomass (d) of *S. camporum* plants at 0 and 91 days after planting (DAP) in nutrient solutions containing 0 and 1480 μM Al. For the same treatment, distinct letters indicate significant differences ($P < 0.05$) between 0 and 91 DAP. Absence of asterisks indicates non-significant difference ($P > 0.05$) between treatments at 91 DAP (vertical bars = s.d.).

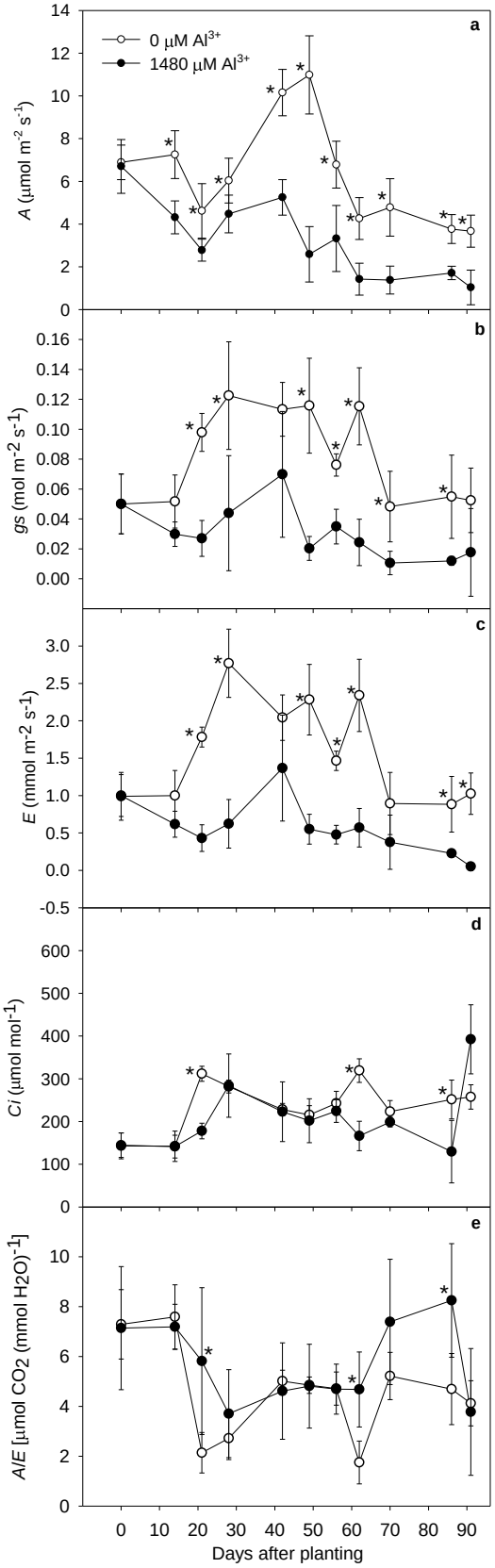

Fig 4 Mean values (n = 5 plants) of gas exchange rates (a, b, c), intercellular CO_2 (d), and water use efficiency (e) of *S. camporum* plants grown for 91 days in nutrient solutions containing 0 and 1480 μM Al. Asterisks indicate significant difference ($P < 0.05$) between treatments at each evaluation date. (vertical bars = s.d.).

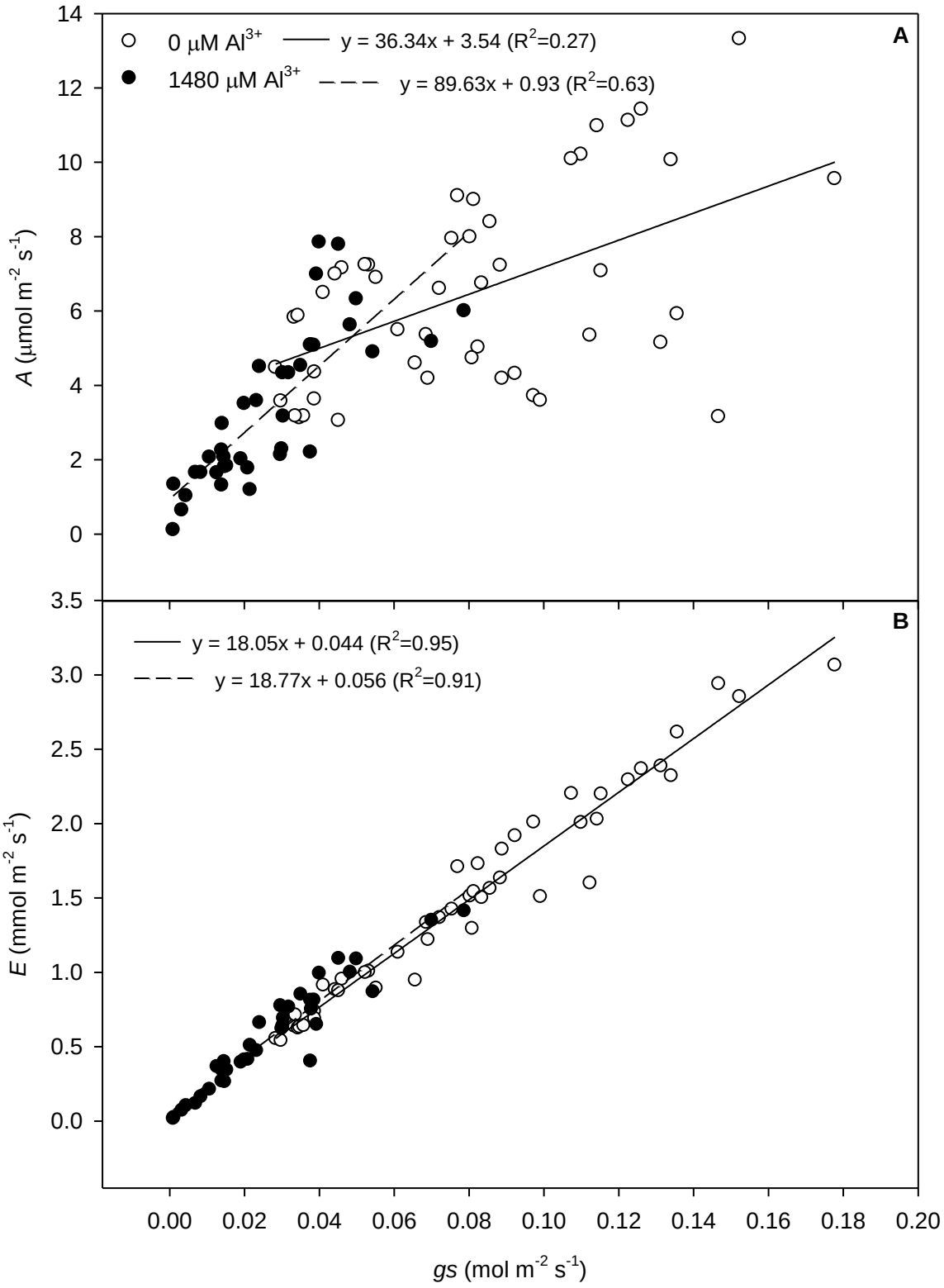

Fig 5 Bivariate correlations between *A* x *gs* (a) and *E* x *gs* (b) for *S. camporum* plants grown for 91 days in nutrient solutions containing 0 and 1480 μM Al. Each plot represents reading performed on one plant measured throughout the experimental time.

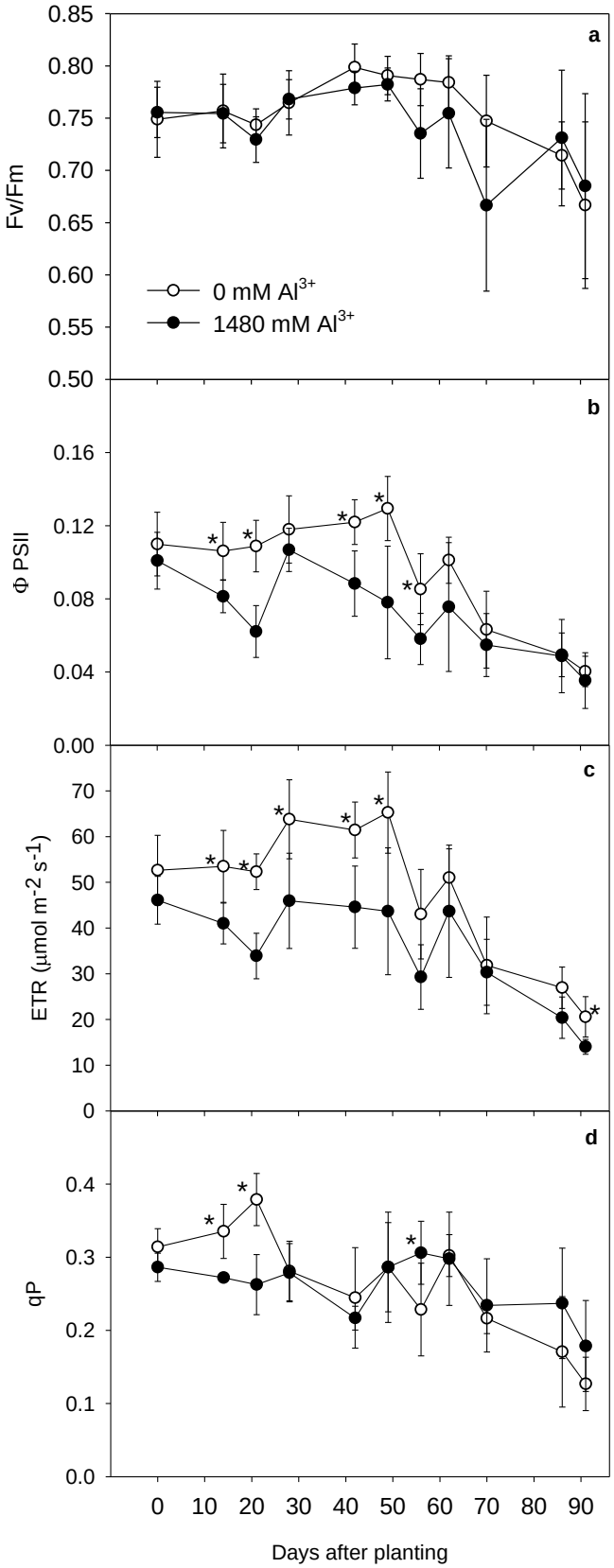

Fig 6 Mean values (n = 5 plants) of chlorophyll a fluorescence parameters of *S. camporum* plants grown for 91 days in nutrient solutions containing 0 and 1480 µM Al. Asterisks indicate significant difference ($P < 0.05$) between treatments at each evaluation date. (vertical bars = s.d.).

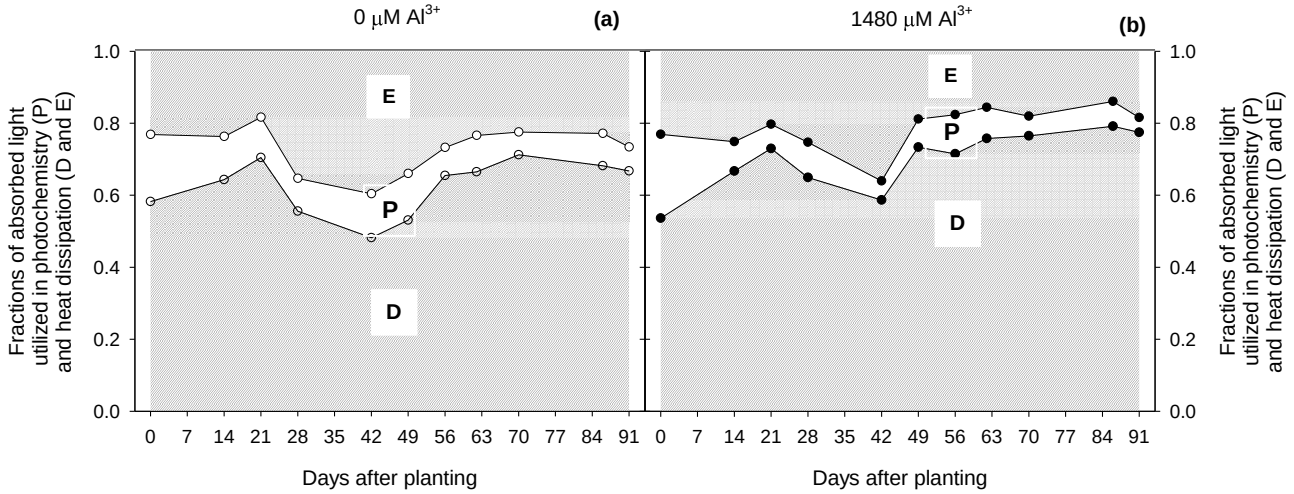

Fig 7 Variations in fractions of absorbed light utilized in photochemistry (P), heat dissipation in the antenna (D) and in reaction centers (E) of PSII in *S. camporum* grown for 91 days under 0 (a) and 1480 μM Al (b). P values for each parameter between both treatments are presented below.

	Days after planting										
Photochemical parameter	**0**	**14**	**21**	**28**	**42**	**49**	**56**	**62**	**70**	**86**	**91**
E	0.921	0.330	0.040	0.016	0.267	0.041	0.015	0.015	0.185	0.072	0.033
P	0.651	0.002	0.001	0.161	0.010	0.016	0.433	0.187	0.509	0.947	0.924
D	0.365	0.195	0.050	0.053	0.128	0.019	0.101	0.043	0.227	0.054	0.031

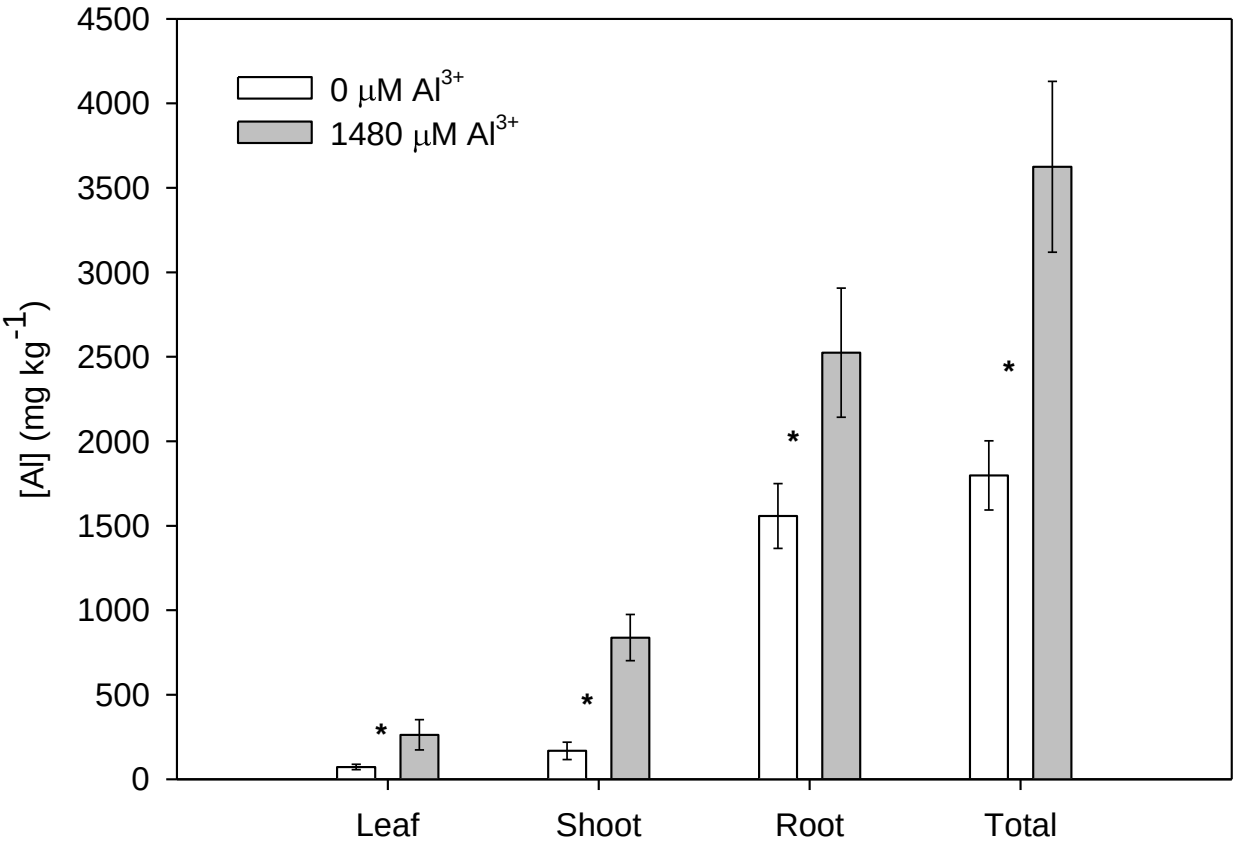

Fig 8 Mean values (n = 5 plants) of Al concentration in leaves, shoots, roots, and in the whole plant of *S. camporum* grown for 91 days in nutrient solutions containing 0 and 1480 μM Al. Asterisks indicate significant difference ($P < 0.05$) between treatments at 91 DAP (vertical bars = s.d.).