WILKA MESSNER DA SILVA BISPO

PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF THE MANGO-*Ceratocystis fimbriata* INTERACTION

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, para obtenção do título de Doctor Scientiae.

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APROVADA: 19 de fevereiro de 2014.

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Prof. Fábio Murilo DaMatta
(Coorientador)

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Prof. Fabrício Ávila Rodrigues
(Orientador)

Aos meus pais, Secundino e Nilzete, e ao
meu irmão, Adolpho, pelo apoio e amor
incondicional. Tudo por vocês.
Ao meu namorado, Rodrigo, por todo amor,
paciência e auxílio.
OFEREÇO e DEDICO.

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... e a Tabatã!

Muito obrigada!

BIOGRAFIA

WILKA MESSNER DA SILVA BISPO, filha de Secundino Bispo da Silva Filho e Nilzete Messner da Silva Bispo, nasceu em 24 de outubro de 1985, em Vitória, Estado do Espírito Santo. Ingressou em 2004, no curso de Ciências Biológicas da Universidade Federal do Espírito Santo (UFES), Vitória-ES, concluindo-o em janeiro de 2008. Logo em seguida, iniciou o curso de Mestrado do Programa de Pós-Graduação em Biologia Vegetal no Centro de Ciências Humanas e Naturais da Universidade Federal do Espírito Santo (CCHN-UFES), Vitória, concluindo-o em fevereiro de 2010. Em março de 2010, ingressou no curso de Doutorado do Programa de Pós-Graduação em Fisiologia Vegetal na Universidade Federal de Viçosa (UFV), Viçosa-MG, submetendo-se à defesa em 19 de fevereiro de 2014.

SUMÁRIO

RESUMO

BISPO, Wilka Messner da Silva, D.Sc., Universidade Federal de Viçosa, Fevereiro de 2014. **Aspectos fisiológicos e bioquímicos da interação mangueira-Ceratocystis fimbriata.** Orientador: Fabrício Ávila Rodrigues. Coorientador: Fábio Murilo DaMatta.

A seca-da-mangueira, causada por Ceratocystis fimbriata Ellis & Halsted, tem grande impacto econômico, sendo uma das doenças que mais afeta a produção de manga no mundo. Observações empíricas têm mostrado grande variabilidade entre as cultivares em termos de seu nível basal de resistência à seca-da-mangueira, no entanto, poucos esforços tem sido realizados no sentido de elucidar os mecanismos fisiológicos envolvidos na resposta do hospedeiro ao processo de infecção. Considerando a lacuna de informações existente na literatura acerca da fisiologia da doença, este estudo teve como objetivo principal, investigar alterações fisiológicas nas plantas das cultivares de manga Ubá, Tommy Atkins e Palmer, as quais apresentam diferentes níveis de resistência basal à seca-da-mangueira. As relações entre a severidade da doença e as mudanças fisiológicas do hospedeiro foram avaliadas em três experimentos distintos, em que a cv. Ubá, considerada resistente, confrontou as cvs. Tommy Atkins (moderadamente resistente) e Palmer (suscetível). Os dois primeiros experimentos compararam o comportamento das cvs. Ubá e Tommy Atkins em relação às possíveis alterações no status hídrico das plantas e na ativação do sistema antioxidativo em resposta a infecção por C. fimbriata. Em suma, diminuições mais pronunciadas na condutância hidráulica aparente, na assimilação líquida de CO_2 e na condutância estomática foram observadas na cv. Tommy Atkins, bem como a diminuição na concentração de clorofila e aumentos na concentração de aminoácidos livres totais, prolina e peroxidação lipídica. Plantas da cv. Tommy Atkins também apresentaram aumentos mais significativos na atividade das enzimas dismutase do superóxido (SOD), peroxidases não-específicas (POX), peroxidase do ascorbato (APX), peroxidase daglutationa (GPX) e redutase da glutationa (GR), e concentrações de metabolitos (peróxido de hidrogênio e compostos fenólicos totais) relacionados às respostas ao estresse oxidativo. Essas modificações foram mínimas na cv. Ubá. Maior severidade da doença foi também encontrada nas plantas da cv. Palmer durante o terceiro experimento, quando comparada com a cv. Ubá. Além de reduzido desempenho fotossintético, a cv. Palmer mostrou a diminuição das concentrações foliares de amido e aumento na concentração de hexoses, em consonância com a reduzida atividade da pirofosforilase da ADP-glucose (AGPase) e um aumento na atividade de ambas as invertases,

ácida e alcalina, o que sugere a necessidade osmólitos para a manutenção da turgescência celular e proteção de membranas e proteínas devido aos maiores danos sofridos por esta cultivar. As amostras foliares da cv. Ubá não apresentaram alterações nas concentrações de carboidratos e atividade de enzimas, como as envolvidas na síntese de amido (AGPase) e sacarose (sintase da sacarose fosfato, SPS). No entanto, ao longo do experimento, observou-se redução na concentração de amido nos tecidos caulinares, sugerindo um aumento da remobilização de reservas para a produção de compostos de defesa no local da infecção. Conclui-se, então, que os sintomas da doença desenvolveram-se mais rapidamente nas cvs. Tommy Atkins e Palmer e foram associados às maiores restrições nas relações hídricas e trocas gasosas, além de sintomas de estresse oxidativo, quando em estádios mais avançados da infecção fúngica. Em contraste, a cv. Ubá mostrou-se capaz de atuar diretamente no local da infecção, adiando a colonização do patógeno pelos tecidos e o desenvolvimento dos sintomas de murcha.

ABSTRACT

BISPO, Wilka Messner da Silva, D.Sc., Universidade Federal de Viçosa, February, 2014. **Physiological and biochemical aspects of the mango-Ceratocystis fimbriata interaction.** Advisor: Fabrício Ávila Rodrigues. Co-advisor: Fábio Murilo DaMatta.

The mango (Mangifera indica L.) wilt, caused by Ceratocystis fimbriata Ellis & Halsted, has great economic impact, being one of the most important diseases affecting mango production worldwide. Empirical observations have shown great variability among mango cultivars in terms of their basal level of resistance to the mango wilt; nevertheless, to date, few attempts have been made to elucidate the physiological mechanisms underlying how mango respond to fungal infection. Considering the lack of information about the mango wilt physiology, this study aimed to investigate some physiological alterations in mango plants from cultivars Ubá, Tommy Atkins and Palmer, which present different levels of basal resistance to the mango wilt. The relationships between disease severity and the host physiological changes were assessed in three distinct experiments, in which cv. Ubá, considered resistant, confronted both cvs. Tommy Atkins (moderately resistant) and Palmer (susceptible). The first two experiments confronted cvs. Ubá and Tommy Atkins for the possible changes in plant water relations and the differences in the antioxidative responses upon fungal inoculation. The more pronounced decreases in apparent hydraulic conductance, net CO_2 assimilation rate and stomatal conductance were observed in cv. Tommy Atkins relative to cv. Ubá upon fungal inoculation, as well as decreases in total chlorophyll concentration and increases in total free amino acids, proline concentration and lipid peroxidation. Plants from cv. Tommy Atkins also presented more prominent increases in the activity of the superoxide dismutase (SOD), non-specific peroxidases (POX), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and glutathione reductase (GR) enzymes and concentrations of metabolites (hydrogen peroxide and total phenolics) related to the oxidative stress responses. These modifications were minimal in cv. Ubá. Greater disease severity was also found for plants from cv. Palmer in the third experiment when compared to cv. Ubá. In addition to reduced photosynthetic performance, leaves from cv. Palmer showed decreased concentrations of starch and increases in hexoses concentrations as disease progressed, in accordance with the reduced activity of ADP-glucose pyrophosphorylase (AGPase) and increased activity of both acid and alkaline invertases, suggesting the need for osmolytes to maintain cell turgor and the protection of membranes and proteins due to the higher damages. By contrast, leaf samples from cv. Ubá

showed no changes in carbohydrates concentration and on the activity of enzymes, such as those involved in the synthesis of starch (AGPase) and sucrose (sucrose phosphate synthase, SPS). Nevertheless, throughout the experiment, reduced starch concentrations were found in the stem tissues of cv. Ubá suggesting an increased remobilization of reserves for the production of defense compounds at the infection sites. It can be concluded that disease symptoms developed faster on plants from cv. Tommy Atkins and Palmer and were associated with a more pronounced impairment of water relations and gas exchange coupled with symptoms of oxidative stress at advanced stages of fungal infection. In sharp contrast, cv. Ubá was better able to act locally at the site of infection, postponing pathogen spread and the development of wilt symptoms.

GENERAL INTRODUCTION

Due to favorable environmental conditions, Brazil is one of the largest producers of fruits worldwide, and the mango (Mangifera indica L.) stands out among the several cultivated and traded fruit species, receiving growing appreciation, due to the enhanced acceptance of internal and foreign markets (Oliveira et al, 2002; Poll et al, 2011). The Brazilian fruit production, as also observed in several countries, is intended, primarily, to the in natura market and there is great interest in producing and marketing exportable cultivars at the expense of those used for industrialization purposes. Among the different cultivars the cv. Tommy Atkins is the most demanded by the international market and also by the large consuming centers of the Centre-South (Souza et al., 2002), concentrating about 90% of the cropped area in Brazil. This cultivar presents great hardiness, attractive color, good conservation in the post-harvest period, in addition to production stability for consecutive years (Braz et al., 2008). Although not the main focus, great attention has been given to the use of the fruit in industrial processing and manufacturing of fruit pulp, being preferred for this destination, varieties which present high pulp yield, high soluble solids and absence of fibers (Braz et al., 2008). Among the Brazilian cultivars, the Ubá represents the most widespread as raw material for the processing industries (Benevides et al., 2008).

Despite the successful development of mango in several production centers for both fresh consumption and industry, the occurrence of diseases causes many losses in total yield. Additionally, diseases such as anthracnose and floral and vegetative malformation are becoming relevant in many producing regions (Araújo et al., 2004). The mango wilt, caused by Ceratocystis fimbriata Ellis and Halsted (Halsted, 1890), is among the most important mango tree diseases. This disease causes the death of plants in different growth stages from seedlings to mature trees, with the infection starting from the crown, with the aid of a beetle vector (Hypocryphalus mangiferae) or from the root system (Gallo et al., 2002; Masood et al., 2009). The gum extrusion from the points of damage in affected branches, necrosis and browning of vascular tissue are the initial symptoms of the disease (AL Adawi et al., 2006; Al-Sadi et al., 2010). The most characteristic symptom is the progressive drying of the plant, which initiates in branches of small caliber and progresses throughout the entire canopy, provoking the yellowing of leaves, wilting and drying of affected branches (Cunha et al., 2000; Batista et al., 2008).

Al-Sadi et al. (2010) showed the presence of mycelia in the vascular system of infected plants. This observation, coupled with the significant correlation between the movement of

the fungus within the stem tissues and progress of wilt symptoms, gives evidence of the contribution of the pathogen in the blockage of the vascular system and consequent death of the plants; in part due to embolism or a reduction in the useful area of the conducting vessels (Nogués et al., 2002; McElrone et al., 2003). The water deprivation substantially alter the plant metabolism and mechanisms able to circumvent this condition and/or mitigate their consequences can function as an alternative to minimize the presence of the pathogen, favoring the normal plant physiology and contributing for the production of defense compounds. It is known by empirical results that distinct mango cultivars respond differently to C. fimbriata infection ranging from extremely susceptibility to resistance. Given this variability coupled with the need to strengthen the mango production, this study aimed to understand some traits of mango wilt on mango physiology and to raise some questions about the mechanisms involved in the differential responses of some mango cultivars to fungal infection since there is a gap in the literature about how mango respond against C. fimbriata infection.

LITERATURE CITED

Al-Adawi, A.O.; Deadman, M. L.; Al-Rawahi, A. K.; Al-Maqbali, Y. M.; Al-Jahwari, A. A.; Al-Saadi, B. A.; Al-Amri, I. S.; Wingfield, M. J. Aetiology and causal agents of mango sudden decline disease in the Sultanate of Oman. European Journal of Plant Pathology, v. 116, p. 247-254, 2006.

Al-Sadi, A. M.; Al-Ouweisi, F. A.; Al-Shariani, N. K.; Al-Adawi, A. O.; Kaplan, E. J.; Deadman, M. L. Histological changes in mango seedlings following infection with Ceratocystis manginecans, the cause of mango decline. Journal of Phytopathology, v. 158, p. 738–743, 2010.

Araújo, J. L. P.; Correia, R. C.; Guimarães, J.; Araújo, E. P. Production cost analysis and commercialization of mangos for exporting produced in the Sub-middle São Francisco Region, Brazil. Acta Horticulturae, v. 645, p. 379-381, 2004.

Batista, D. C.; Terao, D.; Barbosa, M. A. G.; Barbosa, F. R. Seca-da-mangueira: detecção, sintomatologia e controle. Petrolina: Embrapa Comunicado Técnico 138, 2008.

Benevides, S. D.; Ramos, A. M.; Stringheta, P. C.; Castro, V. C. Qualidade da manga e polpa da manga Ubá. Ciência e Tecnologia de Alimentos, v. 28, p. 571-578, 2008.

Braz, V. B.; Nunes, E. S.; Vieira, G.; Ribeiro Júnior, J. I.; Bertini, L. A.; Couto, F. L. D. Indução do amadurecimento de mangas cv. Tommy Atkins e cv. Ubá pela aplicação de ethephon pós-colheita. Bragantia, v.67, p.225-232, 2008.

Cunha, M. M. da; Santos Filho, H. P.; Nascimento, A. S. do. (Org.). Manga: fitossanidade. Brasília: Embrapa Comunicação para Transferência de Tecnologia, 2000. p. 25-47 (Frutas do Brasil; 6).

Gallo, D.; Nakano, O.; Silveira Neto, S.; Carvalho, R. P. L.; Baptista, G. C. de; Berti Filho, E.; Parra, J. R. P.; Zucchi, R. A.; Alves, S. B.; Vendramin, J. D.; Lopes, J. R. S.; Omoto, C. Entomologia Agrícola. 2.ed., Piracicaba: FEALQ, 2002. 920 p.

Halsted, B. D. Some fungous diseases of the sweet potato. New Jersey Agricultural College Experiment Station, Bulletin 76, pp. 25-27, 1890.

Masood, A.; Shafqat, S.; Asif, S.; Mudassar, A. Life cycle and biology of mango bark beetle, Hypocryphalus mangifera Stebbing: as a possible vector of sudden death disease of mango. Pakistan Journal of Zoology, v. 41, p. 281-288, 2009.

Mcelrone, A. J.; Sherald, J. L.; Forseth, I. N. Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. Journal of Experimental Botany, v. 54, p. 419-430, 2003.

Nogués, S.; Cotxarrera, L.; Alegre, L.; Trillas, M. I. Limitations to photosynthesis in tomato leaves induced by Fusarium wilt. New Phytologist, v. 154, p. 461-470, 2002.

Oliveira, F. C.; Coelho, E. F.; Vasconcelos, L. F. L.; Araújo, E. C. E.Produção de manga sob diferentes regimes de irrigação, em condições subúmidas. Revista Brasileira de Engenharia Agrícola e Ambiental, v. 6, p. 390-396, 2002.

Poll, H; Vencato, A. Z.; Kist, B. B.; Santos, C.; Carvalho, C.; Reetz, E. R.; Beling, R. R. Anuário Brasileiro da Fruticultura. Santa Cruz do Sul: Editora Gazeta Santa Cruz, 2011, 128 p.

Souza, J. da S.; Almeida. C.; Araujo, J. L. P.; Cardoso, C. E. L. Aspectos socioeconômicos. In: Genú, P. J. de C.; Pinto, A. C. de Q.(Ed.). A cultura da mangueira. Brasília: Embrapa Informação Tecnológica, 2002. p. 19-29.

CHAPTER 1

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Water relations and photosynthetic performance of mango cultivars with different levels of resistance to Ceratocystis fimbriata infection

Wilka M. S. Bispo, Leonardo Araújo, Isaías S. Cacique, Fábio M. DaMatta and Fabrício A. Rodrigues

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ABSTRACT

Bispo, W. M. S., Araújo, L., Cacique, I. S., DaMatta, F. M., and Rodrigues, F. A. Water relations and photosynthetic performance of mango cultivars with different levels of resistance to Ceratocystis fimbriata infection. Phytopathology 104:xx-xx

The mango (Mangifera indica L.) wilt is caused by Ceratocystis fimbriata Ellis & Halsted and it is of great economic importance in mango production. Considering that the physiological effects of this vascular disease are poorly characterized, this study aimed to investigate the physiological alterations in mango (Mangifera indica L.) leaves from cultivars Ubá and Tommy Atkins, which present different levels of resistance to the mango wilt. The relationships of disease severity to water relations, gas exchange and chlorophyll a fluorescence parameters were evaluated over 30 days. Leaf samples were collected to determine the lipid peroxidation, total pigments, total free amino acids and proline concentration. Briefly, more pronounced decreases in apparent hydraulic conductance as well as in the net CO_2 assimilation rate and stomatal conductance were observed in cv. Tommy Atkins relative to cv. Ubá upon inoculation. Decreases in total chlorophyll concentrations and increases in total free amino acids, proline concentration and lipid peroxidation were found for cv. Tommy Atkins at 30 days after inoculation in comparison to the non-inoculated plants from the same cultivar. It can be concluded that disease symptoms developed faster in cv. Tommy Atkins and were associated with a more pronounced impairment of water relations

and gas exchange coupled with symptoms of oxidative stress at advanced stages of fungal infection. In sharp contrast, cv. Ubá (resistant) was better able to postpone pathogen spread and xylem occlusion, and as a result, it suffered less from the dehydration imposed by fungal infection compared with the most susceptible cultivar.

Additional keywords: gas exchange; chlorophyll a fluorescence; mango wilt; osmotic adjustment, vascular pathogen; water potential.

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INTRODUCTION

Due to the great variety of climatic conditions, Brazil is one of the largest producers of tropical fruits in the world, with a harvested area bigger than 2.5 million hectares (49). Among several cultivated and traded fruit species, mango (Mangifera indica L.) has experienced a growing domestic demand and increasing forecasts in activities related to production following the acceptance of the international market (48). The cultivar 'Tommy Atkins' is the most in-demand by the international market and also by the high consumption centers of Mid-South Brazil (10,57), where approximately 80% of the cultivated area is concentrated. The remaining areas are used for the production of other varieties such as 'Haden', 'Palmer' and 'Keitt'. Some Brazilian cultivars are also cultivated such as Ubá (46), which is widely used as a raw material for processing industries because of its characteristics like high pulp yield, high soluble solid contents, and low fibers (7,10).

In addition to the successful development of various production hubs for both fresh consumption and industry, diseases cause significant yield losses and, consequently, reduced producer profit. Mango wilt is caused by the fungus Ceratocystis fimbriata Ellis & Halsted (27), and it is one of the most important diseases affecting mango trees worldwide (47). This disease causes death in plants at different development stages, from seedlings to mature trees (25). The initial disease symptoms are gummosis from the bark, bark splitting, streaking and necrosis of the vascular tissues beneath the gummosis (36,37). The most characteristic symptom of this disease is the progressive drying of the plant, which begins in small branches and progresses towards the trunk up to the canopy (6,17).

Tree death is associated with the blockage of vascular tissues by physical impairments such as gels, tyloses and fungal material, which may result in xylem embolism and a reduction in the useful conducting area of the xylem vessels (43,44,58). These dysfunctions usually lead to decreases in hydraulic conductivity and ultimately impair the flow of water and nutrients to the upper canopy (44,54). Usually, these alterations result in decreases in leaf water potential, thereby leading to internal water deficits (39,41). Water stress substantially impairs plant growth in addition to altering the photosynthetic activity and metabolism as a whole. Overall, decreases in photosynthetic performance under water deficit conditions have, to a large extent, been attributed to diffusion limitations through the stomata and mesophyll; however, only severe drought conditions leading to biochemical limitations in the CO_2 fixation can help impair the photosynthetic capacity (23,24).

Empirical observation has shown that there is great variability among mango cultivars in terms of their basal level of resistance to mango wilt. To the best of our knowledge, this variability has not been explored; furthermore, virtually nothing is known about how C. fimbriata affects mango tree physiology. To fill in this gap, this study was carried out with the aim of characterizing traits of the physiological performance of two mango cultivars with different levels of basal resistance to mango wilt, and great economic importance. It was hypothesized that the more resistant cultivar would preserve its hydraulic conductivity to a better extent in comparison to its less resistant counterpart and would thus be better able to maintain an adequate water status and photosynthetic performance during the C. fimbriata infection process.

MATERIALS AND METHODS

Plant material. Mango plants of approximately 1 year old from cultivars Ubá and Tommy Atkins, which are known to be resistant and moderately resistant, respectively, to C. fimbriata (14,50), were obtained from a commercial orchard (Dona Euzébia, Minas Gerais State, Brazil). Both cultivars were grafted onto plants from cv. Imbú, widely used as rootstock in the Zona da Mata region, Minas Gerais State, Brazil. The saplings were transplanted into plastic pots containing 8 kg of substrate consisting of a mixture of soil, sand and manure in a 2:1:1 proportion. The plants were kept in a greenhouse (temperature of 30 ± 2°C and relative humidity of 70 ± 5%) for two months before the beginning of the experiments. Plants were irrigated and fertilized as needed.

Inoculation procedure. The C. fimbriata isolate CEBS15, used to inoculate the plants, was obtained from symptomatic mango plants collected in the city of Brejo Santo (07° 29' 34" S, 38° 59' 06" W), Ceará State, Brazil. The isolate was preserved by Castellani's method (19). Plugs of malt extract agar medium containing fungal mycelia were transferred to Petri dishes containing potato dextrose agar (PDA). After three days, PDA plugs containing fungal mycelia were transferred to new Petri dishes containing the same culture medium and were maintained in an incubator chamber (at a temperature of 25°C with a 12 h photoperiod) for 14 days.

Plants were inoculated according to Al-Sadi et al. (3) with a few modifications. Stem disks (10 mm in diameter and approximately 2 mm in width) were removed from the stems with the aid of a punch at approximately 5 cm above the graft scar. A PDA plug (10 mm in diameter) obtained from a 14-day-old colony of each C. fimbriata isolate was carefully placed in the punch hole. Each hole containing a PDA plug with fungal mycelia was carefully covered with a piece of moistened cotton and then wrapped with parafilm to maintain adequate moisture for fungal infection. The disks used to inoculate each plant were taken from the middle portion of each fungal colony to make the inoculation as homogeneous as possible. Holes on the stems of plants receiving only PDA medium plugs served as the control treatment.

Relative lesion indices. Disease progress in the inoculated stem tissues was evaluated at 2, 10, 20 and 30 days after inoculation (dai). The upward, downward and radial colonization of the stem tissues by fungal hyphae from the C. fimbriata isolate were evaluated by measuring

the length (in cm) of the internal necrotic tissue using digital calipers. The upward relative lesion length (URLL) and the downward relative lesion length (DRLL) were determined as the ratio between the length from the graft scar to the top of the stem (LGST) and the lesion length (LL) in the same interval (upward and downward) from the inoculation point according to the following formula: URLL or DRLL = LL × 100/LGST. The plants were standardized to a length of 20 cm (the distance from the graft scar to the top of the stem). The radial fungal colonization (RFC) was determined as the length of the necrotic tissue in relation to the total stem diameter × 100.

Water potential and apparent hydraulic conductance. The predawn leaf water potential (Ψ_{pd}) was determined at 2, 10, 20 and 30 days after inoculation (dai) with a Scholander-type pressure chamber (model 1000, PMS Instruments, Albany, NY, USA). One single leaf from ten replications (plants) of each treatment was collected from the same plant portion for the measurements. Leaf samples were stuck through the petiole to the pressure chamber, which was slowly pressurized until a droplet of a translucent liquid appeared on the cut surface. The displayed pressure at the moment of liquid surfacing was recorded as a negative value of leaf water potential. As previously reported by Castro Neto et al. (15), the measurements of leaf water potential on mango leaves using a pressure chamber is problematic due to the presence of latex and this was especially observed for cv. Tommy Atkins. Thus, prior to the measurements, the latex drops were carefully removed with the aid of a piece of filter paper. The apparent hydraulic conductance (K_L) was expressed as the ratio between the total plant transpiration (gravimetrically estimated) between predawn and midday and the differences in water potential ($\Delta\Psi_w$) at the same interval (45,56). The soil surface in the plastic pots was covered with a plastic film to minimize evaporation.

Leaf gas exchanges and chlorophyll a fluorescence measurements. The net carbon assimilation rate (A), stomatal conductance to water vapor (g_s), internal-to-ambient CO_2 concentration ratio (C_i/C_a) and transpiration rate (E) were measured in fully expanded leaves with an infrared CO_2/H_2O gas analyzer (LI 6400, Li-Cor, Lincoln, NE) equipped with a blue/red light source (Li-6400-02B). The measurements were conducted at ambient temperature and CO_2 conditions under artificial light (1000 µmol photons m^{-2} s^{-1}) from approximately 0800 to 1200 h at 2, 10, 20 and 30 dai. Measurements were performed in six replications from the initial group of 10 plants, being selected those that had the most visible symptoms of the disease at each evaluation time. The evaluations were done twice per

replication, yielding similar values, but only one data set was recorded. The intrinsic (A/g_s) and instantaneous water use efficiency (A/E) at the aforementioned level of irradiance was also calculated.

Chlorophyll a fluorescence measurements were determined using a portable pulse amplitude modulation fluorometer (MINIPAM, Heinz Walz GmbH, Effeltrich, Germany) on the same leaves used for the gas exchange measurements. After 40 min of dark adaptation, the leaf tissues were exposed to a weak modulated measuring beam (0.03 µmol m^{-2} s^{-1}) to determine the initial fluorescence (F_0). Next, a saturating white light pulse of 6,000 µmol m^{-2} s^{-1} was applied for 0.8 s to ensure maximum fluorescence emission (F_m). From these initial measurements, the maximum quantum efficiency of PSII photochemistry for dark-adapted leaves was calculated as follows: (F_v/F_m) [(F_m - F_0)/F_m)]. The steady-state fluorescence yield (F_s), the light-adapted maximum fluorescence (F_m'), which was measured after 0.8 s of saturating white light pulse (6,000 µmol m^{-2} s^{-1}), and the light-adapted initial fluorescence (F_0') estimated according to Oxborough and Baker (42) were determined in light-adapted leaves. From these parameters, the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m') was calculated [(F_m' - F_0'/F_m')]. The estimated fraction of open PSII centers (q_L) was calculated as [(F_m' - F_s)*F_0'/ (F_m' - F_0')*F_s] (30), and the non-photochemical quenching coefficient (NPQ) was calculated as [(F_m/F_m') - 1)] (9). The actual quantum yield of PSII electron transport (Φ_{PSII}) was computed as [(F_m' – F_s)/F_m'], from which the electron transport rate (ETR) was calculated as (Φ_{PSII}*PPFD*f*α), where f is a factor that accounts for the partitioning of energy between PSII and PSI and is assumed to be 0.5, which indicates that the excitation energy is equally distributed between the two photosystems; α is the leaf absorbance by the photosynthetic tissues and is assumed to be 0.84 (38).

Determination of photosynthetic pigments. The concentrations of chlorophylls (Chl) a and b and carotenoids were determined using dimethyl sulfoxide (DMSO) as an extractor (59). Five leaf disks (10 mm in diameter) were punched from each leaf that had been previously used for gas exchange and Chl a fluorescence measurements at 2, 10, 20 and 30 dai. The collected disks were immersed in glass tubes containing 6 ml of a saturated DMSO solution and calcium carbonate ($CaCO_3$) (5 g l^{-1}) (53) and kept in the dark for 48 h. The absorbances of the extracts were read at 480, 649.1 and 665.1 nm using a saturated solution of DMSO and $CaCO_3$ as a blank.

Total free amino acids (TFA) and proline (Pro) determinations. For each replication, four to five fully expanded leaves exposed to sunlight, including those used for gas exchange and Chl a fluorescence measurements, were collected from the same portion of the plant. The midrib region and the leaf ends were avoided. A composite sample was obtained and used for the biochemical analysis. From this sample, approximately 120 mg of leaf tissue were homogenized in 80% (v/v) ethanol as described by Robbins and Pharr (51). The total free amino acids concentration was determined by following the methodology of Moore and Stein (40). Aliquots of 100 µl from the ethanolic extract were diluted in water to 2 ml in glass tubes. A total of 1.5 ml of the ninhydrin reagent was added to the tubes, and the set was stirred [the ninhydrin reagent was prepared by the 1:1 (v/v) mixture (25°C) of a 1.6 mg/mL $SnCl_2.2H_20$/citrate buffer (pH 5.0) solution and a 40 mg/mL ninhydrin/ethylene glycol monomethyl ether solution]. The mixture was incubated at 100°C for 20 min and then cooled in running water. Under agitation, 8 ml of 50% (v/v) ethanol were added to the tubes, and the readings were performed after 10 minutes at 570 nm in spectrophotometer (Thermo Scientific Multiskan GO UV/Vis). Amino acid concentrations were estimated using a standard curve with an equimolecular mixture of glycine, glutamic acid, phenylalanine and arginine in 50% (v/v) methanol.

The same ethanolic extract used to determine TFA concentration was used to quantify the proline concentration using the acid-ninhydrin method as described by Bates et al. (5) with a few modifications. A total of 150 µl of the ethanolic extract was diluted to 3 ml in water and mixed with a 4 ml solution of acid ninhydrin and acetic acid (1:1, v/v), and the mixture was incubated at 100°C for 50 min (the ninhydrin reagent was composed by a 1:25:17 (w/v/v) solution of ninhydrin/acetic acid/orthophosphoric acid). The reaction was stopped in an ice bath, and the chromophores were extracted with 4 ml of toluene. The resulting upper pink phase was collected, and the absorbance was read at 515 nm in spectrophotometer (Thermo Scientific Multiskan GO UV/Vis), using toluene as a blank. The proline concentration was determined from a standard curve of 50µM proline prepared in 70% (v/v) ethanol.

Lipid peroxidation assay. The oxidative damage to lipids was estimated as the concentration of the total amount of 2-thiobarbituric acid (TBA) reactive substances and expressed as equivalents of malondialdehyde (MDA) according to Cakmak and Horst (13) with a few modifications. A total of 200 mg of leaf tissues was homogenized in 2 ml of 0.1 % (w/v) trichloroacetic acid (TCA) solution at 4°C following centrifugation at 10,000 g for 15 min. The supernatant (0.5 ml) was reacted with 1.5 ml of TBA (0.5% in 20% TCA) for 20

min in a boiling water bath. The reaction was stopped by immersion in an ice bath. The samples were centrifuged at 13,000 g for 4 min, and the absorbance of the supernatant was recorded at 532 nm in spectrophotometer (Thermo Scientific Multiskan GO UV/Vis). The nonspecific absorbance was estimated at 600 nm and subtracted from the specific absorbance values. An extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate the MDA concentration.

Vein density. Two leaves from six non-inoculated plants of each cultivar were collected at the end of the experiment. Fragments from the central portion of those leaves (400 mm^2 in size) were collected and fixed in 70% ethanol for seven days. Leaf fragments were cleared and stained according to Berlyn and Miksche (8) with a few modifications. Initially, the fragments were cleared in 5% aqueous NaOH for 5 days, rinsed several times in distilled water and then exposed to an ethanolic series (30, 50, 70 and 100%) for 3 min per step. Leaf fragments were stained with safranin 1% (1 g safranin in 100 mL of 100% ethanol), submitted to a reverse ethanolic series and washed in distilled water to remove the excess stain. One stained fragment was mounted abaxial side up on glass slides containing 2-3 drops of 70% glycerol solution. The glass slides were then individually examined under a Carl Zeiss Axio Imager A1 microscope (50 × magnification) with phase contrast optics. The images were analyzed using Image-Pro Plus software (version 4.5, Media Cybernetics, Silver Spring, USA). The vein density (mm mm^{-2}) was expressed as the sum of the length of all segments (mm) per unit area (mm^2).

Experimental design and statistics. Data from all variables were subjected to analysis of variance (ANOVA). For ANOVA, the design was considered to be a 2 × 2 × 4 factorial experiment consisting of two cultivars, non-inoculated or inoculated plants and four sampling times (2, 10, 20 and 30 dai). The experiment was arranged in a completely randomized design. Each experimental unit consisted of one plastic pot with one mango sapling. At each evaluation time, ten plants from each treatment were used for the disease assessments and the Ψ_{pd} and K_L measurements. From this initial group of plants, six plants were used for gas exchange and Chl a fluorescence assessments, TFA, Pro, MDA and pigment concentrations. Means from non-inoculated and inoculated plants for each cultivar as well as between cultivars for non-inoculated and inoculated plants at each sampling time were compared by the t-test ($P \leq 0.05$) using SAS (Release 8.02 Level 02M0 for Windows, SAS Institute, Inc., 1989, Cary, NC, USA).

RESULTS

A preliminary experiment was carried out and, overall, yielded similar trends for the evaluated parameters in each treatment (data not shown) as discussed below.

Analysis of variance. The factors cultivar, plant inoculation and sampling times were all significant for the disease indices DRLL, URLL and RFC. At least one of the factors and some of their interactions were significant for A, g_s, C_i/C_a, E, A/E, A/g_s, Ψ_{pd}, K_L, F_v/F_m, F_v'/F_m', ETR, NPQ, q_L, Chl(a+b), Chla/b, Chl(a+b)/car, MDA, TFA and Pro (Table 1).

Disease assessments. The URLL, DRLL and RFC increased overtime in both Ubá and Tommy Atkins cultivars, but they reached the greatest values for cv. Tommy Atkins (Fig. 1A-C). Plants from cv. Tommy Atkins had higher values for all three disease indices than cv. Ubá at 20 and 30 dai. DRLL, URLL and RFC were, respectively, 35, 51 and 35% higher at 20 dai. At 30 dai, the differences were, respectively, of 25% for DRLL, 56% for URLL and 24% for RFC between cultivars.

Water relations variables. There were no significant differences between non-inoculated and inoculated plants from both cultivars for Ψ_{pd} (Figs. 2A and B). In comparison to the non-inoculated, inoculated plants from cv. Tommy Atkins presented a significant decrease around 26% for K_L at 30 dai, while inoculated plants from cv. Ubá presented a decrease around 17% at 30 dai (Figs. 2C and D). Overall, neither Ψ_{pd} nor K_L differed significantly for non-inoculated plants when comparing cultivars, except for the 64% lower K_L for cv. Ubá at 2 dai (Figs. 2A-D). Inoculated plants from cv. Tommy Atkins showed values for Ψ_{pd} about 25% higher than those obtained for inoculated plants from cv. Ubá at 20 dai (Figs. 2A and B). Inoculated plants from cv. Tommy Atkins presented K_L about 85% higher than those from inoculated Ubá plants at 2 dai (Figs. 2C and D).

Gas exchanges and chlorophyll a fluorescence. Inoculated plants from cv. Tommy Atkins presented average decreases of 55% for A, 73% for g_s, 65% for E (considering the data from 10, 20 and 30 dai) and a 17% average decrease for C_i/C_a (considering 20 and 30 dai) in comparison to the non-inoculated plants. For the inoculated plants from cv. Ubá, average decreases of 34% for A, 57% for g_s, and 49% for E were found at 20 and 30 dai, while a decrease of 17% for C_i/C_a was found at 20 dai in relation to the non-inoculated plants.

Average decreases of 19, 24 and 27% were found for A, g_s and E, respectively, in inoculated plants from cv. Tommy Atkins in relation to the inoculated plants from cv. Ubá. Overall, inoculated plants from both cultivars showed higher A/g_s and A/E ratios, suggesting that water use efficiency coupled to hydraulic impairments was improved. The differences between non-inoculated and inoculated plants were more evident when considering A/g_s in cv. Tommy Atkins. Significant differences in Chl a fluorescence parameters were observed between non-inoculated and inoculated plants at 20 (for ETR and q_L) and 30 dai (for F_v'/F_m', ETR and NPQ) for Tommy Atkins but not for Ubá (Table 2). Decreases on the order of 17 and 22% were found for F_v'/F_m' and q_L, and an average decrease of 28% in the ETR was found for the inoculated plants, and the NPQ increased by 19% at 30 dai. Non-inoculated plants from cv. Tommy Atkins presented a higher F_v/F_m ratio along the evaluated time course when compared to cv. Ubá.

Concentrations of pigments, TFA, proline and lipid peroxidation. Significant differences between non-inoculated and inoculated cv. Tommy Atkins plants occurred in the concentrations of Chl (a+b), Chl (a+b)/Car, TFA, Pro and MDA (Table 3). There were significant decreases of 10 and 19%, respectively, for the Chl (a+b) and Chl (a+b)/Car concentrations and increases of 24, 25 and 24% for the concentrations of TFA, Pro and MDA, respectively, in inoculated cv. Tommy Atkins plants in relation to its non-inoculated counterparts. Significant differences between cultivars occurred in inoculated plants (Table 3); relative to cv. Ubá, cv. Tommy Atkins displayed decreases (10%) in the Chl (a+b) concentration and increases in TFA (18%), Pro (29%) and MDA (31%).

Vein density. The leaf vein density was significantly higher in cv. Ubá (13.6 mm mm^{-2}) than in cv. Tommy Atkins (12.8 mm mm^{-2}).

DISCUSSION

The results of this study provided new information about the physiological traits of the mango wilt disease as mainly demonstrated by the alterations on water relations and photosynthetic performance arising from the colonization of mango plants by C. fimbriata. In agreement with the working hypothesis of the present study, the C. fimbriata infection of mango stem tissues appears to be associated with impaired plant water relations, as demonstrated by the lower K_L values obtained for inoculated plants. These decreases are most likely coupled with the intense stem tissue necrosis as the disease progressed, associated with a possible blockage of the colonized xylem vessels. In other interactions, such as bitternut hickory-Ceratocystis smalleyi and tanoak-Phytophthora ramorum, the rapid crown decline occurred in association with a reduced sap ascent, which was presumably caused by the obstruction provoked by tyloses or fungal material (43,44). Tyloses are usually formed in response to xylem cavitation and often induced by pathogens that physically block the vessels or secreted substances that alter the intrinsic properties of the sap (43,58).

Regardless of the cultivar, the radial stem tissues colonization (RFC) by C. fimbriata seemed to be of utmost importance for xylem vessel impairment. This finding could largely explain why the cv. Tommy Atkins plants, which displayed greater radial necrosis in the stem tissues compared to cv. Ubá, also experienced earlier and greater reductions in K_L upon fungal infection as can be seen by the noteworthy data tendency, especially from 20 dai onwards, which should ultimately have contributed to increased plant water shortages. The impairments in leaf hydration of cv. Tommy Atkins may have been further facilitated by its lower vein density, given that the water transport efficiency and the length of the hydraulic pathway through mesophyll are closely related to this anatomical variable (11,12). In any case, the occurrence of stem xylem cavitation, reductions in K_L or even eventual changes in leaf or stem water status can trigger hydraulic signals through pressure volume-changes in sensing cells or yet transient cavitation within leaf veins (16,28,52). These signals mediate various acclimation responses to water deficit and result in increased synthesis and concentration of abscisic acid (ABA), ultimately leading to reductions in g_s and water loss by transpiration (16). The increase in ABA concentrations may also be favored by the pathogen itself, since it has been shown that fungal species are capable of producing this hormone, including the ascomycete Ceratocystis fimbriata (21,55). Indeed, remarkable decreases in E and g_s were found for both cultivars. Given that these decreases were coupled with reductions in the C_i/C_a ratio, it can be proposed that the decreases in A were at least partially attributable to diffusive

(stomatal) constraints. In this regard, the disease-induced decrease in A is consistent with the effects of mild water deficits on photosynthesis, as noted in other studies where stomatal rather than biochemical limitations largely accounted for the observed decreases in the A of mango leaves under mild drought stress (18,34). Anyway, A decreased to a lesser extent than g_s (and E) upon C.fimbriata infection, and thus, increases in both A/g_s and A/E ratios were found, which implies that the water use efficiency increased in the infected plants regardless of the cultivar, particularly after 20 dai.

Irrespective of the cultivar, decreases of A in inoculated plants occurred without any apparent alterations in the F_v/F_m, suggesting that primary photochemistry were unlikely to have compromised the CO_2 fixation. Furthermore, during most occasions, the ETR exceeded 90 µmol m^{-2} s^{-1}, which is much higher than the photochemical requirement for the observed photosynthesis rates (35). A high ETR with a relatively low A usually leads to excess reducing power, which can be used to produce reactive oxygen species that can trigger a variety of photoinhibitory and photooxidative effects (32). Notwithstanding, the stronger decreases in A in cv. Tommy Atkins than in cv. Ubá were associated with adjustments at the photochemical level but only in the former cultivar, as demonstrated by the concomitant decreases in both the ETR and the efficiency of excitation energy captured by the open PSII reaction centers (estimated as F_v'/F_m') in parallel to increased NPQ at 30 dai. Collectively, these changes suggest increases in thermal energy dissipation as a mean of protecting against increased excitation pressure preventing photodamage (2,4,31). Nevertheless, despite resorting to mechanisms involved in the dissipation of excess energy, it was observed an increase in lipid peroxidation in parallel to a slight decrease of the Chl pools in the plants selected for the analysis at later stages of disease development. As reported for the chestnut-Phytophthora cinnamomi (20) and tomato-Fusarium oxysporum f.sp. lycopersici (1,22) interactions, the decreases in Chl concentrations may be related not only to an oxidative stress response arising from the water shortage, but also to the release of non-selective toxins produced during the infection process.

In the present study, the declines in K_L on inoculated plants from cv. Tommy Atkins culminated in an increase in the concentrations of total free amino acids, including proline. The accumulation of organic osmolytes for osmotic adjustment is believed to have great importance for physiological acclimation against water stress (33). Proline seems to be particularly able to protect cells from damage by acting as both an osmotic agent and a ROS scavenger, thus conferring protection to membranes in addition to helping to preserve the activities of enzymes such as those involved in the antioxidant pathways and carbon

metabolism (26,29,33). In this regard, the higher levels of proline displayed by plants from cv. Tommy Atkins might be interpreted as an attempt to provide protection against water stress, which seems to be consistent with the higher level of disease severity in plants from this cultivar at advanced stages of fungal infection.

The results of the present study provide new insights into how mango plants respond to C. fimbriata infection at the physiological level. Irrespective of cultivar, the effects of fungal infection on stem tissues were probably associated with alterations in plant water relations. It was demonstrated that in the most susceptible Tommy Atkins cultivar, the disease symptoms developed faster and were associated with a more pronounced impairment of water relations and gas exchanges coupled with symptoms of oxidative stress at advanced stages of fungal infection. In contrast, although the most resistant cv. Ubá was better able to postpone disease development and, as such, suffered less from the stress imposition. In any case, the precise mechanisms underlying the responses to C. fimbriata infection may involve a myriad of physiological and biochemical processes and remain an important question that should be addressed in future studies. Considering that studies with contrasting results are often found for the involvement of water relation on vascular diseases development, it is suggested that this type of experiment must be carried out under natural conditions using direct measurements of the sap flow in order to favor the development of techniques suitable for early detection or control of the disease. Continued efforts in this direction should provide a better understanding of the alterations in plant water relations caused by mango wilt.

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LITERATURE CITED

1. Abdel-Fattah, G. M., and Al-Amri, S. M. 2012. Induced systemic resistance in tomato plants against Fusarium oxysporum f.sp. lycopersici by different kinds of compost. African J. Biotech. 11:12454-12463.

2. Adams, W. W. I., Zarter, C. R., Mueh, K. E., Amiard, V. S. E., and Demmig-Adams, B. 2005. Energy dissipation and photoinhibition: a continuum of photoprotection. In: Demmig-Adams, B., Adams, W. W., and Mattoo, A. K. eds. Photoprotection, Photoinhibition, Gene Regulation and Environment. Springer-Verlag, Berlin, 49-64.

3. Al-Sadi, A. M., Al-Ouweisi, F. A., Al-Shariani, N. K., Al-Adawi, A. O., Kaplan, E. J., and Deadman, M. L. 2010. Histological changes in mango seedlings following infection with Ceratocystis manginecans, the cause of mango decline. J. Phytopathol. 158:738-743.

4. Baker, N. R. 2008. Chlorophyll Fluorescence : A Probe of Photosynthesis In Vivo. Annu. Rev. Plant Biol. 59:89-113.

5. Bates, L. S., Waldren, R. P., and Teare, D. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39:205-207.

6. Batista, D. C., Terao, D., Barbosa, M. A. G., and Barbosa, F. R. 2008. Seca-da-mangueira Detecção, Sintomatologia e Controle. Comunicado Técnico 138, EMBRAPA, Petrolina, PE, Brazil.

7. Benevides, S. D., Ramos, A. M., Stringheta, P. C., and Castro, V. C. 2008. Qualidade da polpa da manga e polpa da manga Ubá. Ciênc. Tecnol. Alim. 28:571-578.

8. Berlyn, G. P., and Miksche, J. P. 1976. Botanical microtechnique and cytochemistry. Iowa State University Press, Ames, Iowa, USA.

9. Bilger, W., and Bjorkman, O. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosyn. Res. 25:173-186.

10. Braz, V. B., Nunes, E. S., Vieira, G., Ribeiro Júnior, J. I., Bertini, L. A., and Couto, F. A. D. 2008. Indução do amadurecimento de mangas cv. Tommy Atkins e cv. Ubá pela aplicação de ethephon pós-colheita. Bragantia 67:225-232.

11. Brodribb, T. J., Feild, T. S., and Jordan, G. J. 2007 Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiol. 144:1890-1898.

12. Brodribb, T. J., Field, T. S., and Sack, L. 2010. Viewing leaf structure and evolution from a hydraulic perspective. Funct. Plant Biol. 37:488-498.

13. Cakmak, I., and Horst, J. 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiol. Plantarum 83:463-468.

14. Carvalho, C. R. L., Rosseto, C. J., Mantovani, D. M. B., Morgano, M. A., Castro, J. V., and Bortoletto, N. 2004. Avaliação de cultivares de mangueira selecionadas pelo instituto agronômico de campinas comparadas a outras de importância comercial. Rev. Bras. Frutic. 26:264-271.

15. Castro Neto, M. T., Reinhardt, D. H., and da S Ledo, C. A. 2004. Determination of water potential on mango trees by pressure chamber. Acta Hort. 645:425-427.

16. Christmann, A., Grill, E., and Huang, J. 2013. Hydraulic signals in long-distance signaling. Curr. Opin. Plant Biol. 16:293-300.

17. Cunha, M. M., Santos Filho, H. P., and Nascimento, A. S. 2000. Manga: Fitossanidade. EMBRAPA, Brasília, DF, Brazil.

18. Damour, G., Vandame, M., and Urban, L. 2009. Long-term drought results in a reversible decline in photosynthetic capacity in mango leaves, not just a decrease in stomatal conductance. Tree Physiol. 29:675-684.

19. Dhingra, O. D., and Sinclair, J. B. 1995. Basic Plant Pathology Methods. Lewis Publisher, Boca Raton, FL, USA.

20. Dinis, L., Peixoto, F., Zhang, C., Martins, L., Costa, R., and Gomes-Laranjo, J. 2011. Physiological and biochemical changes in resistant and sensitive chestnut (Castanea) plantlets after inoculation with Phytophthora cinnamomi. Physiol. Mol. Plant Pathol. 75:146-156.

21. Dörffling, K., Petersen, W., Sprecher, E., Urbasch, I. and Hanssen, H.-P. 1984. Abscisic acid in phytopathogenic fungi of the genera Botrytis, Ceratocystis, Fusarium, and Rhizoctonia. Z. Naturforsch. 39:683-684.

22. El-Khallal, S. M. 2007. Induction and modulation of resistance in tomato plants against Fusarium Wilt disease by bioagent fungi (arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 1 - changes in growth, some metabolic activities and endogenous hormones related to defence mechanism. Aust. J. Basic Appl. Sci. 1:691-705.

23. Flexas, J., Bota, J., Cifre, J., Escalona, J. M., Galmés, J., Gulías, J., Lefi, E., Martínez-Cañellas, S. F., Moreno, M. T., Ribas-Carbó, M., Riera, D., Sampol, B., and Medrano, H. 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. Ann. Appl. Biol. 144:273-283.

24. Flexas, J., Diaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbo, M. 2007. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. Plant Cell Environ. 30:1284-1298.

25. Gallo, D., Nakano, O., Silveira Neto, S., Carvalho, R. P. L., Baptista, G. C., Berti Filho, E., Parra. J. R. P., Zucchi, R. A., Alves, S. B., Vendramin, J. D., Lopes, J. R. S., and Omoto, C. 2002. Entomologia Agrícola. 2nd Ed., FEALQ, Piracicaba, SP.

26. Hessini, K., Martínez, J. P., Gandour, M., Albouchi, A., Soltani, A., and Abdelly, C. 2009. Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in Spartina alterniflora. Environ. Exp. Bot. 67:312-319.

27. Halsted, B. D. 1890. Some fungous diseases of the sweet potato. New Jersey Agricultural College Experiment Station, Bulletin 76, pp. 25-27

28. Hubbard, R., Ryan, M., Stiller, V., and Sperry, J. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ. 24:113–121.

29. Kaushal, N., Gupta, K., Bhandhari, K., Kumar, S., Thakur, P., and Nayyar, H. 2011. Proline induces heat tolerance in chickpea (Cicer arietinum L.) plants by protecting vital enzymes of carbon and antioxidative metabolism. Physiol. Mol. Biol. Plants 17:203-213.

30. Kramer, D. M., Johnson, G., Kiirats, O., and Edwards, G. E. 2004. New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosyn. Res. 79:209-218.

31. Krause, G. H., and Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.

32. Lima, A. L., DaMatta, F. M., Pinheiro, H. A., Totola, M. R., and Loureiro, M. E. 2002. Photochemical responses and oxidative stress in two clones of Coffea canephora under water deficit conditions. Environ. Exp. Bot. 47:239-247.

33. Liu, H., Yang, W., Liu, D., Han, Y., Zhang, A., and Li, S. 2011. Ectopic expression of a grapevine transcription factor VvWRKY11contributes to osmotic stress tolerance in Arabidopsis. Mol. Biol. Rep. 38:417-427.

34. Lu, P., Chacko, E. K., Bithell, S. L., Schaper, H., Wiebel, J., Cole, S., and Müller, W. J. 2012. Photosynthesis and stomatal conductance of five mango cultivars in the seasonally wet-dry tropics of northern Australia. Sci. Hort. 138:108-119.

35. Martins, S. C. V., Galmés, J. G., Molins, A., and DaMatta, F. M. 2013. Improving the estimation of mesophyll conductance: on the role of electron transport rate correction and respiration. J. Exp. Bot. 64:3285-3298.

36. Masood, A., Saeed, S., Malik, M. T., Iqbal, N., and Kazmi, M. R. 2010. Methodology for the evaluation of symptoms severity of Mango Sudden Death Syndrome in Pakistan. Pakistan J. Bot. 42:1289-1299.

37. Masood, A., Saeed, S., Silveira, S. F., Akem, C. N., Hussain, N., and Farooq, M. 2011. Quick decline of mango in Pakistan: survey and pathogenicity of fungi isolated from mango tree and bark beetle. Pakistan J. Bot. 43:1793-1798.

38. Maxwell, K., and Johnson, G. N. 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51:659-668.

39. McElrone, A. J., Sherald, J. L., and Forseth, I. N. 2003. Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. Exp. Biol. 54:419-430.

40. Moore, S., and Stein, W. H. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem. 176:367-388.

41. Nogués, S., Cotxarrera, L., Alegre, L., and Trillas, M. I. 2002. Limitations to photosynthesis in tomato leaves induced by Fusarium wilt. New Phytol. 154:461-470.

42. Oxborough, K., and Baker, N. R. 1997. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components: calculation of q_P and F_v'/F_m' without measuring F_0'. Photosyn. Res. 54:135-142.

43. Park, J. H., Juzwi, J., and Cavender-Bare, J. 2013. Multiple Ceratocystis smalleyi infections associated with reduced stem water transport in bitternut hickory. Phytopathology 103:565-574.

44. Parke, J. L., Oh, E., Voelker, S., Hansen, E. M., Buckles, G., and Lachenbruch, B. 2007. Phytophthora ramorum colonizes tanoak xylem and is associated with reduced stem water transport. Phytopathology 97:1558-1567.

45. Pinheiro, H. A., DaMatta, F. M., Chaves, A. R. M., Loureiro, M. E., and Ducatti, C. 2005. Drought tolerance is associated with rooting depth and stomatal control of water use in clones of Coffea canephora. Ann. Bot. 96:101-108.

46. Pinto, A. C. Q., Andrade, S. E. M., Amaro, A. A., and Gomes, U. 2004. Mango industry in Brazil. Acta Hort. 645:37-50.

47. Ploetz, R. C. 2003. Diseases of mango. In: Ploetz, R. C., ed. Diseases of Tropical Fruit Crops. CABI Publishing, Wallingford, 327-363.

48. Poll, H., Vencato, A. Z., Kist, B. B., Santos, C., Carvalho, C., Reetz, E. R., and Beling, R. R. 2011. Anuário Brasileiro da Fruticultura. Editora Gazeta, Santa Cruz do Sul, RS, Brazil.

49. Pommer, C. V., and Barbosa, W. 2009. The impact of breeding on fruit production in warm climates of Brazil. Rev. Bras. Frutic. 31:612-634.

50. Ribeiro, I. J. A., Lourenção, A. L., Pilho, O. P., and Soares, N. B. 1984. Seca-da-mangueira. Vii. Resistência de cultivares de mangueira ao fungo Ceratocystis fimbriata Ell. & Halst. Bragantia 43:237-243.

51. Robbins, N. S., and Pharr, D. M. 1988. Effect of restricted root growth on carbohydrate metabolism and whole plant growth of Cucumis sativus L. Plant Physiol. 87:409-413.

52. Salleo, S., Nardini, A., Pitt, F., Lo Gullo, M. A. 2000. Xylem cavitation and hydraulic control of stomatal conductance in laurel (Laurus nobilis L.). Plant Cell Environ. 23:71-79.

53. Santos, R. P., Cruz, A. C. F., Iarema, L., Kuki, K. N., and Otoni, W. C. 2008. Protocolo para extração de pigmentos foliares em porta-enxertos de videira micropropagados. Ceres 55:356-364.

54. Schenk, H. J., Espino, S., Mendez, A. N., and McElrone, A. J. 2013. Limitations in the hydraulic pathway: effects of xylem embolisms on sap velocity and flow. Acta Hort. 991:323-332.

55. Siewers, V., Kokkelink, L., Smedsgaard, J., and Tudzynski, P. 2006. Identification of an abscisic acid gene cluster in the grey mold Botrytis cinerea. Appl. Environ. Microbiol. 72:4619-4626.

56. Silva, P. E. M., Cavatte, P. C., Morais, L. E., Medina, E. F., and DaMatta, F. M. 2013. The functional divergence of biomass partitioning, carbon gain and water use in Coffea canephora in response to the water supply: Implications for breeding aimed at improving drought tolerance. Environ. Exp. Bot. 87:49-57.

57. Souza, J. S., Almeida, C., Araujo, J. L. P., and Cardoso, C. E. L. 2002. Aspectos sócio-econômicos. In: Genú, P.J.C., and Pinto, A.C.Q. eds. A cultura da mangueira. Embrapa, Brasília, Brazil, 19-29.

58. Tyree, M. T., and Sperry, J. S. 1989. Vulnerability of xylem to cavitation and embolism. Annu. Rev. Plant Physiol. Mol. Biol. 40:l9-38.

59. Wellburn, A. R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144:307-314.

LIST OF TABLES AND FIGURES

Table 1. Analysis of variance (ANOVA) of the effects of cultivar (C), plant inoculation (I), sampling time (ST) and the interactions between these factors on disease severity and some physiological and biochemical parameters.

Parameters	Sources of variation						
	Cultivar (C)	Inoculation (I)	Sampling time (ST)	C × I	I × ST	C × ST	C × I × ST
	F-based P values						
A	0.3823	<0.0001	0.0039	<0.0001	<0.0001	0.5435	0.0023
g_s	0.0221	<0.0001	0.0034	0.0001	0.0201	0.9772	0.0182
$C_{i/}C_a$	0.1803	<0.0001	0.0002	0.1124	0.1036	0.7995	0.3405
E	0.0037	<0.0001	<0.0001	0.0001	<0.0001	0.8297	0.0206
A/E	0.0441	0.0366	0.0035	0.9617	0.6146	0.3982	0.9077
A/g_s	0.0297	<0.0001	0.0166	0.2082	0.0113	0.2595	0.1809
F_v/F_m	0.7493	0.0440	0.0044	0.3964	0.2914	0.2629	0.2083
F_v'/F_m'	0.2815	0.0054	<0.0001	0.0169	0.6971	0.0799	0.4256
ETR	0.4834	0.0112	0.0124	0.0189	0.7951	0.1940	0.8390
NPQ	0.2581	0.4569	0.0014	0.1820	0.7950	0.0005	0.9394
q_L	0.2660	0.4994	0.0003	0.5610	0.9815	0.0085	0.4981
Ψ_{pd}	0.0139	0.0281	0.0025	0.8707	0.7156	0.2117	0.7969
K_L	0.0002	0.0482	0.0029	0.3920	0.2791	0.0001	0.5771
Pro	<0.0001	0.7967	<0.0001	0.6745	0.3077	<0.0001	0.0071
TFA	0.0012	0.2646	<0.0001	0.8956	0.1317	0.7368	0.0002
Chl(a+b)	0.1187	0.2817	0.0114	0.4581	0.6252	0.0025	0.9157
Car	0.0423	0.5054	0.0066	0.7183	0.8913	0.0046	0.6712
Chla/b	0.0005	0.7999	0.1706	0.0178	0.0887	0.0627	0.9654
Chl(a+b)/Car	0.7451	0.3879	0.7105	0.9186	0.0088	0.4511	0.1687
MDA	0.1160	0.3088	<0.0001	0.4160	0.0270	<0.0001	<0.0001
URLL	0.0061	<0.0001	<0.0001	0.0061	<0.0061	0.0036	0.0036
DRLL	0.0269	<0.0001	<0.0001	0.0269	<0.0001	0.1746	0.1746
RFC	0.0007	<0.0001	<0.0001	0.0007	<0.0001	0.0143	0.0143
DF	1	1	3	1	3	3	3

Table 2. Maximum quantum efficiency of PSII photochemistry in dark-adapted leaves (F_v/F_m), the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m'), electron transport rate through PSII (ETR),non-photochemical quenching (NPQ) and fraction of open PSII reaction centers (q_L) determined in the leaves of plants from cultivars Ubá and Tommy Atkins non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata.

Parameters	Ubá				Tommy Atkins			
	20 dai		30 dai		20 dai		30 daí	
	NI	I	NI	I	NI	I	NI	I
F_v/F_m	0.79 ± 0.002	0.79 ± 0.007	0.80 ± 0.005	0.80 ± 0.004	0.81 ± 0.003 b	0.80 ± 0.003	0.82 ± 0.003 b	0.79 ± 0.081
F_v'/F_m'	0.57 ± 0.02	0.55 ± 0.01	0.55 ± 0.02	0.56 ± 0.02	0.59 ± 0.01	0.53 ± 0.02	0.59 ± 0.01	0.46 ± 0.06 a
ETR	83.1 ± 11.2	79.6 ± 4.90	116 ± 6.40	112 ± 5.10	108 ± 8.30	78.9 ± 8.70 a	130 ± 5.00	91.2 ± 21.7 a
NPQ	1.94 ± 0.20	2.11 ± 0.17	2.29 ± 0.16	2.18 ± 0.21	1.92 ± 0.10	2.52 ± 0.29	2.08 ± 0.18	2.58 ± 0.27 a
q_L	0.18 ± 0.03	0.19 ± 0.02	0.3 ± 0.02	0.28 ± 0.03	0.23 ± 0.02	0.19 ± 0.02 a	0.28 ± 0.02	0.27 ± 0.04

Means of NI and I plants within each cultivar followed by the letter a at each evaluation time are significantly different according to the Student's t test ($P \leq 0.05$). The letter b, when shown, indicates differences ($P \leq 0.05$) between cultivars for NI plants at each evaluation time according to the Student's t test ($P \leq 0.05$). Values are means ± SE (n = 6).

Table 3. Concentrations of total chlorophyll [Chl(a+b)] and carotenoids (Car), Chla/b ratio, Chl (a+b)/Car ratio and the concentrations of total free amino acids (TFA), proline (Pro) and malondialdehyde (MDA) equivalents determined in the leaves of plants from cultivars Ubá and Tommy Atkins non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata.

Parameters	Ubá		Tommy Atkins	
	NI	I	NI	I
Chl(a+b) (g m^{-2})	0.67 ± 0.03	0.67 ± 0.03	0.67 ± 0.03	0.60 ± 0.02 ac
Car (g m^{-2})	0.11 ± 0.003	0.11 ± 0.005	0.10 ± 0.005	0.12 ± 0.007 c
Chla/b	3.08 ± 0.14	3.03 ± 0.04	3.05 ± 0.46	3.05 ± 0.31
Chl(a+b)/Car	6.04 ± 0.07	6.07 ± 0.06	6.7 ± 0.16	5.4 ± 0.15 a
TFA (mmol m^{-2})	11.26 ± 0.66	14.59 ± 2.95	13.53 ± 1.29	17.72 ± 0.99 ac
Pro (mmol m^{-2})	0.81 ± 0.09	0.75 ± 0.03	0.79 ± 0.05	1.06 ± 0.06 ac
MDA (µmol m^{-2})	26.12 ± 0.54	23.87 ± 1.22	26.47 ± 0.31	34.63 ± 0.84 ac

Means of NI and I plants within each cultivar followed by the letter a at each evaluation time are significantly different according to the Student's t test ($P \leq 0.05$). The letter c, when shown, indicates differences ($P \leq 0.05$) between cultivars for I plants at each evaluation time according to the Student's t test ($P \leq 0.05$). Values are means ± SE (n = 6).

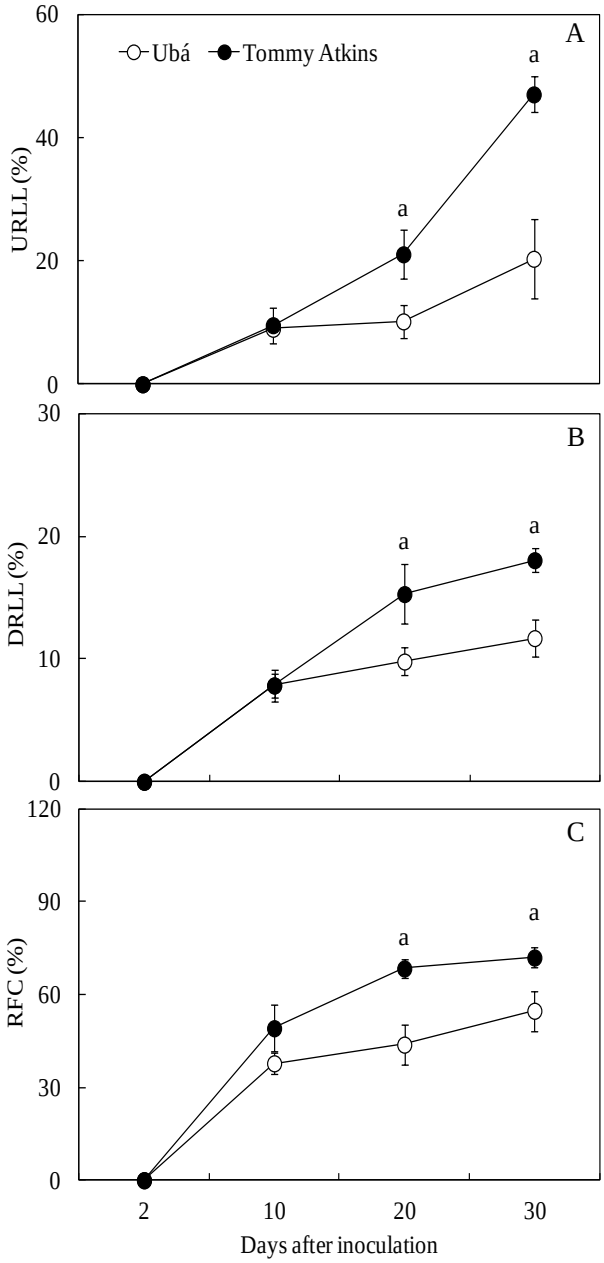

Figure 1. The upward relative lesion length (URLL), downward relative lesion length (DRLL) and the radial fungal colonization (RFC) on the stem tissues of mango plants from cultivars Ubá and Tommy Atkins inoculated with Ceratocystis fimbriata. Means followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean (n = 6).

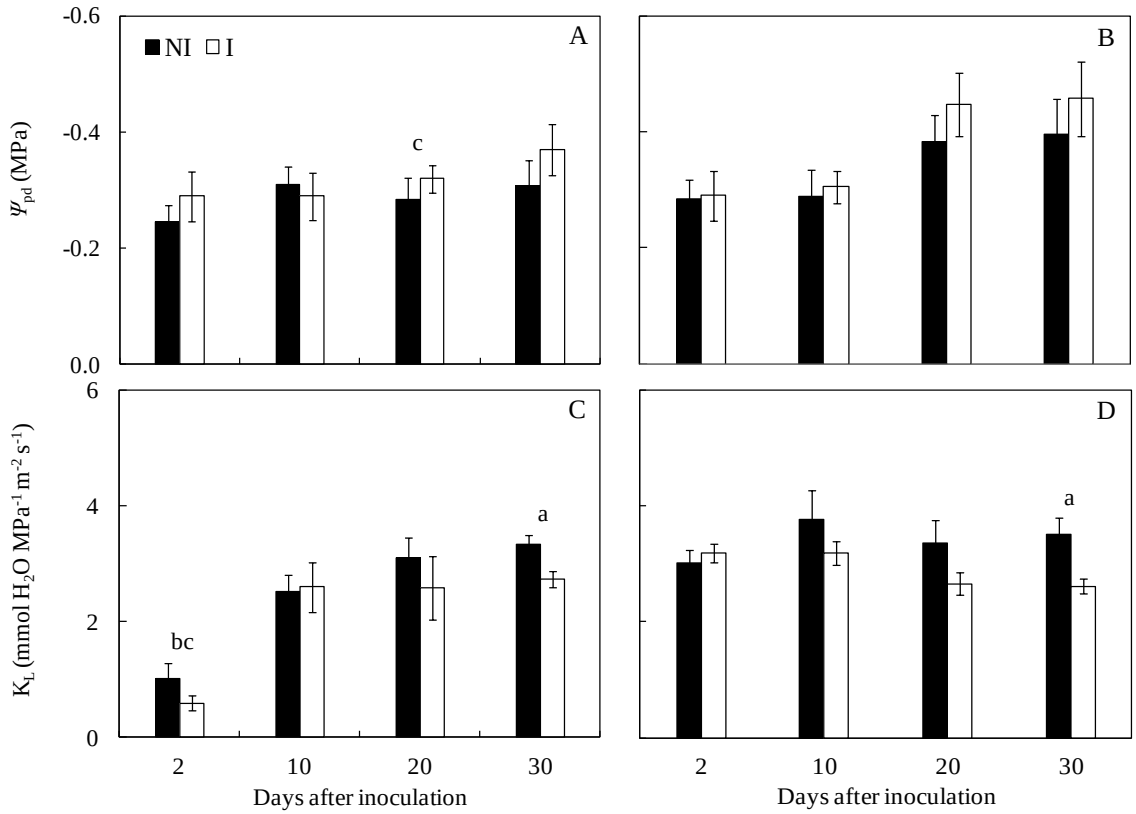

Figure 2. Predawn leaf water potential (Ψ_{pd}) and apparent hydraulic conductance (K_L) on mango plants from cultivars Ubá (A and C) and Tommy Atkins (B and D) non-inoculated (NI) and inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letter c indicates differences for I plants between the cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean (n = 6).

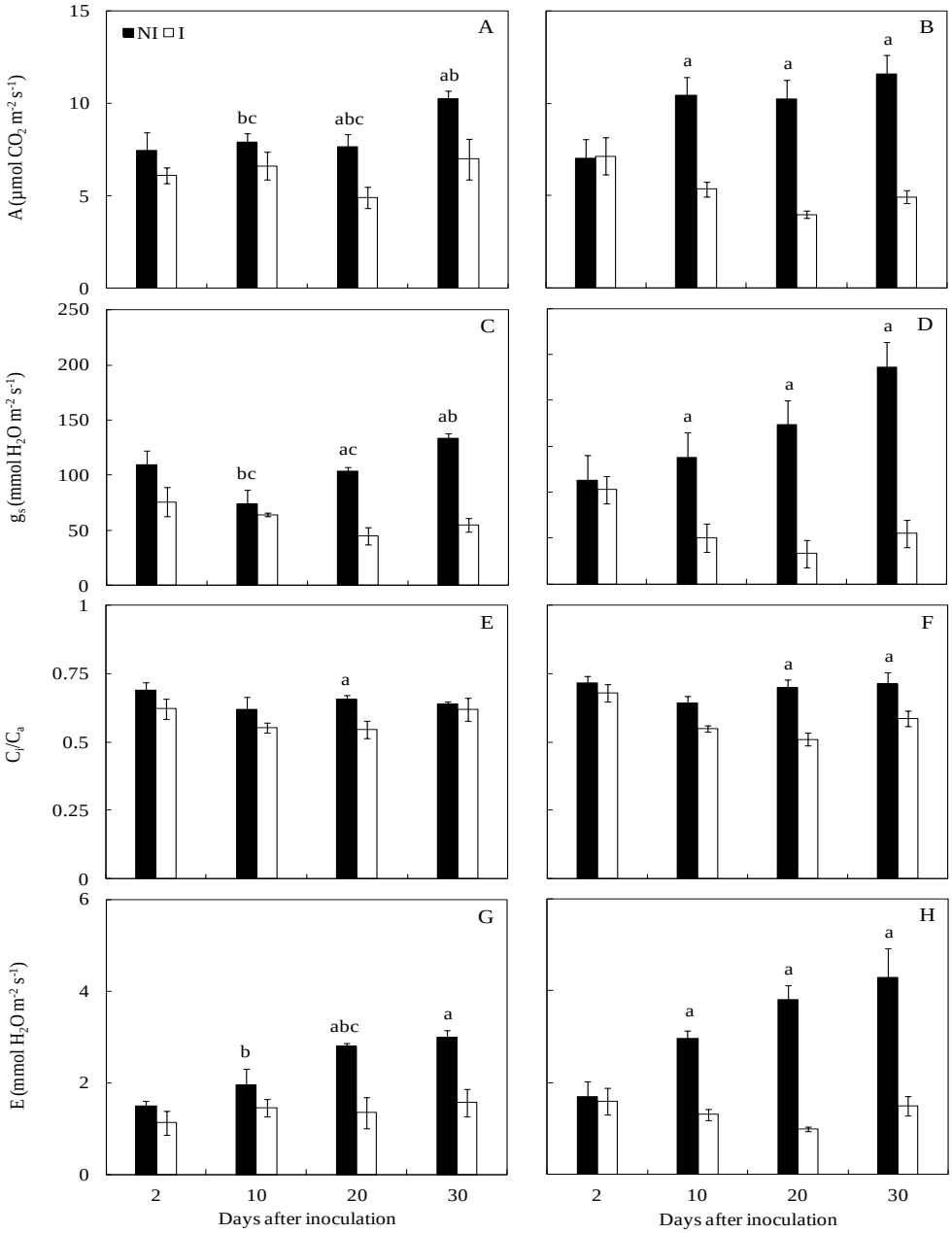

Figure 3. Net carbon assimilation rate (A) (A and B), stomatal conductance to water vapor (g_s) (C and D), internal to ambient CO_2 concentration ratio (C_i/C_a) (E and F) and transpiration rate (E) (G and H) determined in the leaves of mango plants from cultivars Ubá (left column) and Tommy Atkins (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter (a) at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences for, respectively, NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean (n = 6).

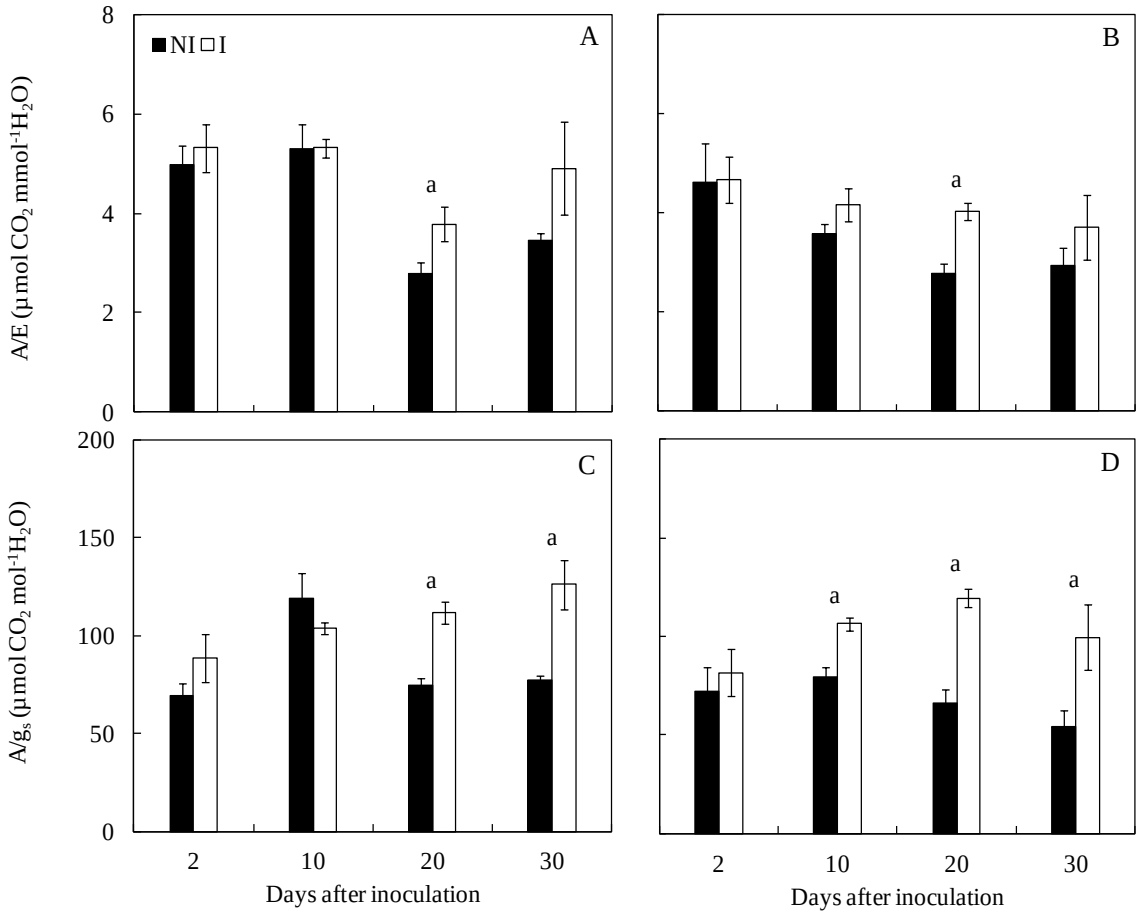

Figure 4. Instantaneous water use efficiency (A/E) (A and B) and intrinsic water use efficiency (A/g_s) (C and D) determined in the leaves of mango plants from cultivars Ubá (left column) and Tommy Atkins (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean (n = 6).

CHAPTER 2

Pathogen-induced alterations in the antioxidative system of mango cultivars with different levels of resistance to Ceratocystis fimbriata infection

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ABSTRACT

Bispo, W. M. S., Araújo, L., Bermudez C., M. B., Cacique, I. S., DaMatta, F. M., and Rodrigues, F. A. Pathogen-induced alterations in the antioxidative system of mango cultivars with different levels of resistance to Ceratocystis fimbriata infection.

The mango wilt, caused by Ceratocystis fimbriata Ellis & Halsted, is one of the most important diseases affecting mango production worldwide. Nevertheless, to date, few attempts have been made to elucidate the physiological mechanisms underlying host responses to the infection process. It was herein hypothesized that the attenuation of disease severity displayed by some mango cultivars might be related to a higher antioxidant capacity on the leaf level. To test this hypothesis, the activities of a range of enzymes (e.g., SOD, CAT, POX, GST, APX, GPX and GR), metabolites (e.g., ascorbate, total glutathione and total phenolics) involved in the antioxidant system as well as some markers for oxidative stress (e.g., malondealdehyde and hydrogen peroxide), were evaluated for over 30 days after inoculation (dai) of mango cultivars with different levels of resistance to C. fimbriata infection. At 30 dai, the disease severity was more pronounced in the less resistant cv. Tommy Atkins (that displayed higher values for the upward (34%), downward (51%) and radial colonization of the stem tissues (44%) than the more tolerant cv. Ubá. In contrast to the working hypothesis, plants from cv. Tommy Atkins presented more prominent increases in the activity of enzymes and concentrations of metabolites related to the oxidative stress responses. These increases were minimal, if any, in cv. Ubá. It is suggested that the cv. Tommy Atkins upon fungal

infection, by less effectively restraining the spread of the pathogen in the vascular tissues, needed to trigger its antioxidant system at the leaf level to cope with the more rapid stress development when compared with the cv. Ubá.

Additional keywords: gas exchange; oxidative stress, phenolics, vascular pathogen.

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INTRODUCTION

The mango (Mangifera indica L.) industry is one of the largest tropical fruit industries comprising approximately 100 countries in tropical and subtropical regions worldwide with a production of over 34.3 million tons (Bally, 2011). Brazil stands out as one of the chief producers of mango along with Mexico and Asian countries such as China and India, the major world producers (Bally, 2011). Among the mango cultivars commercially exploited in Brazil, the cv. Tommy Atkins concentrates around 90% of the cropped area mainly due to their hardiness, attractive color and good postharvest conditions besides the relative stability of production between consecutive years (Almeida et al., 2001). Some Brazilian cultivars are also cropped such as Ubá (Pinto et al., 2004), that is widely used as raw material for processing industries due to some characteristics of interest such as high pulp yield, high soluble solids content, and few fibers (Benevides et al., 2008; Braz et al., 2008). The greatest success in the development of various production hubs facing both industry and the fresh market is, however, confronted by a range of pests and diseases that can appreciably reduce fruit production and quality (Ploetz, 2003; Bally, 2011). The mango wilt, caused by the fungus Ceratocystis fimbriata Ell. & Halst. (Halsted, 1890), is one of the most important diseases affecting mango production worldwide because it causes the decline of entire orchards and thus ultimately cause losses in production and profits (Ploetz, 2003; Batista et al., 2008).

The genus Ceratocystis includes various plant pathogens affecting herbaceous and, especially, woody plants causing symptoms that include necrosis of the vascular tissues, cankers and leaf wilting (Upadhyay, 1993; van Wyk et al., 2007). Ceratocystis fimbriata is primarily considered a xylem pathogen (Harrington, 2000) causing the obstruction of the xylem vessels and dysfunctions on plant sap flow (Al-Sadi et al., 2010; Park et al., 2013). As a consequence of fungal infection, the whole-plant hydraulic conductivity is expected to decrease with the increasing tissue colonization by the pathogen (Fradin and Thomma, 2006; Parke et al., 2007, Park et al., 2013). The fungal structures such as mycelia and clamydospores and tissue necrosis, in addition to the promptly activation of defense structures (e.g., tyloses, gums and gels) by the hosts are closely related to the sealing of the vessels (Rahman et al., 1999; Parke et al., 2007; Al-Sadi et al., 2010; Yadeta and Thomma, 2013).

The disruption in the upward water movement in association with a complex interaction of enzyme secretion, non-selective toxins and hormones contributes to changes in leaf water status and to imbalances in the cellular homeostasis (Nogués et al., 2002). Actually, under

conditions of water deprivation, disproportionate increases in the production and accumulation of reactive oxygen species (ROS) as byproducts of various cellular processes may occur and may therefore lead to dramatic damages to cell structures and disruption of the metabolic activity including photosynthetic performance (Nogués et al., 2002; Chaves et al., 2003; Sharma et al., 2012). Fortunately, in order to restrain the deleterious action of the ROS, plant species are endowed with a complex antioxidant system which comprises metabolites and enzymes that can act over those oxidant compounds located in different organelles of the plant cells (Petridis et al., 2012). Some of the components involved in this cell scavenging process are the enzymes superoxide dismutases (SOD), that catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2; catalases (CAT), responsible for the removal of H_2O_2, and the enzymes and metabolites of the ascorbate/glutathione cycle, as well as phenolic compounds such as the flavonols (Chaves et al., 2003; Petridis et al., 2012).

A wealth of studies involving oxidative stress and metabolism of phenolic compounds in host-pathogen interactions have been reported, though most of these studies have centered their attention in evaluating the localized infection of biotrophic or necrotrophic pathogens in leaf tissues (Stevenson et al., 1997; Vanacker et al., 1998; García-Limones et al., 2002). By contrast, metabolic modifications in response to vascular pathogens have barely been investigated (García-Limones et al., 2002; Pomar et al., 2004), specially when concerning the disease effects on the leaf level. Given that the tolerance to a great variety of stressful conditions, including water deprivation, has been associated with an improved activity of the antioxidant system on the leaf level (Terzi and Kadioglu, 2006; Rivero et al., 2007; Chugh et al., 2011; Zhang et al, 2013), it was hypothesized that plants from the most resistant mango cultivar should have an untimely and/or higher capacity to cope with a possible leaf oxidative stress arising from the vascular tissues colonization by C. fimbriata. Accordingly, considering the importance of mango wilt as part of the problems associated with the decrease on mango production worldwide and that more information is needed to better understand how this disease affects the mango physiology, the main goal of this study was to evaluate if the antioxidant system might be related to the reduction or postponement of the disease symptoms. This innate ability should so keep a relatively low concentration of ROS and cell integrity, providing, therefore, conditions for the host to mount mechanisms of resistance against C. fimbriata infection.

MATERIALS AND METHODS

Plant material. Mango plants of approximately 1 year old from cultivars Ubá and Tommy Atkins, which are known to be resistant and moderately resistant, respectively, to C. fimbriata (Ribeiro et al., 1984; Carvalho et al., 2004), were obtained from a commercial orchard (Dona Euzébia, Minas Gerais State, Brazil). Both cultivars were grafted onto plants from cultivar Imbú, widely used as rootstock in the Zona da Mata region, Minas Gerais State, Brazil. The saplings were transplanted into plastic pots containing 8 kg of substrate consisting of a mixture of soil, sand and manure in a 2:1:1 proportion. The plants were kept in a greenhouse (temperature of 30 ± 2°C and relative humidity of 70 ± 5%) for two months before the beginning of the experiments. Plants were irrigated and fertilized as needed.

Inoculation procedure. The isolate CEBS15 of C. fimbriata was used to inoculate the plants. This isolate was obtained from symptomatic mango plants collected in the city of Brejo Santo, Ceará State (07° 29' 34" S, 38° 59' 06" W), Brazil. The isolate was preserved by Castellani's method (Dhingra and Sinclair 1995). Plugs of malt extract-agar medium containing fungal mycelia were transferred to Petri dishes containing potato-dextrose-agar (PDA). After three days, the PDA plugs containing fungal mycelia were transferred to new Petri dishes containing the same culture medium and were maintained in an incubator chamber (temperature of 25°C and 12-h photoperiod) for 14 days.

Plants were inoculated according to Al-Sadi et al. (2010) with a few modifications. Stem disks (10-mm in diameter and approximately 2-mm in width) were removed from the stems with the aid of a punch at, approximately, 5 cm above the graft scar. A PDA plug (10-mm in diameter) obtained from a 14-days-old fungal colony was carefully placed in the punch hole. Each hole containing a PDA plug with fungal mycelia was carefully covered with a piece of moistened cotton and then wrapped with parafilm to maintain adequate moisture for fungal infection. The disks used to inoculate each plant were taken from the middle portion of each fungal colony to make the inoculation as homogeneous as possible. Holes on the stems of plants receiving only PDA medium plugs served as the control treatment.

Relative lesion indices. Disease progress was evaluated at 30 days after inoculation (dai). The upward, downward and radial colonization of the stem tissues by fungal hyphae was evaluated by measuring the length (in cm) of the internal necrotic tissue using a digital caliper. The upward relative lesion length (URLL) and the downward relative lesion length

(DRLL) were determined as the ratio between the length from the graft scar to the top of the stem (LGST) and the lesion length (LL) in the same interval (upward and downward) from the inoculation point according to the following formula: URLL or DRLL = LL × 100/LGST. The plants were standardized to a length of 20 cm (the distance from the graft scar to the top of the stem). The radial fungal colonization (RFC) was determined as the length of the necrotic tissue in relation to the total stem diameter × 100. Measurements were performed in six replications from a initial group of 10 plants, being selected those that had the most visible symptoms of the disease at each evaluation time.

Leaf gas exchanges. The net carbon assimilation rate (A), stomatal conductance to water vapor (g_s), internal-to-ambient CO_2 concentration ratio (C_i/C_a) and transpiration rate (E) were measured in fully expanded leaves with an infrared CO_2/H_2O gas analyzer (LI 6400, Li-Cor, Lincoln, NE) equipped with a blue/red light source (Li-6400-02B). The measurements were conducted at ambient temperature and CO_2 conditions under artificial light (1000 µmol photons m^{-2} s^{-1}) from approximately 0800 to 1200 h at 2, 10, 20 and 30 dai. Measurements were performed in six replications from an initial group of 10 plants, being selected those that had the most visible symptoms of the disease at each evaluation time.

Biochemical assays. Each treatment consisted of ten plants (replicates) per each sampling time and the five most representative ones concerning the development of the symptoms were selected for sampling. For each replicate, four to five fully expanded leaves exposed to sunlight were collected from the same portion of the plant. The midrib region and the leaf ends were discarded. A composite sample was obtained to be used in the following biochemical assays.

Lipid peroxidation assay. The oxidative damage to lipids was estimated as the concentration of the total amount of 2-thiobarbituric acid (TBA) reactive substances and expressed as equivalents of malondialdehyde (MDA) according to Cakmak and Horst (1991) with a few modifications. A total of 0.2 g of leaf tissues was ground into a fine powder and homogenized in a 2 mL 0.1% (w/v) trichloroacetic acid (TCA) solution at 4°C following centrifugation at 10,000 g for 15 min. The supernatant was used for the MDA assay. A total volume of 500 µL was added to 1.5 ml of TBA (0.5% in 20% TCA) and the mixture was incubated for 20 min in a mixer (300 rpm) with control temperature set to 95°C. The reaction was stopped by immersion in an ice bath. The samples were centrifuged at 13,000 g for 4 min

and the absorbance of the supernatant was recorded at 532 nm. The non-specific absorbance was estimated at 600 nm and subtracted from the specific absorbance values. An extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate the MDA concentration.

Determination of hydrogen peroxide (H_2O_2) concentration. A total of 0.2 g of leaf tissues was ground in liquid nitrogen into a fine powder and homogenized in 2 mL of a mixture containing 50 mM potassium phosphate buffer (pH 6.5) and 1 mM hydroxylamine. The homogenate was centrifuged at 10,000 g for 15 min at 4°C (Kuo and Kao, 2003) and the supernatant was used as the crude extract. A total of 100 µL of the supernatant was then added to a reaction mixture containing 100 µM ferric ammonium sulfate ($FeNH_4[SO_4]$), 25 mM sulfuric acid, 250 µM xylenol orange and 100 mM sorbitol in a final volume of 2 mL (Gay et al., 1999). After 30 min of dark incubation at room temperature, the absorbance of the samples was determined at 560 nm. The controls for the reagents and crude extracts were prepared under the same conditions and subtracted from the sample. A standard curve of H_2O_2 (Sigma-Aldrich, São Paulo, Brazil) was used to determine the H_2O_2 concentration.

Determination of ascorbate (AsA) concentration. A total of 0.2 g of leaf tissues was ground in liquid nitrogen into a fine powder and homogenized in 2 mL of a 6% trichloroacetic acid (TCA) (w/v) solution. The homogenate was centrifuged at 15,000 g for 5 min at 4°C (Kampfenkel et al., 1995). An aliquot of 50 µL from the supernatant was then added to a 0.02 M sodium phosphate buffer (pH 7.4) solution and the resulting mixture was incubated at 42°C for 15 min. Subsequently, a reaction mix consisting of 0.02 M sodium phosphate buffer (pH 7.4) solution, 5% TCA (w/v), 8.4% H_3PO_4 (v/v), 0.8% 2,2'-dipyridyl (w/v) and 0.3% $FeCl_3$ (w/v) was added to the mixture and brought to a final volume of 1 mL. After agitation, the solution was again incubated at 42°C for 40 min and the reaction was stopped in ice bath. The absorbance was recorded at 525 nm and the concentration of AsA was determined according to a calibration curve of AsA (Sigma-Aldrich, São Paulo, Brazil).

Determination of total glutathione concentration (GSH+GSSG). A total of 0.2 g of leaf tissues was ground in liquid nitrogen and the obtained powder was homogenized in 2 ml of a mixture consisting of 0.1 M HCl and 1 mM EDTA. The homogenate was centrifuged at 12,000 g for 10 min at 4°C (Anderson, 1985). In order to determine the total glutathione (GSH+GSSG) concentration, 50 µL of the supernatant was added to a reaction mixture consisting of 125 mM sodium phosphate buffer (pH 7.5), 6.3 mM EDTA, 0.3 mM NADPH

and 6 mM 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) in a total volume of 1 mL. After incubation for 5 min at 30°C, 10 µL of glutathione reductase enzyme (50 U ml^{-1}) was added to the mix and the absorbance was determined at 412 nm during 2 min. The concentration of GSH+GSSG was determined by using a calibration curve of glutathione (Sigma-Aldrich, São Paulo, Brazil) according to Griffith (1980).

Determination of total phenolics (TP) concentration. A total of 0.2 g of leaf tissues was ground into a fine powder using a pestle and mortar with liquid nitrogen. The fine powder was homogenized with 1.5 mL of 80% methanol and extracted overnight on a mixer (300 rpm) with control temperature set to 25°C. The methanolic extract was then centrifuged at 12,000 g for 10 min and the supernatant was used to determine the total phenolics concentration according to Zieslin and Ben-Zaken (1993). A volume of 150 µL of the extract was added to 150 µL of 0.25 N Folin-Ciocalteau's Phenol reagent (Sigma-Aldrich, São Paulo, Brazil) and the mixture was homogenized and kept at room temperature for 5 min. Subsequently, 150 µL of 1 M Na_2CO_3 were added to the mixture that was incubated at room temperature for 10 min., A total of 1 mL of sterile water was added to the resulting blend followed by a new incubation at room temperature for 1 h. The development of a blue color was recorded at 725 nm and a standard curve was produced by relating catechin concentration to the solution absorbance. Sample absorbance for TP concentration was estimated and expressed as catechin (Sigma-Aldrich, São Paulo, Brazil) equivalents.

Enzyme extractions and assays. Leaf samples of inoculated and non-inoculated plants from cultivars Ubá and Tommy Atkins collected at 2, 10, 20 and 30 dai were flash frozen in liquid nitrogen and subsequently stored at -80°C until further analysis. For the assays of superoxide dismutases (SOD, EC 1.15.1.1), catalases (CAT, EC 1.11.1.6), non-specific peroxidases (POX, EC 1.11.1.7), glutathione-S-transferases (GST, EC 2.5.1.18), ascorbate peroxidases (APX, EC 1.11.1.11) and glutathione peroxidases (GPX, EC 1.11.1.9), a total of 0.2 g of leaf tissues was ground in liquid nitrogen into a fine powder and homogenized in a 2 mL solution containing 50 mM potassium phosphate buffer (pH 6.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 2% (w/v) polyvinylpyrrolidone (PVP). For glutathione reductases (GR, EC 1.8.1.7), the fine powder was homogenized in 2 mL of a solution containing 100 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA, 1 mM DL-dithiothreitol (DTT), 1 mM PMSF and 2% (w v^{-1}) PVP. For phenylalanine ammonia-lyases (PAL, EC 4.3.1.5) and polyphenoloxidases (PPO,

EC 1.10.3.1), 2 mL of 50 mM sodium phosphate buffer (pH 6.5) and potassium phosphate buffer (pH 6.8), respectively, containing 1 mM PMSF and 0.1 mM EDTA were used for homogenization. The extracts were centrifuged at 12,000 g at 4°C for 15 min for SOD, CAT, POX, GST, APX, GPX and GR and at 20,000 g at 4°C for 25 min for PAL and PPO. The supernatants were used for the enzymes activities assays. All steps were performed at 4°C.

The total SOD activity was determined by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). The reaction was started after the addition of 50 µL of the crude enzyme extract to 1.95 mL of a mixture containing 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NTB, 0.1 mM EDTA and 2 µM riboflavin. The samples were exposed to 10 min of light and the production of formazan blue, resulting from the photoreduction of NBT, was measured at 560 nm. The absorbance at 560 nm of a reaction mixture with the same composition, but kept in the dark for 10 min, served as a blank. One unit of SOD was defined as the amount of enzyme necessary to inhibit NBT photoreduction by 50%.

The CAT activity was determined by adding 50 mL of the crude enzyme extract to 1.95 ml of a reaction mixture consisting of potassium phosphate buffer 50 mM (pH 7.0) and 12.5 mM hydrogen peroxide (H_2O_2) (Havir and McHale, 1987). The decrease in absorbance at 240 nm was measured for 2 min and the enzyme activity was calculated from the initial rate of the enzyme using the extinction coefficient of 40 mM^{-1} cm^{-1} at 240 nm.

The POX activity was assayed using the colorimetric method by determining the pyrogallol oxidation as proposed by Kar and Mishra (1976). The reaction was started after the addition of 15 µL of the crude enzyme extract to 1.985 mL of a reaction mixture containing 25 mM potassium phosphate (pH 6.8), 20 mM pyrogallol and 20 mM H_2O_2. The activity was determined through the absorbance of colored purpurogallin recorded for 2 min at 420 nm and the enzyme activity was calculated from the initial rate of the enzyme using the extinction coefficient of 2.47 mM^{-1} cm^{-1} (Chance and Maehley, 1955).

The GST-like activity was determined using the methodology proposed by Habig et al. (1974). A total of 20 µL of the crude enzyme extract was added to 1.98 mL of the mixture containing 97 mM potassium phosphate buffer (pH 6.5), 0.97 mM EDTA, 2.5 mM reduced glutathione (GSH) and 1.0 mM 1-chloro-2,4-dinitrobenzene (CDNB). The absorbance was measured at 340 nm over 5 min. The extinction coefficient of 9.6 mM^{-1} cm^{-1} was used to determine the enzyme activity.

The APX activity assay was conducted as described by Nakano and Asada (1981). A total of 15 µL of the crude enzyme extract was added to 1.985 mL of the mixture containing 50

mM phosphate buffer (pH 7.0), 1 mM H_2O_2 and 1 mM sodium ascorbate. The rate of ascorbate oxidation recorded by the decrease in the absorbance at 290 nm was measured for 2 min and the enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate.

The GR activity was assayed according to Carlberg and Mannervik (1985). The reaction was started after the addition of 20 µL of the crude enzyme extract to a volume of 1.98 mL of a mixture containing 100 mM potassium phosphate (pH 7.5), 1 mM EDTA, 2mM DTT, 1 mM PMSF, PVPP 2%, 1 mM oxidized glutathione (GSSG) and 0.1 mM NADPH (prepared in 0.5 mM Tris-HCl buffer, pH 7.5). The decrease in absorbance was determined at 340 nm for 2 min and GR activity was calculated from the initial rate of its activity using an extinction coefficient of 6.22 mM^{-1} cm^{-1} (Foyer and Halliwell, 1976).

The GPX activity was estimated according to Lawrence and Burke (1976). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), 10 mM EDTA, 1.14 mM NaCl, 0.2 mM NADPH, 1 U/ml GSSG-reductase, 1 mM GSH, 0.25 mM H_20_2 and the enzyme source in a total volume of 1 mL. All components were combined at the beginning of the assay, except for the enzyme source and H_20_2. A total of 10 µL of the crude extract was added to the above mixture and allowed to incubate for 5 min at 25°C and the reaction was initiated by the addition of 100 µL of a H_20_2 solution. The decrease in absorbance was measured at 340 nm for 5 min and the extinction coefficient of 6.22 mM^{-1} cm^{-1} (Anderson and Davis, 2004) was used to calculate GPX activity.

The PPO activity was determined by the oxidation of pyrogallol according to the method of Kar and Mishra (1976). The reaction mixture was composed of 100 mM potassium phosphate buffer (pH 6.8) and 100 mM pyrogallol in a total volume of 0.98 mL added to 20 µL of the enzyme source. The absorbance was measured at 420 nm for 3 min after addition of the crude enzyme extract. An extinction coefficient of 2.47 mM^{-1} cm^{-1} was used to calculate PPO activity (Chance and Maehley, 1955).

The PAL activity was determined by adding 50 µL of the extract to a mixture containing 25 mM Tris-HCl (pH 8.8) and 100 mM L-phenylalanine in a total volume of 1 mL. The reaction mixture was incubated in a water bath at 30°C for 1 h and then finalized by the addition of 60 µL of 6 N HCl. The absorbance of the trans-cinnamic acid derivatives was measured at 290 nm and the molar extinction coefficient of 10^4 mM^{-1} cm^{-1} (Zucker, 1965) was used to calculate PAL activity.

All enzyme activities were expressed on a protein basis and the soluble protein concentrations of the extracts were measured by the method of Bradford (1976) using bovine serum albumin as the standard protein.

Experimental design and statistics. A 2 × 2 factorial experiment consisting of two mango cultivars (Ubá and Tommy Atkins) and non-inoculated or inoculated plants with C. fimbriata were arranged in a completely randomized design with six replications for gas exchange measurements and five replications for the biochemical assays. Each experimental unit consisted of one plastic pot containing one mango plant. The experiment was repeated once. Data were analyzed by an analysis of variance (ANOVA) and within each sampling time treatment means were compared by Student's t test ($P \leq 0.05$) using SAS (Release 8.02 Level 02M0 for Windows, SAS Institute, Inc., 1989, Cary, NC, USA).

RESULTS

Relative lesion indices. At 30 dai, URLL, DRLL and RFC were much higher for plants of cv. Tommy Atkins (34, 51 and 44%, respectively) than for plants of cv. Ubá.

Leaf gas exchanges. Inoculated plants from cv. Tommy Atkins presented average decreases of 45% for A, 62% for g_s, 12% for C_i/C_a, and 50% for E (for 20 and 30 dai) in comparison to the non-inoculated plants (Figs. 1B,D,F,H). For the inoculated plants from cv. Ubá, decreases of 24% for A, 40% for g_s, 5% for C_i/C_a, and 37% for E were found at 30 dai in relation to the non-inoculated plants (Figs. 1A,C,E,G).

Lipid peroxidation and H_2O_2 concentration. The concentrations of H_2O_2 significantly increased by 13% for inoculated plants from cv. Tommy Atkins at 30 dai in comparison to the non-inoculated plants (Table 2). There were no changes for H_2O_2 and MDA concentrations during the evaluation times for plants from cv. Ubá, regardless of plant inoculation. The concentrations of H_2O_2 and MDA were, respectively, 21 and 20% higher for inoculated plants from cv. Tommy Atkins in comparison to inoculated plants from cv. Ubá at 30 dai. At 20 dai, inoculated plants from cv. Tommy Atkins also showed a higher H_2O_2 concentration (30%) in comparison to inoculated plants from cv. Ubá. Non-inoculated plants from cv. Tommy Atkins showed a higher concentration of H_2O_2 (35%) at 20 dai when compared to its counterpart from cv. Ubá.

Ascorbate and total glutathione concentrations. There was significant difference between non-inoculated and inoculated plants for concentration of AsA only from cv. Tommy Atkins at 30 dai (Table 2). There were no significant differences between non-inoculated and inoculated plants for total glutathione regardless of the cultivar (Table 2). There were no significant differences for ascorbate and glutathione concentrations during the evaluation times between cultivars regardless of plant inoculation.

Total phenolics concentration. At 30 dai, the TP concentration increased by 4% for inoculated plants from cv. Ubá in comparison to the non-inoculated plants (Fig. 2E) while there were no difference for plants from cv. Tommy Atkins (Fig. 2F). The TP concentration for inoculated plants from cv. Ubá was 4% higher in comparison to inoculated plants from cv. Tommy Atkins at 30 dai.

Enzyme activities. Overall, the activities of most of the assessed enzymes involved in ROS scavenging significantly increased for plants from cv. Tommy Atkins as disease progressed (Table 1). Significant differences between non-inoculated and inoculated plants occurred mostly at 30 dai. There were increases of 10% for SOD, 33% for POX, 31% for APX, 25% for GR and 23% for GPX on inoculated plants in comparison to the non-inoculated ones. At 10 dai, SOD activity significantly increased by 22% on inoculated plants in comparison to the non-inoculated ones. For cv. Ubá, SOD activity was 8% higher at 30 dai on inoculated plants in comparison to the non-inoculated ones (Table 1). PAL significantly increased by 20 and 32% on inoculated plants from cv. Ubá at 2 and 30 dai, respectively, in comparison to the non-inoculated ones (Fig. 1A). Differences between non-inoculated and inoculated plants from cv. Tommy Atkins occurred only at 20 dai when inoculated plants presented higher PAL activity (22%) (Fig. 1B). Concerning the differences between cultivars (Table 1), the SOD activity was 9 and 5% higher, respectively, at 20 and 30 dai for inoculated plants from cv. Tommy Atkins in comparison to inoculated plants from cv. Ubá. Inoculated plants from cv. Tommy Atkins showed higher POX activity at 20 dai (34%) and 30 dai (63%) while the APX activity was 39% higher for inoculated plants from cv. Tommy Atkins in comparison to inoculated plants from cv. Ubá at 30 dai. For non-inoculated plants from cv. Tommy Atkins, POX activity was 8% lower in comparison to their counterparts from cv. Ubá at 20 dai. CAT activity was 21% higher for cv. Ubá in comparison to non-inoculated plants from both cultivars at 20 dai.

DISCUSSION

The results from the present study bring novel information about the intricate alterations on mango tree physiology arising from the infection by the vascular pathogen C. fimbriata and mainly attempt to demonstrate the involvement of broader mechanisms in disease resistance, not linked to host localized responses against fungal infection. Thus, in order to evaluate the involvement of leaf mechanisms on the attenuation of disease symptoms, the activities of enzymes and metabolites involved in antioxidant mechanisms were examined. Based on the URLL, DRLL and RFC values, plants from cv. Tommy Atkins were more susceptible to the C. fimbriata infection than plants from cv. Ubá. As reported for many interactions involving a range of plant species and vascular pathogens and observed in our previous results for the interaction mango-Ceratocystis fimbriata (data not shown), the colonization of the host stem tissues upon fungal infection often increase the resistance to xylem sap flow and develop leaf water shortages and wilt symptoms (Al-Sadi et al., 2010; Parke et al., 2007, Park et al., 2013). Under such condition, hydraulic signals may be translated into increased levels of abscisic acid in the leaf tissues, mediating acclimative responses such as stomatal closure, in order to save water from the transpiration loss (Christmann et al., 2013). In fact, substantial declines were found for g_s and E in plants from both cultivars over time, although in a greater extent in plants from cv. Tommy Atkins. The coupled decreases in C_i/C_a ratio suggests that, at least initially, the decreases in A were related to stomatal constraints. Limited A may decrease the consumption of electrons released from water and ensure an excess of excitation energy (Lawlor, 2002), that help in increasing ROS formation and accumulation, thereby generating a potential oxidative stress (Nogués et al., 2002; Chaves et al., 2003). Indeed, data from the present study show a greater concentration of H_2O_2, a very stable oxidant (Boominathan and Doran, 2002; Upadhyaya et al., 2007), and a tendency of an increase in the MDA concentration as a secondary end product of the oxidation of polyunsaturated fatty acids (del Rio et al., 1992), as particularly noted in cv. Tommy Atkins at 30 dai.

As disease progressed, alterations in cell metabolism, such as an increased electron flow to the Mehler reaction and/or the enhancement of the photorespiratory pathway, may be expected and, as a consequence, molecular oxygen could act as an electron acceptor contributing, therefore, to the unbalanced formation of $O_2^{\bullet-}$, a moderately reactive ROS that is usually the first to be generated (Noctor et al., 2002; Gill and Tuteja, 2010). The increased formation of $O_2^{\bullet-}$ may have favored the increase on SOD activity on leaves of plants from cv.

Tommy Atkins and, presumably, contributed to the increase in the H_2O_2 concentration at advanced stages of fungal infection, since SOD catalyzes the dismutation of $O_2^{\bullet-}$ into H_2O_2 and O_2. SOD is the front-line enzyme in ROS attack; however, the catalyzed reaction only converts one ROS to another that also needs to be counteracted by other scavenging systems (Noctor and Foyer, 1998; Resende et al., 2012). Among the enzymes involved in the removal of the excess of H_2O_2 generated either spontaneously or by dismutation of $O_2^{\bullet-}$ through the activity of SOD, peroxidases play a key role in conjunction with CAT (Mittler, 2002; Debona et al., 2013). Actually, data from the present study showed that POX, APX and GPX activities increased on the inoculated plants from cv. Tommy Atkins, although CAT activity was held in check between non-inoculated and inoculated plants.

The ascorbate-glutathione (AsA-GSH) cycle, regarded as one of the most important antioxidant pathways (Noctor and Foyer, 1998), is also largely responsible for the scavenging of H_2O_2, preventing the molecule action itself and the subsequent formation of the highly reactive hydroxil radical (•OH) (Wang et al., 2012). Among the enzymes that are part of this cycle, APX and GR were assessed in the present study and both showed increased activity during the evaluated time course, which suggests an enhanced activity of the AsA-GSH cycle. In its scavenging action over H_2O_2, APX utilizes AsA as an electron donor due to its high affinity for H_2O_2 (Smirnoff, 2000; Sharma and Dubey, 2005; Pyngrope et al., 2013). The NADPH-dependent GR, in turn, is a crucial enzyme of the cycle since it catalyzes the reduction of GSH from GSSG, maintaining sustained levels of GSH, fostering the regeneration of AsA and the consequent functionality of the AsA–GSH cycle (Foyer and Shigeoka, 2011; Hossain et al., 2013). In agreement with the data from the present study, reports of increases in the activity of APX and GR under conditions of water privation and oxidative stress are often found in the literature (Pastori and Trippi, 1992; Sharma and Dubey, 2005; Koussevitzky et al., 2008; Nazarli et al., 2011; Cruz et al., 2013). It is noteworthy that both AsA and GSH are metabolites linked through the AsA-GSH cycle and are also important low molecular weight non-enzymatic antioxidants that dramatically contribute to the maintenance of the cell redox status (Asada, 1999; Smirnoff and Wheeler, 2000). Besides acting as enzyme substrate and cofactor, AsA can directly quench $^1O_2$, $O_2^{\bullet-}$ and •OH and regenerate α-tocopherol, providing itself protection to the cell wall membranes (Thomas et al., 1992; Wang et al., 2012). Then, it is expected that the concentration of AsA reach enough levels to help the offset of the oxidative stress and regulate other plant metabolic processes (Smirnoff, 2000; Athar et al., 2008). Data from the present study, however, indicated that despite a tendency of increase in AsA concentration at 20 dai, there was a slight, but

significant decline at advanced stages of fungal infection, which may be faced as a transitory response, possibly related to its increased exploitation as substrate in antioxidant reactions, like those involving APX activity (Behnamnia et al., 2009). The concentration of the total glutathione pool, in its turn, remained unchanged as the disease progressed. In any case, enhanced activity of enzymes with antagonistic actions over the GSH pool may account for a reduction in its reduced form, as observed for enhanced GPX activity that comprises a family of isoenzymes that use GSH to reduce H_2O_2 and lipid hydroperoxides (Quan et al., 2008; Foyer and Noctor, 2011).

Despite no differences were noticed between non-inoculated and inoculated plants from cv. Ubá for the activities of most enzymes and metabolites involved in the oxidative stress, slight differences were found for TP concentration at 30 dai. According to Petridis et al. (2012), phenolic compounds, besides acting as non-enzymatic antioxidants, exhibit high UV absorbance and may act as agents against the damaging effects of UV-B radiation that are usually coupled with alterations in plant water status. The higher concentration of TP is in accordance with higher PAL activity, the key enzyme involved in the phenylpropanoid pathway. The increase in PAL activity without the increase on PPO activity can be seen as a strategy launched by plants to favor the antioxidant activity of phenols since PPO is involved in the oxidation of phenolic compounds (Ben Ahmed et al, 2010; Beritognolo et al, 2011).

Although the intrinsic symptoms of vascular diseases involve a complex network of metabolic interactions, probably with responses coming from different tissues and metabolic pathways, rather than an increase in the activity of enzymes and metabolites associated with the antioxidant system, the host resistance to C. fimbriata seems to be related to a higher capacity of the attacked plants to cope with the pathogens directly at the infection sites, providing substrates and stimulating defense mechanisms. The meager and, perhaps, initial responses of the antioxidant system seems in close agreement with the moderate resistance presented by the cv. Tommy Atkins. This alterations probably reflected in an increase in their antioxidant capacity due to a lower utilization of reducing power in photosynthesis, for instance, situation probably raised by the reduced ability in evolve localized strategies to halt pathogen growth. So, the less expressive tissue colonization on plants from cv. Ubá by C. fimbriata, appears therefore crucial for the successful resistance against the pathogen. The delay in the onset of the oxidative stress on ‘Ubá’ plants also probably kept the metabolic regularity, ensuring the production of substrates for the synthesis of defense-related compounds, directed to the colonized cells.

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LITERATURE CITED

1. Almeida, C.O., Souza, J.S., Mendes, L.N., and Pereira, R.J. 2001.Tendências do mercado internacional de manga. Rev. Econ. Nor. 32: 112-120.

2. Al-Sadi, A.M., Al-Ouweisi, F.A., Al-Shariani, N.K., Al-Adawi, A.O., Kaplan, E.J., and Deadman, M.L. 2010. Histological changes in mango seedlings following infection with Ceratocystis manginecans, the cause of mango decline. J. Phytopathol. 158: 738-743.

3. Anderson, J.V., and Davis, D.G. 2004. Abiotic stress alters transcript profiles and activity of glutathione-S-transferase, glutathione peroxidase, and glutathione reductase in Euphorbia esula. Physiol. Plantarum 120: 421-433.

4. Anderson, M.E. 1985. Determination of glutathione and glutathione disulfide in biological samples. Meth. Enzymol. 113: 548-55.

5. Asada, K. 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant. Mol. Biol. 50: 601-639.

6. Athar, H.R., Khan, A., and Ashraf, M. 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot. 63: 224-231.

7. Bally, I.S.E. 2011. Advances in research and development of mango industry. Rev. Bras. Frut. Volume especial: 57-63.

8. Batista, D.C., Terao, D., Barbosa, M.A.G., and Barbosa, F.R. 2008. Seca-da-mangueira: detecção, sintomatologia e controle. Petrolina, BR: Embrapa Semi-Árido: Comunicado Técnico no. 138.

9. Beauchamp, C., and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.

10. Behnamnia, M., Kalantari, K.H.M., and Rezanejad, F. 2009. Exogenous application of brassinosteroid alleviates drought-induced oxidative stress in Lycopersicon esculentum L. Gen. Appl. Plant Physiol 35: 22-34.

11. Ben Ahmed, C., Rouina, B.B., Sensoy, S., Boukhriss, M., and Ben Abdullah, F. 2009. Saline water irrigation effects on antioxidant defense system and proline accumulation in leaves and roots of field-grown olive. J. Agr. Food Chem. 57: 11484-11490.

12. Benevides, S.D., Ramos, A.M., Stringheta, P.C., and Castro, V.C. 2008. Qualidade da polpa da manga e polpa da manga Ubá. Ciênc. Tecnol. Aliment. 28: 571-578.

13. Beritognolo, I., Harfouche, A., Brilli, F., Prosperini, G., Gaudet, M., Brosché, M., Salani, F., Kuzminsky, E., Auvinen, P., Paulin, L., Kangasjärvi, J., Loreto, F., Valentini, R., Mugnozza, G.S., and Sabatti, M. 2011. Comparative study of transcriptional and physiological responses to salinity stress in two contrasting Populus alba L. genotypes. Tree Physiol. 31: 1335-55.

14. Boominathan, R., and Doran, P.M. 2002. Ni-induced oxidative stress in roots of the Ni hyper-accumulator, Alyssum bertolonii. New Phytol. 156: 205-215.

15. Bradford. M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72: 248-254.

16. Braz ,V.B., Nunes, E.S., Vieira, G., Ribeiro Júnior, J.I., Bertini, L.A., and Couto, F.A.D. 2008. Indução do amadurecimento de mangas cv. Tommy Atkins e cv. Ubá pela aplicação de ethephon pós-colheita. Bragantia 67: 225-232.

17. Cakmak, I., and Horst, W.J. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiol. Plantarum 83: 463-468.

18. Carlberg, I., and Mannervik, B. 1985. Glutathione reductase assay. Meth. Enzymol. 113: 484-495.

19. Carvalho, C.R.L., Rossetto, C.J, Mantovani, D.M.B, Morgano, M.A., De Castro, J.V., and Bortoletto N. 2004. Avaliação de cultivares de mangueira selecionadas pelo Instituto Agronômico de Campinas comparadas a outras de importância comercial. Rev. Bras. Frut. 26: 264-271.

20. Chance, B., and Maehly, A.C. 1955. Assay of catalase and peroxidases. Meth. Enzymol. 2: 764-775.

21. Chaves, M.M., Maroco, J.P., and Pereira, J.S. 2003. Understanding plant responses to drought - from genes to the whole plant. Funct. Plant Biol. 30: 239-264.

22. Chugh V, Kaur K, Gupta AK, 2011. Evaluation of oxidative stress tolerance in maize (Zea mays L.) seedlings in response to drought. Indian J. Biochem. Bio. 48: 47-53.

23. Cruz, F.J.R., Castro, G.L.S, Silva Júnior, D.D., Festucci-Buselli, R.A., and Pinheiro, H.Á. 2013. Exogenous glycine betaine modulates ascorbate peroxidase and catalase activities and prevent lipid peroxidation in mild water-stressed Carapa guianensis plants. Photosynthetica 51: 102-108.

24. Christmann, A., Grill, E., and Huang, J. 2013. Hydraulic signals in long-distance signaling. Curr. Opin. Plant Biol. 16:293-300.

25. del Río, L.A., Sandalio, L.M., Palma, J.M., Bueno, P., and Corpas. F.J. 1992. Metabolism of oxygen radicals in peroxisomes and cellular implications. Free Radical Bio. Med. 13: 557-580.

26. Debona, D., Rodrigues, F. Á., Rios, J. A., and Nascimento, K J T. 2012. Biochemical changes in the leaves of wheat plants infected by Pyricularia oryzae. Phytopathology 102: 1121–9.

27. Dhingra OD, Sinclair JB, eds, 1995. Basic Plant Pathology Methods. Boca Raton, FL, USA: Lewis Publisher.

28. Fradin, E.F., and Thomma, B.P.H.J. 2006. Physiology and molecular aspects of Verticillium wilt diseases caused by V. dahlia and V. albo-atrum. Mol. Plant Pathol 7: 71-86.

29. Foyer, C.H., and Halliwell, B. 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133: 21-25.

30. Foyer, C.H., and Noctor, G. 2011. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155: 2-18.

31. Foyer, C.H., and Shigeoka, S. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol.155: 93-100.

32. García-Limones, C., Hervás, A., Navas-Cortés, J.A., Jiménez-Diáz, R.F., and Tena, M. 2002. Induction of an antioxidant enzyme system and other oxidative markers associated with compatible and incompatible interactions between chickpea (Cicer arietinum L.) and Fusarium oxysporum f.sp. ciceris. Physiol. Mol. Plant Pathol. 61: 325-337.

33. Gay C, Gebicki JM, 2000. A critical evaluation of the effect of sorbitol on the ferric-xylenol orange hydroperoxide assay. Analytical Biochemistry 284, 217-220.

34. Gill, S.S., and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48: 909-930.

35. Habig, W.H., Pabst, M.J., and Jakoby, W.B. 1974. Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130-7139.

36. Halsted, B.D.,1890. Some fungal diseases of the sweet potato. New Jersey, USA: New Jersey Agricultural College Experiment Station: Bulletin 76, 25-27.

37. Harrington, T.C. 2000. Host specialization and speciation in the American wilt pathogen Ceratocystis fimbriata. Fitopatol. Bras. 25: 262-263.

38. Havir, E.A., and McHale, N.A. 1987. Biochemical and developmental that lack of expression of the T-CAT form in the mutant characterization of multiple forms of catalase in

tobacco is caused by the inability to upregulate expression of leaves. Plant Physiol. 84: 450-455.

39. Hossain, M.A., Ismail, M.R., Uddin ,K., Islam, M.Z., and Ashrafuzzaman, M. 2013. Efficacy of ascorbate-glutathione cycle for scavenging H_2O_2 in two contrasting rice genotypes during salinity stress. Aust. J. Crop Sci. 7: 1801-1808.

40. Kampfenkel, K., Van Montagu, M., and Inzé, D. 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Anal. Biochem. 225: 165-167.

41. Kar, M., and Mishra, D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physio. 57: 315-319.

42. Koussevitzky, S., Suzuki, N., Huntington, S., Armijo, L., Sha, W., Cortes, D., Shulaev, V., and Mittler, R. 2008. Ascorbate peroxidase 1 plays a key role in the response of Arabidopsis thaliana to stress combination. J. Biol. Chem. 283: 34197-34203.

43. Kuo, M.C., and Kao, C.H. 2003. Aluminum effects on lipid peroxidation and antioxidative enzyme activities in rice leaves. Biol. Plantarum 46: 149-152.

44. Lawlor, D.W. 2002. Limitations to photosynthesis in water-stressed leaves: stomata vc. Metabolism and the role of ATP. Ann. Bot. 89: 871-885.

45. Lawrence, RA, Burk, R.F. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Bio. Res. Com. 71: 952-958.

46. Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7: 405-410.

47. Nakano, Y., and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22: 867-880.

48. Nazarli, A., Faraji, F., and Zardashti, M.R. 2011. Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower. Agron. Res. Moldavia 44: 35-42.

49. Nogués, S., Cotxarrera, L., Alegre, L., and Trillas, M.I. 2002. Limitations to photosynthesis in tomato leaves induced by Fusarium wilt. New Phytol. 154: 461-470.

50. Noctor, G., and Foyer, C.H. 1998. Ascorbate and glutathione: keeping active oxygen under control. Ann. Rev. Plant Physiol. Plant Mol. Biol. 49:, 249-279.

51. Noctor ,G., Veljovic-Jovanovic. S., Driscoll, S., Novitskaya, L., and Foyer, C.H. 2002. Drought and oxidative load in wheat leaves: A predominant role for photorespiration? Ann. Bot. 89: 841-850.

52. Park, J.H, Juzwi, J., and Cavender-Bare, J. 2013. Multiple Ceratocystis smalleyi infections associated with reduced stem water transport in bitternut hickory. Phytopathology 103: 565-574.

53. Parke, J.L., Oh, E., Voelker, S., Hansen, E.M., Buckles, G., Lachenbruch, B. 2007. Phytophthora ramorum colonizes tanoak xylem and is associated with reduced stem water transport. Phytopathology 97: 1558-1567.

54. Pastori, G.M., and Trippi, V.S. 1992. Oxidative stress induces high rate of glutathione reductase synthesis in a drought-resistant maize strain. Plant Cell Physiol. 33: 957-961.

55. Petridis, A., Theriosa, I., Samouris, G., and Tananakic, C. 2012. Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (Olea europaea L.) and their relationship to antioxidant activity. Environ.Exp. Bot. 79: 37-43.

56. Pinto, A.C.Q., Andrade, S.R.M., Amaro, A.A., and Gomes, U. 2004. Mango industry in Brazil. Acta Hort. 645: 37-50.

57. Ploetz, R.C. 2003. Diseases of Mango. In: Ploetz, RC, ed. Diseases of Tropical Fruit Crops. Wallingford, UK: CABI Publishing, 327-363.

58. Pomar, F., Novo, M., Bernal, M.A., Merino, F., Ros Barceló, A. 2004. Changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during Verticillium wilt in Capsicum annuum. New Phytol. 163: 111-123.

59. Pyngrope, S., Bhoomika, K., and Dubey, R.S. 2013. Reactive oxygen species, ascorbate-glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (Oryza sativa L.) seedlings subjected to progressing levels of water deficit. Protoplasma 250: 585-600.

60. Quan, L.J., Zhang, B., Shi, W.W., Li, H.Y. 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J. Int. Plant Biol. 50: 2-18.

61. Rahman, M.A., Abdullah, H., Vanhaecke, M. 1999. Histopathology of susceptible and resistant Capsicum annuum cultivars infected with Ralstonia solanacearum. J. Phytopathol. 147: 129-140.

62. Resende, R.S., Rodrigues, F.A., Cavatte, P.C., Martins, S.C.V., Moreira, W.R., Chaves, A.R.M., and DaMatta, F.M. 2012. Leaf gas exchange and oxidative stress in sorghum plants supplied with silicon and infected by Colletotrichum sublineolum. Phytopathology 102: 892-898.

63. Ribeiro, I.J.A., Lourenção, A.L., Paradela Filho, O., and Soares, N.B. 1984. Seca da mangeira. VII. Resistência de cultivares de mangueira ao fungo Ceratocystis fimbriata Ell. & Halst. Bragantia 43: 237-243.

64. Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., and Blumwald, E. 2007. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci 104: 19631-19636.

65. Sharma, P. and Dubey, R.S. 2005. Lead toxicity in plants. Braz. J. Plant Physiol. 17: 35-52.

66. Sharma, P., Jha, A.B., Dubey, R.S., and Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot.2012: 1-26.

67. Smimoff, N. 2000. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 1125: 27-58.

68. Smirnoff, N., and Wheeler, G.L. 2000. Ascorbic acid in plants: biosynthesis and function. Crc. Cr. Rev. Plant Sci. 19: 67-290.

69. Stevenson, P.C., Turber, H.C., and Haware, M.P. 1997. Phytoalexin accumulation in the roots of chickpea (Cicer arietinum L.) seedlings associated with resistance to Fusarium wilt (Fusarium oxysporum f.sp. ciceris). Physiol. Mol. Plant Pathol. 39: 79-93.

70. Terzi, R., and Kadioglu, A. 2006. Drought stress tolerance and the antioxidant enzyme system in Ctenanthe setosa. Acta Biol. Cracov. Series Botanica 48: 89-96.

71. Thomas, C.E., McLean, L.R., Parker, R.A., and Ohlweiler, D.F. 1992. Ascorbate and phenolic antioxidant interactions in prevention of liposomal oxidation. Lipids 27: 543-550.

72. Upadhyaya, H., Khan, M.H., and Panda, S.K. 2007. Hydrogen peroxide induces oxidative stress in detached leaves of Oryza sativa L. Gen. App. Plant Physiol. 33: 83-95.

73. Upadhyay, H.P. 1993. Classification of the Ophiostomatoid fungi. In: Wingfield MJ, Seifert KA, Webber JA, eds. Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity. St. Paul, Minnesota: APS press, 7-14.

74. Vanacker, H., Foyer, C.H., and Carver, T.L.W. 1998. Changes in apoplastic antioxidants induced by powdery mildew attack in oat genotypes with race non-specific resistance. Planta 208: 444-452.

75. van Wyk, M., Pegg, G., Lawson, S., Wingfield, M.J. 2007. Ceratocystis atrox sp. nov. associated with Phoracanthta acanthocera infestations on Eucalyptus in Australia. Aust. J. Plant Pathol. 36: 407-414.

76. Wang, S., Liang, D., Li, C., Hao, Y., Maa, F., and Shu, H. 2012. Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. Plant Physiol. Biochem. 51: 81-89.

77. Yadeta, K.A., Thomma, B.P.H.J. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front. Plant Sci. 4: 1-12.

78. Zhang, W., Tian, Z., Pan, X., Zhao, X., and Wang, F. 2013. Oxidative stress and non-enzymatic antioxidants in leaves of three edible Canna cultivars under drought stress. Hort. Environ. Biotech. 54: 1-8.

79. Zieslin, N., and Ben-Zaken, R. 1993. Peroxidase activity and presence of phenolic substances in peduncles of rose flower. Plant Physiol. Biochem. 31: 333-339.

80. Zucker, M. 1965. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. 40: 779-784.

LIST OF TABLES AND FIGURES

Table 1. Activities of superoxide dismutases (SOD) (unit min^{-1} mg^{-1} protein), catalases (CAT) (mmol min^{-1} mg^{-1} protein), peroxidases (POX) (µmol min^{-1} mg^{-1} protein), ascorbate peroxidases (APX) (µmol min^{-1} mg^{-1}protein), glutathione reductases (GR) (µmol min^{-1} mg^{-1} protein), glutathione peroxidases (GPX) (µmol min^{-1} mg^{-1} protein) and glutathione-S-transferases (GST-like) (µmol min^{-1} mg^{-1} protein) on leaves of mango plants from cultivars Ubá and Tommy Atkins non-inoculated (NI) and inoculated (I) with Ceratocystis fimbriata.

Enzymes	Ubá				Tommy Atkins			
	20 daí		30 dai		20 daí		30 dai	
	NI	I	NI	I	NI	I	NI	I
SOD	22.0 ± 0.5	22.0 ± 0.2	20.1 ± 0.3	21.8 ± 0.2 a	21.2 ± 0.37	24.0 ± 0.7 c	20.7 ± 0.8	23.0 ± 0.5 ac
CAT	1.04 ± 0.2	1.01 ± 0.1	0.86 ± 0.1	0.90 ± 0.1	0.82 ± 0.2 b	1.25 ± 0.2	0.80 ± 0.1	1.04 ± 0.2
POX	54.5 ± 2.5	50.3 ± 4.40	33.4 ± 8.10	30.1 ± 4.20	68.5 ± 12.8 b	72.2 ± 9.3 c	54.1 ± 4.1	80.6 ± 7.68 ac
APX	19.4 ± 2.3	17.9 ± 1.9	16.9 ± 1.0	17.8 ± 2.0	22.5 ± 1.2	25.0 ± 0.8	20.3 ± 1.4	29.3 ± 1.4 ac
GR	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.04	0.17 ± 0.02	0.20 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.20 ± 0.01 a
GPX	0.18 ± 0.02	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.21 ± 0.02 a
GST	0.60 ± 0.1	0.60 ± 0.1	0.50 ± 0.16	0.41 ± 0.12	0.39 ± 0.13	0.39 ± 0.12	0.29 ± 0.10	0.50 ± 0.13

The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences for, respectively, NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). Values are means ± SE. n = 5.

Table 2. Concentrations of hydrogen peroxide (H_2O_2) (µmol m^{-2}), malondialdehyde (MDA) (µmol m^{-2}), ascorbate (ASC) (mmol m^{-2}) and glutathione (GLU) (mmol m^{-2}) on leaves of mango plants from cultivars Ubá and Tommy Atkins non-inoculated (NI) and inoculated (I) with Ceratocystis fimbriata.

Metabolites	Ubá				Tommy Atkins			
	20 dai		30 dai		20 daí		30 dai	
	NI	I	NI	I	NI	I	NI	I
H_2O_2	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01 b	0.2 ± 0.01 c	0.1 ± 0.01	0.2 ± 0.01 ac
MDA	22 ± 0.6	21 ± 0.8	19 ± 2.7	20 ± 1.3	18 ± 2.1	19 ± 1.6	21 ± 1.0	24 ± 0.5 c
ASC	8.6 ± 0.4	8.0 ± 0.1	8.3 ± 0.2	7.9 ± 0.2	8.0 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	7.9 ± 0.2 a
(GSH+GSSG)	3.0 ± 0.1	3.2 ± 0.02	3.6 ± 0.1	3.3 ± 0.2	3.3 ± 0.3	3.4 ± 0.2	3.5 ± 0.3 c	2.8 ± 0.5

The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences for, respectively, NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). Values are means ± SE. n = 5.

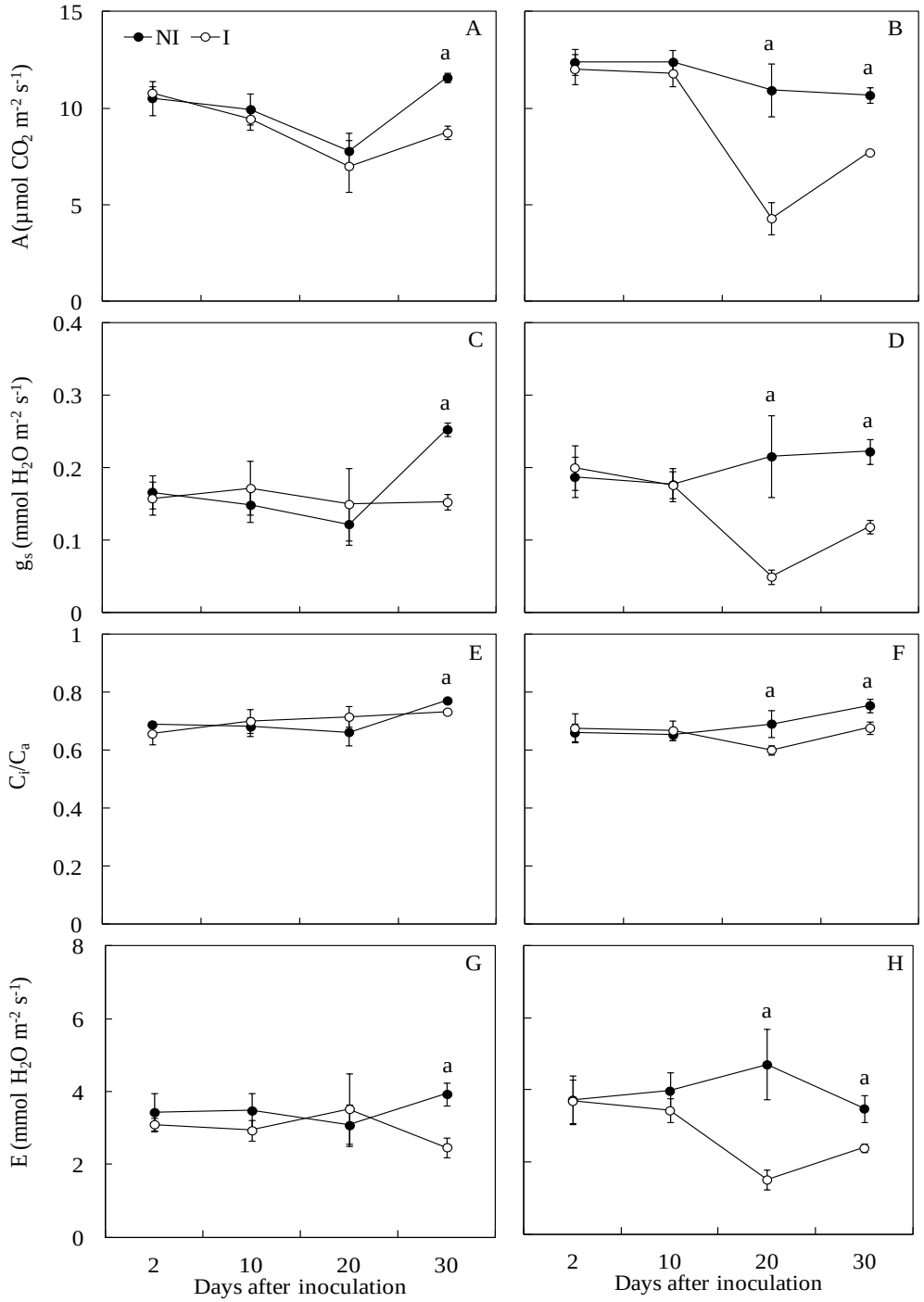

Figure 1. Net carbon assimilation rate (A) (A and B), stomatal conductance to water vapor (g_s) (C and D), internal to ambient CO_2 concentration ratio (C_i/C_a) (E and F) and transpiration rate (E) (G and H) determined in the leaves of mango plants from cultivars Ubá (left column) and Tommy Atkins (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 6.

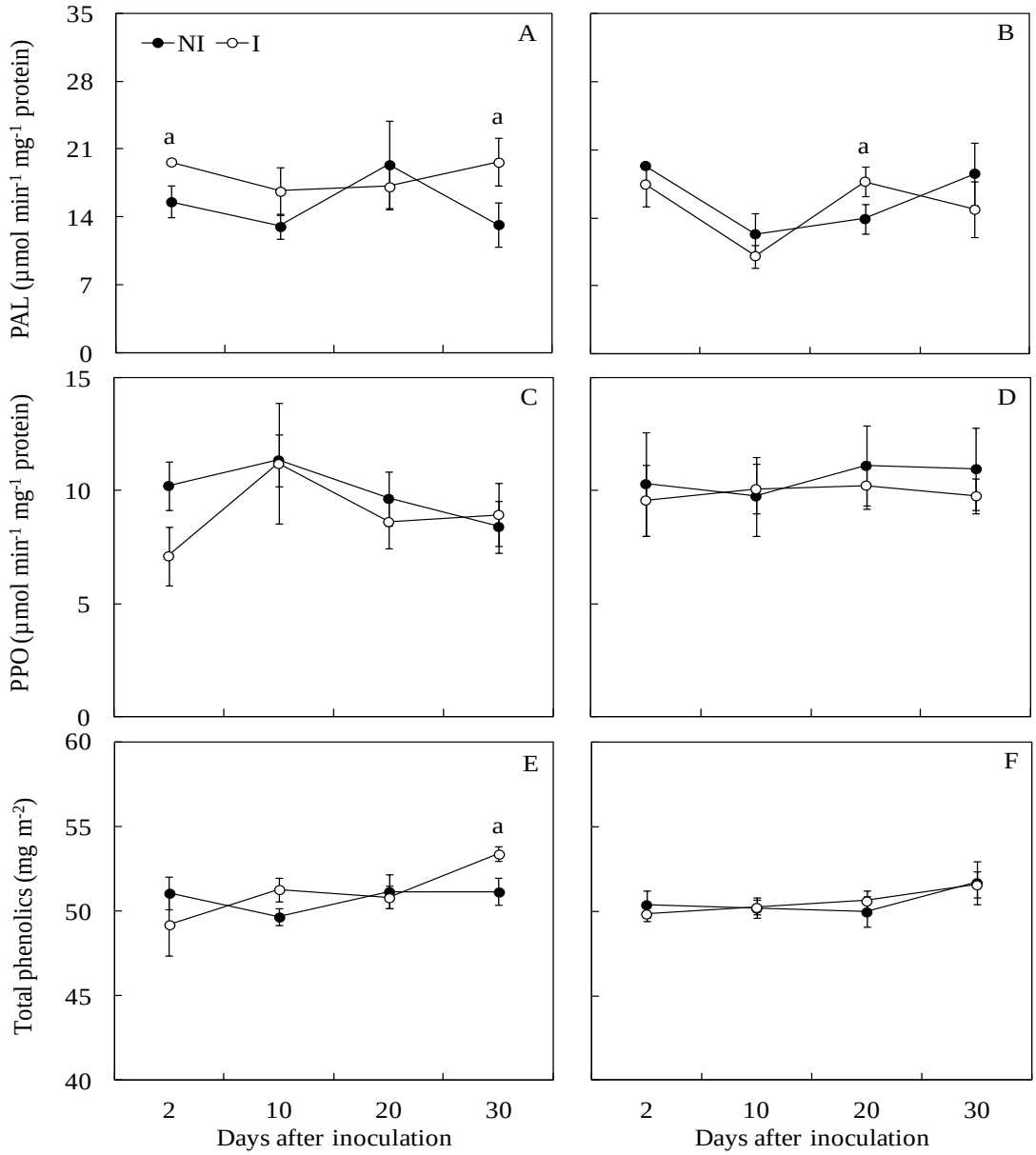

Figure 2. Activities of phenylalanine ammonia-lyases (PAL) (A and B) and polyphenoloxidases (PPO) (C and D) as well as concentration of total phenolics (E and F) on leaves of mango plants from cultivars Ubá (left column) and Tommy Atkins (right column) non-inoculated (NI) and inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences for, respectively, NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

CHAPTER 3

Photosynthetic performance and carbohydrate metabolism on leaves and stems of mango cultivars with different levels of basal resistance to Ceratocystis fimbriata infection

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ABSTRACT

Bispo, W. M. S., Araújo, L., Ávila, R. T., DaMatta, F. M., and Rodrigues, F. A. Photosynthetic performance and carbon metabolism on leaves and stems of mango cultivars with different levels of basal resistance to Ceratocystis fimbriata infection.

The mango wilt, caused by the vascular pathogen Ceratocystis fimbriata Ellis & Halsted is one of the most problematic diseases affecting mango (Mangifera indica L.) production worldwide. Since empirical observations have shown great variability among mango cultivars in terms of their basal level of resistance to the mango wilt, this study aimed to assess the changes in photosynthetic and carbon metabolism performance on leaves and stems of plants inoculated with C. fimbriata that may be related to the differential resistance responses. The relationships between disease severity, gas exchanges and chlorophyll a fluorescence parameters were evaluated over 21 days. Leaves and stem tissues were also collected to determine the activity of enzymes involved in the carbon metabolism and carbohydrate concentrations. The greater disease severity for plants from cv. Palmer contributed to the reduced photosynthetic performance and changes at photochemical level, in addition to decreased leaf concentrations of starch and increases in sucrose and hexoses content as disease progressed. Those alterations in carbohydrates concentrations were in accordance with the reduced activity of ADP-glucose pyrophosphorylase and increased

activity of invertases. By contrast, leaves from cv. Ubá showed no alterations in carbohydrate concentrations and enzyme activity. Nevertheless, throughout the experiment, reduced starch concentrations were found in stem tissues, suggesting an increased remobilization of reserves for the production of defense compounds at the site of infection. The greater ability of plants from cv. Ubá to act locally at the site of infection appears to be crucial for the greater resistance presented by this cultivar, favoring the postponement of the xylem colonization and the onset of the symptoms.

Additional keywords: gas exchange; chlorophyll a fluorescence; mango wilt; starch; soluble sugars

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INTRODUCTION

Mango (Mangifera indica L.) is an economically important fruit crop recognized as the most important in the tropical and subtropical areas worldwide (Masood and Saeed, 2012). The mango industry is one of the largest tropical fruit industries comprising approximately 100 producing countries, with a production of over 34.3 million tons (Bally, 2011). In recent years, nonetheless, both mango production and quality has suffered declines due to the occurrence of a variety of abiotic and biotic factors able to cause the sudden death of trees, affecting entire orchards (Masood et al., 2009; Masood et al., 2011). The mango production is hampered by many significant disease, among which the mango wilt is regarded as one of the most serious threats all over the mango growing regions (Ploetz, 2003; Masood and Saeed, 2012). The symptoms presented by the diseased trees include gum exudation from the trunks, wilting and loss of the dark green foliage that causes the browning of leaves on single branches (Ribeiro, 1997; Al-Subhi et al., 2006). Depending on tree size, hardiness and disease severity, complete tree death may occurs until about six months after the first appearance of the symptoms (Ribeiro, 1997; Al-Subhi et al., 2006; Masood and Saeed, 2012).

Ceratocystis species are often found in association with mango trees developing disease symptoms, as reported by Ribeiro (1980) and Al-Adawi et al. (2006) in Brazil and Oman, where C. fimbriata and C. omanensis were respectively isolated from diseased trees. Malik et al. (2010) also reported the confirmation of the capability of C. fimbriata to cause mango decline in Pakistan and, as highlighted by Masood and Saeed (2012), the mango tree decline is primarily caused by C. fimbriata infection (van Wyk et al., 2007). Ceratocystis fimbriata is a typical xylem pathogen (Harrington, 2002), based on the cross sections examinations of woody tissues, and the colonization of the stem tissues can be observed in the form of radially disposed necrotic lesions (Ferreira and Milani, 2002). By virtue of its infectious process, C. fimbriata may trigger the obstruction of the xylem vessels and provoke dysfunctions on plant sap flow (Clérivet et al., 2000; Al-Sadi et al., 2010; Park et al., 2013). Fungal material accumulation, mycelia growth, tissue necrosis and the establishment of structures associated with host responses (e.g., tyloses, gums and gels) can interfere with the upward movement of water, thereby leading to wilt symptoms in the foliage (Rajput and Rao 2007; Parke et al., 2007; Al-Sadi et al., 2010; Yadeta and Thomma, 2013).

Plants are able to cope with the infection process of pathogens by activating many strategies of defense (Bolton, 2009). A panoply of defense mechanisms are distinguished during a host-pathogen interaction including constitutive barriers (e.g. strong cell wall and thick waxy and bark layers) and the inducible defenses generally associated with physical changes (e.g., cell wall thickening and callose deposition) or the synthesis and accumulation of secondary metabolites such as phytoalexins and various phenolic compounds (van Loon et al. 2006, Guérard et al., 2007; Freeman and Beattie, 2008). Much of the ability of plants to contain the pathogens infection depends on how quick the synthesis of secondary metabolites are, which, at least in part, depend directly on their ability to mobilize carbon sources close to, or distal to the infection site, then being considered a costly process in terms of energy (Christiansen et al., 1987; Guérard et al., 2007).

Due to the alterations in water relations and leaf metabolism, photosynthesis is one of the key physiological processes affected by the vascular diseases, including the mango wilt, especially due to decreases on CO_2 diffusion from the atmosphere to the chloroplast (Pinheiro and Chaves, 2011). The increased demand for assimilates for mounting defenses very often leads to changes in carbon metabolism and alterations of the source-sink patterns (Berger et al., 2007). Some reports emphasize that the ability of trees to reduce disease severity may have some relation to the level of soluble carbohydrates around the infection sites and that the carbon used to mount the induced-reactions may originate from the hydrolysis of the stored starch (Guérard et al., 2007). When under severe pathogen attacks, however, the carbohydrates available around the site of infection may be rapidly depleted and the transport of soluble sugars from the crown may be as well required to support defenses (Christiansen et al., 1976). Alterations in carbon metabolism were found in leaves of pepper plants inoculated with Verticillium dahlie and the accumulation of soluble sugars was noted and suggested as a sensor of the deleterious effect caused by fungal infection (Goicoechea et al., 1999). Changes in the activities of enzymes involved in the carbon metabolism were also reported for tomato plants, in which the increase in the spatial and temporal distribution of the extracellular invertase was showed in resistant root tissues infected with Fusarium oxysporum f. sp. racidis-lycopersici (Benhamou et al., 1991).

There is great variability among mango cultivars in terms of their basal level of resistance to the mango wilt. Because plant defense responses may substantially alter the primary metabolism in order to adjust it to the cell demands, then, differences

between resistant and susceptible plants should exist regarding those alterations in the pool size of many metabolic intermediates. It was hypothesized that the more resistant cultivar has greater ability to provide carbon reserves for the production of defense-related compounds, thus favoring greater containment of C. fimbriata cells in the stem tissues and minor alterations to the mango physiology as a whole. The aim of this study was to assess some of the modifications on carbohydrate concentrations and the activities of some enzymes involved in carbon metabolism on leaves and stems of mango plants during the infection process of C. fimbriata.

MATERIALS AND METHODS

Plant material. Mango plants of approximately 1 year old from cultivars Ubá and Palmer, which are known to be resistant and susceptible, respectively, to C. fimbriata (Ribeiro et al., 1984; Carvalho et al., 2004), were obtained from a commercial orchard (Dona Euzébia, Minas Gerais State, Brazil). Both cultivars were grafted onto plants from cultivar Imbú, widely used as rootstock in the Zona da Mata region, Minas Gerais State, Brazil. The saplings were transplanted into plastic pots containing 8 kg of substrate consisting of a mixture of soil, sand and manure in a 2:1:1 proportion. The plants were kept in a greenhouse (temperature of 30 ± 2°C and relative humidity of 70 ± 5%) for two months before the beginning of the experiments. Plants were irrigated and fertilized as needed.

Inoculation procedure. The isolate CEBS15 of C. fimbriata was used to inoculate the plants. This isolate was obtained from symptomatic mango plants collected in the city of Brejo Santo, Ceará State (07° 29' 34" S, 38° 59' 06" W), Brazil. The isolate was preserved by Castellani's method (Dhingra and Sinclair 1995). Plugs of malt extract-agar medium containing fungal mycelia were transferred to Petri dishes containing potato-dextrose-agar (PDA). After three days, the PDA plugs containing fungal mycelia were transferred to new Petri dishes containing the same culture medium and were maintained in an incubator chamber (temperature of 25°C and 12-h photoperiod) for 14 days.

Plants were inoculated according to Al-Sadi et al. (2010) with a few modifications. Stem disks (10-mm in diameter and approximately 2-mm in width) were removed from the stems with the aid of a punch at approximately 5 cm above the graft scar. A PDA plug (10-mm in diameter) obtained from a 14-days-old fungal colony was carefully placed in the punch hole. Each hole containing a PDA plug with fungal mycelia was carefully covered with a piece of moistened cotton and then wrapped with parafilm to maintain adequate moisture for fungal infection. The disks used to inoculate each plant were taken from the middle portion of each fungal colony to make the inoculation as homogeneous as possible. Holes on the stems of plants receiving only PDA medium plugs served as the control treatment.

Relative lesion indices. Disease progress was evaluated at 7, 14 and 21 days after inoculation (dai). The upward, downward and radial colonization of the stem tissues by fungal hyphae was evaluated by measuring the length (in cm) of the internal necrotic tissue using a digital caliper. The upward relative lesion length (URLL) and the downward relative lesion length (DRLL) were determined as the ratio between the length from the graft scar to the top of the stem (LGST) and the lesion length (LL) in the same interval (upward and downward) from the inoculation point according to the following formula: URLL or DRLL = LL × 100/LGST. The plants were standardized to a length of 20 cm (the distance from the graft scar to the top of the stem). The radial fungal colonization (RFC) was determined as the length of the necrotic tissue in relation to the total stem diameter × 100.

Leaf gas exchanges and chlorophyll a fluorescence measurements. The leaf gas exchange parameters were simultaneously determined with the measurements of Chl a fluorescence by using a portable open-flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, USA) equipped with an integrated fluorescence chamber head (LI-6400-40, LI154 COR Inc.). The net carbon assimilation rate (A), stomatal conductance to water vapor (g_s), internal-to-ambient CO_2 concentration ratio (C_i/C_a) and transpiration rate (E) were measured in fully expanded leaves of six plants from each treatment at 7, 14 and 21 dai. The measurements were conducted at ambient temperature and CO_2 conditions under artificial light (1000 µmol photons m^{-2} s^{-1}) from approximately 0800 to 1200 h. Dark-adapted leaf tissues were illuminated with a weak modulated measuring beam (0.03 µmol m^{-2} s^{-1}) to obtain the initial fluorescence (F_0). Saturating white light pulses of 8000 µmol photons m^{-2} s^{-1} were applied for 0.8 s to ensure maximum fluorescence emission (F_m). From these initial measurements, the maximum quantum efficiency of PSII photochemistry for dark-adapted leaves was calculated as follows: (F_v/F_m) $[(F_m - F_0)/F_m)]$. The steady-state fluorescence yield (F_s) and the light-adapted maximum fluorescence (F_m') were measured after 0.8 s of saturating white light pulse (8,000 µmol m^{-2} s^{-1}). The actinic light was then turned off and the far-red illumination was applied (2 µmol m^{-2} s^{-1}) to measure the light-adapted initial fluorescence (F_0'). From these parameters, the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m') was calculated $[(F_m' - F_0'/F_m')]$. The non-photochemical quenching coefficient (NPQ) was calculated as $[(F_m/F_m') - 1)]$ (Bilger and Bjorkman, 1990). The actual quantum yield of PSII electron transport (Φ_{PSII}) was computed as

[(F_m' - F_s)/F_m'], from which the electron transport rate (ETR) was calculated as (Φ_{PSII}*PPFD*f*α), where f is a factor that accounts for the partitioning of energy between PSII and PSI and is assumed to be 0.5, which indicates that the excitation energy is equally distributed between the two photosystems; α is the leaf absorbance by the photosynthetic tissues and is assumed to be 0.84 (Maxwell and Johnson, 2000).

Biochemical assays. For each replication, three fully expanded leaves exposed to sunlight (including those used for gas exchanges and Chl a fluorescence measurements) were sampled from the same portion of the plant and immediately frozen in liquid nitrogen. The midrib region and the leaf ends were discarded. A composite sample was obtained and then stored at -80°C until used for the biochemical assays. For the determination of carbohydrates on the stem tissues, stem segments adjacent to the inoculation point (5 cm above and below the inoculation point) were sampled from each replication (a total of five plants) of each treatment after disease evaluations. For all enzymes, the linearity of the enzyme activities over time and the proportionality between the rate and amount of extracts were tested.

Extraction and determination of carbohydrates. Leaf and stem tissues were lyophilized (-48°C) and ground in a cell disruptor with the aid of metal balls of 3.2 mm diameter (Mini-Bead beater-96, Bio Spec Products, Bartlesville, OK, USA). About 15 mg of each lyophilized material were homogenized using 80% (v/v) aqueous ethanol. The powdered material was shaken and incubated at 80ºC for 30 min and then centrifuged at 15,000 g for 10 min. The pellets were extracted further two times with 80% ethanol. The supernatants obtained from the three extractions of each sample were combined and stored at -20ºC for soluble sugar determinations. Glucose, fructose and sucrose were measured through the reduction of NAD^+ by glucose-6-P dehydrogenase after the sequential addition of hexokinase, phosphoglucose isomerase and invertase (Trethewey et al., 1998). Reduction of NAD^+ was continuously followed at 340 nm using an ELISA reader (Tunable Microplate Reader, VERSAmax, Sunnyvale, USA). The ethanol-insoluble pellet was used for starch determination. The pellet was suspended in NaOH 0.1 M, incubated at 95^{o}C for 40 min, neutralized with 1 M acetic acid and centrifuged at 15,000 g for 10 min. Starch was then hydrolyzed in 100 mM citrate buffer (pH 4.6) containing amyloglucosidase (Trethewey et al., 1998) and the released glucose was measured as described above.

Enzyme extraction and assays. Frozen leaf tissues (± 50 mg) were ground in a cold mortar and pestle with 2 mL of HEPES-KOH extraction buffer at pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 2 mM benzamidine, 2 mM ε-amino-n-caproic acid, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), 0.1% (w/v) bovine serum albumin (BSA), 10% (v/v) glycerol and 0.1% (v/v) Triton X-100. Following centrifugation at 15,000 g for 15 min (4ºC) (Geigenberger & Stitt, 1993). The supernatant were then stored at -80ºC until required for the enzyme assays.

Sucrose-phosphate synthase (SPS) (EC 2.4.1.14) was assayed according to Doehlert and Huber (1983) under saturating (non-selective assay; V_{max}) and limiting substrate conditions (selective assay; V_{sel}). The V_{max} assay consisted of 40 µL of the crude extract, 50 mM HEPES-KOH at pH 7.4, 12 mM $MgCl_2$, 1 mM DTT, 12 mM UDP-glucose, 36 mM glucose-6-P and 12 mM fructose- 6-P. The V_{sel} assay followed the same procedure described above, except that it contained 6 mM glucose-6-P and 2 mM fructose-6-P and 5 mM Pi. Assays were incubated for 15 min at 27°C and then the reaction was stopped and neutralized rapidly with 70 µL 30% KOH and boiled for 10 min. Subsequently, 1 mL of anthrone in concentrated sulfuric acid (H_2SO_4) (0.14% w/v anthrone in H_2SO_4 28 N) was added and the reaction mixture was incubated for 20 min at 40ºC. Sucrose was determined at 620 nm in a spectrophotometer (Thermo Scientific Multiskan GO UV/Vis). The activation state of SPS was calculated as the ratio between V_{max} and V_{sel} (%).

ADP-glucose pyrophosphorylase (AGPase) (EC 2.2.7.27) was assayed in a reaction containing 30 µL of the extract, 80 mM HEPES-KOH at pH 7.9, 2 mM magnesium chloride ($MgCl_2$), 10 µM glucose-1,6-BP, 10 mM phosphoglyceric acid, 5 mM DTT, 0.6 mM NAD^+, 1 mM ADP-glucose, 10 mM sodium fluoride (NaF), 1 unit mL^{-1} phosphoglucomutase, 2.5 unit mL^{-1} glucose-6-P dehydrogenase and 1.5 mM Na-PPi to start the reaction (Müller-Röber et al., 1992).

The activity of invertases were measured in a medium containing the respective enzyme extract, 20 mM sucrose and 100 mM sodium acetate buffer at pH 4.7 for acid invertases (acid INV) (EC 3.2.1.26) and 100 mm HEPES-NaOH (pH 8.0) for alkaline invertases (alkaline INV) (EC 3.2.1.153) (Zrenner et al., 1996; Essmann et al., 2008). The amount of hexoses that was released was measured according to the method of Trethewey et al. (1998).

Sucrose synthase (SuSy) (EC 2.4.1.13) activity was assayed as described by Geigenberger and Stitt (1993). A reaction mixture contained 40 µL of the extract, 20 mM HEPES-KOH at pH 7.0, 100 mM sucrose and 4 mM UDP. The mixture was incubated for 30 min in a mixer (300 rpm) with control temperature set to 25°C and then boiled for 5 min to stop the reaction. Following centrifugation for 1 min at 15,000 g, the concentration of UDP-glucose formed was assessed enzymatically by adding 40 µL of the reaction mixture into a second medium containing 200 mM glycine at pH 8.9, 5 mM $MgCl_2$, 2 mM NAD^+ and 0.02 unit mL^{-1} UDP-glucose dehydrogenase. The reduction of NAD^+ was continuously followed at 340 nm using an ELISA reader (Tunable Microplate Reader, VERSAmax, Sunnyvale, USA).

The starch phosphorilase activity (SPase) (EC 2.4.1.1) was determined according to Harada and Ishizawa (2003). A reaction mixture contained 40 µL of the extract, 75 mM MES-NaOH at pH 6.0, 0.25% soluble starch, 5 mM NaF and 75 mM Na_2HPO_4. The mixture was incubated for 90 min in a mixer (300 rpm) with control temperature set to 37°C and then boiled for 5 min to stop the reaction. Then, 115 µL of the reaction mixture were added into a second medium containing 50 mM HEPES-KOH at pH 7.4, 5 mM $MgCl_2$, 0.5 mM NAD^+, 1 mM EDTA, 25 µM glucose-1,6-bisphosphate and 2 unit mL^{-1} phosphoglucomutase. The reduction of NAD^+ was continuously followed at 340 nm using an ELISA reader (Tunable Microplate Reader, VERSAmax, Sunnyvale, USA).

Experimental design and statistics. A 2 × 2 factorial experiment consisting of two mango cultivars (Ubá and Palmer) and non-inoculated or inoculated plants with C. fimbriata were arranged in a completely randomized design with five replications. Each experimental unit consisted of one plastic pot with one mango plant. The experiment was repeated once. Data were analyzed by an analysis of variance (ANOVA) and within each sampling time treatment means were compared by Student's t test ($P \leq 0.05$) using SAS (Release 8.02 Level 02M0 for Windows, SAS Institute, Inc., 1989, Cary, NC, USA).

RESULTS

Disease assessments. The URLL, DRLL and RFC increased overtime on plants from both Ubá and Palmer cultivars, but significant differences between cultivars occurred only for RFC (Fig. 1A-C). For plants from cv. Palmer, RFC was 32 and 24% greater in comparison to plants from cv. Ubá, respectively, at 14 and 21 dai (Fig. 1C).

Gas exchanges and chlorophyll a fluorescence. Inoculated plants from cv. Palmer presented average decreases of 46% for A, 54% for g_s, 50% for E (considering the data from 7, 14 and 21 dai) and a 22% decrease for C_i/C_a at 7 dai in comparison to the non-inoculated plants (Fig. 2B, D, F and H). There were no significant differences between non-inoculated and inoculated plants from cv. Ubá regarding the gas exchange parameters (Fig. 2A, C, E and G). At 7 dai, inoculated plants from cv. Ubá displayed higher values of about 32% for A, 56% for g_s, 48% for E and 19% for C_i/C_a in comparison to inoculated plants from cv. Palmer. At 21 dai, increases of 67% for A, 56% for g_s and 64% for E occurred for inoculated plants from cv. Ubá in comparison to its counterparts from cv. Palmer (Fig. 2). Alterations between non-inoculated and inoculated plants for Chl a fluorescence parameters were observed only in plants from cv. Palmer at 21 dai (Table 1). Inoculated plants from cv. Palmer displayed decreases of 14% for F_v'/F_m' and of 28% for ETR and an increase of 25% for NPQ when compared to the non-inoculated plants. Inoculated plants from cv. Palmer showed higher NPQ values (30%) than those from cv. Ubá at 21 dai. The F_v/F_m ratio did not respond to the treatments in either cultivar.

Leaf starch concentration. The starch concentration on leaves of inoculated plants from cv. Palmer was about 46% lower than the concentration found for non-inoculated plants at 21 dai (Fig. 3B). Non-inoculated plants from cv. Palmer displayed higher starch concentration than that of non-inoculated plants from cv. Ubá at 7 (47%), 14 (41%) and 21 (77%) dai (Fig. 3A and B).

Stem starch concentration. The stem tissues of inoculated plants from cv. Ubá presented lower starch concentration than that from non-inoculated plants at 7 (70%), 14 (66%) and 21 (80%) dai (Fig. 3C). The stem tissues of inoculated plants from cv. Palmer presented lower starch concentration than that from non-inoculated plants at 14

dai (around 80%) (Fig. 3D). There were no significant differences between cultivars for starch concentration, regardless of the inoculation treatment (Fig. 3C and D).

Leaf glucose, fructose and sucrose concentrations. Higher leaf concentrations of fructose occurred at 7 (56%) and 21 (45%) dai on inoculated plants from cv. Palmer in comparison to the non-inoculated plants (Fig. 4D). For inoculated plants from cv. Palmer at 7 and 14 dai, glucose and fructose concentrations were, on average, 51 and 54% higher, respectively, in comparison to the inoculated plants from cv. Ubá (Fig. 4A-D). There were no significant differences between cultivars for the sucrose concentration regardless of the inoculation treatment (Fig. 4E and F).

Stem glucose, fructose and sucrose concentrations. Higher concentration of glucose (36%) occurred on the stem tissues of inoculated plants from cv. Ubá at 7 dai and higher concentrations of fructose at 7 (59%) and 21(67%) dai, in comparison to the non-inoculated plants (Fig. 5A and C). For both cultivars, the sucrose concentrations were greater at 7 dai (37 and 18% for cvs. Ubá and Palmer, respectively) and 14 dai (23 and 20% for cvs. Ubá and Palmer, respectively) on inoculated plants in comparison to the non-inoculated ones (Fig. 5E and F).

Enzyme activity. There were no significant differences between non-inoculated and inoculated plants from cv. Ubá for all the enzymes that were assessed (Fig. 6A, C, E, G, I and K). in sharp contrast, inoculated plants from cv. Palmer displayed reduced AGPase activity (25%) at 21 dai in comparison to the non-inoculated plants (Fig. 6B). Both acid INV and alkaline INV had higher activities (22 and 27%, respectively) for inoculated plants from cv. Palmer at 21 dai in comparison to the non-inoculated ones (Fig. 6J and L). The alkaline INV activity also increased by 37% on the inoculated plants at 14 dai. There were no significant differences between non-inoculated and inoculated plants for SPase, SuSy (Fig. 6D and H) and SPS (V_{sel} and V_{max}) (data not shown), except for a slight decrease (approx.. 10%) in the activation state of SPS for inoculated plants from cv. Palmer at 21 dai (Fig. 6F). Both non-inoculated and inoculated plants from cv. Palmer displayed higher SPase activity (61 and 53%, respectively) than its counterparts from cv. Ubá at 21 dai (Fig. 6C and D). At 21 dai, non-inoculated and inoculated plants from cv. Ubá presented higher SuSy activity (62 and 55%, respectively) than those from cv. Palmer (Fig. 6G and H). Inoculated plants

from cv. Palmer had approximately 50% higher acid INV activity than the inoculated plants from cv. Ubá at 21 dai (Fig. 6I and J). Non-inoculated plants from cv. Palmer displayed, on average, higher acid INV activity (40%) than the inoculated plants from cv. Ubá at 14 and 21 dai (Fig. 6I and J). The alkaline INV activity was higher in non-inoculated (40%) and inoculated plants (42%) from cv. Ubá than in its counterparts from cv. Palmer at 7 dai (Fig. 6K and L).

DISCUSSION

To the best of our knowledge, the results of this study provide new information on the role of carbohydrate metabolism during the infection process of Ceratocystis fimbriata and insert some first insights into the resistance mechanisms involved in the differential behavior of mango cultivars against fungal attack. Plants from cv. Palmer were more susceptible to the mango wilt based on the disease indices, which indicated greater proportion of necrotic lesions in the stem tissues of plants from this cultivar. As evaluated in a myriad of interactions such as banana-Fusarium oxysporum f. sp. cubense (Dong et al., 2012), tomato-Fusarium oxysporum f. sp. lycopersici (Nogués et al., 2002), potato-Verticillium dahliae (Goicoechea et al., 2000), Bitternut Hickory-Ceratocystis smalleyi (Park et al., 2013) and also mango-Ceratocystis fimbriata (WMS Bispo and FA Rodrigues, unpublished), the greater xylem colonization and the major extent of damage to the vascular tissues appears to increase the resistance of xylem sap flow and may result in alterations in leaf water status, ultimately leading to reductions in photosynthetic performance, transpiration rates and leaf longevity and integrity (Nogués et al., 2002). Indeed, decreases in A, g_s and E were found on plants from the most susceptible cv. Palmer. Although initially stomatal constraints may have influenced CO_2 uptake and carbon availability, the reductions in g_s without equivalent reductions in C_i/C_a ratio during the time course of the experiment suggest possible limitations for A on mesophilic or biochemical level. Adjustments at the photochemical level, as suggested by the reductions in ETR and F_v'/F_m' and NPQ increase were also observed in cv. Palmer, avoiding, therefore, photodamages. In fact, the maximum quantum yield of PSII photochemistry, as evaluated by the F_v/F_m ratio, did not decrease during the stress imposition, suggesting that photoinhibition was not evident in this current study. The unchanged photosynthetic performance observed in cv. Ubá (despite a minor, although significant, increase in stem tissue colonization in comparison to cv. Palmer) suggests that the damages on the xylem vessels were less pronounced and the ascent of the xylem sap was not drastically constrained as could be expected to occur in the more sensitive cv. Palmer.

The production and consumption of photoassimilates are greatly affected by the alterations in plant water status that inevitably change carbon partitioning at the leaf and whole plant levels (Chaves, 1991). Accordingly, inoculated plants from cv. Palmer presented reductions in leaf starch concentration as disease progressed. As reported by

others authors, starch hydrolysis could partially buffer fluctuations in sugar concentrations when photosynthesis is limiting and favor the accumulation of soluble sugars in leaves (Chaves, 1991; Geigenberger et al., 1997). In fact, increases in glucose and fructose concentrations were observed on leaves of inoculated plants from cv. Palmer. The higher concentration of soluble sugars may function as osmolytes to maintain cell turgor and the protection of membranes and proteins from stress damage (Kaplan and Guy, 2004; Krasensky and Jonak, 2012). The decrease in AGPase activity, the primary regulatory enzyme for the starch synthesis, was observed in consonance with the decreases in leaf starch concentration, despite the maintenance of the SPase activity. This response seems to be common in a range of plants under the attack of vascular pathogens (Goicoechea et al., 2000; Dinis et al., 2011), when marked declines in leaf relative water content in pepper plants infected with Verticillium dahliae and chestnut plantlets inoculated with Phytophthora cinnamomi also coincided with a drastic mobilization of starch and a higher soluble sugars concentration. The accumulation of soluble sugars observed in inoculated Palmer plants may also have resulted from the increased activities of acid and alkaline INV over its substrate sucrose. As highlighted by Praxedes et al. (2006), it is unlikely that SuSy has played a chief role in sucrose degradation since its activity was maintained constant as the disease progressed. Despite the higher activity of both acid and alkaline INV, the leaf sucrose concentration remained unchanged in leaves from inoculated plants from cv. Palmer. This behavior suggests that sucrose synthesis should have been maintained in some extent, since only a slight reduction in the activation state of the SPS was found. Also, due to the apparent changes in the plant water status, a reduced phloem loading and sucrose exportation may have occurred (Dinis et al, 2011; Woodruff, 2013). Phloem sap viscosity influences phloem sieve cell conductivity, being largely determined by the relative concentrations of water within the phloem sap (Woodruff, 2013).

Depending on when the plant is infected by the pathogen and on the level of disease severity, some authors suggest that the amount of reserves available around the infection sites may become critical and, at this point, plants may rely more on current assimilates from the foliage (Christiansen et al., 1986; Guérard et al., 2007). Nevertheless, based on the possible impairments in exportation observed for plants from cv. Palmer, the ability to obtain carbon sources from the leaves seems, however, to be modest in the most susceptible plants, which may also favor disease development and bring additional difficulties at later stages of disease development. In any case,

considering that no alterations were found both for carbohydrates concentration and enzyme activities in leaves from cv. Ubá, the direct assistance from distant tissues seems to have a late role, and the success in disease resistance seems to be initially attributable to the mobilization of carbon from the tissues adjacents to the infection point. Indeed, the cv. Ubá, which showed to be more resistant to C. fimbriata infection, presented lower starch concentration in the stem tissues at all evaluation times, suggesting an increased mobilization. This statement is in agreement with Guérard et al. (2007), who concluded that starch is readily mobilized for the construction of defense reactions and the contribution of the currently assimilated carbon play a minor role in the process. In consonance with the greater mobilization of starch, higher concentrations of glucose, fructose and sucrose were found in the stem tissues of 'Ubá' plants. As discussed by Rosa et al. (2009), sucrose and glucose, in addition to acting as osmolytes to maintain cell homeostasis, are also substrates for cellular respiration and carbon skeletons that may be used in the biosynthesis of various metabolites (Bolton et al., 2009). Fructose, however, seems to be related to secondary metabolites biosynthesis, being involved into lignin and phenolic compounds synthesis (Dinis et al., 2007; Rosa et al., 2009). Yadeta and Thomma (2013) raised the question that plants accumulate different phenolic compounds in the xylem in response to infection of vascular pathogens and cites the example of olive trees, which accumulate phenolic compounds such as rutin and oleuropein on the infection sites of Verticillium dahliae, having toxic effects against this pathogen (Báidez et al., 2007). Accordingly, recent results from our research group highlight the importance of phenolic compounds on pathogen containment and show that the accumulation at the infection site has deleterious effects against Ceratocystis fimbriata infecting mango plants (L Araújo and FA Rodrigues, unpublished)

Although being impractical, based on data from the present study, to predict the fate and the contribution of the mobilized carbon to the diverse range of compounds that may be involved in curbing fungal growth in the stem tissues, it is tempting to suggest that the resistance responses against C. fimbriata infection were directly associated with an increased mobilization of starch from the areas around the infection sites and, at least in the early stages of fungal infection, the support of carbon sources from leaves seems minimal. Notably, plants from cv. Ubá, known to be resistant to the mango wilt, probably had greater success in mobilizing reserves and committing them to the production of defense compounds. Therefore, less stem tissues colonized by C.

fimbriata avoided drastic changes in plant water status and probably allowed the regular plant functioning for a longer time. In sharp contrast, failures or delays in mobilizing and targeting carbon for the production of defense-related compounds may have accounted for the lower resistance level to C. fimbriata presented by cv. Palmer.

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LITERATURE CITED

1. Adams, W. W. I., Zarter, C. R., Mueh, K. E., Amiard, V. S. E., and Demmig-Adams, B. 2005. Energy dissipation and photoinhibition: a continuum of photoprotection. In: Demmig-Adams, B., Adams, W. W., and Mattoo, A. K. eds. Photoprotection, Photoinhibition, Gene Regulation and Environment. Springer-Verlag, Berlin, 49-64.

2. Al-Adawi, A. O., Deadman, M. L., Al-Rawahi, A. K., Al-Maqbali, Y. M., Al-Jahwari A. A., Al-Saadi, B. A., Al-Amri I. S., and Wingfield, M. J. 2006. Aetiology and causal agents of mango sudden decline disease in the Sultanate of Oman. Eur. J. Plant Pathol. 116: 247–254.

3. Al-Sadi, A. M., Al-Ouweisi, F. A., Al-Shariani, N. K., Al-Adawi, A. O., Kaplan, E. J., and Deadman, M. L. 2010. Histological changes in mango seedlings following infection with Ceratocystis manginecans, the cause of mango decline. J. Phytopathol. 158: 738-743.

4. Al-Subhi, A. M., Al-Adawi, A. O., van Wyk M., Deadman, M. L., Wingfield, M. J. 2006. Ceratocystis omanensis, a new species from diseased mango trees in Oman. Mycol. Res. 110: 237-245.

5. Báidez, A. G., Gómez, P., Del Río, J. A., and Ortuño, A. 2007. Dysfunctionality of the xylem in Olea europaea L. plants associated with the infection process by Verticillium dahlia Kleb.: role of phenolic compounds in plant defense mechanism. J. Agric. Food Chem. 55: 3373–3377.

6. Baker, N. R. 2008. Chlorophyll Fluorescence : A Probe of Photosynthesis In Vivo. Annu. Rev. Plant Biol. 59:89-113.

7. Bally, I.S.E. 2011. Advances in research and development of mango industry. Rev. Bras. Frut. Volume especial: 57-63.

8. Benhamou, N., Grenier, J., and Chrispeels, M. J. 1991. Accumulation of β-fructosidase in the cell walls of tomato roots following infection by a fungal wilt pathogen. Plant Physiol. 97: 739-750.

9. Berger, S., Sinha, A. K., and Roitsch, T. 2007. Plant physiology meets phytopathology : plant primary metabolism and plant – pathogen interactions. J. Exp. Bot. 58: 4019–4026.

10. Bilger, W., and Bjorkman, O. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosyn. Res. 25:173-186.

11. Bolton, M. D. 2009. Primary metabolism and plant defense - fuel for the fire. Mol. Plant Microbe. In. 22: 487–97.

12. Carvalho, C. R. L., Rosseto, C. J., Mantovani, D. M. B., Morgano, M. A., Castro, J. V., and Bortoletto, N. 2004. Avaliação de cultivares de mangueira selecionadas pelo instituto agronômico de campinas comparadas a outras de importância comercial. Rev. Bras. Frutic. 26:264-271.

13. Chaves, M. M. 1991. Effects of water deficits on carbon assimilation. J. Exp. Bot. 42: 1-16.

14. Chaves, M. M., Maroco, J. P., and Pereira, J. S. 2003. Understanding plant responses to
drought - from genes to the whole plant. Func. Plant Biol. 30: 239–264.

15. Christiansen, E., Waring, R. H., and Berryman, A. A. 1987. Resistance of conifers to bark beetle attack: searching for general relationships, For. Ecol. Manage. 22: 89–106.

16. Clérivet, A., Déon, V., Lopez, I. A. F., Geiger, J. P., and Nicole, M. 2000. Tyloses and gels associated with cellulose accumulation in vessels are responses of plane tree

seedlings (Platanus × acerifolia) to the vascular fungus Ceratocystis fimbriata f. sp platani. Trees. 15:25–31.

17. Dhingra, O. D., and Sinclair, J. B. 1995. Basic Plant Pathology Methods. Lewis Publisher, Boca Raton, FL, USA.

18. Dinis, L., Peixoto, F., Zhang, C., Martins, L., Costa, R., and Gomes-Laranjo, J. 2011. Physiological and biochemical changes in resistant and sensitive chestnut (Castanea) plantlets after inoculation with Phytophthora cinnamomi. Physiol. Mol. Plant Pathol. 75:146-156.

19. Doehlert, D. C., and Huber, S. C. 1983. Regulation of spinach leaf sucrose phosphate synthase by glucose-6-phosphate, inorganic phosphate and pH. Plant Physiol. 73: 989-994.

20. Dong, X., Ling, N., Wang, M., Shen, Q., and Guo, S. 2012. Fusaric acid is a crucial factor in the disturbance of leaf water imbalance in Fusarium-infected banana plants. Plant Physiol. Bioch. 60: 171-179.

21. Essmann, J., Schmitz-Thom, I., Schön, H., Sonnewald, S., Weis, E., and Scharte, J. 2008. RNA Interference-Mediated Repression of Cell Wall Invertase Impairs Defense in Source Leaves of Tobacco. Plant Physiol. 147: 1288–1299.

22. Ferreira, F. A.; Milani, D. Diagnose visual e controle das doenças abióticas e bióticas do Eucalipto no Brasil. (Visual diagnosis and control of abiotic and biotic Eucalyptus diseases in Brasil). International Paper, Mogi-Guaçu. 104 p.

23. Freeman, B.C., and Beattie, G. A. An Overview of Plant Defenses against Pathogens and Herbivores. 2008. The Plant Health Instructor. DOI: 10.1094/PHI-I-2008-0226-01.
https://www.apsnet.org/edcenter/intropp/topics/Pages/OverviewOfPlantDiseases.aspx (accessed Nov 2013).

24. Geigenberger, P., and Stitt, M. 1993. Sucrose synthase catalyses a readily reversible reaction in vivo in developing potato tubers and other plant tissues. Planta 189: 329-339.

25. Goicoechea, N., Aguirreolea, J., Cenoz, S., and Garc, J. M. 2000. Verticillium dahliae modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. Eur. J. Plant Pathol. 106: 19–25.

26. Guérard, N., Maillardb, P., Bréchet, C., Lieutiera, F., and Dreyerb, E. 2007. Do trees use reserve or newly assimilated carbon for their defense reactions? A ^{13}C labeling approach with young Scots pines inoculated with a bark-beetle-associated fungus (Ophiostoma brunneo ciliatum). Ann. For. Sci. 64: 601–608.

27. Harada, T., Ishizawa, K. 2003. Starch degradation and sucrose metabolism during anaerobic growth of pondweed (Potamogeton distinctus A. Benn.) turions. Plant Soil 253: 125–135.

28. Harrington, T. C. 2000. Host specialization and speciation in the American wilt pathogen Ceratocystis fimbriata. Fitop. Bras. 25: 262–263.

29. Kaplan, F., and Guy, C. L. 2004. beta-Amylase induction and the protective role of maltose during temperature shock. Plant Physiol. 135: 1674–1684.

30. Krasensky, J., and Jonak, C. 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot. 63: 1593–1608.

31. Lima, A. L. S., DaMatta, F. M., Pinheiro, H. A., Totola, M. and Loureiro, M. E. 2002. Photochemical responses and oxidative stress in two clones of Coffea canephora under water deficit conditions. Environ. Exp. Bot. 47: 239-247.

32. Malik, M. T., Akem, C., Khan, S. M., Khan, M. A., Dasti, A. A., Kazmi, Grewal, A. G., and Awan, M. Z. 2010. Confirmation of the capability of Ceratocystis fimbriata to cause mango sudden death syndrome in Pakistan. Pakistan J. Phytopathol. 22:120-125.

33. Martín, J. A., Solla, A., Domingues, M. R., Coimbra, M. A., and Gil, L. 2008. Exogenous phenol increase resistance of Ulmus minor to Dutch elm disease through formation of suberin-like compounds on xylem tissues. Environ. Exp. Bot. 64: 97–104.

34. Masood, A., and Saeed, S. 2012. Bark beetle, Hypocryphalus mangiferae Stebbing (Coleoptera: Curculionidae: Scolytinae) is a vector of mango sudden death disease in Pakistan. Pakistan J. Bot. 44: 813-820.

35. Masood, A., Saeed, S., Ali, M., and Sajjad, M. A. 2009. Life cycle and biology of mango bark beetle, H. mangiferae, a possible vector of mango sudden death disease in Pakistan. Pakistan J. Zoology. 41: 281-288.

36. Masood, A., Saeed, S., Silveira, S. F., Akem, C. N., Hussain, N., and Farooq, M. 2011. Quick decline of mango in Pakistan: survey and pathogenicity of fungi isolated from mango tree and bark beetle. Pakistan J. Bot. 43:1793-1798.

37. Maxwell, K., and Johnson, G. N. 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51:659-668.

38. Müller-Rober, B., Sonnewald, U., and Willmitzer, L. 1992. Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. EMBO J. 11: 1229-1238.

39. Nogués, S., Cotxarrera, L., Alegre, L., and Trillas, M. I. 2002. Limitations to photosynthesis in tomato leaves induced by Fusarium wilt. New Phytol. 154:461-470.

40. Park, J. H., Juzwi, J., and Cavender-Bare, J. 2013. Multiple Ceratocystis smalleyi infections associated with reduced stem water transport in bitternut hickory. Phytopathology. 103: 565-574.

41. Pinheiro, C., and Chaves, M. M. 2011. Photosynthesis and drought: can we make metabolic connections from available data? J. Exp. Bot. 62: 869–882.

42. Ploetz, R. C. 2003. Diseases of mango. In: Ploetz, R. C., ed. Diseases of Tropical Fruit Crops. CABI Publishing, Wallingford, 327-363.

43. Praxedes, S. C., DaMatta, F. M., Loureiro, M. E., Ferrão, M. A. G., and Cordeiro, A. T. 2006. Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (Coffea canephora Pierre var. kouillou) leaves. Environ. Exp. Bot. 56: 263–273.

44. Rajput, K. S., and Rao, K. S. 2007. Death and decay in the trees of Mango (Mangifera indica L.). Microbiol Res. 162: 229-237.

45. Reid R. W.,Whitney H. S.,Watson J. A. 1967. Reactions of the Lodgepole pine to attack by Dendroctonus ponderosae Hopkins and blue stain fungi. Can. J. Bot. 45: 1115–1125.

46. Ribeiro, I. J. A. 1980. Seca da mangueira: agentes causais e estudo da moléstia. In: Simpósio brasileiro sobre a cultura da mangueira. Anais. UNESP, Jaboticabal, 123-130.

47. Ribeiro, I. J. A. 1997. Doenças da mangueira (Mangifera indica L.). In: Kimati, H., Amorim, L., Bergamin Filho, A., Camargo, L. E. A., Rezende, J. A. M, eds. Manual de fitopatologia: doenças das plantas cultivadas. Agronômica Ceres, São Paulo, 511-524.

48. Ribeiro, I. J. A., Lourenção, A. L., Pilho, O. P., and Soares, N. B. 1984. Seca-da-mangueira. Vii. Resistência de cultivares de mangueira ao fungo Ceratocystis fimbriata Ell. & Halst. Bragantia 43:237-243.

49. Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., and Prado, F. E. 2009. Soluble sugars - Metabolism, sensing and abiotic stress. A complex network in the life of plants. Plant Signal. Behav. 4: 388-393.

50. Trethewey, R.N., Geigenberger, P., Riedel, K., Hajirezaei, M.R., Sonnewald, U., Stitt, M., Riesmeier, J. W., and Willmitzer, L. 1998. Combined expression of glucokinase and invertase in potato tubers leads to a dramatic reduction in starch accumulation and a stimulation of glycolysis. Plant J. 15: 109-118.

51. van Loon, L. C., Rep, M., and Pieterse, C. M. 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44:135-162.

52. van Wyk, M., Pegg, G., Lawson, S., and Wingfield, M. J. 2007. Ceratocystis atrox sp. nov. associated with Phoracanthta acanthocera infestations on Eucalyptus in Australia. Australas. Plant. Path. 36: 407–414.

53. Woodruff, D. R. 2013. The impacts of water stress on phloem transport in Douglas-fir trees. Tree Physiol. 00: 1–10.

54. Yadeta, K. A., and Thomma, B. P. H. J. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Front. Plant. Sci. 4: 1-12.

55. Zrenner, R., Schüler, K., and Sonnewald, U. 1996. Soluble acid invertase determines the hexose-to-sucrose ratio in cold-stored potato tubers. Planta 198: 246–252.

LIST OF TABLES AND FIGURES

Table 1. Maximum quantum efficiency of PSII photochemistry in dark-adapted leaves (F_v/F_m), the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m'), electron transport rate through PSII (ETR) and the non-photochemical quenching (NPQ) determined on leaves of mango plants from cultivars Ubá and Palmer non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata.

Parameters	Ubá				Palmer			
	14 dai		21 dai		14 dai		21 daí	
	NI	I	NI	I	NI	I	NI	I
F_v/F_m	0.80 ± 0.004	0.79 ± 0.002	0.80 ± 0.002	0.79 ± 0.002	0.81 ± 0.001	0.80 ± 0.00	0.82 ± 0.002	0.81 ± 0.081
F_v'/F_m'	0.60 ± 0.01	0.55 ± 0.01	0.59 ± 0.02	0.56 ± 0.01	0.57 ± 0.02	0.60 ± 0.01	0.59 ± 0.01	0.50 ± 0.02 a
ETR	100 ± 4.96	88 ± 2.79	93 ± 4.27	78 ± 5.87	106 ± 5.89	99 ± 8.23	101 ± 5.23	73 ± 8.30 a
NPQ	0.92 ± 0.11	1.31 ± 0.20	1.06 ± 0.17	1.11 ± 0.10	1.07 ± 0.11	0.92 ± 0.11	1.19 ± 0.12	1.57 ± 0.13

Means of NI and I plants within each cultivar followed by the letter a at each evaluation time are significantly different according to the Student's t test ($P \leq 0.05$). The letters b and c indicate differences ($P \leq 0.05$) between cultivars for, respectively, NI and I plants at each evaluation time according to the Student's t test ($P \leq 0.05$). Values are means ± SE. n = 5.

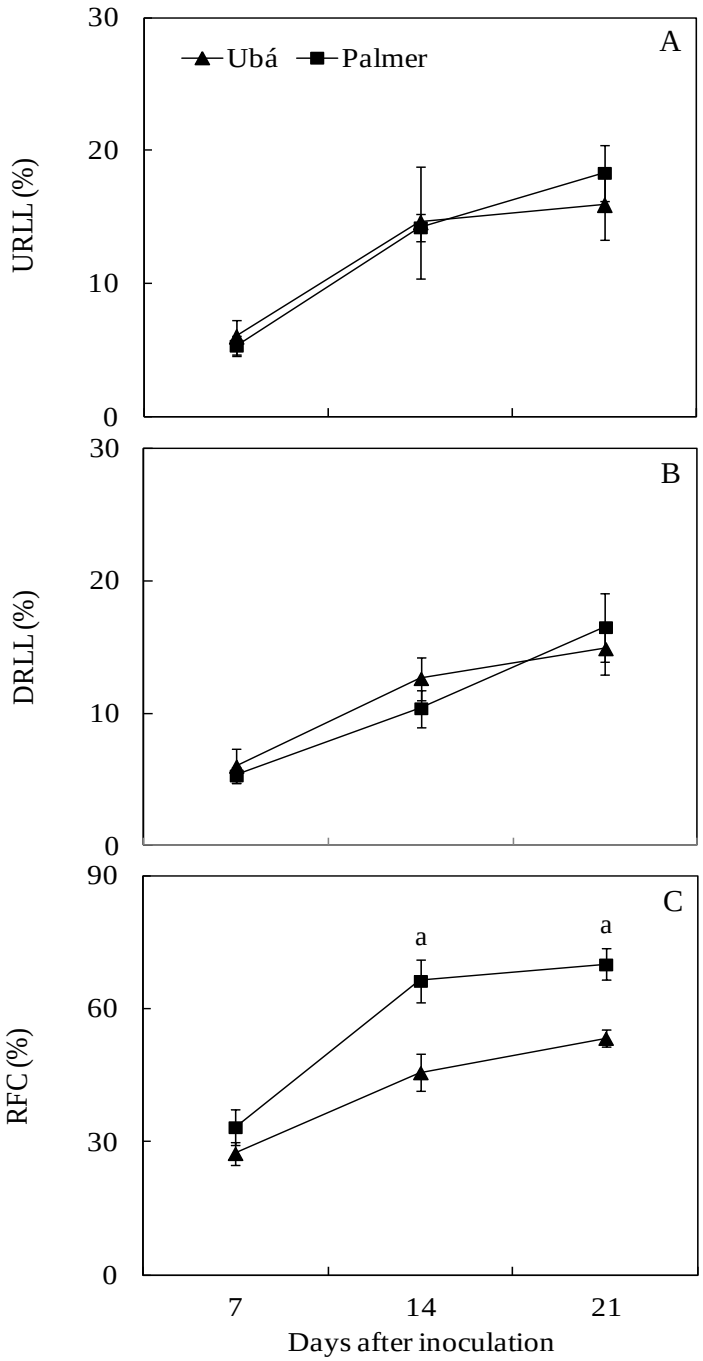

Figure 1. The upward relative lesion length (URLL), downward relative lesion length (DRLL) and the radial fungal colonization (RFC) on the stem tissues of mango plants from cultivars Ubá and Palmer inoculated with Ceratocystis fimbriata. Means followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

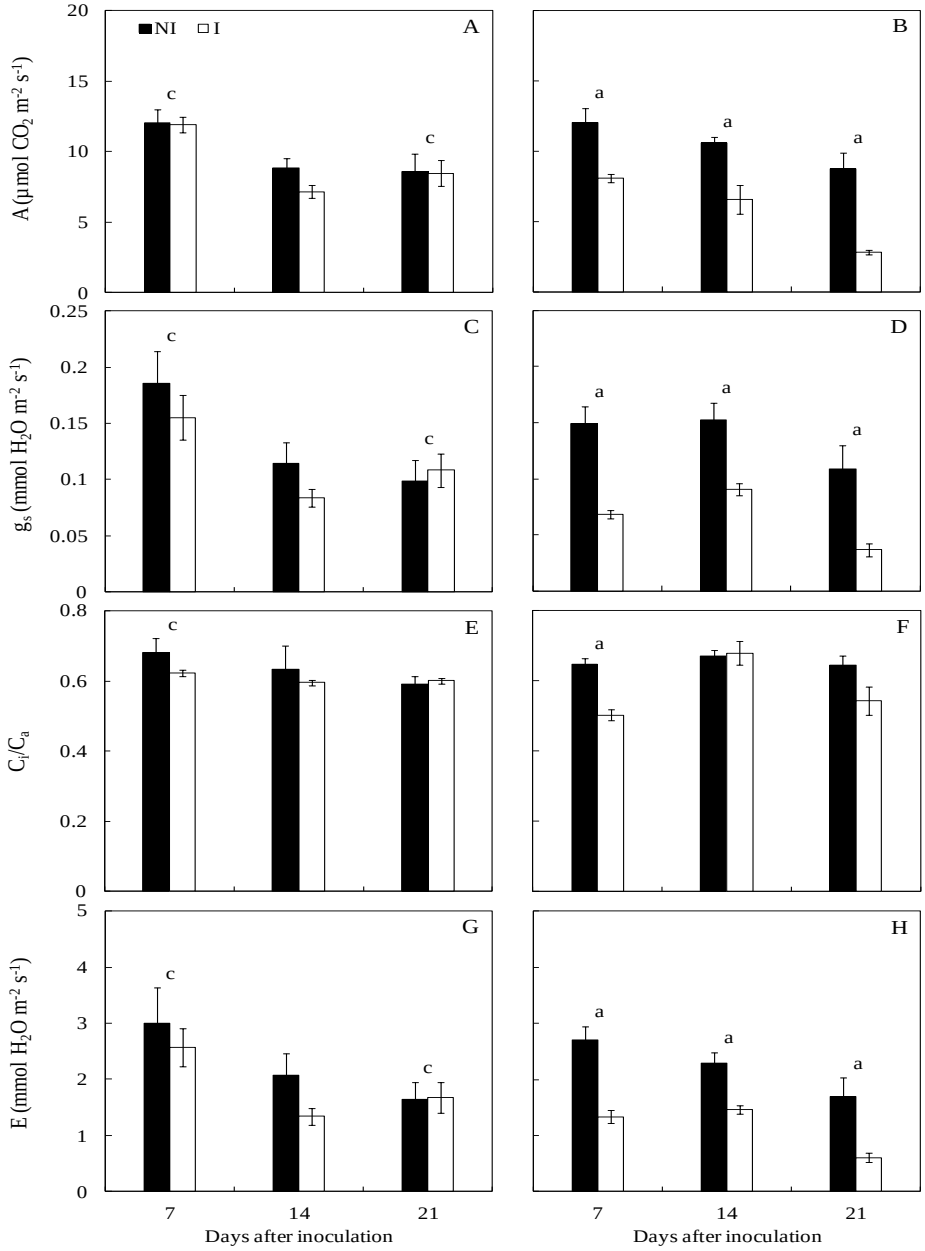

Figure 2. Net carbon assimilation rate (A) (A and B), stomatal conductance to water vapor (g_s) (C and D), internal to ambient CO_2 concentration ratio (C_i/C_a) (E and F) and transpiration rate (E) (G and H) determined in the leaves of mango plants from cultivars Ubá (left column) and Palmer (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences for, respectively, NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

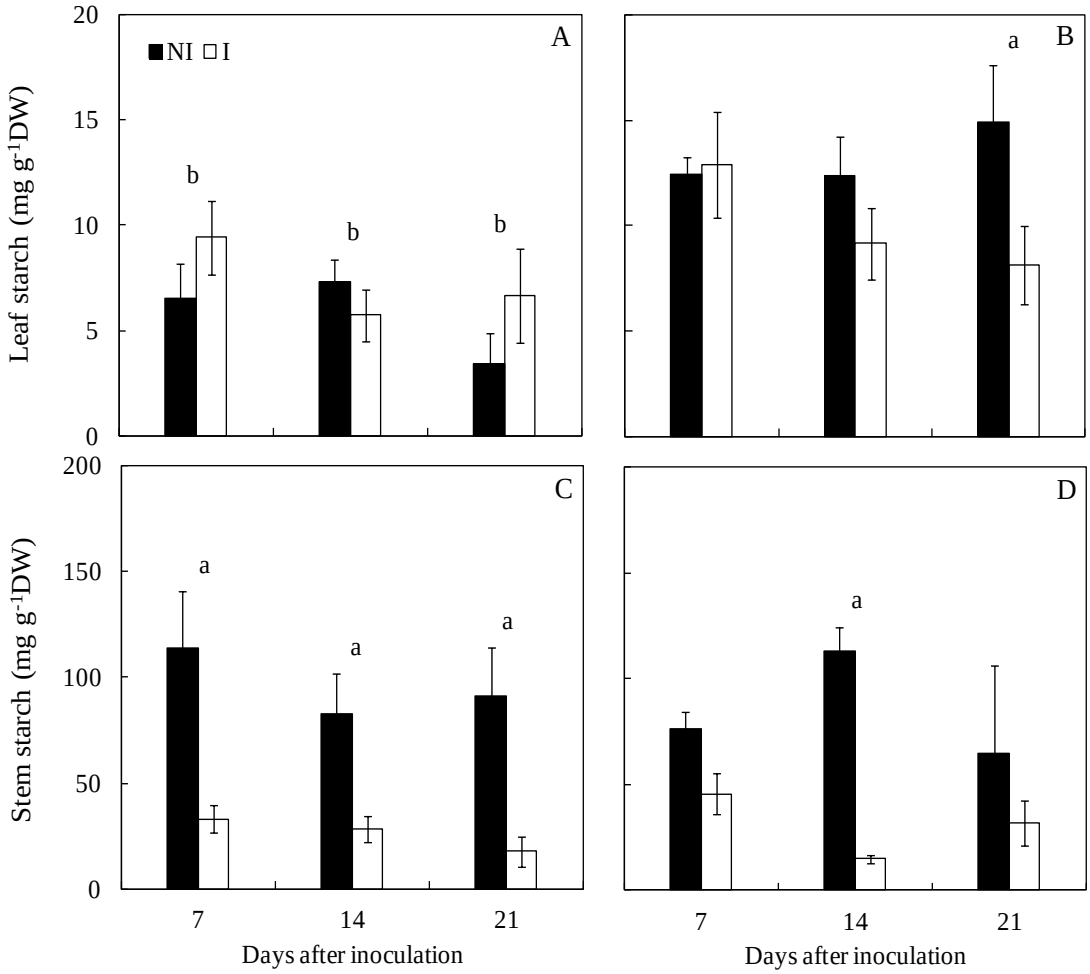

Figure 3. Leaf starch concentration (mg g^{-1} DW) (A and B) and stem starch concentration (mg g^{-1} DW) (C and D) from mango plants from cultivars Ubá (left column) and Palmer (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letter b indicates differences for NI plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

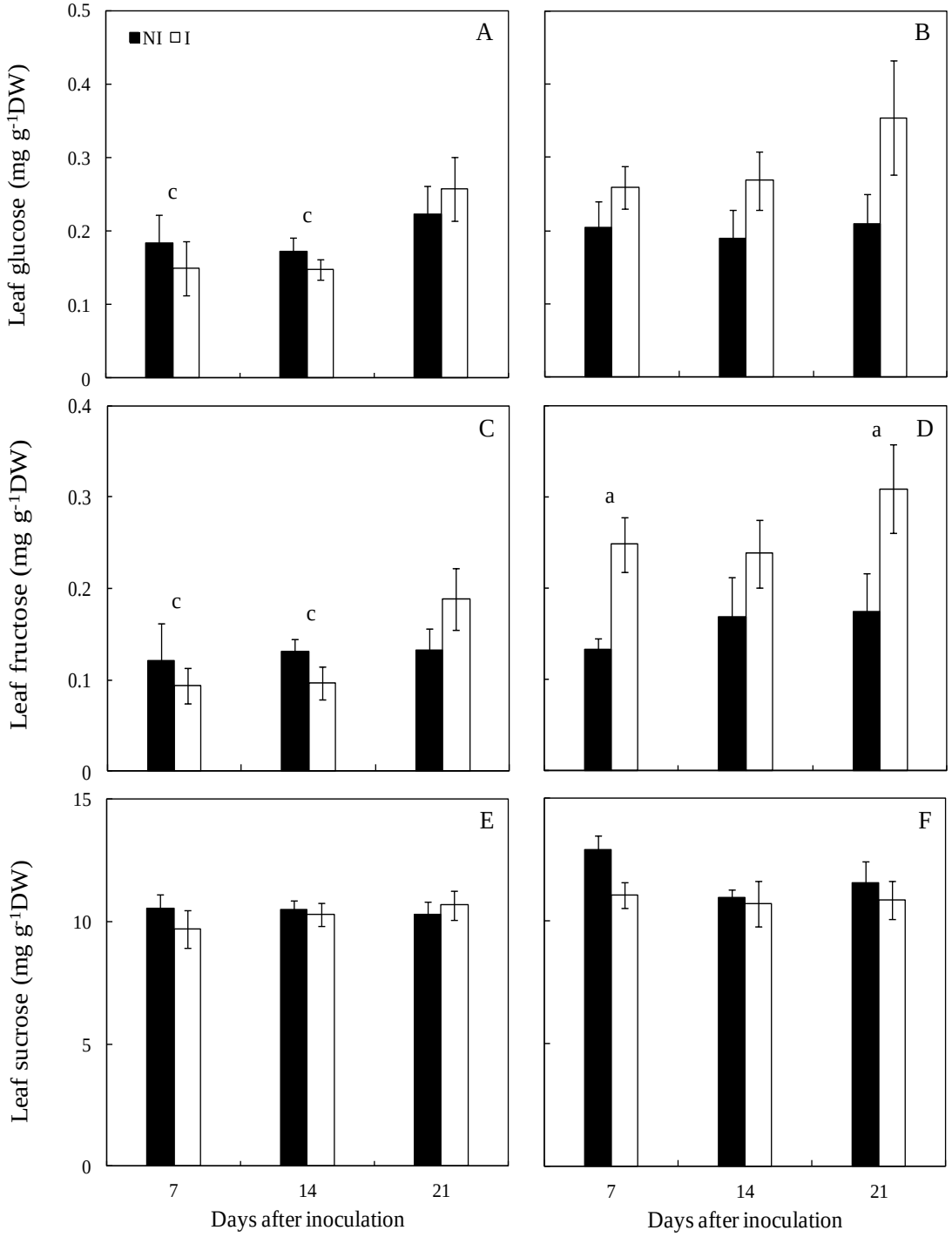

Figure 4. Concentrations of glucose (A and B), fructose (C and D) and sucrose (E and F) (mg g^{-1} DW) on leaves of mango plants from cultivars Ubá (left column) and Palmer (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letter c indicates differences for I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

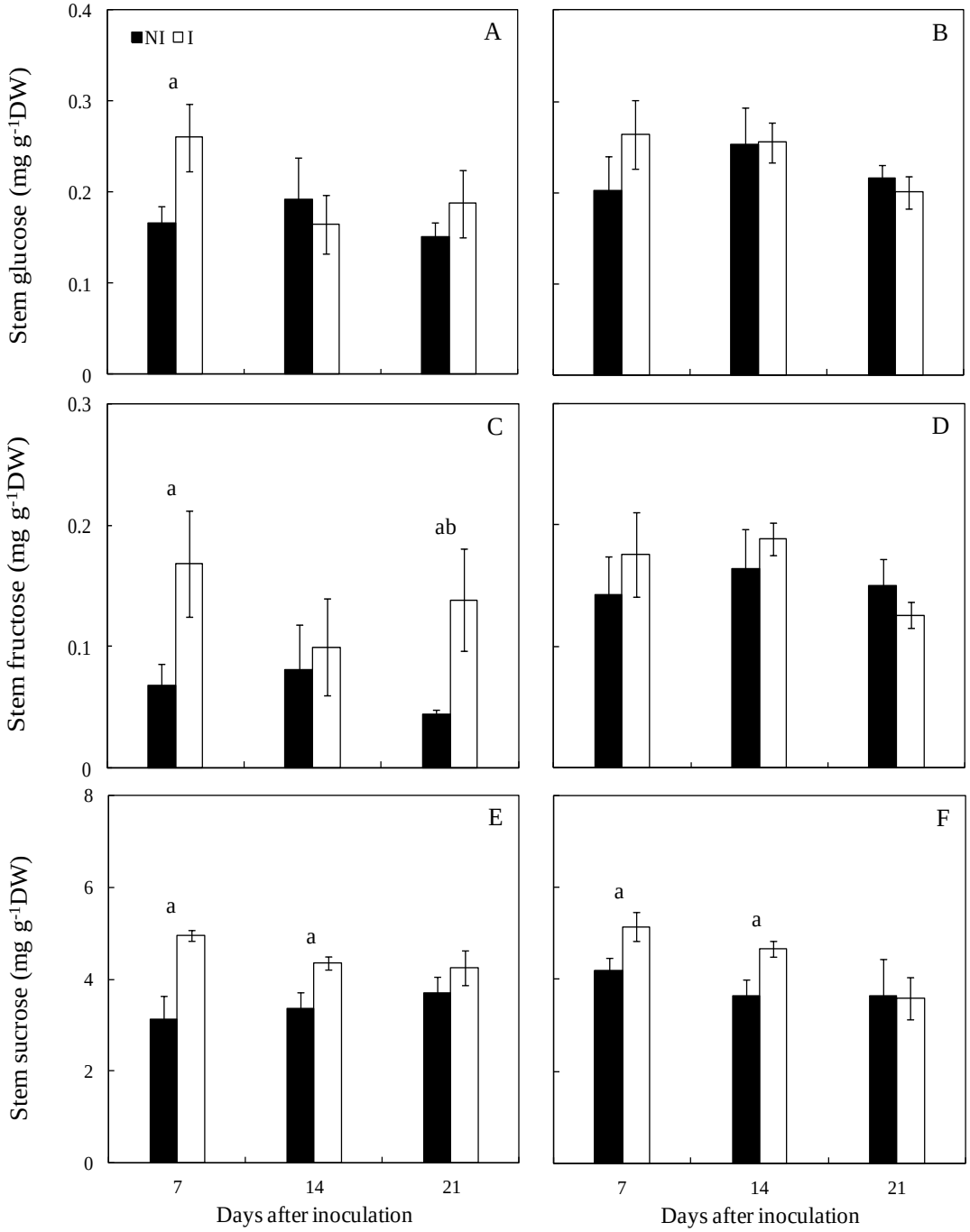

Figure 5. Concentrations of glucose (A and B), fructose (C and D) and sucrose (E and F) (mg g^{-1} DW) on the stem tissues of mango plants from cultivars Ubá (left column) and Palmer (right column) non-inoculated (NI) or inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences respectively for NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

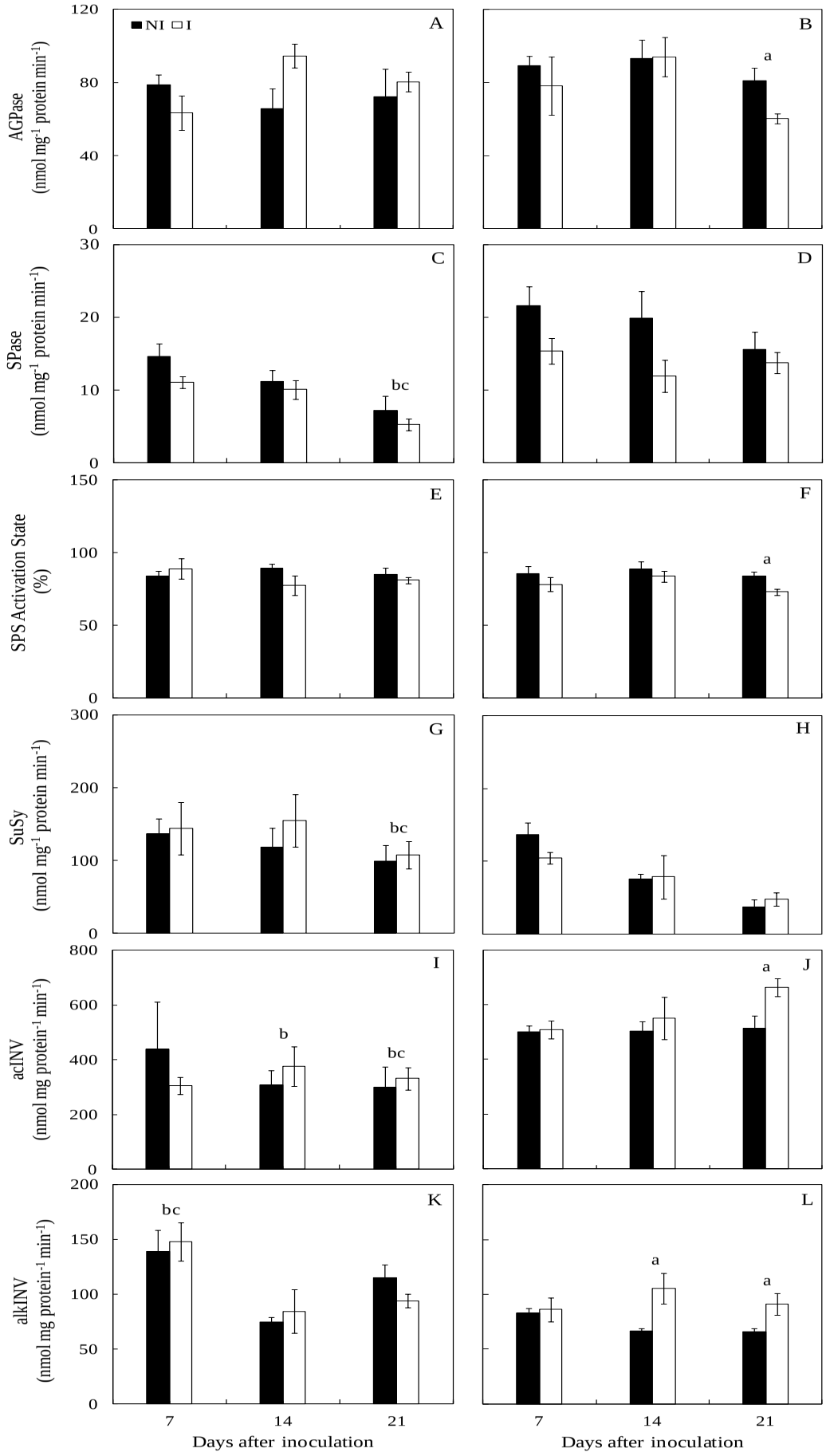
NI I
A
B
C
D
E
F
G
H
I
J
K
L
AGPase (nmol mg-1 protein min-1)
SPase (nmol mg-1 protein min-1)
SPS Activation State (%)
SuSy (nmol mg-1 protein min-1)
acINV (nmol mg protein-1 min-1)
alkINV (nmol mg protein-1 min-1)
a
bc
b
7
14
21
Days after inoculation

Figure 6. Activities of ADP-glucose pyrophosphorylase (AGPase, nmol mg^{-1} protein min^{-1}) (A and B), starch phosphorylase (SPase, nmol mg^{-1} protein min^{-1}) (C and D), sucrose-phosphate synthase activation state (SPS, %) (E and F), sucrose synthase (SuSy, nmol mg^{-1} protein min^{-1}) (G and H), acid invertase (acINV, nmol mg^{-1} protein min^{-1}) (I and J) and alkaline invertase (alkINV, nmol mg^{-1} protein min^{-1}) (K and L) on leaves of mango plants from cultivars Ubá (left column) and Palmer (right column) non-inoculated (NI) and inoculated (I) with Ceratocystis fimbriata. The means of NI and I treatments within each cultivar followed by the letter a at each evaluation time are significantly different according to Student's t test ($P \leq 0.05$). The letters b and c indicate differences for, respectively, NI and I plants between cultivars at each evaluation time according to Student's t test ($P \leq 0.05$). The error bars represent the standard error of the mean. n = 5.

GENERAL CONCLUSION

It can be concluded from the obtained results that the disease symptoms observed on mango plants upon inoculation with Ceratocystis fimbriata developed faster on plants from cv. Tommy Atkins and Palmer, known to be, respectively, moderately resistant and susceptible to the mango wilt. The symptoms appearance were associated with a more pronounced impairment of water relations and alterations in whole plant water status, leading to reduced photosynthetic performance coupled with symptoms of oxidative stress at advanced stages of fungal infection. In sharp contrast, cv. Ubá was better able to act locally at the site of infection, proving to be more efficient in the remobilization of reserves for the promotion of defense mechanisms, postponing the pathogen spread through the stem tissues and the development of the wilt symptoms.